www.TurkJBiochem.com

TÜRK BİYOKİMYA DERGİSİ

Turkish Journal of Biochemistry

ULUSLARARASI BİYOKİMYA KONGRESİ 2023
INTERNATIONAL BIOCHEMISTRY CONGRESS 2023

34. ULUSAL BİYOKİMYA KONGRESİ
34th NATIONAL BIOCHEMISTRY CONGRESS

29 Ekim - 1 Kasım 2023, Fethiye, Türkiye

29 October - 1 November 2023, Fethiye, Türkiye

Türk Biyokimya Derneği'nin yayın organıdır.
[Published by the Turkish Biochemical Society]

2023

Cilt [Volume] 48

Ek Sayı [Supplement] 1

YER ALDIĞI İNDEKSLER [INDEXED BY]

SCI Expanded, Journal Citation Reports/Science Edition, Chemical Abstracts, Index Copernicus, Embase, Scopus, Ulakbim Türk Tıp Dizini, Ulrich's Periodical Directory, EBSCO, Türkiye Atıf Dizini

2023 · VOLUME 48 · ISSUE 6

TURKISH JOURNAL OF BIOCHEMISTRY
TÜRK BİYOKİMYA DERGİSİ

OFFICIAL JOURNAL OF THE TURKISH BIOCHEMICAL SOCIETY

DE GRUYTER

Turkish Journal of Biochemistry (TJB), official journal of Turkish Biochemical Society, is issued electronically every 2 months. Research articles, reviews, short communications, technical reports, case presentations, opinions, and letters to the editor, that have not published elsewhere, on biochemistry, clinical biochemistry, molecular biology, molecular genetics, biotechnology, bioinformatics, bioengineering, and their educational disciplines are published in the journal.

The main aim of the journal is to support the research and publishing culture by ensuring that every published manuscript has an added value and thus providing international acceptance of the "readability" of the manuscripts published in the journal.

ABSTRACTED/INDEXED IN Baidu Scholar · Cabells Journalytics · CABI (over 50 subsections) · Chemical Abstracts Service (CAS): CAplus; SciFinder · CNKI Scholar (China National Knowledge Infrastructure) · CNPIEC: cnpLINKer · Dimensions · DOAJ (Directory of Open Access Journals) · EBSCO (relevant databases) · EBSCO Discovery Service · EMBASE · FSTA: Food Science & Technology Abstracts · Genamics JournalSeek · Google Scholar · Index Copernicus · Japan Science and Technology Agency (JST) · J-Gate · Journal Citation Reports/Science Edition · JournalGuide · JournalTOCs · KESLI-NDSL (Korean National Discovery for Science Leaders) · MyScienceWork · Naver Academic · Naviga (Softweco) · Primo Central (ExLibris) · Publons · QOAM (Quality Open Access Market) · ReadCube · Scilit · SCImago (SJR) · SCOPUS · Semantic Scholar · Sherpa/RoMEO · Summon (ProQuest) · TDNet · TEMA Technik und Management · TÜBITAK/ULAKBIM · TÜRK MEDLINE-ULUSAL ATIF INDEKSI ve TÜRKIYE ATIF DIZINI · Ulrich's Periodicals Directory/ulrichsweb · WanFang Data · Web of Science: Science Citation Index Expanded · WorldCat (OCLC) · X-MOL · Yewno Discover

e-ISSN 1303-829X

All information regarding notes for contributors, subscriptions, open access, back volumes and orders is available online at www.degruyter.com/view/j/tjb.

RESPONSIBLE EDITOR Doğan Yücel PhD, Professor of Biochemistry, Department of Medical Biochemistry, Faculty of Medicine, University of Lokman Hekim, Söğütözü Mh. 2179 Cd. No:6, Çankaya, 06510, Ankara, Turkiye, Tel: +90 4448548, Email: doyucel@yahoo.com

JOURNAL COORDINATOR Heike Jahnke, De Gruyter, Genthiner Straße 13, 10785 Berlin, Germany. Tel.: +49 (0) 30 260 05–220, Email: heike.jahnke@degruyter.com

ADVERTISEMENTS e-mail: anzeigen@degruyter.com

TYPESETTING TNQ Technologies, Chennai, India

CONTENTS

WELCOME MESSAGE

Dear Colleagues, Dear Friends,

As a nation, we are going through a very tough period. We lost almost fifty thousand of our citizens in the Kahramanmaraş centered earthquakes. We are carrying tens of thousands wounded, still in treatment. Against all efforts, shortage of shelter, food, hygiene and production is still a big problem. We hope that the wounds will be healed quickly and the suffering will end soon.

On the other hand, life goes on despite this great disaster. We will be organizing our 34th congress, as an international congress before, on the 100th anniversary of our republic, starting with the celebration of the 100th Republic Day between 29 October – 1 November 2023. We have selected Liberty Lykia Hotel in Fethiye/Muğla as our congress center.

As usual we will provide scholarship for our young colleagues who actively attend the congress and also work on providing even more advantages for our young colleagues affected from the earthquakes. The abstracts will be published in the Turkish Journal of Biochemistry as a supplement indexed in SCI-E.

We invite you to join us in International Biochemistry Congreess 2023 // 34th National Biochemistry Congress to share the extensive scientific program, to get together in this nice corner of our country and celebrate the 100th anniversary of the Republic of Turkiye.

Best regards,

Dr. Doğan Yücel
President, Turkish Biochemical Society

HONORARY PRESIDENTS

- Nazmi Ozer
- Serafettin Ozkurt

ORGANIZING COMMITTEE

- Doğan Yücel - Kongre Başkanı
- Ferhan Girgin Sağın - II. Başkan
- Oytun Portakal - Genel Sekreter
- Mehmet Şenes - Sayman
- Aylin Sepici Dinçel
- Ali Ünlü
- Muhittin A. Serdar
- Oğuzhan Zengi
- Güneş Özhan
- Hilal Koçdor
- Abdullah Tuli
- Abdurrahman Coşkun
- Sedef Yenice

SCIENTIFIC COMMITTEE

- Fehime Aksungar
- Diler Aslan
- Banu Başok
- Berrin Berçik İnal
- Serkan Bolat
- Murat Cihan
- Abdurrahman Coşkun
- Hikmet Can Çubukçu
- Z. Günnur Dikmen
- Halef Okan Doğan
- Zübeyde Erbayraktar
- Ferhan Girgin Sağın
- Sergei M. Gryaznov
- Fatih Gültekin
- Gökhan Hotamışlıgil
- Hüseyin Kayadibi
- Deryanur Kılıç
- Hilal Koçdor
- Giusseppe Lippi
- Qing H. Meng
- Kristin Moberg Aakre
- Tomris Özben
- Bilgen Özdemir
- Habib Özdemir
- Güneş Özhan
- Aysun Pabuçcuoğlu
- Mario Plebani
- Oytun Portakal
- Ebru Saatçi
- Konrad Sandhoff
- Roger Sandhoff
- Aylin Sepici Dinçel
- Muhittin Serdar
- Çiğdem Sönmez
- Mehmet Şeneş
- Deniz İlhan Topçu
- Abdullah Tuli
- Kamil Taha Uçar
- Ali Ünlü
- Sedef Yenice
- Fatma Hümeyra Yerlikaya
- Fatma Meriç Yılmaz
- Doğan Yücel
- Oğuzhan Zengi
- Figen Zihnioğlu

SCIENTIFIC PROGRAM

October 29th, Sunday

09:00-16:00 Workshops

17:00-17:45 Opening Ceremony
Chair: Z. Günnur Dikmen
Hacettepe University, Türkiye

Doğan Yücel
Turkish Biochemical Society President Lokman Hekim University, Türkiye
Tomris Özben
EFLM President, IFCC President-Elect Akdeniz University, Türkiye

17:45-19:00 IUBMB Plenary Lecture
Chair: Z. Günnur Dikmen
Hacettepe University, Türkiye
Subcellular Molecular Architecture as a Critical Determinant of Metabolic Programming
Gökhan Hotamışlıgil
Harvard T.H. Chan School of Public Health, USA

21:00-23:30 100th Anniversary Celebration of the Turkish Republic
Arnna Event Hall

SCIENTIFIC PROGRAM

October 30th, Monday

09:00-10:30 Biological Clocks and Chronobiology Panel
Chairs: Aysel Özpınar, Alev Kural

09:00-09:25 Rhythmic and Non-rhythmic Variations of Laboratory Measurands
Abdurrahman Coşkun
Acıbadem Mehmet Ali Aydınlar University, Türkiye

09:20-09:50 New Target "Circadian Rhythm" for Treatment of Various Diseases
İbrahim Halil Kavaklı
Koç University, Türkiye

09:50 - 10:15 Harnessing Biological Clock for Biotechnological Applications
Nuri Öztürk
Gebze Technical University, Türkiye

10:15 - 10:30 Q & A

10:30 - 11:15 Keynote Lecture 1
Chair: Muhittin Serdar
Acıbadem Mehmet Ali Aydınlar University, Türkiye
Integrated Diagnostics: The Future of Laboratory Medicine
Mario Plebani
University of Padova, Italy

11:15 - 11:45 Coffee Break

11:45 - 13:15 New Therapeutic Approach to Pan-Cancer Treatment: Direct Telomer-Targeting Molecules and Immune Check Point Inhibitors
Chairs: Z. Günnur Dikmen, Hilal Koçdor

11:45 - 12:10 The Role of Immunotherapy in the Treatment of Lung Cancer from the Clinician's Perspective
Saadettin Kılıçkap
İstinye University, Türkiye

12:10 - 12:35 Targeting Immune Resistance in Lung Cancer
Esra Akbay
The University of Texas, USA

12:35 - 13:00 Telomerase-Driven Telomeric DNA Modification as Potential Broad Cancer Treatment Platform
Sergei M. Gryaznov
MAIA Biotechnology, USA

SCIENTIFIC PROGRAM

October 30th, Monday

13:00 - 13:15 Q & A

13:15 - 14:30 Lunch Break

14:30 - 15:15 Satellite Symposium
Chair: Cihan Coşkun

14:30 - 15:15 Evaluation of Primary Hemostasis in Patients with Bleeding Diathesis
Reyhan Küçükkaya
Istanbul Bilim University, Turkey

16:00 - 16:15 Coffee Break

16:15 - 17:00 Keynote Lecture 3
Chair: Sedef Yenice
Gayrettepe Florence Nightingale Hospital, Türkiye
Current Advances in Clinical Application of Tumor Biomarkers: Utility, Challenge and Perspective
Qing H. Meng
University of Texas, USA

17:00 - 17:45 Satellite Symposuim
Chair: Mehmet Şeneş
Ankara Teaching and Research Hospital, Türkiye

17:00 - 17:45 Standardization and Harmonization in Immunoassays: Quality and Comparability are Needed
Mario Plebani
University of Padova, Italy

20:30 - 21:30 Prof. Emrah Safa Gürkan

SCIENTIFIC PROGRAM

October 31st, Tuesday

09:00 - 10:30 Updates to Clinical and Medical Laboratory Guidelines and International Standards
Chairs: Sabahattin Muhtaroğlu, Mutay Aslan

09:00 - 09:25 Current Update of the IFCC Committee on Clinical Application of Cardiac Biomarkers Educational Recommendations
Kristin Moberg Aakre
University of Bergen, Norway

09:25 - 09:40 The Risks and Opportunities Presented by ISO 15189:2022 towards Achieving Continuous Improvement in Medical Laboratory Practices
Canan Karadağ
Eskişehir City Hospital, Türkiye

09:40 - 09:55 Charting the Path from Past Studies to a Visionary Future with the New Structure of the EFLM Quality and Regulation Committee
Hikmet Can Çubukçu
The Ministry of Health, Health Services General Directorate, Türkiye

09:55 - 10:10 Overview of Recent CLSI Guidelines: Enhancing Laboratory Medicine Practices
Sedef Yenice
Gayrettepe Florence Nightingale Hospital, Türkiye

10:10 - 10:30 Q & A

10:30 - 11:15 Keynote Lecture 4
Oturum Başkanı: Oytun Portakal
Harmonizing The Post-Analytical Phase
Mario Plebani
University of Padova, Italy

11:15 - 11:45 Coffee Break

11:45 - 13:15 Integrative Approaches to Molecular Interactions and Drug Design
Chairs: Özlem Dalmızrak, Güneş Özhan

11:45 - 12:10 The Role of Protein-structure-function Relationship in Disease Pathogenesis in Autoimmune Diseases: Example of AS
Günseli Bayram Akçapınar
Acıbadem Mehmet Ali Aydınlar University, Türkiye

12:10 - 12:35 How AI Will Revolutionize Structure-based Drug Design
Ezgi Karaca
İzmir Biomedicine and Genome Center, Türkiye

SCIENTIFIC PROGRAM

October 31st, Tuesday

12:35 - 13:00 The Bright Future of Structural Biology in Türkiye
Hasan Demirci
Koç University, Türkiye

13:00 - 13:15 Q & A

13:15 - 14:30 Lunch Break

14:30 - 15:15 Satellite Symposium
Chair Erhan Palaoğlu
American Hospital, Türkiye
Digital Transfomation in Clinical Laboratories : Reliable Sample Monitoring, Efficient Data Analysis, Improved TAT and Clinical Benefits
Oğuzhan Zengi
İstanbul Başakşehir Çam ve Sakura City Hospital, Türkiye
Said İncir
Koç University, Türkiye

15:15 - 16:15 Forum 1
Residency Training in Türkiye: Before, During and After
Ali Ünlü
Selçuk University, Türkiye
Z. Günnur Dikmen
Hacettepe University, Türkiye

16:15 - 16:30 Coffee Break

SCIENTIFIC PROGRAM

October 31st, Tuesday

16:30 - 17:15 Satellite Symposium
Chair: Filiz Akbıyık
Ankara Bilkent City Hospital Lab., Türkiye
Tomorrow's Labs: Innovations & Trends
Fatih Küçükali
Siemens Healthineers, Türkiye
Ekrem Yıldırım
Siemens Healthineers, Türkiye

17:15 - 18:45 Forum 2
Young Investigator Roadmap
Chairs: Ferhan Girgin Sağın, Aylin Sepici Dinçel
Career Development for Young Scientist In Clinical Biochemistry and Laboratory Medicine
Qing H. Meng
University of Texas, USA
Establish Your Own Research - A Roadmap for Young Investigators
Kristin Moberg Aakre
University of Bergen, Norway
What FEBS Offers to Young Scientists?
Ferhan Girgin Sağın
Chair, FEBS Education Committee
Ege University, Türkiye

SCIENTIFIC PROGRAM

November 1st, Wednesday

09:00 - 10:30 New Laboratory Techniques
Chairs: F. Hümeyra Yerlikaya Aydemir, Mine Ergüven

09:00 - 09:25 Biochemical, Genetic and Phenotypic Characterization of Lysosomal Sulfatide Degradation Disorders
Asuman Özkara
Hacettepe University, Türkiye

09:25 - 09:50 Skin Barrier Ceramide Metabolism in Health and Disease
Roger Sandhoff
German Cancer Research Center, Germany

09:50 - 10:15 NMR in Clinical Laboratory Diagnostics: Lipoprotein Profile Analysis
Mustafa Serteser
Acıbadem Mehmet Ali Aydınlar University, Türkiye

10:15 - 10:30 Q & A

10:30 - 11:15 Keynote Lecture 6 - FEBS National Lecture
Chair: Asuman Özkara
Hacettepe University, Türkiye
My Journey into the Field of Sphingolipids and Sphingolipidosis
Konrad Sandoff
Bonn University, Germany

11:15 - 11:45 Coffee Break

11:45 - 12:30 Satellite Symposium
Chair: Berrin Berçik İnal
DxI 9000 as a New Beckman Coulter's Approach to Immunochemistry – Dubrava University Hospital Validation Experience And Results
Marko Žarak
Dubrava University Hospital, Croatia

12:30 - 13:15 Keynote Lecture 7
Chair: Pınar Eker
Secrets and Mysteries in the Preanalytical Phase
Giusseppe Lippi
University of Verona

SCIENTIFIC PROGRAM

November 1st, Wednesday

12:30 - 13:15 ARTED - Association of Research Based Medical Technologies Manufacturers Session (Hall B)
Digital World in Laboratory Medicine
Murat Cihan
Ordu University, Türkiye

13:15 - 14:30 Lunch Break

14:30 - 16:00 Where Are We Going in Diagnosis, Follow-up and Treatment?: Current and Near Future
Chairs: Diler Aslan, Mehmet Hicri Köseoğlu

14:30 - 14:55 The Future of Medicine and Health Sciences: The Invasion of Engineering
Engin Ulukaya
İstinye University, Türkiye

14:55 - 15:55 Clinical Decision Tools in the New Era of Healthcare
Value of Clinical Laboratories in Clinical Decision Making
Merve Sibel Güngören
MedxThera Consultancy, Türkiye
Integrated Diagnostics Approach and Radiology
Ali Murat Koç
İzmir Katip Çelebi University, Türkiye
Clinical Decision Support Systems and Integrated Diagnostics
Deniz İlhan Topçu
HSU İzmir Tepecik Teaching and Research Hospital, Türkiye

15:55 - 16:00 Q & A

16:00 - 16:30 Coffee Break

16:30 - 18:00 Nazmi Özer Science Awards and Closing Ceremony
Chair: Ferhan Girgin Sağın
Chair, FEBS Education Committee
Ege University, Türkiye
Science Award Winner's Presentation
Award Ceremony
- Nazmi Özer Science Award
- FEBS Open Bio Poster Award
- TJB Innovation Award
- TBS2023 Poster Award

Closing Ceremony

ORAL PRESENTATION PROGRAM

October 30th, Monday

09:00-10:30 Session 1 - Hall B - Chair: Kübranur Ünal, Serkan Bolat

S001
THE EFFECT OF AEROBIC EXERCISE ON SERUM IRISIN AND PREPTINE LEVELS İN OVERWEIGHT SEDENTARY WOMEN
Oğuzhan Özcan, Serdar Doğan, Güner Çiçek, Filiz Kaçmaz, Hamdi Oğuzman, Abdullah Arpacı

S002
THE STUDY OF THE PLASMA AMINO ACIDS PROFILE IN PATIENTS WITH HASHIMOTO THYROID DISEASE
Nihayet Bayraktar, Mehmet Ali Eren, Mustafa Bayraktar

S003
TRIMETHYLAMINE N-OXIDE AND LIPOPOLYSACCHARIDE BINDING PROTEIN AS POTENTIAL BIOMARKERS IN VITAMIN D, VITAMIN B12, AND IRON DEFICIENCY
Sadinaz Akdu, Ummugulsum Can, Serdar Şahinoğlu

S004
THE EFFECT OF FUNCTIONAL TRAINING ON INSULIN, HDL, AND LDL LEVELS IN SEDENTARY WOMEN
Sedat Özdemir, Musa Şahin

S005
EVALUATION OF THE RELATIONSHIP BETWEEN HBA1C AND VITAMIN D LEVELS IN TYPE 2 DIABETES AND PREDIABETIC CASES
Marwa Abdelmageed, Figen Güzelgül, Merve Çatak

S006
INVESTIGATION OF THE EFFECT OF KRILL OIL ON IRISIN AND UCP1 LEVELS IN A HIGH-FAT DIET-INDUCED OBESE RAT MODEL
Esra Pınarbaş, Eda Yılmaz Kutlu, Ayşegül Sümer, Hülya Kılıç Yılmaz

S007
THE TEST THAT TOUCHED HUMAN LIFE ‘’TETRAHYDROBIOPTERIN LOADING TEST IN HYPERPHENYLALANINEMIA’’
Ayla Yıldız, Merve Aslantas, Hasan Onal

S008
ANTIGLICATION EFFECT OF PYRIDOXAMINE, COMBINATION WITH CHILI PEPPER EXTRACT AND CAPSAICIN IN STZ-INDUCED DIABETIC RATS
Cemal Nas, Nesrin İnceören, Göksel Kızıl, Feryal Zeliha Akay, Bircan Çeken Toptancı, Murat Kızıl

ORAL PRESENTATION PROGRAM

October 30th, Monday

S009
COMPARISON OF GENE EXPRESSIONS IN INDIVIDUALS WITH G6PD MEDITERRANEAN MUTATION AND HEMOLYTIC ANEMIA
Başak Günaştı, Abdullah Tuli

S060
EVALUATION OF IMMUNOLOGICAL TESTS PERFORMED BEFORE KIDNEY TRANSPLANTATION
Basak Celtikci

S057
MEASURING THE INHIBITION ACTIVITY OF HYPERGLYCEMIC ENZYMES WITH ZnO NANOPARTICLES
Nilgün Güler

09:00 - 10:30 Session 2 - Hall C - Chair: Deniz İlhan Topçu, Hikmet Can Çubukçu

S013
DEVELOPMENT OF LC-MS/MS BASED METHODS FOR 2ND TIER ANALYSIS OF SUAC, MMA AND BIOMARKERS OF MSUD
Murat Emrah Mavis, Gokce Goksu Gursu, Sule Yalcin

S014
THE EFFECT OF EDTA ON FLOW CYTOMETRY CROSSMATCH OUTCOMES
Rasime Derya Güleç, Fatma Demet Arslan

S015
REVIEW OF THE DIAGNOSIS OF DIABETES ACCORDING TO GLUCOSE MEASUREMENT UNCERTAINTY
Öznur Asil, Kaan Kuzu, Giray Bozkaya

S016
COMPARISON OF CREATININE LEVELS MEASURED BY JAFFE AND LC/MS/MS METHOD
Saadet İbiş, Turan Akdağ, Ali Ünlü, Hüseyin Dost, Menekşe Kuzu, Firdevs Sak

S017
INTERCHANGEABILITY OF K3-EDTA AND SODIUM CITRATE TUBES FOR HBA1C MEASUREMENT BY CAPILLARY ELECTROPHORESIS
Semih Tek, Kamil Taha Uçar

S018
THE PERFORMANCE ASSESSMENT OF HEMOGRAM PARAMETERS: SIGMA VALUES WITH DIFFERENT TOTAL ALLOWABLE ERROR GOALS
Mehmet Akif Bildirici

ORAL PRESENTATION PROGRAM

October 30th, Monday

S019
EVALUATION OF PERFORMANCE CHARACTERISTICS OF ROCHE COBAS T511 COAGULATION DEVICE
Şükran Bıçakcı, Semih Fazlı Kayahan, Ayşe Betül Demir, Mehmet Şeneş

S020
ANALYTICAL VERIFICATION OF THE HEMATOLOGY ANALYZER DYMIND DH615
Raziye Yıldız, Muammer Yücel

S021
CALCULATION OF MEASUREMENT UNCERTAINTY OF SERUM IMMUNOGLOBULINS ACCORDING TO ISO/TS 20914 GUIDELINE
Emine Feyza Yurt, Medine Alpdemir

S022
EVALUATION OF THE DIAGNOSTIC USEFULNESS OF TRIGLYCERIDE GLUCOSE INDEX AS A GLYCEMIC CONTROL MARKER
Muzaffer Katar, Osman Demir

S023
DETERMINATION OF MEASUREMENT UNCERTAINTY FOR ESTIMATED SERUM OSMOLALITY BASED ON DATA OBTAINED FROM DIFFERENT ANALYSERS (ISO/TS 20914)
Mehmet Fatih Alpdemir, Sezen Tutar

S024
METHOD VERIFICATION AND METHOD COMPARISON FOR ELECTROCHEMILUMINESCENCE AND LC-MS/MS METHODS FOR SALIVARY CORTISOL MEASUREMENT
Fatih Serin, Medine Alpdemir, Mehmet Şeneş

11:45 - 13:15 Session 3 - Hall B - Chair: Emre Avcı, Medine Alpdemir

S025
EFFECT OF ANTIRERETROVIRAL THERAPY ON LYMPHOCYTE SUBGROUP AND CD38 AND HLADR IN HIV PATIENTS
Gamze Keşre, Hamdi Oğuzman, Mehmet Çabalak, Oğuzhan Özcan

S026
IDENTIFICATION OF THERAPEUTIC MOLECULES THAT WILL INTERACT COVALENTLY AGAINST SARS-COV2 IN SILICO APPROACHES
Murat Serilmez, Serdar Durdağı

ORAL PRESENTATION PROGRAM

October 30th, Monday

S027
ADENOSİNE DEAMİNASE: AS AN İNFLAMMATORY AND PROGNOSTİC MARKER İN THEEARLY PHASE OF THE DİSEASE İN COVID-19 PATİENTS?
Senay Eren, Gulcin Alp Avcı, Mustafa Eren, Emre Avcı

S028
THE EFFECT OF TAURINE ON PRESEPSIN AND INTERLEUKIN-6 LEVELS IN A LIPOPOLYSACCHARIDE-INDUCED SEPSIS MODEL
Yasemin Atıcı, Nida Aslan Karakelle, Safiye Göçer

S029
RETURNED ASSESSMENT OF SERUM VITAMIN B12 LEVELS OF PATIENTS DIAGNOSED WITH COVID-19
Gökçe Güven Açık, Burcu Gürer Giray

S030
CHANGES OF PLATELET-RELATED BIOMARKERS IN PULMONARY TUBERCULOSIS
Muammer Özdemir, Aslı Şule Tıpırdamaz Yurteri, Sedat Abuşoğlu

S031
DISCOVERY OF FIRST-IN-CLASS PLASMODIUM OTU INHIBITORS AND UNVEILING NOVEL PATHWAYS
Pınar Siyah, Serdar Durdağı, Fatih Kocabaş

S033
THE ROLE OF MICROBIOTA AND OXIDATIVE STRESS IN FOOD ALLERGIES
Gulcin Alp Avcı, Ulku Irem Yılmaz, Emre Avcı, Cumhur Bılgı

S035
PLASMA LIPID PROFILE IS ALTERED IN CHRONIC MIGRAINE PATIENTS AND CORRELATED WITH MIGRAINE RELATED DISABILITY
Hale Gök Dağıdır, Merve Hilal Ceren Akgör, Doğa Vurallı, Hayrunnisa Bolay Belen

S036
INVESTIGATION OF THE EFFECTS OF WHITE TEA ON THE DEVELOPMENT OF ATEROSCLEROSIS IN MICE FEED WITH ATEROGENIC DIET
Merve Hüner Yiğit, Mehtap Atak, Ertuğrul Yiğit, Tolga Mercantepe, Mehmet Kıvrak, Hüseyin Avni Uydu

ORAL PRESENTATION PROGRAM

October 30th, Monday

11:45 - 13:15 Session 3 - Hall C - Chair: Hamit Hakan Alp, Mehmet Fatih Alpdemir

S037
INVESTIGATION OF BCL11A (rs1427407) and HMIP (rs9399137) SNPs ASSOCIATED WITH HIGH HbF LEVELS IN β-THALASSEMIA
Yasemin Özküçük, Ebru Dündar Yenilmez, Göksel Leblebisatan, Abdullah Tuli

S039
THE IMPORTANCE OF PANEL DESING FOR FLOW CYTOMETRY
Cigdem Sonmez, Barıs Boral, Nuran Ahu Baysal

S040
FLOW CYTOMETRIC DIAGNOSIS OF MULTIPLE MYELOMA: A CASE REPORT
Alpaslan Öztürk, Lale Aydın Kaynar

S041
EVALUATION OF HEMOGLOBINOPATHIES WITH CAPILLARY ZONE ELECTROPHORESIS
Nazife Doğan

S042
THE IMPACT OF TRANSPORTING TUBES WITH A PNEUMATIC SYSTEM ON ROUTINE COAGULATION TESTS
Nur Benil Yabacı, Feyza Yağmur Tekeli, Seçkin Özgür Tekeli

S043
THE EFFECTS OF THE TRANSPORT CONDITION ON STEM CELLS
Oğuzhan Zengi

S045
LINEAR REGRESSION MODELS CAN PREDICT THE DIRECT LDL-C TEST LEVELS WITH 74% ACCURACY: MACHINE LEARNING-BASED COMPUTING OF CHOLESTEROL
Süheyl Uçucu

S046
EVALUATION OF KNOWLEDGE LEVELS, ATTITUDES AND BEHAVIOURS OF MEDICAL BIOCHEMISTRY SPECIALISTS ABOUT GREEN AND SUSTAINABLE MEDICAL LABORATORY
Emine Feyza Yurt, Medine Alpdemir, Mehmet Şeneş

S047
BLOOD COLLECTION TRAINING STATUS OF LABORATORY TECHNICIANS
Pınar Eker

S048
MICROBIOLOGICAL EVALUATION OF DISPOSABLE HOLDERS USED IN THE BLOOD COLLECTION UNIT IN MULTIPLE USES
Nilgün Işıksaçan, Ramazan Korkusuz, Duygu Teksöz, Pınar Kasapoğlu

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

09:00 - 10:30 Session 1 - Hall B - Chair: Soycan Mızrak, Yasemin Atıcı

S049
INVESTIGATION OF THE RELATIONSHIP OF OXIDATIVE STRESS, INFLAMMATION, AND CELL ADHESION MARKERS WİTH CLINICAL PARAMETERS IN FABRY DISEASE
Berna Kuş, Menderes Yusuf Terzi, Faruk Hilmi Turgut, Abdullah Arpacı

S050
THE IMPORTANT ROLE OF MULTIPLEX PCR IN THE DIAGNOSIS OF DUCHENNE MUSCULAR DYSTROPHY
Fatimazahra Smaili, Khawla Zerrouki, Fatima Ezzahra Aouni, Ayad Ghanam, Abddeladim Babakhouya, Mariam Tajir

S051
THE INVESTIGATION OF EXPRESSION LEVEL DIFFERENCES OF CARDIAC AQUAPORINS IN H2O2 INDUCED H9c2 CARDIOMYOCYTES
Ayca Bostanoglu, Elif Merve Avcu, Beril Erdem Tuncdemir, Emel Saglar Ozer

S052
INVESTIGATION OF HNF1A GENE POLYMORPHISMS AND THEIR METABOLIC EFFECTS ON MODY AND TYPE 2 DIABETES
Deniz Kanca Demirci, Nurdan Gul, Bengu Tokat, Ilhan Satman, Oguz Ozturk, Hulya Yilmaz Aydogan

S053
IMMUNOGOLD LABELING OF MUTANT AVP PRECURSORS AT ELECTRON MICROSCOPY
R. Dilara Vaizoglu, Beril Erdem Tuncdemir, Emel Saglar Ozer, Ceren Acar, Mehmet Gül, Hatice Mergen

S054
COMPARATIVE EVALUATION OF SILICA MEMBRANE AND PHENOL-CHLOROFORM DNA EXTRACTION TECHNIQUES FOR ANCIENT SAMPLES
Hazal Tahravi, Yeşim Doğan, Hatice Mergen

S055
AUTOPHAGIC CELL DEATH STUDIES LACK EVIDENCE OF CAUSALITY
Yasmin Ghaseminejad, Ali Burak Özkaya

S059
IMPORTANCE OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN CHRONIC LUNG AND LIVER DISEASES
Fatma Sengul, Fikret Akyurek

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

S146
ANALYTICAL PERFORMANCE VALIDATION OF THE NEONATAL IRT FEIA KIT DEVELOPED FOR NEWBORN CYSTIC FIBROSIS SCREENING
Ceyhan Ceran Serdar, Kübra Almacıoğlu, Emrah Kömürcü

S139
RELATIONSHIP BETWEEN SERUM EMPAGLIFLOZIN AND METFORMIN LEVELS AND HEMATOLOGICAL PARAMETERS
Menekşe Kuzu, Duygu Eryavuz Onmaz, Firdevs Sak, Sedat Abuşoğlu, Ali Ünlü
Fatma Hümeyra Yerlikaya Aydemir, Gülsüm Abuşoğlu

S125
TRIMETHYLAMINE-N-OXIDE AND UNCOUPLING PROTEIN-1 LEVELS IN GESTATIONAL DIABETES MELLITUS
Sara Çıbık, Hüsamettin Vatansev, Muslu Kazım Körez, Esranur Turgut, Özlem Seçilmiş

09:00 - 10:30 Session 6 - Hall C - Chair: Gülnihal Kulaksız Erkmen, Halide Edip Temel

S061
PROTECTIVE EFFECT OF NEUROPEPTIDE-S ON A CELLULAR MODEL OF PARKINSON'S DISEASE
Aleyna Öztüzün, Fatma Gonca Koçancı, Ebral Çubukcu, İrem Akçalı, Tuğçe Çeker, Mutay Aslan, Aysel Ağar, Mehmet Bülbül

S062
PROTECTIVE EFFECTS OF NEUROPEPTIDE-S IN A RAT MODEL OF PARKINSON'S DISEASE
Tuğçe Çeker, Aleyna Öztüzün, İrem Akçalı, Fatma Gonca Koçancı, Mutay Aslan, Aysel Ağar, Mehmet Bülbül

S063
INVESTIGATION OF THE EFFECTS OF SPARSTOLIN B ON APOPTOTIC PATH ACTIVATION IN COLORECTAL CANCER CELLS
Bürke Çırçırlı, Çağatay Yılmaz, Esma Kırımlıoğlu, Mutay Aslan

S065
INVESTIGATION OF THE EFFECT OF MANGOSTIN FRUITS ON BLADDER CANCER CELL LINE
Ayşegül Dalmızrak, Esra Demir, Ayla Solmaz Avcıkurt

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

S066
INVESTIGATION OF THE RELATIONSHIP BETWEEN TRYPTOPHAN METABOLISM AND PD1/PDL-1 SIGNALING PATHWAY IN NON-SMALL CELL LUNG CANCER TUMOR MICROENVIRONMENT
Mehmet Tolgahan Hakan, Dilara Sönmez Zor, Cem Horozoğlu, Özlem Küçükhüseyin, Akif Turna, İlhan Yaylım

S067
SALVIA CADMICA BOISS. VAR. CADMICA RESIN ON PROLIFERATION AND APOPTOSIS LEVELS IN HUMAN COLON CANCER CELL LINE
Eissa Almaghrebi, Fatma Akat, Hakan Vatansev, Hüsamettin Vatansev

S068
THE PROTECTIVE EFFECTS OF MORIN OR TARAXASTEROL AGAINST THE CYTOTOXIC EFFECTS OF CISPLATIN/DOXORUBICIN IN RAT SPERMATOGONIA CELL
Ecem Kaya Sezginer, Omer Faruk Kirlangic, Taner Ozgurtas, Cengiz Karakaya

S071
INVESTIGATION OF NEUROTROPHIN LEVELS IN MULTIPAROUS WOMEN WITH STRESS URINARY INCONTINENCE
Kübranur Ünal, Musa Latif Çöllüoğlu, Elif Erdem, Cansu Özbaş, Leyla İbrahimkhanlı, Özhan Özdemir

S072
EFFECTS OF VANILLIC ACID ON ACETAMINOPHEN-INDUCED ENDOPLASMIC RETICULUM STRESS, AUTOPHAGY AND CYTOTOXICITY IN AML12 CELLS
Tugce Karabas, Yagmur Ucar Yagci, Mervenur Barakli Kocabas, Gulben Sayilan Ozgun

S147
EFFECT OF SILYMARIN ON ACRYLAMIDE-INDUCED APOPTOSIS AND CYTOTOXICITY IN MOUSE HEPATOCYTE CELL LINE
Mervenur Barakli Kocabas, Tugce Karabas, Yagmur Ucar Yagci, Gulben Sayilan Ozgun

S069
THE IMPACT OF THE VITAMIN D AND RESVERATROL ADMINISTRATION T2DM RAT AORTA TRACE ELEMENT AND MINERAL LEVELS
Duygu Aydemir, Merve Anapalı, Fatma Kaya Dağıstanlı, Turgut Ulutin, N. Nuray Ulusu

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

11:45 - 13:00 Session 7 - Hall B - Chair: Tevfik Noyan

S073
COMPOSITIONAL AND FUNCTIONAL DIVERSITY OF COLON MICROBIOTA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE
Sibel Kucukyildirim, H. Ozgur Ozdemirel, Beril Erdem Tuncdemir, Saadet Alan, Ceren Acar, Hatice Mergen

S074
EVALUATING THE BACTERIAL DNA LOAD IN BLOOD AS A POTENTIAL COLORECTAL CANCER BIOMARKER
H. Özgür Özdemirel, Sibel Küçükyıldırım, Beril Erdem Tunçdemir, Hatice Mergen

S075
DETERMINATION THE EFFECT OF BIOCHANIN A ON T98G CELLS THROUGH THE CHANGE OF INTRACELLULAR CALCIUM LEVEL
Ezel Demir, Beril Erdem Tuncdemir, Emel Saglar Ozer

S076
PROTEOMIC INVESTIGATION OF THE EFFECTS OF DINUTUXIMAB BETA IN INSULINOMA INS-1 CELLS
Ayse Karatug Kacar

S077
THE EFFECT OF TRANSURETHRAL RESECTION AND BCG THERAPY ON CYTOKINE LEVELS IN NON-MUSCLE INVASIVE BLADDER CANCER
Oktay Üçer, Gökhan Temeltaş, Talha Müezzinoğlu, Zeki Arı, Funda Kosova

S078
6-HYDROXY-L-NICOTINE ACTS AGAINST AMYLOID BETA 1-42 AGGREGATION IN HUMAN CANCER CELLS
Lucian Hritcu, Adrian Tiron, Crina Elena Tiron, Paula Alexandra Stache, Marius Mihasan, Razvan Stefan Boiangiu

S079
INDUCTION OF APOPTOTIC CELL DEATH IN HUMAN LUNG CANCER CELLS THROUGH ENHANCED OXIDATIVE STRESS CAUSED BY RUBUS TERETICAULIS LEAVES EXTRACTS
Gamze Nur Öter, Ezgi Durmuş, Ali Şen, Abdürrahim Koçyiğit

S080
Pllans-II: A PROMISING PROTOTYPE FOR TARGETED THERAPY IN CERVICAL CANCER
Eliécer Jiménez-charris, Alejandro Montoya-gómez, María Jose Sevilla-sánchez, Mildrey Mosquera-escudero

S081
CYTOTOXIC EFFECT OF NEWLY SYNTHESIZED BENZIMIDAZOLE DERIVATIVES ON LUNG AND BREAST CANCER CELL LINE
Taner İlker Gümrükçüoğlu, Hakan Akgün, Bahar Bilgin Sökmen, Hakan Bektaş, Emine Gülçeri Güleç Peker

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

S083
INVESTIGATION OF SERUM TRYPTOPHAN AND KYNURENINE METABOLITES IN BREAST CANCER PATIENTS
Mohammad Ahmad Bik, Karam Mazın Kamıl Gharab, Duygu Eryavuz Onmaz, Sedat Abuşoğlu, Ali Ünlü

S084
INVESTIGATION OF THE IN VITRO AND IN VIVO EFFECTS OF TELOMERE-TARGETED NEW DRUG CANDIDATE COMPOUNDS IN DIFFERENT CANCER CELL LINES
Merve Yilmaz, Sibel Goksen, Gunes Esendagli, Sefik Evren Erdener, Ates Kutay Tenekeci, Larisa L. Birichevskaya, Ilgen Mender, Sergei M. Gryaznov, Z. Gunnur Dikmen

S143
THE ROLE OF DOXORUBISINE-LOADED MESENCHYMAL STEM CELLS IN ANAPLASTIC THYROID CANCER TREATMENT
Ayşe Özlem Silistreli, Hilal Koçdor, Arzu Yıldırım, Ezel Bildik, Halil Ateş, Erdem Erinç Silistreli, Mehmet Ali Koçdor

11:45 - 13:15 Session 8 - Hall C - Chair: Ayfer Çolak, Fatma Demet Arslan

S085
THE EFFECT OF AUTOVERIFICATION ON TURNAROUND TIME FOR CLINICAL CHEMISTRY AND IMMUNOASSAY TESTS
Havva Yasemin Çinpolat

S086
DETERMINATION OF SIX SIGMA LEVEL OF SAMPLE REJECTION REASONS IN PUBLIC HEALTH LABORATORY IN A SIX MOUNTH PERIOD IN ANKARA
Pınar Koyuncu, Gökçe Güven Açık

S087
EVALUATION OF 25-HYDROXY VITAMIN D3 TEST REQUESTS IN THE CONTEXT OF RATIONAL LABORATORY PRACTICES
Gülnur Tekin, Ferdi Bişkin, Gülsüm Abuşoğlu

S089
CALCULATION OF MEASUREMENT UNCERTAINTY OF CARBAMAZEPIN AND VALPROIC ACID TESTS
Ayşenur Macun Ayan

ORAL PRESENTATION PROGRAM

October 31st, Tuesday

S090
CAN REFERENCE CHANGE VALUES FOR BIOCHEMISTRY ANALYTES BE ESTIMATED FROM EXTERNAL QUALITY ASSESSMENT AND AVAILABLE BIOLOGICAL VARIATION DATA?
Gizem Yılmaz Çalık, Mehmet Şeneş

S091
EVALUATION OF UNNECESSARY CK-MB(MASS) TEST REQUEST ACCORDİNG TO CHANGES IN CURRENT GUIDELINES
Hüseyin Yaman

S092
CALCULATED TRANSFERRIN: IS IT NECESSARY TO CALCULATE NEW FORMULA AND NEW REFERENCE INTERVAL?
Kezban Çavdar Yetkin, Semih Fazlı Kayahan, Hasan Alp Turgut, Hacer Doğan, Mehmet Şeneş

S093
USE OF ARTIFICIAL INTELLIGENCE FOR REFERENCE INTERVAL CALCULATION IN THYROID FUNCTION TESTS: FUZZIFICATION OF TRANSITIONS AND GROUPING
Semih Fazlı Kayahan, Muhammed Fatih Alaeddinoğlu, Mehmet Şeneş

S094
RELIABILITY OF PROTEIN ANALYSIS IN COMPLETE URINE ANALYSIS
Fikret Akyurek, Fatma Sengul

S095
THE EFFECT OF URINE PH VALUE ON URINE BIOCHEMICAL ANALYSES
Kübra Kılıç Kartal, Güzin Aykal, Hamit Yaşar Ellidağ

S096
DETERMINATION OF POTENTIAL DRUG TYPES FOR NERVOUS SYSTEM DISEASES USING MULTI-TASK LEARNING TECHNIQUE
Aytun Onay

S148
POTENTIAL BENEFICIAL EFFECTS OF BROMELAIN AGAINST OXIDATIVE DAMAGE CAUSED BY METHOTREXATE IN RAT TESTICULAR TISSUE
Kürşat Kaya

ORAL PRESENTATION PROGRAM

November 1st, Wednesday

09:00 - 10:30 Session 9 - Hall B - Chair: Giray Bozkaya, Uğur Fahri Yürekli

S099
DEVELOPMENT OF A DRUG TO PREVENT MASTITIS PATHOLOGY THAT REDUCES MILK YIELD IN DAIRY COWS
Fatma Akat, Ali Şahin, Huseyn Babayev, Eissa Almaghrebi, Hüsamettin Vatansev

S100
INVESTIGATION OF RE1-SILENCING TRANSCRIPTION FACTOR-COREST REPRESSOR COMPLEX BY MOLECULAR MODELING
Fulya Çağlar Çirkin, Cenk Selçuki

S101
HOW MACHINE LEARNING READS CLINICAL FEATURES: A FOCUS ON A1c
Ferhat Demirci, Deniz İlhan Topcu

S102
CAN CHATGPT BE A "RELIABLE FACILITATOR" İN THE PREPARATION OF A BIOCHEMISTRY LABORATORY TECHNICAL SPECIFICATION?
Saliha Uysal

S103
INVESTIGATION OF THE EFFECT OF GEL USED IN SERUM SEPARATOR TUBES ON VITAMIN B12 ANALYSIS
Tuba Batur, Halil İbrahim Akbay, Serap Sezer

S104
IS TOTAL CHOLESTEROL CAUSE THE DIFFERENCE BETWEEN THE PAINT BINDING METHOD AND THE ALBUMIN CONCENTRATIONS MEASURED BY CAPILLARY ELECTROPHORESIA?
Elif Nihal Başer, Semih Fazlı Kayahan, Mehmet Şeneş

S131
THE IMPORTANCE OF URİNE INTEGRİTY TESTS İN DRUG SUBSTANCE ANALYSİS USİNG LC/MS-MS:AN EVALUATİON OF REJECTED SAMPLES
Ömer Faruk Çakmak, Özge Yakar, Mehmet Şeneş

ORAL PRESENTATION PROGRAM

November 1st, Wednesday

S105
A PRELIMINARY STUDY ON INTERFERENCE OF THE URINARY PROTEIN ASSAY BY POVIDONE IODINE SOLUTION CONTAMINATION
Halil İbrahim Büyükbayram, İlter İlhan, Fevziye Burcu Şirin, Duygu Kumbul Doğuç

S106
EVALUATION OF LIPEMIA INTERFERENCE WITH NATURAL ULTRALIPEMIC MATERIAL AND INTRAVENOUS LIPID EMULSION IN HEMOGLOBIN VARIANT ANALYSIS
Elmas Öğüş, Gül Kırtıl, Medine Alpdemir, Emel Çolak Samsum, Mehmet Fatih Alpdemir, Mehmet Şeneş

S107
DOES TURBIDITY CAUSE INTERFERENCE IN QUANTITATIVE URINE TOTAL PROTEIN MEASUREMENT?
Ayşe Betül Demir, Kezban Çavdar Yetkin, Mehmet Şeneş

S142
QUERCETIN REDUCES AXL LEVELS, AN IMPORTANT THERAPEUTIC TARGET, IN LX2 CELL LINE
Merve Özel, Gülden Başkol

09:00 - 10:30 Session 10 - Hall C - Chair: Sedat Abuşoğlu, Muammer Yücel

S109
A CONDITION THAT CAUSES LOW HBA1C MEASUREMENT BY HPLC METHOD
Seydi Ali Peker, Yunus Emre Haskılıç, Abdulkadir Tekin, Cihan Demir

S110
BENIGN TRANSIENT HYPERPHOSPHATASEMIA IN CHILDREN
Emir Matpan, Aysun Toker, Mustafa Serteser

S111
PSEUDOHYPERKALEMIA CASE RELATED TO TROMBOSITOSIS
Ayşen Caniklioğlu, Rumeysa Betül Kılınçer

S112
WHAT IS A NON-REFRIGERATED CENTRIFUGE CAPABLE OF?
Hatice Bozkurt Yavuz

S113
THE PLACE OF FLOW CYTOMETRY IN DIAGNOSIS OF MYELODYSPLASTIC SYNDROME IN A PATIENT WITH PANCYTOPENIA: A CASE REPORT
İlknur Alkan Kuşabbi

ORAL PRESENTATION PROGRAM

November 1st, Wednesday

S114
CAN REFLEX TESTING UTILIZE THE HEMATOLOGY ANALYZER FLAG?
Gülsüm Feyza Türkeş, Ekin Kırcalı

S115
FALSELY HIGH FREE TRIIODOTHYRONINE VALUE DUE TO MONOCLONAL ANTIBODY THERAPY
Didem Barlak Keti, İlknur Uzun, Sabahattin Muhtaroğlu

S116
CAN GLUCOSE, AND ELECTROLYTES OBTAINED ON BLOOD GAS ANALYSER BE USED INSTEAD OF BIOCHEMISTRY ANALYSER RESULTS IN ADULT PATIENTS?
Ahmet Burak Gürpınar, Abdullah Üner, Tevfik Noyan, Murat Cihan

S117
COMPARISON OF ADVANCED GLYCATION END PRODUCT (AGE) AND ZINC LEVELS IN PATIENTS WITH DIABETES MELLITUS AND DIABETIC NEPHROPATHY
Alev Kural, Nazlı Helvacı, Özgür Can, Kürşad Nuri Baydili

S118
INVESTIGATION OF APELIN, ELABELA, ENDOGLIN AND IMA BLOOD LEVELS IN ACUTE MYOCARDIAL INFARCTION
Irem Arslanturk, Huseyin Fatih Gul

S119
WNT SIGNALING INHIBITORS FOR INCREASING OSSEOINTEGRATION
Rabia Şemsi, Erdal Ergünol, Duygu Dayanır, Remzi Orkun Akgün, Okan Ekim, Oral Cenk Aktaş, Altay Uludamar, Ayhan Özkul, Aylin Sepici Dinçel

S120
GOJIBERRY POTENTIATES THE EFFECT OF L-CARNITINE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA IN VITRO
Mine Ergüven, Haniyeh Behrouzizad, Esin Çetin Aktaş, Ayhan Bilir

14:30 - 16:00 Session 11 - Hall B - Chair: Ayşenur Macun

S082
METABOLOMIC PROFILING IN DISTINCT TYPES OF LEUKEMIA
Ayşe Zehra Gül, Şahabettin Selek, Somer Bekiroğlu, Metin Demirel, Fatma Betül Çakır, Bülent Uyanık

S121
THE EFFECT OF ENRİCHMENT OF BLACK TEA AND YOGHURT DRİNK WİTH VİTAMİN D3 ON SERUM 25(OH)D3 LEVELS İN RATS
Eda Selçuk, Özgür Baykan, Hayrettin Kara, Saliha Uysal, Elif Aksöz

ORAL PRESENTATION PROGRAM

November 1st, Wednesday

S122
THE PROGNOSTIC NUTRITIONAL INDEX (PNI) USED TO PREDICT OUTCOMES IN ISCHEMIC STROKE PATIENTS BY ASSESSING THEIR NUTRITIONAL STATUS
Naile Mısırlıoğlu, Defne Ozen

S123
EFFECT OF NUTRITIONAL DISORDER ON SERUM TRYPTOPHAN-KYNURENINE PATHWAY METABOLITE LEVELS
Asli Parlakcerez, Fatma Humeyra Yerlikaya Aydemir, Duygu Eryavuz Onmaz, Ali Kandeger, Sedat Abusoglu

S124
USING DIFFERENT BLOOD SAMPLES HAS A SIGNIFICANT EFFECT ON AMINO ACID CONCENTRATIONS OBTAINED BY LC-MS/MS
Ceylan Bal, Esra Özyurt, Gülsen Yılmaz

S126
EVALUATION OF TRISOMY 21 RISK WITH DOUBLE AND TRIPLE SCREEN TESTS: A CASE REPORT
Mine Busra Pehlivan, Berna Seyhan

S127
OXİDATİVE DNA DAMAGE OF ACETHAMİPRİD İN ZEBRAFİSH (Danio rerio), VERTEBRATE MODEL ORGANİSM
Gülsüm Koçak, Burcu Eser, Rabia Şemsi, Göktuğ Gül, Pınar Arslan, Aysel Çağlan Günal

S128
EFFECT OF STORAGE PERİOD ON BLOOD ETHANOL LEVELS
Tuba Çakmak, Giray Bozkaya

S129
EVALUATION OF MONOCYTE COUNT TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN AMPHETAMINE USERS
Kadriye Akpınar

S130
METABOLIC EFFECTS OF ORAL TITANIUM DIOXIDE IN JUVENILE RATS: INSIGHTS FROM NMR-BASED METABOLOMICS ANALYSIS
Fatmanur Köktaşoğlu, Metin Demirel, Kübra İzler, Fatih Gültekin, Eray Metin Güler, Somer Bekiroğlu, Şahabettin Selek

14:30 - 16:00 Session 12 - Hall C - Chair: Fikret Akyürek, Ceyhan Ceran Serdar

ORAL PRESENTATION PROGRAM

November 1st, Wednesday

S133
INVESTIGATION OF THE EFFECTS OF KRILL OIL ON TESTICULAR TISSUE IN HIGH FAT DIET-INDUCED OBESE RATS
Eda Yılmaz Kutlu, Mehtap Atak, Zehra Topal Suzan

S134
INVESTIGATION OF THE TREATMENT EFFECTIVENESS OF SIMULTANEOUS INHIBITION OF TRPC3/6 ION CHANNELS IN THE MOUSE LIVER FIBROSIS MODEL
Ebru Onalan, Cemil Demir, Tugce Kaymaz, Ibrahim Hanifi Ozercan, Arzu Etem

S135
ISCHEMIA MODIFIED ALBUMIN LEVELS IN MIGRAINE PATIENTS
Gözde Ülfer, Burcu Polat, Çağrı Çakıcı

S136
THE EFFECT OF EUGENOL ON PARAOXONASE-1 AND PARAOXONASE-3 LEVELS IN AML12 MOUSE HEPATOCYTES
Yagmur Ucar Yagci, Tugce Karabas, Mervenur Barakli Kocabas, Eray Ozgun

S137
INVESTIGATION OF THE L-ARGININE-NITRIC OXIDE-ASIMETRIC DIMETHYL ARGINE PATHWAY IN AUTISM
Şükriye Yabancıün, Fatma Hümeyra Yerlikaya Aydemir, Sedat Abuşoğlu, Ali Ünlü

S138
EVALUATION OF THE EFFECT OF LITHIUM USE ON THYROID HORMONES AND INFLAMMATORY MARKERS
Rabia Zülal Bilge, Aslıhan Bilge Bektaş, Giray Bozkaya

S140
DEVELOPMENT OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR OPIPRAMOL
Beyza Nur Ozkan, Firdevs Sak, Fatma Hümeyra Yerlikaya Aydemir, Sedat Abuşoğlu, Abdullah Sivrikaya, Ali Unlü

S141
BIOLOGICAL EFFECTS OF 6-HYDROXY-L-NICOTINE AND COTININE IN VARIOUS EXPERIMENTAL MODELS OF DEMENTIA: IN SILICO AND IN VIVO STUDY
Razvan Stefan Boiangiu, Ion Brinza, Marius Mihasan, Lucian Hritcu

POSTER PRESENTATION PROGRAM

30 October - 1 November

P001
ULTRASENSITIVE HIV-ENVELOPE PROTEIN GP41 DETECTION WITH MACHINE LEARNING BY ANTIMICROBIAL BIOSENSOR
Ergin Taşkın, Zihni Onur Uygun

P003
EVALUATION OF PARAMETERS USED IN COMPLETE URINE EXAMINATION TO DIAGNOSE URINARY TRACT INFECTION
Duygu Öcal, Özlem Doğan, Beril Yazıcı, Haluk Furkan Hanedan, Melis Demir, Elif İrem Arslan, Atalay Mert Yüksel, Yade Sultan Kurt, İsmail Utku Bilge

P004
COMPARISON OF SERUM FREE LIGHT CHAIN AND PARAPROTEIN LEVELS IN MONOCLONAL GAMMOPATHIES
Raneen Sliman, Sammar Nafafaa

P005
ROTATIONAL THROMBOELASTOMETRY AND CONVENTIONAL COAGULATION TESTS IN PATIENTS UNDERGOING CARDIAC SURGERY
Isabel Rodriguez Martin

P006
IMPORTANCE OF MEDICAL BIOCHEMISTRY SPECIALIST MANAGEMENT IN LABORATORY QUALITY : A BEFORE/AFTER STUDY
Hilmi Furkan Arslan

P007
NOISE EXPOSURE OF MEDICAL LABORATORY EMPLOYEES
Metin Özdemir, Tülay Özdemir

P008
MANAGEMENT OF INTERNAL QUALITY CONTROL IN HAEMOSTASIAS AT HEMATOLOGY LABORATORY FARHAT HACHED HOSPITAL, SOUSSE
Khalil Atıg, Néjia Brahem

P009
OBJECTIVE: THIS IS THE FIRST TIME YOU HAVE HEARD THE TERM ''CORRECTED FASTING PLASMA GLUCOSE''.
Ahmet Coşkun, Atilla Ünal

P010
DEVELOPMENT OF 3-METHOXY-L-TYROSINE (3-OMD) LC-MS/MS METHOD IN DRIED BLOOD SPOT (DBS) SAMPLES
Hazal Yilmaz, Murat Emrah Mavis, Gokce Goksu Gursu

POSTER PRESENTATION PROGRAM

30 October - 1 November

P011
DEVELOPMENT OF A SIMPLE LC-MS/MS METHOD FOR MEASUREMENT OF URINARY 8-HYDROXY-2-DEOXYGUANOSINE (8-OHDG) AS OXIDATIVE STRESS MARKER
Hazal Yilmaz, Murat Emrah Mavis, Gokce Goksu Gursu

P012
PRECISION OF COBAS C311 ROCHE ANALYZER IN DETERMINING THE VALUES OF TOTAL CHOLESTEROL – VERIFICATION STUDY
Melda Emin, Hristina Ampova, Irena Kostovska, Julijana Brezovska - Kavrakova, Svetlana Cekovska, Sonja Topuzovska, Jasna Bogdanska, Danica Labudovic, Katerina Tosheska - Trajkovska

P013
ESTIMATING INTRA- AND INTER- ASSAY COEFFICIENTS OF VARIABILITY FOR LACTATE DEHYDROGENASE ENZYME ON COBAS c311 ROCHE ANALYZER
Hristina Ampova, Melda Emin, Irena Kostovska, Katerina Tosheska-trajkovska, Svetlana Cekovska, Julijana Brezovska-kavrakova, Jasna Bogdanska, Danica Labudovic, Sonja Topuzovska

P014
A PILOT PERFORMANCE EVALUATION OF CEREBROSPINAL FLUID BIOMARKERS USED FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE
Tuba Saadet Deveci Bulut, Nigar Esra Erkoç Ataoğlu, Ümit Özgür Akdemir, Lütfiye Özlem Atay, Özlem Gülbahar

P015
EVALUATION OF THE QUALITY PERFORMANCE OF THE COBAS 8000 ANALYZER
Özge Cindemir

P016
INVESTIGATION OF METABOLIC CHANGES AND AP2 PROTEIN IN PROLONGED FASTING MODEL
Meltem Pak Demir, Musa Çelik, Nilay Topal, Ayse Aydanur Kulaç Ersen, Bulut Yurtsever, Devrim Öz Arslan, Fehime Aksungar

P017
DETERMINATION OF TOCOPHEROL PROFILE AND QUANTITIES IN EXTRACTS OF RESIN OF DICLE CRAB IN DIFFERENT SOLVENTS.
Eissa Almaghrebi, Hakan Vatansev, Engie Aydin Kamal Alabbas, Fatma Akat, Hüsamettin Vatansev

P018
MEASUREMENT UNCERTAINTY OF THE CALCULATED TRANSFERRIN PARAMETER
Elif Nihal Başer, İbrahim Yangın, Mehmet Şeneş

P019
ASSESSMENT OF MEASUREMENT UNCERTAINTY OF ROUTINE BIOCHEMICAL TESTS
Levent Deniz

POSTER PRESENTATION PROGRAM

30 October - 1 November

P020
EVALUATION OF THE DIAGNOSTIC USEFULNESS OF TRIGLYCERIDE/ HDL-C RATIO AS A GLYCEMIC CONTROL MARKER
Muzaffer Katar, Osman Demir

P021
CALCULATION OF ALBUMIN TEST MEASUREMENT UNCERTAINTY VALUE ACCORDING TO ISO/TS 201914 GUIDELINE
Meltem Yardım, Nilüfer Çelik

P022
A COMPARISON OF BIOINFORMATICS PIPELINES FOR MICROBIOME RESEARCH
Sibel Kucukyildirim

P023
CALCULATION OF THE MOLECULAR DESCRIPTORS FOR THE NERVOUS SYSTEM DRUG DATASET
Aytun Onay

P024
COMPUTATIONAL İNVESTIGATION OF STRUCTURE AND DYNAMICS OF AMYLOID BETA FIBRILS FOR DISCOVERY OF PEPTIDES THAT PREVENT PLAQUE FORMATION
Fulya Çağlar Çirkin, Sevgi Gülyüz, Özgür Yılmaz, Sefer Baday, Özge Şensoy

P025
INVESTIGATION OF DNA APTAMER MODIFICATIONS ASSOCIATED WITH GLIOBLASTOMA THROUGH MOLECULAR MODELING
Hasip Cirkin

P026
INVESTIGATION OF PCSK9 AND CETP POLYMORPHISMS IN DIABETIC NEPHROPATHY
Nazlı Helvacı, Alev Kural, Sibel Kuraş, Özgür Can, Fatih Hacımustafaoğlu

P027
THE SANGER SEQUENCING IN THE DETECTION OF THE RECURRENT MUTATION IN PRIMARY HYPEROXALURIA TYPE I
Jihane Ahmıdı, Khawla Zerrouki, Fatima Ezzahra Aouni, Fatimazahra Smaili, Sara Meziane, Intissar Haddiya, Yassamine Bentata, Mariam Tajir

P028
COULD GENE REGION VARIANTS ENCODING HYDROLASE ACTIVITY OF THE SOLUBLE EPOXIDE HYDROLASE ENZIME BE ASSOCIATED WITH TYPE 2 DIABETES?
Esma Ozmen, Dilara Fatma Akın, Durmuş Ayan, Çağatay Emir Onder, Burcu Kose, İsmail Sarı, Cevat Yazıcı

POSTER PRESENTATION PROGRAM

30 October - 1 November

P029
THE INVESTIGATION OF OXIDATIVE STRESS IN H2O2 INDUCED H9c2 CARDIOMYOCYTES
Ayca Bostanoglu, Beril Erdem Tuncdemir, Emel Saglar Ozer

P030
INTRACELLULAR LOCALIZATION AND STRUCTURE OF MUTANT AVP PRECURSOR AGGREGATES IN AUTOSOMOL DOMINANT NEUROHYPOPHYSEAL DIABETES INSIPIDUS
R. Dilara Vaizoglu, Beril Erdem Tuncdemir, Emel Saglar Ozer, Ceren Acar, Mehmet Gül, Hatice Mergen

P031
ROLE OF VITAMIN D RECEPTOR GENE POLYMORPHISMS ON NORMAL AND OVERWEIGHT PATIENTS WITH POLYCYSTIC OVARY SYNDROME (PCOS)
Zümrüt Mine Işık Sağlam, Vuslat Lale Bakır, Merve Nur Ataş, İlhan Yaylım, H. Arzu Ergen

P032
EXPLORING DIET-RELATED BIOMOLECULAR ALTERATIONS IN MEDULLA OBLONGATA WITH IR SPECTROSCOPY AND BIOCHEMICAL ANALYSIS
Göktuğ Kaçıra, Şebnem Garip Ustaoğlu, Ayça Doğan

P033
CAN BLOOD GAS ANALYSER RESULTS BE USED INSTEAD OF BIOCHEMISTRY AUTO-ANALYSER RESULTS IN PATIENTS TREATED NEONATAL INTENSIVE CARE UNIT?
Ahmet Burak Gürpınar, Tevfik Noyan, Murat Cihan, Abdullah Üner

P034
CHANGES IN THE LEUKOGRAM OF COVID-19 PATIENTS
Lajla Halilović, Berina Hasanefendić, Emir Šeherčehajić, Armina Dedić, Suzana Tihić Kapidžić, Ahmed Velić, Lejla Ibričević Balić

P035
COMPARISON OF LEUKOCYTE SUB-GROUPS (LYMPHOCYTE, MONOCCYTE AND NEUTROPHIL) MEASURED IN THE MINDRAY DEVICE WITH FLOW CYTOMETRIC MEASUREMENTS
Tuğçe Acar, İbrahim Türk, Zahide Adıyaman, Hatice Topaç, Derya Sönmez

P036
5-YEAR EXPERIENCE OF FLOW CYTOMETRY
Alev Lazoglu Ozkaya, İbrahim Yazıcı, Konca Altınkaynak, Kadriye Akpınar

P037
MULTIPLE MYELOMA WITH TRICLONAL GAMMOPATHY: CASE REPORT
Nazife Doğan

P038
cFGF-23, sKLOTHO, AND 1,25 (OH)2 D3 VITAMIN LEVELS IN KIDNEY TRANSPLANT PATIENTS
Hatice Paşaoğlu, Ayşe Şenelmiş, Özge Tuğçe Paşaoğlu, Ülver Derici, Özant Helvacı

POSTER PRESENTATION PROGRAM

30 October - 1 November

P039
COMPARISON ROUTINE OF LABORATORY PARAMETERS IN PATIENTS WITH SCHIZOPHRENIA AND BIPOLAR DISORDER
Elif Menekşe, Muhammed Emin Düz, Burak Yasin Avcı, Ecem Avcı

P040
PREVALENCE OF DIABETIC NEPHROPATHY IN TREATED DIABETICS: SINGLE-CENTER STUDY
Berina Hasanefendić, Snežana Unčanin, Emir Šeherčehajić, Suzana Tihić-kapidžić, Velda Smajlbegović, Lajla Halilović, Denis Hasković

P041
EVALUATION OF ADROPIN AND PHOENIXIN-14 LEVELS IN WOMEN WITH EUTHYROID HASHIMOTO'S THYROIDITIS
Sadinaz Akdu, Ummugulsum Can, Ahmet Hamdi Aktan

P042
THE INVESTIGATION of KISSPEPTIN, SPEXIN and GALANIN in EUTHYROID WOMEN with HASHIMOTO'S THYROIDITIS
Ümmügülsüm Can, Sadinaz Akdu, Ahmet Hamdi Aktan

P043
TYPE 1 COLLAGEN C-TERMINAL TELOPEPTID, OSTEOCALCIN AND VITAMIN D LEVELS IN PATIENTS WITH OSTEOPOROSIS
Gülşen Yalçın, Mehmet Çağatay Taşkapan, Harun Şentürk, Cengizhan Katırcı

P044
OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE BIOMARKERS IN PATIENTS WITH GRAVES' ORBITOPATHY
Marija Sarić Matutinović, Miloš Žarković, Jasmina Ćirić, Svetlana Ignjatović, Biljana Nedeljković Beleslin

P045
EFFECT OF AEROBIC EXERCISE ON FRUCTOSE INDUCED METABOLIC SYNDROME COMPONENTS THROUGH LIVER FGF21 AND PGC1α EXPRESSION
Belkis Narli, Kardelen Kocaman Kalkan, Fatmanur Er, Erdal Zorba, Mehmet Gunay, Mitat Koz, Canan Yilmaz

P046
INCREASED SERUM GLUTAMINE/GLUTAMATE AND AMELIORATION OF NEUROPATHY OF MICE WITH STREPOZOTOCIN-INDUCED DIABETES BRED IN ENRICHED ENVIRONMENT
Pavlina Gateva, Natasha Ivanova, Milen Hristov, Zafer Sabit

P047
EFFECT OF GROUND GRAPE SEED ON NONALCOHOLIC FATTY LIVER DISEASE
Hüsamettin Vatansev, Fatma Akat, Esranur Turgut, Eissa Almaghrebi, Ali Habacı, Muslu Kazım Körez

POSTER PRESENTATION PROGRAM

30 October - 1 November

P048
THE EFFECT OF TWO-WEEK HIGH-INTENSITY INTERVAL TRAINING ON INSULIN RESISTANCE
Sabahattin Muhtaroğlu, Tuğba Sarıtaş, Didem Barlak Keti

P049
EVALUATION OF ADIPOCYTE FATTY ACID BINDING PROTEIN IN PREDIABETIC PATIENTS
Aydan Taka Kucuk, Murat Aksıt, Ayfer Colak

P050
EVALUATION OF ADIPOCYTE FATTY ACID BINDING PROTEIN IN PREDIABETIC PATIENTS
Aydan Taka Kucuk, Murat Aksıt, Ayfer Colak

P051
PREDICTION OF MICROBIAL FUNCTIONAL DIFFERENCES IN COLON CANCER PATIENTS BY USING FAPROTAX
H. Özgür Özdemirel, Sibel Küçükyıldırım, Beril Erdem Tunçdemir, Ceren Acar, Hatice Mergen

P052
INCREASED EXPRESSION LEVEL OF ATF GENE IN T98 GLIOBLASTOMA CELLS AFTER TREATMENT WITH BIOCHANIN A
Ezel Demir, Elif Merve Avcu, Beril Erdem Tuncdemir, Emel Saglar Ozer

P053
THE EFFECT OF HOGNA RADIATA VENOM IN HUMAN BREAST CANCER CELL LINE
Hazal Tahravi, Refika Dilara Vaizoğlu, Hatice Mergen

P054
NOVEL PHOSPHOLIPID DERIVATIVES OF TELOMERE TARGETED ANTICANCER NUCLEOSIDES
Merve Yilmaz, Ates Kutay Tenekeci, Z. Gunnur Dikmen, Larisa L. Birichevskaya, Sergei M. Gryaznov

P055
A RARE CASE WITH AN UNEXPECTEDLY HIGH CA125 LEVEL
Feyza Yağmur Tekeli, Işın Üreyen

P056
INVESTIGATION OF THE ANTITUMOREGENIC EFFECT OF CITRUS AURANTIUM ON PANCREAS, THYROID AND PROSTATE CANCER CELLS
Arzu Yıldırım, Ezel Bildik, Mehmet Ali Koçdor, Hilal Koçdor

P057
EVALUATION OF ALTERATIONS IN THE HDL SUBFRACTION AND HDL-RELATED ENZYMES IN PATIENTS WITH LIVER FAILURE AND IN HEALTHY DONORS
Çağatay Yılmaz, Tuğçe Çeker, Aleyna Öztüzün, Bürke Çırçırlı, Bora Dinç, İlker Öngüç Aycan, Mutay Aslan

POSTER PRESENTATION PROGRAM

30 October - 1 November

P058
EFFECT OF 4-PHENYLBUTYRATE ON COBALT CHLORIDE-INDUCED CYTOTOXICITY IN AML12 CELLS
Yagmur Ucar Yagci, Tugce Karabas, Mervenur Barakli Kocabas, Gulben Sayilan Ozgun

P059
THE EFFECT OF ACETYLSALICYLIC ACID ON ACRYLAMIDE-INDUCED CELLULAR HEPATOTOXICITY
Mervenur Barakli Kocabas, Tugce Karabas, Yagmur Ucar Yagci, Gulben Sayilan Ozgun

P060
INTERCELLULAR COMMUNICATION IN ATHEROSCLEROSIS: EFFECT OF DIFFERENT ENDOTHELIAL EXOSOME DOSES ON UPTAKE-DRIVEN CHANGES IN VASCULAR SMOOTH MUSCLE CELLS
Joumana Al Akhdar, Melike Nur Yangın, Fatima Hacer Kurtoğlu Babayev, Kemal Baysal

P061
MACROPHAGE GENE EXPRESSION CHANGES OBSERVED IN IN VITRO CO-CULTURES WITH ENDOTHELIAL OR VASCULAR SMOOTH MUSCLE CELLS
Melike Nur Yangın, Joumana Al Akhdar, Fatıma Hacer Kurtoğlu Babayev, Kemal Baysal

P062
RETROSPECTIVE COMPARISON OF CARDIAC TROPONIN-T VALUES IN PATIENTS WITH AND WITHOUT CHRONIC KIDNEY DISEASE UNDIAGNOSED OF ACUTE MYOCARDIAL INFARCTION
Zahide Adıyaman, İbrahim Türk, Tuğçe Acar, Büşra Üresin Yazlak, Mevlüde Ayyıldız, Berrin Berçik İnal

P063
THE IMPORTANT ROLE OF SANGER SEQUENCING IN THE DETECTION OF THE MAGHREBIAN MUTATION C.525DELT IN THE SGCG GENE IN LIMB-GIRDLE MUSCULAR DYSTROPHY
Fatimazahra Smaili, Khawla Zerrouki, Fatima Ezzahra Aouni, Ayad Ghanam, Abddeladim Babakhouya, Mariam Tajir

P064
LABORATORY PERSPECTIVE TO ELEVATED SERUM AMYLASE: A CASE REPORT
Irem Acilan, Sedat Abusoglu, Abdullah Sivrikaya, Ali Unlu

P065
HIGH B12 LEVEL AS ACUTE PHASE REACTANT IN CHOLESTATIC LIVER DISEASES
Sabahattin Muhtaroğlu, Naci Tektaş, Semanur Doğan, Didem Barlak Keti

POSTER PRESENTATION PROGRAM

30 October - 1 November

P066
THE SIGNIFICANCE OF SERUM TOTAL BILE ACID AS A PREGNANCY BIOMARKER: A CRUCIAL INDICATOR FOR OBSTETERIC CHOLESTASIS
Zubaır Ahmed Rahojo

P067
INDIRECT PANCREATIC FUNCTION TEST- UTILITY OF FECAL PANCREATIC ELASTASE FOR DIAGNOSIS OF EXOCRINE PANCREATIC INSUFFICIENCY IN CHILDREN
Shagufta Nısar Alı

P068
BONE TURNOVER MARKERS IN SYSTEMIC SCLEROSIS: IS THERE AN INCREASED RISK FOR OSTEOPOROSIS?
Nazlı Ecem Dal Bekar, Gamze Tuna, A. Merih Birlik, Gül Güner Akdoğan, G. Hüray İşlekel

P069
STRUCTURAL AND COMPUTATIONAL-DRIVEN RECOGNITION OF PHOSPHOINOSITIDE DERIVATIVE FOR HIV ERADICATION
Halilibrahim Ciftci, Belgin Sever, Nurettin Tokay, Masami Otsuka, Hiroshi Tateishi, Mikako Fujita, Hasan Demirci

P070
EVALUATION SERUM LEVELS OF NRF-2, HEMOXYGENASE-1 AND NEOPTERIN LEVELS IN BRUCELLOSIS PATIENTS
Nihayet Bayraktar, Büşra Öztürk, Mehmet Çelik, Mehmet Reşat Ceylan, Mehmet Bayraktar

P071
NUMERICAL CHANGES IN COVID-19 CASES AND CORRELATION WITH AGE AND BLOOD GROUP : A CROSS SECTIONAL STUDY IN . HYDERABAD , PAKISTAN
Zubaır Ahmed Rahojo

P072
INVESTIGATION OF FECAL CALPROTECTIN TEST USED FOR INFLAMMATORY BOWEL DISEASE
Kübranur Ünal, Leyla İbrahimkhanlı

P073
THE ROLE OF INFLAMMATORY MARKERS DERIVED FROM HEMATOLOGICAL PARAMETERS IN FEMALE MIGRAINE PATIENTS
Yasemin Atıcı, Esra Eruyar, Nida Aslan Karakelle, Safiye Göçer

P074
INVESTIGATION OF THE EFFECT OF THYMOQUINONE ON CLAUDIN-5 PROTEİN EXPRESSION IN A CELL CULTURE INTESTINAL INFLAMMATION MODEL
Öznur Yaşar, Ebru Kanımdan, Abdurrahim Koçyiğit

POSTER PRESENTATION PROGRAM

30 October - 1 November

P075
DETERMINATION OF THE FREQUENCY OF DISCORDANT C-REACTIVE PROTEIN AND ERYTHROCYTE SEDIMENTATION RATE RESULTS IN ADULTS
Caner Yılmaz, Giray Bozkaya

P076
INVESTIGATION OF TRYPTOPHAN AND KYNURENINE IN PATIENTS WITH RHEUMATOID ARTHRITIS
Karam Mazın Kamıl Gharab, Mohammad Ahmad Bik, Duygu Eryavuz Onmaz, Sedat Abuşoğlu, Ali Ünlü

P077
ANTI-INFLAMMATORY EFFECTS OF KV1.3 CHANNEL BLOCKER BIOMOLECULE ON LIPOPOLYSACCHARIDE-INDUCED PULMONARY INJURY
Nesrine Ahras-sifi, Amina Ladjel-mendil, Fatima Laraba-djebari

P078
IL33 GENE VARIANTS AND EXPRESSION IN CHILDHOOD ASTHMA
Rihab Charrad, Anissa Berraies, Jamel Ammar, Kamel Hamzaoui, Agnés Hamzaoui

P079
CRP-ALBUMIN RATIO AND NEUTROPHIL-LYMPHOCYTE RATIO IN ACUTE ISCHEMIC STROKE
Veli İyilikçi, Eyyüp Kanat, Hande Turan, İnanç Karakoyun, Ayfer Çolak

P080
SPURIOUS CARDIAC TROPONIN-T ELEVATION BY ASSAY INTERFERENCE: CLINICIANS CONSULTING THE LABORATORY FOR PATIENT ADVICE
Sena Türkmen, Okşan Demiröz Güleryüz, Ayla Akca Çağlar, Ercan Demir, Özlem Gülbahar

P081
ENZYME-CATALIZED SYNTHESIS OF NEW PHOSPHOLIPID DERIVATIVES OF ANTICANCER NUCLEOSIDES
Larysa L. Birycheuskaya, Marharita A. Vinter, Anatoli I. Zinchenko, Z. Günnur Dikmen, Sergei M. Gryaznov

P082
COMPARISON OF BIOCHEMICAL MARKERS BEFORE AND AFTER BARIATRIC SURGERY
Kübranur Ünal, Leyla İbrahimkhanlı, Hüseyin Göbüt, Ahmet Çağrı Büyükkasap

P083
ANALYSIS OF SPHINGOSINE-1–PHOSPHATE BY LC−MS/MS
Burcu Eser, Aylin Sepici Dincel

POSTER PRESENTATION PROGRAM

30 October - 1 November

P084
DETERMINATION OF ALPHA-AMYLASE/TRYPSIN INHIBITORS IN TURKISH WHEATS BY TARGETED HPLC-MS/MS
Haydar Özpınar, Sorel Tchewonpı Sagu, Aysel Ozpınar, Harshadraı M. Rawel, Florıan Schweıgert

P085
IRREGULAR NUTRITION HABITS EVALUATION OF THE EFFECT ON CARDIOVASCULAR RISK PARAMETERS
Demet Nikbay, Fatma Humeyra Yerlikaya Aydemir, Duygu Eryavuz Onmaz, Ali Kandeger, Sedat Abusoglu

P086
BORON ESTERS AS FOOD SUPPLEMENTS ENHANCE IN VITRO OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STEM CELLS
Serap Topsoy Kolukısa, Sema Akbaba, Resul Biberoğlu, Dursun Ali Köse

P087
RBC FOLATE LEVELS IN CHILDREN WITH GROSS MOTOR FUNCTION CLASSIFICATION SYSTEM (GMFCS) IV-V CEREBRAL PALSY: A CASE-CONTROL STUDY
Özlem Tezol, Belgin Bayram, Sıddıka Songül Yalçın, Oytun Portakal

P088
THE INFLUENCE OF THE GENETIC POLYMORPHISM OF CATECHOL O METHYLTRANSFERASE ON POSTTRAUMATIC STRESS DISORDER APPEARANCE
Valdete Haxhibeqiri, Shpend Haxhibeqiri, Mimoza Hoxha Muhaxhiri, Vlora Kutllovci, Ervehe Myrtaj Dalipi

P089
MORUS ALBA L. EVALUATION OF THE CYTOTOXIC ACTIVITY OF LEAF EXTRACTS ON CACO-2 CELLS TOGETHER WITH ANTIOXIDANT ACTIVITY
Elif Senturk, Gulcin Alp Avcı, Emre Avcı, Cumhur Bılgı

P090
THE EFFECT OF WHITE TEA ON LEPTIN AND ASPROSIN LEVELS IN OBESE RATS-INDUCED BY HIGH-FAT DIET
Merve Nur Toraman, Adnan Yılmaz, Sibel Karakaş

P091
SYNTHESIS OF A SERIES OF BENZIMIDAZOLE COMPOUNDS, DETERMINATION OF ANTIUREASE AND ANTIOXIDANT ACTIVITIES
Taner İlker Gümrükçüoğlu, Hakan Akgün, Bahar Bilgin Sökmen, Hakan Bektaş

P092
EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON MEMBRANE PROTEINS RESPONSIBLE FOR NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE OXIDASE ACTIVITY
Berkay Saraymen, Esma Saraymen, Aysun Çetin, Recep Saraymen

POSTER PRESENTATION PROGRAM

30 October - 1 November

P093
EFFECT OF CAFFEIC ACID ON HYDROGEN PEROXIDE-INDUCED CYTOTOXICITY IN AML12 CELLS
Tugce Karabas, Yagmur Ucar Yagci, Mervenur Barakli Kocabas, Gulben Sayilan Ozgun

P094
RETROSPECTIVE INVESTIGATION OF THYROID HEALTH IN BREAST CANCER PATIENTS
Bengisu Dönmez, Muhittin Serdar, Aysel Özpınar

P095
DETERMINATION OF TAMOXIFEN METABOLITES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY
Abdullah Sivrikaya, Esma Menevse, Fatma Zehra Erbayram, Hamiyet Kose, Oguzhan Tok

P096
DETERMINATION OF THE TOXIC EFFECTS OF MALATHION ON FRESHWATER MUSSELS
Pınar Arslan, Aysel Çağlan Günal

P097
INVESTIGATION OF THE TOXIC EFFECTS OF EMAMECTIN BENZOATE ON FRESHWATER MUSSELS
Meryem Bayrak, Pınar Arslan, Aysel Çağlan Günal

P098
THE EFFECT OF GENDER, AGE AND SEASON ON VİTAMİN D LEVELS
Ertuğrul Avcı, Giray Bozkaya

P099
INVESTIGATION OF HEAVY METAL LEVELS IN BLOOD AND URINE SAMPLES OF YOUNG ADULTS (18-24 AGES)
Nilay Topal, Meltem Pak Demir, Ayşe Aydanur Kulaç Ersen, Bulut Yurtsever, Fehime Aksungar

P100
CORRELATION OF BLOOD, SERUM, AND URINE HEAVY METAL LEVELS
Ayşe Aydanur Kulaç Ersen, Nilay Topal, Meltem Pak Demir, Bulut Yurtsever, Fehime Aksungar

P101
EFFECTS OF KB-R7943 ON INSULINE/GLUCOSE METABOLISM AND MOTOR ACTIVITY IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS
Natasha Ivanova, Pavlina Gateva

P102
COMPARISON OF HEMOGRAM PARAMETERS IN BLOOD SAMPLES TAKEN DURING PERIODIC EXAMINATION OF HEALTH WORKERS
Nilüfer Alpaslan, Fatma Akat, Hüsamettin Vatansev

POSTER PRESENTATION PROGRAM

30 October - 1 November

P103
KINETICS OF SARS-CoV-2 NEUTRALIZING ANTIBODIES AFTER TWO DOSES OF BNT162b2(BioNTech/Pfizer)VACCINE
Katerina Tosheska-trajkovska, Melda Emin, Hristina Ampova, Irena Kostovska, Sonja Topuzovska

P104
ADENOSINE DEAMINASE ACTIVITY IN CHILDREN WITH RESISTANT EPILEPSY
Nurdan Şener, Didem Barlak Keti, Ayten Güleç, Mehmet Canpolat, Sabahattin Muhtaroğlu

P105
EVALUATION OF ANXIETY AND DEPRESSION LEVELS IN MALE PARTNERS OF INFERTILE COUPLE
Sondos Meksi, Amira Sallem, Rim Kooli, Dhekra Chebil, Manel Boussabbeh, Mouna Zarred, Hayet Rebai, Hana Messadi, Fadhila Chebil, Boutheina Kilani, Meriam Mehdi

P106
Identification of Dickkopf-1/Kremen Antagonist Candidates using Virtual Screening
Sahra Setenay Baran, Yusuf Şimşek, Aylin Sepici Dinçel

P107
EVALUATION OF BLOOD SAMPLE REJECTION RATE IN CLINICAL LABORATORY
Müjgan Ercan Karadağ, Ayşe Ertekin, Mehmet Özcan, Rumeysa Duman, Berika Şahin, Esra Tunç, İsmet Enis Çeri, Gürkan Aydın, Mehmet Ali Alkan, Yasemin Engin

P108
Distinguishing Bone Marrow Cells in Tissue Slide Images with AutoKeras Deep Learning System
Hikmet Can Çubukçu, Onur Burak Dursun

INVITED SPEAKER ABSTRACTS

IS001

WHAT IS THE REPUBLIC?

Doğan Yücel

Lokman Hekim Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Ankara, Türkiye

Mustafa Kemal summarizes the struggle from May 1919 to 1927 in a speech he made in the Assembly on October 10-15, 1927. In the introduction of this speech (Nutuk), he evaluates the situation of the country on May 19, 1919, as follows: "On the 19th day of May 1919, I landed in Samsun. General situation and appearance: The community within the Ottoman State had been defeated in the General War (World War I), the Ottoman army was weakened everywhere, and a harsh armistice agreement had been signed. Throughout the long years of the Great War, the nation is tired and impoverished. Those who dragged the nation and the country into the General War, worrying about their own lives, have fled the homeland. Vahdettin, who is both the Sultan and the Caliph (sitting in the seat of the Sultanate and caliphate), has degenerated, seeking despicable measures to protect himself and his throne. The government led by Damat Ferit Pasha is weak, dishonorable, cowardly, and has submitted to the desires of the Sultan alone, bowing to any situation that could protect themselves with him. The army is deprived of its weapons and ammunition, and this continues. The Allied states do not see it necessary to comply with the terms of the armistice agreement. … Officers, officials, and private individuals from foreign states are working everywhere. Later, four days before the date we took as the starting point for our speech, on May 15, 1919, the Greek army is landed in Izmir with the approval of the Allied Powers for various reasons." Shortly after this evaluation, with the signing of the Treaty of Sèvres on August 24, 1920, Türkiye (then the Ottoman Empire) lost at least 75% of its territories. But more importantly, the state lost its political, economic, legal, judicial, and military independence. This essentially meant that there would not be a state named Türkiye in a short time. The palace considered Mustafa Kemal an enemy and was preparing rebellions with the support of the Allied powers against the National Forces in Anatolia. They even wanted the foreign forces that had occupied the country to prevail and destroy the National Forces. In this context, they also issued a death warrant using religion against Mustafa Kemal and his comrades. At the same time, representatives of the Allied powers were trying to discourage Mustafa Kemal and the members of the National Forces from the War of Independence. In this dark environment, a great soldier and intellectual like Mustafa Kemal emerged, starting the War of Independence with the fire of his love for the homeland and people, risking his life. This is truly a miracle. There is no other example of this miracle in the world. It is the first successful national liberation war in the world. However, victory alone does not mean anything. Mustafa Kemal sees this reality and initiates revolutions for full independence both in the economy and in the superstructure (education, culture, art, women's rights, law, health, etc.). He conducts these revolutions, as always, on a legitimate basis. In summary, thanks to the Republic, two reactionary and outdated approaches were overcome: the invaders (Western conservatism) and the Sultanate (Eastern conservatism). If we can say today that we are citizens of the Republic of Türkiye, it is thanks to that great man, Gazi Mustafa Kemal and those who supported him. We live as citizens in these lands thanks to the courage and foresight of this great leader. Mustafa Kemal's those words will always guide us: "For everything in the world, for material and spiritual matters, for success, the truest guide is knowledge and science. Seeking a guide outside of knowledge and science is negligence, ignorance, and deviation. It is necessary to understand and follow the development of the stages of knowledge and science in every minute of our lives." "As a spiritual legacy, I leave no rigid, dogmatic, frozen, and crystallized rules. My spiritual legacy is knowledge and reason. Those who want to adopt me after me will be my spiritual heirs if they accept the guidance of reason and knowledge on this fundamental axis." In a word, we are grateful!

IS002

INVESTIGATING THE IMPACT OF TW68 ON GLUCONEOGENESIS AND FASTING BLOOD SUGAR REGULATION VIA CRYPTOCHROME STABILIZATION

Saliha Sürme[1], Çağla Ergün[4], Şeref Gül[2], Yasemin Akyel[3], Zeynep Melis Gül[1], Onur Özcan[1], Özgecan Şavluğ Ipek[5], Büşra Aytül Akarlar[1], Nurhan Özlü[1], Ali Cihan Taşkın[6], Metin Türkay[7], Ahmet Ceyhan Gören[8], Ibrahim Barış[1], Nuri Öztürk[8], Mustafa Güzel[5], Cihan Aydın[9], Alper Okyar[10], Halil Kavaklı[4]

[1] Koç Üniversitesi, Moleküler Biyoloji ve Genetik Bölümü, Istanbul, Türkiye

[2] Istanbul Üniversitesi, Biyoloji I Bölümü, Istanbul, Türkiye

[3] Istanbul Medipol Üniversitesi,tıp Fakültesi, Medikal Farmakoloji, Istanbul, Türkiye

[4] Koç Üniversitesi, Kimya ve Biyoloji Mühendisliği Bölümü, Istanbul, Türkiye

[5] Istanbul Medipol Üniversitesi, Eczacılık Fakültesi, Temel Eczacılık Bilimleri Bölümü, Istanbul, Türkiye

[6] Istanbul Üniversitesi, Laboratuvar Hayvanları Bilimi Abd, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, Istanbul, Türkiye

[7] Koç Üniversitesi, Endüstri Mühendisliği Bölümü, Istanbul, Türkiye

[8] Gebze Teknik Üniversitesi, Kimya Bölümü, Kocaeli, Türkiye

[9] Istanbul Medeniyet Üniversitesi, Moleküler Biyoloji ve Genetik Bölümü, Istanbul, Türkiye.

[10] Istanbul Üniversitesi, Eczacılık Fakültesi, Farmakoloji Bölümü, Istanbul, Türkiye.

Objectives: Circadian rhythms, the inherent 24-hour cycles that govern biological processes in organisms, are essential for synchronizing an organism's internal functions with the external day-night cycle as Earth rotates on its axis. In mammals, circadian rhythms are orchestrated by intricate transcriptional and translational feedback loops at themolecular level. The core components of this molecular clock include the BMAL1:CLOCK dimer, which initiates the expression of clock-controlled genes, and the CRY:PER dimer, which inhibits the expression of these genes. Disruption of the circadian clock has been implicated in various diseases, spanning metabolic disorders, sleep disturbances, psychological conditions, and diabetes. this comprehensive study, we aimed to elucidate the effects of a novel molecule, TW68, on the degradation and modulation of CRY proteins and its consequent impact on gluconeogenesis and blood sugar levels. Our investigation spanned computational, in vitro, and preclinical studies, employing both cell line models and mouse models. Methods: We aimed to identify molecules that bind to the primary packaging of CRY proteins, specifically focusing on CRY2, using its three-dimensional structure. Our initial screening identified a set of non-toxic molecules that demonstrated the potential to enhance the stability of CRY proteins. This assessment was carried out through Western blot analysis. Subsequently, we investigated the effects of these stabilizing molecules on the circadian rhythm using the U2OS-Bmal1-dLuc cell line. Among the compounds tested, only TW68 exhibited remarkable efficacy and demonstrated the ability to modulate the circadian rhythm in a dose-dependent manner. Intriguingly, we further enhanced the utility of TW68 by conjugating it with biotin, allowing us to establish a physical interaction between TW68 and the primary packaging of CRY proteins. This development provided valuable insights into the mechanistic basis of TW68's circadian-modulating effects. To advance our research towards potential therapeutic applications, we conducted comprehensive studies involving toxicity assessments, pharmacokinetics, and pharmacodynamics in animal models. Substantial quantities of TW68 were synthesized for these investigations. Results: Computational studies led us to identify 100 molecules with binding energies below -7.0 kcal/mol, and we selected 70 of these molecules from the Ambinter library for further investigation. In our in vitro experiments, we found that the TW68 molecule significantly extended the half-lives of both CRY1 and CRY2 proteins when compared to the control DMSO, implying its potential to stabilize these crucial circadian clock proteins. Using the Hep2G cell line, we observed that TW68 effectively

suppressed the expression of *Pck1* and *G6pc* genes, key players in gluconeogenesis regulated through the glucagon-mediated G-protein coupled receptor. This suppression led to a remarkable decrease in glucose production compared to cells exposed to DMSO. In our toxicity studies with mice, a safe dose of 40 mg/kg of TW68 was determined. Pharmacokinetic investigations revealed that TW68›s oral bioavailability surpassed that of intraperitoneal (i.p.) administration, making it a promising oral therapeutic candidate. Furthermore, in diabetic mouse models (*ob/ob* and fat-induced), TW68 demonstrated its ability to control fasting blood sugar levels by suppressing the gluconeogenesis pathway. Collectively, our findings suggest that TW68 holds great promise as a potential treatment for metabolic disorders, particularly diabetes. CONCLUSION: The results have shown that the TW68 molecule is the first molecule that regulates fasting blood sugar by prolonging the degradation of CRY proteins and shows activity in a mouse model.
Keywords: Circadian Clock, Cryptochrome, diabetic mouse, Drug Discover

IS003

HARNESSING THE BIOLOGICAL CLOCK FOR BIOTECHNOLOGICAL APPLICATIONS

Nuri Öztürk, Fatma Yılmaz-atay, Buket Sünbül, Mehmet Serdar Koca, Gözde Özçelik, Feride Demirhan, Büşra Tiryaki

Department of Molecular Biology and Genetics, Gebze Technical University, Gebze, 41400, Kocaeli, Türkiye

The biological clock exists in almost all organisms and regulates physiology and metabolism in daily rhythmic patterns. In eukaryotes, the molecular clock is generated by a transcription-translation feedback loop (TTFL), where two transcription factors transactivate circadian clock-regulated genes (CCGs), which constitute 10–20% of the transcriptome. The products of the two CCGs then inhibit the activity of these two transcription factors. This transactivation and inhibition between the four clock components generates a 24-hour oscillation. The core oscillator is synchronized to the solar clock by a photoreceptor system. Although the core clock is very similar in animals, the photoreceptor mechanism differs. For example, while the clock is reset mainly by melanopsin pigments in mammals, it is reset by cryptochrome (CRY) protein in *Drosophila*. However, CRYs function in the core clock in mammals. Interestingly, CRYs are also photoreceptors in plants but operate by a different mechanism from that of Drosophila CRY. There is also another photoreceptor called CRY4 in birds, fish, and frogs. The involvement of CRY4s in the circadian clock could not be demonstrated, but a possible involvement in magnetic sensing was suggested instead. This variation in CRYs at mechanical and biochemical levels between organisms creates an opportunity to use them for optogenetic purposes, especially in mammalian cells. In particular, the photosensitive Drosophila CRY (DmCRY) and Arabidopsis CRY (AtCRY) do not interfere with mammalian CRYs, as mammalian CRYs are not photosensitive. Arabidopsis CRY fused to an artificial transcription factor such as VP64 can be directed to DNA sequences to transduce gene expression through dCAS9 fused to AtCRY's light-dependent partner CIB. There are also different designs for AtCRY, such as using AtCRY's light-dependent interactors to guide/tether proteins to a subcellular location. On the other hand, light-dependent interactors of DmCRY, such as JET, TIM and BRWD3, have been known for a long time and light-dependent degradation can occur in mammalian cells, but a DmCRY-based optogenetic tool has not been designed so far. We identified many light-dependent interactors of DmCRY using proximity-dependent labeling coupled with mass spectrometry. We test whether DmCRY and its interactors, including those previously known, regulate gene expression similarly to the AtCRY-VP64/CIB-dCas9 system and in a two-part VP16-GAL4 transcription factor system. Initially, we employed a GFP reporter system to screen the candidates. In another design, we are harnessing the light-dependent degradation of DmCRY to control gene expression. In this system, we fused DmCRY to TetR to repress the Tet-responsive Cas9 in mammalian cells. White light degrades DmCRY-TetR in a dose-dependent manner so Cas9 activity can be limited to a certain period. Because this system does not require any light-dependent interactors of DmCRY, it presents a safe system that does not interfere with the endogenous gene expression.

As a proof of concept, we successfully used the light-dependent degradation of DmCRY as an optogenetic tool, and we have some data with the other tool which is based on light-dependent interactions of DmCRY. A successful tool based on DmCRY, and light-dependent interactors will also provide a framework to test a possible magnetic field effect on DmCRY as it was suggested/shown as a magnetoreceptor. Moreover, CRY4 from birds is another candidate for being an optogenetic tool because there is no homologue of it in mammals, and it is not involved in the core clock regulation. We hope that at the end of this work, DmCRY-based optogenetic systems will be available for the researchers to use for optogenetic applications, to further study the action mechanism of DmCRY as a photoreceptor and/or magnetoreceptor, and to control the temporal activity of active proteins such as Cas9.

Keywords: Cryptochrome, Optogenetics, Photoreceptor

IS004

INTEGRATED DIAGNOSTICS: THE FUTURE OF DIAGNOSTIC MEDICINE?

Mario Plebani[1, 2]

[1] University of Padova, Padova, Italy

[2] University of Texas, Medical Branch, Galveston, Usa

Recent progress in diagnostic testing could enable more accurate diagnosis and improved clinical outcomes. However, diagnostic data are fragmented, being produced and delivered within the “silo” of each diagnostic discipline, and the electronic health record does little to synthesize existing data to be translated in usable and actionable information. Therefore, despite great promise, diagnoses may still be incorrect, delayed, or never made. Integrated diagnostics represents a vision for the future, wherein laboratory, pathology and imaging data, together with clinical information, are aggregated to support through expert systems, algorithms based on machine learning and artificial intelligence the provision to clinicians of a more actionable diagnostic information. The possible convergence of laboratory, pathology and imaging test results within the same medical report is, therefore, a valuable goal to foster earlier and more accurate diagnoses, and personalized medicine. The generation of a vast amount of data from the clinical laboratory, pathology genomics and radiology does not automatically convert to meaningful conclusions and higher effectiveness in both diagnosis and patient treatment. Diagnostic integration and generation of unified medical reports, coupled with machine learning techniques especially suited to analyze large amounts of data in real time, should be now adopted to foster an optimal diagnostic process and more specific, accurate, and complete diagnostic assessment. However, a combination of machine learning and human judgement should be taken for granted.

References

1) Lippi G, PLebani M Integrated diagnostics: the future of laboratory medicine ? Biochem Med 2020; 30: 1-13

2) Plebani M. Laboratory medicine in the COVID-19 era: six lessons for the future. Clin Chem Lab Med. 2021 Apr 7. doi: 10.1515/cclm-2021-0367. Epub ahead of print.

3) Plebani M, Laposata M, Lippi G. A manifesto for the future of laboratory medicine professionals. Clin Chim Acta. 2019;489:49-52.

IS005

DEVELOPMENT OF IMMUNOGENIC AUTOCHTHONOUS MOUSE LUNG CANCER MODELS TO STUDY IMMUNE RESISTANCE IN LUNG CANCER

Esra Akbay

University of Texas Southwestern Medical Center, USA

Objectives: Immune checkpoint blockade has revolutionized cancer treatment especially for non-small cell lung cancer. Biomarkers for therapeutic response to immune checkpoint blockade is unknown. Tumor intrinsic factors such as tumor mutational burden was associated with better responses to ICB in lung cancer. However not all tumors with high TMB respond well to ICB. Role of tumor mutational burden in shaping the tumor immunity and response to immune checkpoint blockade has not

been mechanistically addressed in clinically relevant autochthonous lung cancer models.

Methods: Because mouse models lack antigenic diversity, we induced mutations in lung cancer models by utilizing Polymerase epsilon catalytic subunit mutant mice (Pole P286R). This is an ultra-mutator variant of DNA polymerase-E (POLE)(P286R) detected in human tumors and causes elevation of TMB. We crossed this allele into the well-characterized Kras G12D;p53 L/L alleles, the two most commonly mutated genes in NSCLC.

Results: Addition of Pole significantly increased the TMB of the KP model. However, increasing TMB alone was not sufficient to induce immune responses with immune checkpoint blockade. This was in part due to mutational heterogeneity and in part due to tumor microenvironment as syngeneic models derived from these GEMMs were moderately sensitive to ICB. We also addressed the role of clonal heterogeneity in anti-tumor immunity using these models. In summary, we developed novel lung cancer GEMMs and syngeneic models with high TMB for studying immune resistance mechanisms.

Keywords: immunotherapy, mouse model, resistance

IS006

TELOMERASE-DRIVEN TELOMERIC DNA MODIFICATION AS POTENTIAL BROAD-SPECTRUM CANCER TREATMENT PLATFORM

Sergei Gryaznov[1], Ilgen Mender[1], Merve Yılmaz[2], Ateş Kutay Tenekeci[2], Larisa Birichevskaya[3], Anatoliy Zinchenko[3], Z. Günnur Dikmen[2]

[1] Maia Biotechnology, Inc., Chicago, Il 60606, Usa

[2] Hacettepe University Medical Faculty, Ankara, Türkiye

[3] Institute of Microbiology Nas, Minsk, Belarus

Objectives: Telomeres and telomerase in cancer are highly attractive targets for specific anti-tumor therapy, since telomerase is almost universally expressed in cancer cells, but not in most normal counterparts. Here we present properties of modified nucleoside - 6-thio-2'-deoxyguanosine (6-thio-dG; THIO), and new lipid-conjugated prodrug forms of this pharmacophore, as potential anticancer agents with unexpected and previously undescribed mechanism of action. Methods: *In vitro*, THIO is readily converted into the corresponding nucleoside-5'-triphosphate, which is a substrate for mammalian telomerase. Incorporation of this compound, by telomerase, into *de novo* synthesized cancer cell telomeres leads to their structural modification, followed by a fast induction of telomeric DNA damage responses and apoptosis. Notably, the cancer cell death occurs in telomere length-independent manner. Moreover, THIO treatment leads to the formation of cytosolic and extracellular micronuclei structures, containing cGAS/STING pathway neo-adjuvants - the *de novo* generated THIO-modified telomeric DNA fragments. In addition to activation of innate immunity, these *in situ* produced micronuclei-encapsulated neo-adjuvants along with newly released cancer cell antigens were exported extracellularly, and ultimately, absorbed by the host dendritic cells. This process resulted in enhanced cross-priming and tumor-specific T-lymphocyte activation, (both CD4+ and CD8+ cells). Importantly, treatment with THIO overcomes resistance to checkpoint blockade (by anti-PD-1 or anti-PD-L1 agents) in advanced *in vivo* cancer models, leading to profound anticancer effects, and to potent induction of tumor type specific long-term anticancer memory in mice. Thus, *in vivo* essentially curative activity was observed in murine syngeneic models of multiple tumor types, including colorectal (MC-38), non-small cell lung (LLC) and melanoma (YUMM1.7) cancers, when THIO was used in *sequential combination* with anti-PD-L1 agent (atezolizumab). Combinations with other immune checkpoint inhibitors (*i.e.,* anti-PD-1) with THIO were also highly effective. Currently, THIO is in Phase 2 human clinical trials for NSCLC treatment. Results: New THIO prodrugs - phosphatidyl (C_4 - C_{18}) diglyceride derivatives of THIO were prepared using a Phospholipase D - catalyzed biochemical process in aqueous-organic heterophasic media. *In vitro*, these lipid conjugated compounds were able to induce telomeric DNA damage responses that were similar or more profound than those for THIO, as it was assessed by quantitative Telomere Damage

Induced Foci assay (TIF formation). Efficient formation of micronuclei structures was also observed. The compounds anticancer cytotoxic activity - EC_{50} values were determined in multiple human and murine cancer cell lines - HeLa, A549, Lovo, HT-29, CT26, U-87. The EC_{50} values were in sub-μM to low-nM range, and in general, similar to that for THIO. The optimal fatty acid chain length, chemistry and hydrophobicity of the lipid diglyceride groups that affect compounds solubility and anticancer activity were also determined. *In vivo*, initial evaluation of the anticancer activity that conducted in human xenografts and murine syngeneic models of colorectal cancer, established with human HT-29 and murine CT26 tumor cells, respectively, have demonstrated potent anticancer activity at relatively low dose levels for one of the lead lipid conjugates, (3 mg/kg; 4 injections). Combination with murine anti-PD-1 agent was also evaluated. CONCLUSION: Our findings demonstrate the importance of cancer cell telomeric DNA structural and functional integrity for their survival. We also demonstrated a broad-spectrum therapeutically attractive opportunities for specific telomeric stress inducing treatments, resulting in the increase of innate sensing and adaptive antitumor immunity *via "cancer cell self-produced"* chemical modification of telomeres by THIO. Currently, this telomere-centric approach and the leading molecule (THIO) are being evaluated in Phase 2 clinical trials (named THIO-101 trials) in non-small-cell lung carcinoma (NSCLC) patients. Future clinical trials are pending. The described above early results warrant further *in vivo* in-depth investigation of the phosphatidyl-THIO conjugates as a potential second generation of telomerase-mediated telomere-targeting compounds.

Keywords:6-thio-2-deoxyguanosine, phosphatidyl nucleosides, telomeres, cancer

IS007

IMPLEMENTATION FOR SUSTAINABLE PRACTICES IN MEDICAL LABORATORIES: SWITCHING CLINICAL LABORATORIES TO GREEN LABS

Tomris Ozben

European Federation of Clinical Chemistry and Laboratory Medicine, President; Eflm Task Force-green and Sustainable Labs, Chair

Laboratory medicine should contribute to a sustainable healthcare system ensuring that resources are used efficiently from ecological, social, and economical perspectives, while providing high-quality services to patients and physicians. It will be a challenge for clinical laboratories to achieve sustainable operations. Clinical laboratories use more energy and water than offices and generate huge amounts of hazardous and non-hazardous wastes every year. Clinical laboratories can limit their environmental impact and provide sustainable laboratory services making reductions in four key areas—energy consumption, water consumption, waste production, and use of hazardous chemicals. Establishing sustainable development goals and applying multiple means for reductions in these key areas, clinical laboratories can reduce their environmental impact. By being mindful of the environmental impact of everyday actions in a lab, and by taking steps to minimize energy, water, and hazardous chemical use, as well as waste generation, a clinical lab can be transformed into a safe, sustainable space. Sustainability measures should be a key feature in the rapidly changing healthcare environment to reduce their negative impacts on the environment and economy. Laboratory medicine community should lead the shift to carbon neutrality by decreasing their deleterious environmental impact and implementing efficient approaches to address the effects of climate change and pollution without compromising the quality of healthcare. In order to provide high-quality, effective, and safe healthcare services, sustainable healthcare systems need to overcome major economic and social challenges. Though there will be initial capital costs, there is a long-term cost-saving potential of a more efficient use of energy and other resources in healthcare systems. Despite this, there is a long way to go for environment-friendly hospitals, healthcare structures, and clinical laboratories to become the norm. Good collaboration among the healthcare systems and a common vision for future actions would help to achieve such goals.

Keywords:green laboratories, hazardous chemicals, waste

IS008

CURRENT ADVANCES IN CLINICAL APPLICATION OF TUMOR BIOMARKERS: UTILITY,CHALLENGE, AND PERSPECTIVE

Qing H. Meng

The University of Texas, Md Anderson Cancer Center, Department of Labrotory Medicine, Texas, USA

While traditional tumor biomarkers have long served as essential tools in cancer screening, diagnosis, patient management for cancer patients, new emerging tumor biomarkers have been discovered and used in today's oncology practice. For clinical use, some of the tumor markers are FDA-approved while many traditional tumor markers and emerging biomarkers are non-FDA approved but have been demonstrated to be useful in clinical practice. Despite their clinical significance, there remain significant challenges that hinder their clinical utilities. These include biological heterogeneity, analytical complexity, limitations of clinical sensitivity and specificity, lack of harmonization, and regulatory approval hurdles. Immunoassays are extensively used in tumor marker determination as they are rapid, simple, cost effective and available on most chemistry platforms. However, these immunoassays are compromised by many factors including lack of analytical and clinical sensitivity and specificity, method-related difference, hook effect, and susceptibility to immune interference. These shortcomings can compromise result accuracy, leading to potentially incorrect result interpretation and patient mismanagement. The limitations and challenge of tumor biomarkers will be discussed. Amidst the challenges of traditional tumor biomarkers, molecular diagnostics and liquid biopsy are rapidly advancing and emerging. These innovations, driven by cutting-edge technologies and scientific research, mark a new era in personalized oncology. Liquid biopsy, in particular, has emerged as a noninvasive tool capable of providing real-time insights into the spatial and temporal heterogeneity of tumors. An array of circulating biomarkers, including circulating tumor DNA (ctDNA), microRNA, lncRNA, extracellular vesicles, and metabolites, have been harnessed for clinical applications in cancer patient care. The integration of new technologies spanning genomics, epigenomics, transcriptomics, proteomics, and metabolomics has further accelerated the discovery of novel tumor markers. Molecular diagnostics now take center stage in personalized medicine, particularly within the realm of precision oncology. Liquid biopsy-based biomarkers are being used in characterization of an individual patient's biological characterization and profiling for risk assessment, guiding therapy, monitoring treatment response, and prediction of prognosis. These advancements aim to maximize clinical benefits and outcomes for cancer patients. Given the biological heterogeneity in cancer and analytical complexities in tumor biomarkers, the future lies in the integration of traditional tumor biomarkers, multi-omics, molecular diagnostics, and liquid biopsy biomarkers. Leveraging artificial intelligence models will be pivotal in navigating this complex landscape. The integrated approach holds promise as a desirable strategy for achieving personalized precision oncology in cancer patient care. After this presentation, participants will be able to:

-Understand the clinical utility, limitations, and challenges of traditional tumor. biomarkers

-Identify new insights and utility of tumor biomarkers.

-Learn liquid biopsy-based technologies and biomarkers in precision oncology.

IS009

STANDARDIZATION AND HARMONIZATION IN LABORATORY MEDICINE: DATA COMPARABILITY IS NEEDED

Mario Plebani[1, 2]

[1] University of Padova, Padova, Italy

[2] University of Texas, Medical Branch, Galveston, Usa

In the last decades increasing efforts have been made to promote standardization and harmonization in laboratory medicine, but during the COVID-19 pandemic the issue of comparability of laboratory information received an even increased concern as this may avoid confusion and wrong interpretation of laboratory results. In addition, standardization and harmonization are fundamental issues for improving patient safety and for the accreditation of medical

laboratories according to ISO 19189. The terms standardization and harmonization are often used interchangeably, probably because the aim is the same: to provide the clinicians and patients with laboratory results that are comparable and equivalent among different laboratories and over the time. However, it should be taken into account that the two terms refer to different concepts. Standardization should be used when the results are uniform among routine measurement procedures and traceable to a recognized standard reference material defined by the International System of Units (SI) through a high-order primary reference material and/or a reference measurement procedure. Harmonization is aimed to make the results comparable irrespective of the measurement procedure, mainly because neither a reference measurement procedure nor a primary reference material is available. Notably, this is the case of the vast majority of the measurands determined every day in clinical laboratories. In particular, most measurands that included in the immunoassay area should be harmonized but most standardized being their structure complex and characterized by heterogeneity. Snibe Diagnostics has developed a new assay format for small molecules assay which resents better accuracy and correlation with reference methods. The other important difference between the two terms is that the harmonization process includes all the aspects of the total testing process (TTP) besides the analytical quality, taking into consideration pre-analytical issues such as harmonization of patient preparation, sample collection and handling, and post-analytical aspects such as harmonization of measurement units, reference intervals and decision limits. In the last few years, many interesting contributions have been published to improve the knowledge on the harmonization in laboratory medicine but further efforts are needed.
References

1) Plebani M. Harmonization in laboratory medicine: the complete picture. Clin Chem Lab Med. 2013;51(4):741-51
2) Plebani M. Harmonization in laboratory medicine: Requests, samples, measurements and reports. Crit Rev Clin Lab Sci. 2016;53(3):184-96.
3) Plebani M. Harmonization in laboratory medicine: more than clinical chemistry? Clin Chem Lab Med. 2018;56(10):1579-1586.

IS010

RISKS AND OPPORTUNITIES PROVIDED BY ISO 15189:2022 STANDARD FOR CONTINUOUS IMPROVEMENT OF MEDICAL LABORATORY PRACTICES

Canan Karadağ

Eskişehir City Hospital, Eskişehir, Türkiye

ISO 15189, which is the most widely used standard worldwide for the accreditation of medical laboratories, has been updated to its latest version, ISO 15189:2022 Medical laboratories – Requirements for quality and competence. The previous version was published in 2012. The new standard contains requirements and changes that require attention from medical laboratory experts. The transition period set by ILAC (International Laboratory Accreditation Cooperation) for conversion to the new standard is 3 years from its publication date. This period is for signatory accreditation bodies, such as Turkish Accreditation Agency (TURKAK). Facilities will be assessed against the new standard at the time of their next scheduled visit from January 2024 in Turkey . The structure of the standard is different from the previous version in compliance with CASCO requirements. There is an increased focus on patient welfare by assessing, planning, and implementing actions to address risk.

The main change in the latest edition of ISO 15189 is the adoption of risk-based thinking. The standard requires medical laboratories to have a process for identifying risks that could harm patients and opportunities for improving patient care. Addressing both risks and opportunities can increase the effectiveness of the management system and lead to improved results. The laboratory is responsible for deciding which risks and opportunities to address, and the actions taken should be proportional to their potential impact on laboratory examination results, as well as patient and personnel safety. Also, the standard refers to the ISO 22367 document, which describes risk management for medical laboratories. ISO 15189:2022 provides greater flexibility in the requirements for processes, procedures, documented information, and organizational responsibilities. The standard requires documentation to be as extensive as necessary without being prescriptive on what must be documented.

The standard no longer prescribes a quality policy or specifically a quality manual. Procedures, however, still need to be maintained. Specific reference to a quality manager as covered by ISO 15189:2012 has been removed.
ISO 15189:2022 requires the facility to implement a management system, which can be either a Certified Quality Management System or a non-certified Quality Management System. Non-certified Quality Management Systems require clauses 8.2 to 8.9 of the standard to be addressed.
The standard expands on the requirements relating to confidentiality and management of patient information. Laboratories are required to inform patients if they intend to make information publicly available. Patients' well-being, safety, and rights must be the laboratory's primary consideration.
The laboratory must specify calibration and traceability requirements that are sufficient to maintain consistent reporting of examination results. The measurement uncertainty (MU) must be compared against performance specifications and documented. MU evaluations must be regularly reviewed. The validity of laboratory results must be monitored and recorded so that any trends or shifts are detectable. MU information must be made available to laboratory users upon request. The standard refers to the ISO 20914 document for MU estimation.
Appendix A of ISO 15189:2022 covers all point-of-care testing (POCT) devices that are included under the scope of accreditation, regardless of the type of POCT device. Service agreements between the laboratory and other parts of the organization using laboratory-supported POCT must ensure that respective responsibilities and authorities are specified and communicated.
In summary, the most important goal of the ISO 15189:2022 standard, which focuses on the continuous improvement of medical laboratory services by addressing risks and opportunities, is to increase the contribution of medical laboratories to patient care.

Keywords: ISO 15189, quality management, accreditation, risk management, risks and opportunities

IS011

CHARTING THE PATH FROM PAST STUDIES TO A VISIONARY FUTURE WITH THE NEW STRUCTURE OF THE EFLM QUALITY AND REGULATIONS COMMITTEE

Hikmet Can Çubukçu

General Directorate of Health Services, Rare Diseases Department, Turkish Ministry of Health, Ankara, Turkey

The EFLM Quality and Regulations Committee is responsible for producing guidance documents to increase the applicability of regulations in quality management, supporting the establishment of effective accreditation programs in all European countries, coordinating accreditation issues related to laboratory medicine and cooperating with ISO, CEN and the European Accreditation Cooperation on accreditation issues, representing EFLM as a stakeholder in European and International regulatory and legislative bodies. The Working Group on Accreditation and ISO/CEN standards within the Committee is responsible for representing the EFLM and the interests of European laboratories in ISO TC212 and CEN TC140 & EU regulatory frameworks (EU IVD Regulation, EU Health Policy Group), staying informed of developments and disseminating this information to EFLM National Society Members, addressing legitimate concerns of the profession and collaborating with the diagnostic industry. In order to harmonize accreditation in Europe, the working group conducts international surveys on current practices, develops guidance documents for laboratories, supports the translation and implementation of accreditation standards into practice, and prepares training documents for laboratory medicine professionals and assessors of accreditation bodies on the application of specific professional standards of ISO 15189. In the coming period, the committee structure will focus on quality management and implementation of regulations in clinical laboratories under two separate headings and will focus on activities to facilitate the implementation of regulations in clinical laboratories.

Keywords:EFLM, quality, regulations, ISO 15189

IS012

OVERVIEW OF RECENT CLSI GUIDELINES: ENHANCING LABORATORY MEDICINE PRACTICES

Sedef Yenice

Gayrettepe Florence Nightingale Hastanesi, Istanbul, Türkiye

To ensure the highest standards of quality and consistency in laboratory practices, the Clinical and Laboratory Standards Institute (CLSI) has been at the forefront of developing guidelines for laboratory professionals worldwide. The implementation of CLSI guidelines has had a profound impact on laboratory practices globally. By following these evidence-based recommendations, medical and clinical laboratories can enhance the accuracy, precision, and reliability of their test results and establish effective result communication, ultimately leading to improved patient outcomes. Additionally, compliance with CLSI guidelines enables laboratories to meet regulatory requirements and obtain accreditation, thus further validating their commitment to quality and patient safety. This presentation aims to provide an overview of the recent CLSI guidelines and their impact on enhancing laboratory medicine practices.

Keywords:biochemistry, machine

IS013

HARMONIZING THE POST-ANALYTICAL PHASE

Mario Plebani[1, 2]

[1] University of Padova, Padova, Italy

[2] University of Texas, Medical Branch, Galveston, Usa

A body of evidence has been collected in the last decades to demonstrate that extra-analytical phases of the total testing cycle are much more vulnerable to errors than the analytical phase and, even worse, to errors that may lead to diagnostic errors and patient harm. Great efforts have been done to improve the pre-analytical phase while there is still a window of opportunities for improving and harmonizing the post-analytical phase. While the quality, accuracy and reliability, of analytical results is undoubtedly important, so too is the quality of the final laboratory report which includes measurement units, reference intervals, decision limits and interpretative comments. In fact, if the numerical data are not accompanied by valuable comparators (as psareviously cited) they cannot be “actionable” and valuable for the clinical decision making. The harmonization of the post-analytical phase, however, is a rather complicated issue because it implies an exercise of communication that involves parties speaking different languages (i.e laboratory professionals, physicians, information technology specialists and patients). I will review the main issues regarding the laboratory report and its notification, suggesting future initiatives to provide a better laboratory information to both physicians and patients.

References

1) Plebani M. The detection and prevention of errors in laboratory medicine. Ann Clin Biochem. 2010 Mar;47(Pt 2):101-10

2) Plebani M. Towards a new paradigm in laboratory medicine: the five rights. Clin Chem Lab Med. 2016 Dec 1;54(12):1881-1891

3) Plebani M, Lippi G. Improving the post-analytical phase. Clin Chem Lab Med. 2010, 48:435-6

IS014

THE ROLE OF PROTEIN STRUCTURE-FUNCTION RELATIONSHIP IN THE PATHOGENESIS OF AUTOIMMUNE DISEASES:ANKYLOSING SPONDYLITIS AS AN EXAMPLE

Yunus Emre Dilek[1], Sena Kıvrak[2], Irem Kara[2], Şeyma Çolakoğlu Özkaya[3], Kerem Yiğit Abacar[5], Can Erzik[4], Mehmet Pamir Atagündüz[5], Günseli Bayram Akçapınar[1]

[1] Acıbadem University, Institute of Health Sciences Department of Medical Biotechnology, Istanbul, Turkey

[2] Acıbadem University, Institute of Health Sciences Department of Biostatistics and Bioinformatics,

Istanbul, Turkey

[3] Marmara University, Institute of Health Sciences, Department of Medical Biology, Istanbul, Turkey

[4] Marmara University, Faculty of Medicine, Department of Medical Biology and Genetics, Istanbul, Turkey

[5] Marmara University, Faculty of Medicine, Department of Rheumatology, Istanbul, Turkey

There is an intricate interplay of protein structure-function relationships in the pathogenesis of autoimmune diseases, especially in Ankylosing Spondylitis (AS). the Major Histocompatibility Complex I allele, HLA-B*27, and Endoplasmic Reticulum Associated Aminopeptidase ERAP1 both *have pivotal roles in shaping the immune response and contributing to the development of AS. HLA-B*27*, a well-established genetic risk factor, undergoes structural changes that influence antigen presentation, ultimately triggering autoimmunity. ERAP1, an aminopeptidase involved in peptide processing, influences the peptide repertoire presented by HLA-B*27, thereby contributing to AS susceptibility. In this talk, I will discuss our recent computational findings based on the arthritogenic peptide presentation on HLA-B*27 and ERAP dimerization. Understanding these dynamic relationships at the structural and molecular level sheds light on the mechanisms underlying autoimmune diseases and opens new avenues for therapeutic strategies.

Keywords: Ankylosing spondylatis, molecular dynamics, arthritogenic peptides, peptide presentation, ERAP, HLA-B27

IS015

HOW AI WILL REVOLUTIONIZE STRUCTURE-BASED DRUG DESIGN

Ezgi Karaca [1,2]

[1] Izmir Biomedicine and Genome Center, Dokuz Eylul University, Izmir, Türkiye

[2] Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Türkiye

The understanding of protein interactions at the atomistic scale is crucial for studying cellular function. Experimental techniques like X-ray diffraction, NMR spectroscopy, and cryo-electron microscopy (cryo-EM) provide high-resolution structures of protein complexes. However, in cases where experimental approaches face limitations, modeling becomes valuable. In particular, homology modeling is employed when there is a resolved structure of an evolutionarily related complex, while docking is preferred in the absence of such a template. Various strategies, including coevolution integration and the use of available experimental data, have been implemented to enhance docking accuracy. For intricate cases involving intertwined complexes, fold-and-dock strategies are employed.

The CAPRI blind docking competition has been evaluating the state-of-the-art in assembly modeling since 2002. In 2014, CAPRI joined forces with CASP to assess the prediction of protein complexes on a larger scale. Several rounds of CASP-CAPRI experiments have been conducted, shedding light on the capabilities and limitations of assembly modeling approaches. A major limitation in protein complex modeling has been the absence of reliable templates for modeling the monomer structures of an assembly. This limitation has been alleviated to a large extent with the release of AlphaFold2 (AF2), an artificial intelligence (AI) tool that has made unprecedented progress in tertiary structure prediction. In CASP14, AF2 demonstrated high accuracy in modeling tertiary structure targets regardless of the prediction difficulty. Consequently, the release of AF2 Protein Structure Database in 2022, with over 214 million predicted protein structures covering nearly all UniProt sequences, has significantly impacted the field of structural biology.

Since the release of AF2, scientists have sought to incorporate this framework into their modeling pipelines. The simplest way to employ tertiary structure modeling methods to quaternary structure modeling was to join individual sequences of complex subunits into a longer, artificial sequence by means of adding an artificial glycine linker between monomers or introducing a sequence gap between multiple chains. These approaches showed improvement over classical docking methodologies. In 2021, DeepMind released AlphaFold-Multimer (AF2M, version 2.2), the multimeric version of AF2 specifically retrained on biological interfaces. AF2M outperformed

previously outlined AF2-monomer modifications in the case of heteromers. Although DeepMind did not participate in CASP15, they did so indirectly since the vast majority of assembly groups adopted AF2M in their modeling pipelines.

During CASP14 and CASP15, I acted as the assessor in the biomolecular assembly prediction category. During my talk, I will present our CASP-based analysis to demonstrate the state of AI in protein complex modeling. I will also show how the community could push the boundaries of AI-based assembly modeling forward, together with how AI will revolutionize structure-based drug design.

IS016

AMBIENT TEMPERATURE STRUCTURAL STUDIES OF RIBOSOME COMPLEXES

Hasan Demirci

Koc University

High-resolution ribosome structures determined by cryo X-ray crystallography have provided important insights into the mechanism of translation. Such studies have thus far relied on large ribosome crystals kept at cryogenic temperatures to reduce radiation damage. We use serial femtosecond X-ray crystallography (SFX) with an X-ray free-electron laser (XFEL) to obtain diffraction data from ribosome microcrystals in liquid suspension at ambient temperature. Small 30S ribosomal subunit microcrystals programmed with initiation and decoding complexes and bound to either antibiotic compounds or their next-generation derivatives diffracted to high resolution. Our results demonstrate the feasibility of using SFX to better understand the structural mechanisms underpinning the interactions between ribosomes and other substrates such as antibiotics, initiation and decoding complexes. We have determined the structure of a large (50S) ribosomal subunit in a record-short time by using the record-low amount of sample during an XFEL beamtime. This structure is the largest one solved to date by any FEL source to near-atomic resolution (3 MDa). We expect that these results will enable routine structural studies, at near-physiological temperatures, of the large ribosomal subunit bound to clinically relevant classes of antibiotics targeting it, e.g. macrolides and ketolides, also with the goal of aiding the development of the next generation of these classes of antibiotics. Overall, the ability to collect diffraction data at near-physiological temperatures promises to provide new fundamental insights into the structural dynamics of the ribosome and other medically important drug targets with their functional and inhibitor complexes.

Keywords:X-ray crystallography, near-physiological temperature, Serial Femtosecond X-ray Crystallography

IS017

DIGITAL TRANSFORMATION IN THE CLINICAL LABORATORY: RELIABLE SAMPLE TRACKING, EFFICIENT DATA ANALYSIS, FAST RESULTS AND CLINICAL BENEFITS

Erhan Palaoğlu[1], Said Incir[2], Oğuzhan Zengi[3]

[1] American Hospital, Istanbul, Türkiye

[2] Koç University, Istanbul, Türkiye

[3] Çam ve Sakura City Hospital, Istanbul, Türkiye

Digital transformation offers the potential to reduce errors, improve cost-effectiveness and quality while optimizing workflow in clinical laboratories. It is possible to reduce a significant proportion of errors, especially in the preanalytical process, through automation and integrated systems. This not only enables data analysis and rapid generation of results to improve the efficiency of the laboratory, but also increases the reliability of clinical results. Digital laboratory applications bring both clinical and financial benefits to the healthcare industry. Integration with clinical decision support systems provides unique clinical insights in patient management with the help of evidence-based algorithms and artificial intelligence. Cost/Benefit analysis generally shows a positive trend; automation and data analytics optimize resource utilization by reducing retesting and errors. In terms of sustainability, digital transformation also has positive impacts on carbon emissions and waste management in laboratories. The 'Green Lab' concept offers an approach to optimizing energy and resource use. However, taking this concept from theory to practice requires technological investment and political commitment.

In conclusion, digital transformation can be the key to improving workflow, service quality and sustainability in laboratories, but it is essential that this transformation is managed effectively.

Keywords:digital transformation, sample tracking, data analysis

IS018

CAREER DEVELOPMENT FOR YOUNG SCIENTISTS IN CLINICAL BIOCHEMISTRY AND LABORATORY MEDICINE

Qing H. Meng

The University of Texas, Md Anderson Cancer Center, Department of Labrotory Medicine, Texas, USA

Completing a master's degree, PhD, or post-doctoral training can be an exciting but challenging phase for young scientists. The question often arises: "What's the next step?" Fortunately, there are numerous career avenues to explore. One particularly promising option is a career in Clinical Biochemistry and Laboratory Medicine, offering a diverse array of opportunities in diagnostics, consultation, education, research, administration, healthcare, government agencies, and industry, among others. This session will discuss some of the special training programs and the career opportunities for those individuals who have completed their PhD or postdoctoral training in the field of basic sciences and medicine, with a specific focus on clinical biochemistry and laboratory medicine in the United States and other Western societies. Clinical Biochemists play a vital role as members of laboratory management teams, primarily responsible for setting performance standards in Clinical Biochemistry Laboratories. They leverage a spectrum of skills honed during post-doctoral training to ensure that laboratory services efficiently cater to patient needs. Clinical Biochemists are quality experts who monitor the integrity of testing services. They are also technical experts tasked with method and instrumentation selection and validation. Their unique blend of clinical and technical expertise aids physicians in test selection and interpretation, leading to earlier, more precise diagnoses and personalized patient therapy. Additionally, they actively contribute to the research and teaching endeavors of Clinical Biochemistry Laboratories. The session will also introduce training opportunities in other areas of laboratory medicine, including medical microbiology, medical genetics and genomics, histocompatibility and immunogenetics, and other disciplines relevant to laboratory medicine. Moreover, it will address avenues for professional career development. After this presentation, participants will be able to:

- Review the landscape of Laboratory Professionals in the United States.

- Understand the pathways and processes of education, training, and board certification.

- Identify opportunities for practice and career development in the field of Laboratory Medicine.

IS019

BIOCHEMICAL, GENETIC AND PHENOTYPIC CHARACTERIZATION OF LYSOSOMAL SULFATIDE DEGRADATION DISORDERS

Hatice Asuman Özkara

Hacettepe University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

Sulfatides are sulfated glycosphingolipids found in all organs especially in the central and peripheral nervous systems and kidneys. They are degraded by lysosomal enzyme Arylsulfatase A (ASA) and its activator protein saposin B in lysosomes. ASA is synthesized and posttranslationally modified in ER by formylglycine-generating enzyme (FGE). Deficiency of ASA, saposin B or FGE causes accumulation of sulfatides progressively in cells. In the deficiency of FGE, other sulfated substrates are also accumulated due to multiple sulfatase deficiency (MSD). ASA deficiency is known as Metachromatic Leukodystrophy (MLD). It is characterized by progressive nervous system demyelination. Diagnosis is complicated because of clinical heterogeneity of the disease which ranges from a severe fatal form to a milder adult onset form. Pseudodeficiency of ASA, saposin B deficiency and multiple sulfatase deficiencies are also considered for diagnosis. Deposited sulfatide can also trigger the inflammatory response and affects the clinical phenotype.Laboratory diagnosis is usually made by leukocyte ASA assay. Two problems with the test are: 1) two common pseudodeficiency alleles of ASA that result in low ASA activity, and 2) saposin

B deficiency which has normal ASA activity. Urinary sulfatideanalysis can discriminate saposin B deficient from healthy and pseudodeficient. DNA analysis is helpful for identification of genetic lesion and also for genetic counseling and screening of asymptomatic family members. In some phenotypes differentiation of arylsulfatase isoenzymes is needed. Therefore, comprehensive biochemical, genetic, radiologic and clinical evaluation is required for molecular and phenotypic characterization. In this context individuals with clinically suspected MLD who applied to Hacettepe University Hospitals and clinically unaffected family members were evaluated by lysosomal enzyme assay, arylsulfatase isoenzyme differentiation, urinary sulfatide extraction and analysis, sequencing of ASA, PSAP and SUMF1 exons, Western blotting of ASA proteins, detection of anti-sulfatide and anti-MOG antibodies. In this presentation, evaluation of individuals with suspected MLD by comprehensive clinical, biochemical, radiological, and genetic analyses for molecular and phenotypic characterization will be reviewed. Additionally, a modified diagnostic laboratory algorithm and a novel MRI scoring system for comprehensive evaluation of disease severity in MLD will be presented. Characterization of the phenotype of sulfatide degrading enzyme deficiencies and identification of the presymptomatic cases are important for current and future therapeutic options.

Keywords: Metachromatic leukodystrophy, Arylsulfatase A, Urinary sulfatide, ARSA, Multiple sulfatase deficiency

IS020

SKIN BARRIER CERAMIDE METABOLISM IN HEALTH AND DISEASE

Roger Sandhoff

German Cancer Research Center, Heidelberg, Germany

The epidermis provides terrestrial vertebrates with a pivotal defensive barrier against water loss and harmful pathogens. The lipid-enriched lamellar matrix that embeds the enucleated corneocytes of the stratum corneum is a vital demand for this epidermal permeability barrier. Ceramides are major components of these highly ordered intercellular lamellar structures, in which linoleic acid- and protein-esterified ceramides are crucial for structuring and maintaining skin barrier integrity. These special ceramide structures and topology demand adaptation of ceramide metabolism during keratinocyte maturation and the expression of unique gene combinations. Therefore genetic mutations of genes involved in ceramide metabolism often result especially in skin barrier deficiencies although some of these genes are ubiquitously expressed in our body.

Keywords:ceramides, skin barrier, metabolism, genetic disorders, ichthyosis

IS021

NMR SPECTROSCOPY IN CLINICAL LABORATORIES: LIPOPROTEIN PROFILE ANALYSIS

Mustafa Serteser

Acibadem Mehmet Ali Aydinlar University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Türkiye

Nuclear magnetic resonance (NMR) spectroscopy is an advanced characterization technique to evaluate local magnetic fields around atomic nuclei. It's based on measurement of absorption of electromagnetic radiations in the radio frequency region (4-900 MHz). Although NMR spectroscopy has long been used in academic research fields like; to quantitate compounds in the mixture, to determine molecular structure of compounds, to check sample purity, to evaluate drug structures, it is currently advancing in the field of clinical laboratory diagnostics. Recently, NMR spectroscopy has been featured intensively in the field of metabolomics which provides rewarding data about downstream products of both metabolic and cellular processes. It quantifies metabolite concentrations in several body fluids like; serum, plasma, urine and cerebrosipinal fluid. ^{1}H NMR spectra are used to generate catalogues of profiles of a huge number of metabolites. Development of automated tools for the determination of lipids and lipoproteins is another important advance in NMR-based metabolomics. Lipoproteins contain different concentrations of cholesterol esters, free cholesterol, triglycerides, phospholipids and various different proteins. The at

herogenic part of triglyceride-rich lipoproteins is the cholesterol content which is the marker for remnant cholesterol because of the correlation between these two lipid components of the same lipid particle. NMR-based lipoprotein profiling became the gold standard for lipoprotein analysis since it represents one of the most succesful examples of metabolomics translated into clinical practice. Coronary atherosclerosis is the formation of lipid rich plaque in arterial walls, is an important underlying cause for coronary artery disease (CAD). Since conventional lipid measurements do not provide enough insights about size, density or composition of lipoproteins, known risk factors like high levels of LDL-cholesterol and low levels of HDL-cholesterol fail for the prediction of risk of CAD. NMR spectroscopy provides detailed measurements of cholesterol distribution in different lipoprotein subfractions. Traditionally the classification has based on density and size determination by ultracentrifugation and gel filtration. Exploring lipoprotein subfraction will contribute to understanding how atherosclerosis develops. The continued applications of NMR-based metabolomics led advancements nearly at every scientific field including biomarker discovery and clinical laboratory diagnostics. Several unique characteristics like rapid acquisition and reproducibility of sectra, requirement of little or no sample preperation, preservation of sample integrity are compelling strengths of NMR spectroscopy.

Keywords:NMR, Metabolomics, Lipid, Lipoprotein Particles

IS022

REGULATION OF NEURONAL GANGLIOSIDE DEGRADATION BY GENETIC AND POSTTRANSLATIONAL MODIFIERS

Konrad Sandhoff

Univesity of Bonn, Lımes Institute, Bonn, Germany

Amaurotic Idiocy was the clinical and pathological description of an inherited neurodegenerative disease. My first lipid analysis of the postmortem brain tissue of an infantile patient yielded also known as remnant cholesterol. Plasma triglycerides are two storage compounds. Elucidation of their chemical structures identified them as ganglioside GM2 and its sialic acid free residue GA2. Based on their structure the isoenzymes HexA & B were proposed as candidates for their catabolism. The enzymatic analysis of 3 patients by using radiolabeled storage compounds as substrates yielded controversial results in 1966 and 1967. Brain tissue of one patient had increased HexA & B activities, another one had no activity of both iso-enzymes whereas the third one was deficient of HexA activity only. Studies on the structure of catabolic enzymes, their genes and patient mutations in different laboratories confirmed 3 different genetic diseases, now known as Tay-Sachs disease with HexA deficiency, Sandhoff disease with loss of both, HexA & B activities, and AB-variant of GM2-gangliosidoses with a loss of GM2AP, an essential lipid binding protein for catabolism of GM2. The lipid-cleaving activity of these hydrolases is effectively regulated by secondary lysosomal factors and especially by primary storage compounds, which can induce cascades of secondary storage, e.g. in acid sphingomyelinase (ASM) deficiency disease. Catabolism of complex membrane lipids occurs at the surface of intra-lysosomal luminal vesicles (ILVs). They are degraded by the cooperation of soluble lysosomal hydrolases and soluble lipid-binding and lipid-lifting activator proteins, as there are saposins and the GM2 activator protein (GM2AP). Both, acid hydrolases and lipid binding proteins, are quite unspecific and handle a rather broad spectrum of substrates. Besides saposins and GM2AP, the lipid cleaving activity of acid hydrolases is heavily regulated either by stimulating or by inhibiting membrane lipids of the substrate carrying ILV-membranes. The **primary** genetic defect of an unspecific lysosomal hydrolase can trigger the lysosomal accumulation of several lipid substrates, e.g. of up to 20 different phospholipids (including the growth factor Cer1P and the lysosome-specific stimulator of catabolic pathways, BMP (bis(monoacylglycero)phosphate) in acid sphingomyelinase deficiency (ASMD) disease. The inherited ASMD in Niemann-Pick disease type A and B impairs ma inly, but not only, cellular sphingomyelin catabolism, causing a progressive sphingomyelin accumulation, which triggers a **secondary** accumulation of lipids such as cholesterol, glucosylceramide, ganglioside GM2 by inhibiting

their turnover in late endosomes and lysosomes. ASM appears furthermore to be involved in a variety of major cellular functions with regulatory significance for an increasing number of metabolic disorders. The **primary** lysosomal accumulation of membrane lipids and also of soluble complex glycan molecules can trigger a **secondary** accumulation of complex membrane lipids. This concept will be discussed also for **secondary** lipid accumulation in both, GM2-gangliosidoses and mucopolysaccharidoses like Hurler, Hunter and Sly disease and Sanfilippo-syndrome. In these and in other lysosomal storage disorders catabolic pathways can be inhibited by **primary** lysosomal storage compounds, which often trigger a cascade of **secondarily** accumulating lipids, aggravating the clinical course of the disease. We hypothesize that many membrane lipids have important regulator and signaling functions for various metabolic pathways occurring at organellar membranes and within organellar lumen. Observations obtained so far suppose that membrane lipids may regulate and modify activities of enzymes and receptors in various organelles. Pathological changes of the cellular lipid composition may therefore well mis-regulate cellular metabolism, especially lipid and membrane metabolism in obesity, Alzheimer and Parkinson disease.

Keywords: Discovery and identification of GM1 and GM2 gangliosidoses, Molecular basis of variant LSDs, Secondary lysosomal factors induce cascades of catabolic errors, Catabolism of membrane lipids occurs at intralysosomal luminal vesicles ILVs, Primary defects can trigger cascades of secondary lipid storage in gangliosidoses and mucopolysaccaridoses aggravating the clinical course of the disease

IS023

DxI 9000 AS A NEW BECKMAN COULTER'S APPROACH TO IMMUNOCHEMISTRY – DUBRAVA UNIVERSITY HOSPITAL VALIDATION EXPERIENCE AND RESULTS

Marko Žarak

Dubrava University Hospital, University of Zagreb, Faculty of Pharmacy and Biochemistry, Croatia

Dubrava University Hospital (UHD) is one of the largest hospital institutions in the Republic of Croatia and its capital city of Zagreb. UHD has been working with Beckman Coulter (BEC) for more than 25 years and has built a genuine partnership ever since. The most recent collaboration between BEC and UHD started when UHD was selected to be one of the few hospitals in the world to perform onsite clinical and analytical validation of BEC's new DxI 9000 immunochemical analyzer. Since the installation in March 2023 until today, the analytical validation of the instrument, as well as verification of 23 different assays including brand new Access NT-proBNP, has been performed in the Clinical Department of Laboratory Diagnostics of UHD. This lecture will provide a brief overview of the pre-installation expectations of the instrument, the installation process, validation plan design and execution of all phases, and presentation of all the validation results, including analyzer and assay validation. The lecture will also highlight all the DxI 9000 innovations, comparing them to the previous UniCel DxI 600/800, and provide a brief overview of how those innovations can improve immunochemical diagnostics in laboratories around the world.

Keywords: Beckman Coulter, immunochemistry, validation

IS024

SECRETS AND MYSTERIES IN THE PREANALYTICAL PHASE

Giuseppe Lippi

Section of Clinical Biochemistry and School of Medicine, University of Verona, Verona, Italy

Objectives: There is no doubt that the manual labor-intensive activities of the preanalytical phase (i.e., all the steps and processes that take place before patient samples are analyzed) are those where the overall risk for errors in the entire testing process is highest and can seriously jeopardize patient safety. It is important to note that some of these key issues are still poorly recognized or even completely ignored by healthcare professionals working outside and even inside the clinical laboratory. MATERIALS and METHODS: The steps most prone

to preanalytical errors include patient identification and preparation; specimen collection, management, transport, storage, and preparation for testing; and the presence of a variety of potentially interfering substances in the test specimens that can ultimately lead to biological, chemical, or analytical bias in the measurement. Results: The most reliable approach for contrasting the high vulnerability of the pre-analytical phase encompasses a multifaceted approach, developing through continuous training and education of the healthcare staff (especially that outside the laboratory environment, where most errors occur), elimination of manual steps through automation, development of a total quality management system which may help to systematically identify, record and analyze the various errors that can be encountered throughout the total testing process. CONCLUSION: Improving quality in the preanalytical phase can be certainly regarded as long and windy oad. Attention to detail, adherence to best practices, and effective communication are key to minimizing errors and ensuring the quality of patient care. Proper training and quality control measures play significant roles in this phase, ultimately contributing to better healthcare outcomes and reduction of avoidable costs. Unlike the analytical phase, recent evidence also suggests that preanalytical activities are poorly standardized across clinical laboratories, so that a major degree of harmonization would generate enormous benefits to the global quality of diagnostic testing. To this end, The Working Group for the Preanalytical Phase (WG-PRE) has been officially founded by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) in the year 2013. The main scope of this WG is to reduce the impact of preanalytical variability on quality of testing and improve harmonization in the still manually-intensive activities related to collection, handling, transportation, storage and preparation of biospecimens. Since its birth, the WG-PRE has finalized many valuable projects that will be basically summarized in this lecture.

Keywords:errors, mistakes, preanalytical phase

IS025

THE FUTURE OF BIOLOGICAL AND HEALTH SCIENCES: INVASION OF ENGINEERING

Engin Ulukaya

Istinye University

Biological and medical sciences are on the eve of a serious transformation in the 21st century. All classical educational tools, research methods, treatments etc. are evolving into a new process in which technology is used at the maximum level. The data previously produced on small scales is replaced by a big data. Hypothesis-driven research models are being replaced by comprehensive analysis of whole event-based research models through which big data is obtained. With the increasing penetration of artificial intelligence into science, human arms, legs and organs etc, are being produced and functionalized. It appears that stem cell studies have begun to shape regenerative medicine with the help of technology (e.g. organ productions via bioprinters). A robot will be able to draw blood from the patient or administer medication intravenously to the patient. On the other hand, developments at the molecular level are calling into question the classical methods used in the field of oncology (e.g. pathological/phenotypic evaluation). For example, ten lung cancer patients with the same pathological diagnosis are actually ten different patients because their molecular structures can be very different from each other. Therefore, phenotypic evaluation has begun to be replaced by molecular evaluation. But when it comes to world of molecules, the data must be the big data. Big data, on the other hand, is not something that the human brain can successfully process. So, artificial intelligence, which is the field of engineering, has entered medicine at this point and is about to start taking part in diagnosis and treatment. Therefore, completely patient-based treatments are about to come to the fore. In fact, it has been said many years ago that “there is no disease, there are patients”, but since technology has only recently been able to adequately analyze individuals one by one, it is up to this century to fulfill this saying. At this point, biotechnology, regenerative medicine, transplant surgery, big data and bioinformatics have become the keywords. In this talk, it will be discussed how all these keywords shape biological

and medical sciences.

Keywords:biology, health, transformation, future

IS026

INTEGRATED DIAGNOSTIC APPROACH AND RADIOLOGY

Ali Murat Koc

Izmir Katip Celebi Universitesi

The integrated diagnostic approach signifies the collaboration between clinical branches and diagnostic laboratory branches. In this approach, digital data obtained by radiology, pathology, biochemistry, and genetics branches is merged with analog data from internal medicine and surgical branches. The advantages offered by digital data can be harnessed by leveraging artificial intelligence technology. Within the scope of radiology, the digital data contained in diagnostic images, using AI-supported clinical decision support systems, greatly facilitate the workflow of radiology professionals. Software applications exist that automatically analyze and provide diagnostic or pre-diagnostic information for images containing digital data in conditions such as multiple sclerosis, Alzheimer's disease, tumors, brain aneurysms, and strokes, among others, offered by radiology. In contemporary practice, the diagnosis of diseases largely relies on radiology, biochemistry, and pathology branches. At this juncture, the diversity of reports from different branches can lead to confusion. In the integrated diagnostic approach, one method involves a clinical team serving as an intermediary, organizing and producing a common diagnostic report by collating reports from different branches, as exemplified by a diagnostic medicine department. Another method employs artificial intelligence support in combination with information specialists to generate a common report. Infrastructure-related issues, such as establishing a common interface, hospital integration, and the resistance of different branches to non-conventional systems, are among the challenges of the integrated diagnostic approach. With advancing technology, the sizes of radiological imaging datasets have significantly increased in recent years. This data expansion also translates into an increased workload. Therefore, there is a need for software solutions that process the growing data and integrate it with data from other branches. It is under the umbrella of the integrated diagnostic approach that all these systems can be consolidated.

Keywords:integrated diagnosis, radiology, artificial intelligence

IS027

CLINICAL DECISION SUPPORT SYSTEMS AND INTEGRATED DIAGNOSTICS

Deniz Ilhan Topcu

Health Sciences University Izmir Tepecik Education and Research Hospital, Izmir, Turkey

Clinical Decision Support Systems (CDS) are tools specifically designed to help healthcare professionals make decisions regarding patient care. These systems offer a range of features, including alerts, guidelines order sets, templates, and summaries that provide real-time valuable information to clinicians. CDS systems combine knowledge with patient-specific data to enhance decision-making within the clinical workflow. They play a role in areas such as diagnostics, medication management, and patient monitoring. While CDS systems in laboratory settings address preanalytical, analytical, and postanalytical aspects of diagnostic testing in broader healthcare settings, they primarily assist healthcare providers in making well-informed decisions. The implementation and utilization of CDSSs bring benefits:

- reducing misdiagnosis rates

- improving efficiency and patient care quality

- centralizing relevant clinical information for easy access

- minimizing medication error risk factors

- providing a reliable source of information to aid informed decision-making

Integrated Diagnostics (ID) is an approach that combines various diagnostic processes ranging from radiology to genetics into a unified platform for decision-making. This integration significantly enhances the accuracy of diagnoses and supports personalized medicine. The objective of ID is to gather data from both in vivo and in vitro diagnostics alongside data

from Electronic Health Records (EHR) to improve diagnosis precision and guide appropriate clinical actions. Combining laboratory, pathology, and imaging test results into a medical report may overwhelm healthcare professionals responsible for patient care due to the sheer amount of information. Therefore, it is crucial to reinforce CDS through expert interpretation and guidance to ensure appropriate medical actions are taken. New management approaches can establish a feedback loop by recommending suitable examinations for patients with similar conditions, bridging the gap between the post-analytic and pre-analytic phases. This valuable information can also aid healthcare systems in identifying high-risk patients who require management. However, integrating Integrated Diagnostics (ID) with CDS systems presents challenges such as data privacy, system interoperability, and the constant need for updates and learning. Nevertheless, leveraging the synergies between ID and CDS systems has the potential to revolutionize patient care. Despite these challenges, the outcomes of personalized care, enhanced accuracy, and timely interventions highlight the necessity for continuous innovation and integration in modern healthcare practices.

Keywords: Integrated Diagnostics, Clinical Decision Support Systems, Clinical Laboratory

ORAL PRESENTATION ABSTRACTS

S001

THE EFFECT OF AEROBIC EXERCISE ON SERUM IRISIN AND PREPTINE LEVELS IN OVERWEIGHT SEDENTARY WOMEN

Oğuzhan Özcan[1], Serdar Doğan[1], Güner Çiçek[2], Filiz Kaçmaz[3], Hamdi Oğuzman[1], Abdullah Arpacı[1]

[1] Hatay Mustafa Kemal University, Faculty of Medicine, Medical Biochemistry, Hatay, Turkey

[2] Corum Hitit University, Physical Education and Sport High School, Corum, Turkey

[3] Hatay Mustafa Kemal University, Molecular Biochemistry and Genetics, Hatay, Turkey

Objectives: This study investigated the effect of aerobic exercise on serum irisin, preptin levels in overweight women and their relationship with routine biochemical tests.

Methods: The study included 25 healthy controls and 25 overweight sedentary women (BMI>25). The exercise training was performed 3 days a week for 12 weeks. Anthropometric measurements were performed and fasting blood samples were collected before and after exercise period. All samples were centrifuged at 1500xg for 10 minutes and the serum was separated. Serum irisine and preptin levels were analyzed by ELISA method. Routine biochemical parameters were assayed by spectrophotometric, hsCRP levels by nephelometric and insulin levels by immunoassay method using auto analyzers.

Results: There was a significant decrease in BMI and fat mass in the post-exercise group compared with pre-exercise ($p<0.001$). Serum irisin levels were significantly lower in pre- and post-exercise group compared to controls ($p<0.001$), but no significant change was observed after exercise period. Preptin levels were significantly higher in the pre-exercise group (median/IQR, 558.2 /351,2-756,8 pg/ml) compared to the controls (median / IQR, 137.1/85.2-427.9 pg/ml), and significantly reduced in post-exercise group (medium/IQR, 243.1/179.2-330 pg/ml) compared to pre-exercise group. In the pre-exercise group, a significant negative correlation was observed between irisin and body mass index ($r=-0.443$, $p=0,027$), while a positive correlation was found between preptin and body weight ($r=0.438$, $p=0.029$).

Conclusions: Twelve weeks of aerobic exercise in overweight sedentary women has lowering effect on serum preptin levels but no effect on serum irisin levels.

Supported by HMKU BAP as 21.GAP.048numbered project

Keywords: irisin, preptin, aerobic exercise, overweight

S002

THE STUDY OF THE PLASMA AMINO ACIDS PROFILE IN PATIENTS WITH HASHIMOTO THYROID DISEASE

Nihayet Bayraktar[2], Mehmet Ali Eren[2], Mustafa Bayraktar[3]

[1] Harran Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

[2] Harran Üniversitesi, Tıp Fakültesi, Endekronoloji Bilim Dalı

[3] Yıldırım Beyazit Üniversitesi, Tıp Fakültesi, İç Hastalıklar Anabilim Dalı

Objectives: HT is a chronic autoimmune thyroid disorder characterized by the production of thyroid autoantibodies against thyroid peroxidase and thyroglobulin, In addition to genetic factors, environmental factors also play an important role in the development of Hashimoto's thyroiditis (HT). Infiltration of lymphocytesin thyroid gland and subsequent development of metabolic and inflammatory disorders. Our aim is to determine possiblr role of plasma amino acids in HT and after treatment.

Methods: 60 HT patients and 30 healthy individuals were included in this study. The demographic data of the patients were recorded. Plasma amino acid profile was measured by 8045 LC-MS/MS device. Multivariate statistical analysis of studied parameters was performed.

Results: When HT patients treated with levothyroxine and untreated Euthyroid HT patient groups were compared with the control group, plasma levels of only 1-methyl histidine, alanine, asparagine, histidine, isoleucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, sarcosine, threonine, tyrosine, valine aspartic acid, cystine, glutamic acid, homocitrulline and taurine were found to be lower significantly ($p<0.005$).

Conclusions: Some studied amino acids may be potential biomarkers in the diagnosis, prognosis, treatment and follow up of HT

Keywords: Hashimoto thyroiditis Amino acids LS-MSMS

S003

TRIMETHYLAMINE N-OXIDE AND LIPOPOLYSACCHARIDE BINDING PROTEIN AS POTENTIAL BIOMARKERS IN VITAMIN D, VITAMIN B12, AND IRON DEFICIENCY

Sadinaz Akdu[1], Ummugulsum Can[2], Serdar Şahinoğlu[3]

[1] Fethiye State Hospital, Department of Medical Biochemistry, Muğla, Türkiye

[2] Konya City Hospital, Department of Medical Biochemistry, Konya, Türkiye

[3] Fethiye State Hospital, Department of Internal Medicine, Muğla, Türkiye

Objectives: We aimed to evaluate the levels of trimethylamine N-oxide (TMAO), a gut microbial metabolite, and lipopolysaccharide binding protein (LBP), recommended as a biomarker for intestinal permeability, in patients with vitamin D, vitamin B12, and iron deficiency.

Methods: This study was performed in 33 patients with iron deficiency, 30 with vitamin B12 deficiency, 33 with vitamin D deficiency, 32 with combined deficiency, 24 patients who received vitamin D supplementation and 32 healthy controls. Serum TMAO and LBP levels were determined by enzyme-linked immunosorbent assay.

Results: TMAO values were found lower in patients with iron deficiency and combined deficiency compared with the controls ($p<0.01$). LBP values were lower in the vitamin D suplemented group compared to the controls ($p<0.05$). There was a correlation between TMAO and creatinine ($r= 0.188$, $p<0.05$), ferritin ($r= 0.153$, $p<0.05$) and iron ($r= 0.296$, $p<0.001$) levels. TMAO levels were negatively correlated with glucose levels ($r= -0.174$, $p<0.05$). A negative correlation was found between LBP and ferritin levels ($r= -0.178$, $p<0.05$).

Conclusions: Observation of changes in TMAO and LBP levels and their relationship with biochemical parameters in this study shows that these biomarkers play a role in the pathogenesis of micronutrient deficiencies.

Keywords: Iron deficiency, Vitamin B12 deficiency, Vitamin D deficiency, Trimethylamine N-oxide, Lipopolysaccharide binding protein

S004

THE EFFECT OF FUNCTIONAL TRAINING ON INSULIN, HDL, AND LDL LEVELS IN SEDENTARY WOMEN

Sedat Özdemir[1], Musa Şahin[2]

[1] Ankara Atatürk Sanatorium Training and Research Hospital, Department of Clinical Biochemistry, Ankara, Turkiye

[2] Karabuk University, Hasan Doğan School of Physical Education and Sports, Department of Coaching Education, Karabük, Turkiyr

Objectives: The insulin is the uptake of glucose in the bloodstream by cells for the purpose of energy production.HDL is assists in transporting cholesterol from tissues and arteries to the liver, LDL is responsible for transporting cholesterol from tissues to the areas. The purpose of this study is to examine the acute effects of functional training on insulin, sedentary women and to investigate the acute effects of functional training on HDL and LDL levels.

Methods: 10 sedentary female individuals with an average age of 21.2 ± 1.54 voluntarily participated in the study. After measurements of age height and weight of the participants,

a pre-defined 30-minute functional training session was applied acutely. Insulin, LDL, and HDL levels were measured using the Roche 6000 analyzer series.

Results: The insulin value before exercise was 13.19 ± 2.26, and the insulin value after exercise was 10.72 ± 2.43. Statistically significant in insulin levels was observed after exercise ($p < 0.05$). HDL levels before exercise was 56.60 ± 8.14, and the HDL levels after exercise was 57.90 ± 8.26. The average LDL before exercise was 98.90 ± 24.87, and the average LDL value after exercise was 68.10 ± 19.41. Statistically significant in HDL and LDL levels was observed after exercise ($p < 0.05$).

Conclusions: The results obtained in this study have demonstrated that functional training, as expected, acutely decreases insulin levels. Moreover, acute exercise significantly decreases LDL levels, which are normally expected to decrease in a chronic process after a prolonged exercise, and it also increases HDL levels after acute exercise.

Keywords: Functional Training, Insulin, HDL, LDL

S005

EVALUATION OF THE RELATIONSHIP BETWEEN HBA1C AND VITAMIN D LEVELS IN TYPE 2 DIABETES AND PREDIABETIC CASES

Marwa Abdelmageed[1], Figen Güzelgül[2], Merve Çatak[3]

[1] Tokat Gaziosmanpasa University, Faculty of Medicine, Department of Medical Biochemistry.

[2] Tokat Gaziosmanpasa University, Faculty of Pharmacy, Department of Medical Biochemistry.

[3] Tokat Gaziosmanpasa University, Faculty of Medicine, Department of Internal Medicine.

Objectives: Besides its skeletal effects, the extraskeletal effects of vitamin D, mainly its association with the development of Diabetes Mellitus, attracted considerable attention in several studies. In this study, we aimed to investigate the relationship between vitamin D levels and diabetes mellitus development in prediabetic, and diabetic individuals.

Methods: Patients over 18 years old who applied to the Endocrinology Department of Tokat Gaziosmanpasa University Hospital between June and August 2023 were included in the study. The study population consisted of three groups. The control group consisted of nondiabetic participants with HbA1c< 5.7 (n=46). The prediabetic group included individuals with HbA1c between 5.7 and 6.4 (n=24). Finally, the diabetic group consisted of diabetes mellitus patients with HbA1c> 6.4 (n=28). Vitamin D (25(OH)D3) and glycosylated hemoglobin (HbA1c) were measured in the study individuals, and statistical analysis was performed.

Results: Vitamin D showed lower levels among diabetes mellitus patients 12.7 (5.11-44.81), compared to prediabetic 16.1 (4.86-42.82) and normal individuals 20.2 (5.74-40.5); $p<0.05$. A statistically significant difference was observed in vitamin D levels between the control group 20.2 (5.74-40.5) and the diabetes mellitus group 12.7 (5.11-44.81); $p<0.05$. However, no significant difference was observed in vitamin D levels when comparing the prediabetic group 16.1 (4.86-42.82) with the controls 20.2 (5.74-40.5). Additionally, vitamin D levels were observed to be inversely correlated with HbA1c ($P= 0.03$, $r= -0.219$)

Conclusions: The negative relationship between vitamin D levels and HbA1c suggests that improving vitamin D levels may reduce the risk of developing diabetes mellitus.

Keywords: Diabetes Mellitus, HbA1C, Vitamin D

S006

INVESTIGATION OF THE EFFECT OF KRILL OIL ON IRISIN AND UCP1 LEVELS IN A HIGH-FAT DIET-INDUCED OBESE RAT MODEL

Esra Pınarbaş[1], Eda Yılmaz Kutlu[1], Ayşegül Sümer[2], Hülya Kılıç Yılmaz[1]

[1] Department of Medical Biocehmistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

[2] Department of Nursing, Faculty of Health Sciences, Recep Tayyip Erdogan University, Rize, Turkey

Objectives: Obesity is characterized by an abnormal increase in the number and volume of adipocytes. Adipose tissue is an endocrine organ, stores energy and synthesizes bioactive molecules called adipokines. Irisin is a novel myokine and adipokine that has gained much attention recently due to its mechanisms of action. Irisin is secreted following proteolytic cleavage of its precursor fibronectin typeIII domain containing 5 (FNDC5). Following its release, irisin exerts its major action by increasing the expression of mitochondrial uncoupling protein 1 (UCP 1), which facilitates the conversion of white adipose tissue(WAT) into beige adipose tissue. Krill oil is a dietary supplement derived from Antarctic krill that have been shown to have beneficial effects on lipid metabolism. This study aims to investigate the effect of krill oil on irisin and UCP1 levels.

Methods: In this study, 21 *Sprague Dawley* 6-8 weeks old male rats were divided into three groups as control, high-fat diet, high-fat diet+krill oil. Rats were feed ad libitum with krill oil 600 mg/kg by oral gavage every day for 13 weeks. At the end of the experiment, serum irisin and UCP1 levels were analyzed by ELISA method.

Results: As a result of the analysis, both irisin and UCP1 levels increase in the high-fat diet and krill oil group compared to the control group. However, this increase was not found to be statically significant.

Conclusions: Irisin is the hormone that provides a different approach to globalizing health problems despite conflicting results. The results obtained by conducting more long-term and complex studies should be supported.

Keywords: irisin, UCP1, obesity, krill oil

S007

THE TEST THAT TOUCHED HUMAN LIFE ‘’TETRAHYDROBIOPTERIN LOADING TEST IN HYPERPHENYLALANINEMIA”

Ayla Yıldız[1], Merve Aslantas[2], Hasan Onal[2]

[1] Başakşehir Çam and Sakura City Hospital, Department of Medical Biochemistry, Istanbul, Turkey

[2] Deparment of Pediatric Nutrition and Metebolism Clinic, Istanbul, Turkey

Objectives: The enzymes responsible for the synthesis of tetrahydrobiopterin (BH4) are abnormal in about 3% of phenylketonuria (PKU). In cases where hereditary hyperphenylanin is suspected, the BH4 loading test should be applied for the differential diagnosis of biopterin metabolism disorder. According to the results of this loading test, the treatment plan and management of the patients are determined.

Methods:: BH4 loading tests of 2 sisters who applied to Başakşehir Cam and Sakura City Hospital Pediatric Metabolism Clinic were studied in our laboratory. The patients were administered sapropterin 20 mg/kg. Phenylalanine (FA) measurements were taken at 0, 4, 8, 16 and 24 hours after saptopterin administration. FA levels in the test were studied in Sciex Triple Quadripol 3500 and HPLC Shimadzu devices as new born screening test.

Results: A simultaneous Bh4 loading test was performed in cases aged 4 and 7 years. A decrease of more than 30% in FA level is considered as a BH4 response. Accordingly, it was determined that there was a 96.4% decrease in the 1st case and a 95.5% decrease in the 2nd case.

Conclusions: This loading test is the gold standard for changing the course of diagnosis and treatment. Much more effective and successful results are obtained by both the removal of the lifelong FA-restricted diet and the drug treatment response of the patients with response. Especially with the removal of the dietary requirement, the patient’s lack of food restriction, the cost and social difficulties it brings to the family are overcome.

Keywords: Phenylalanine, Tetrahydrobiopterin

S008

ANTIGLICATION EFFECT OF PYRIDOXAMINE, COMBINATION WITH CHILI PEPPER EXTRACT AND CAPSAICIN IN STZ-INDUCED DIABETIC RATS

Cemal Nas[1], Nesrin İnceören[2], Göksel Kızıl[2], Feryal Zeliha Akay[2], Bircan Çeken Toptancı[2], Murat Kızıl[2]

[1] Department of Biochemistry, Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey

[2] Chemistry Department, Faculty of Science, University of Dicle, Diyarbakır, Turkey

Objectives: The objective of this study was to evaluate the antiglycation effects of red chili pepper extract (RC) and capsaicin (CAPS), the main bitter ingredient in red peppers and pyridoxamine (PM), a vitamin B6, one by one or in combination, in STZ-induced diabetic rats.

Methods:**:** 96 Male Sprague Dawley rats (8 wk old), each weighing 200-300 grams, were divided into 12 groups of 8 rats each. A single dose of STZ (50 mg/kg body weight) was administered to induce experimental diabetes in rats. Group 1 was normal control, group 2 was diabetic control. Groups 3 and 4, 200 and 400 mg/kg RC, groups 5 and 6, 6 and 8 mg/kg CAPS and group 7, 4 mg/mL PM and group 8 were receiving 300 mg/kg metformin. Groups 9 and 10, 200 and 400 mg/kg RC+ 4 mg/mL PM and groups 11 and 12 were diabetic rats treated with 6 and 8 mg/kg CAPS + 4 mg/mL PM.

Results: Our findings indicate that when high doses of KC+PM (400 mg/kg/day + 4 mg/mL/day) and CAPS+PM (8 mg/kg/day + 4 mg/mL/day) are used in combination, Hemoglobin A1C and fructosamine (FA) levels are significantly reduced.

Conclusions: Our findings strongly imply that extract combinations may have powerful antiglycaemic effects on the healing of diabetes-related damage. This is the first study to look at the antiglycation impact of these substances, and as far as we are aware, there have been no prior reports

Keywords: Diabetes, Glycation, Red chili pepper extract, Pyridoxamine, Capcaicin

S009

COMPARISON OF GENE EXPRESSIONS IN INDIVIDUALS WITH G6PD MEDITERRANEAN MUTATION AND HEMOLYTIC ANEMIA

Başak Günaştı, Abdullah Tuli

Çukurova University, Faculty of Medicine, Department of Medical Biochemistry, Adana, Türkiye

Objectives: G6PD enzyme is the most common enzyme deficiency and affects more than 500 million people. Even the patients with Gd-Med mutation are exposed to oxidative agents, some of the patients experience health issues such as hemolytic anemia. In our study, we aimed to clarify the relationship between G6PD enzyme kinetics and mRNA expression levels of the G6PD gene in Gd-Med patients with and without hemolytic anemia.

Methods: The study group consisted of 30 cases with Gd-Med mutation and 30 cases with enzyme activity levels in the reference range. G6PD enzyme was partially purified and its kinetic parameters were studied. Gd-Med mutation was genotyped and the expression levels were calculated.

Results: In our study, a significant difference was found between the Km_{NADP^+} and Km_{G6P} values of the cases with Gd-Med mutation and the control group. There was no significant difference between Km_{NADP^+} and Km_{G6P} values in Gd-Med mutated patients with and without hemolytic anemia. Gene expression of 18 patients without hemolytic anemia were significantly higher than 12 patients with hemolytic anemia. In addition, there was a significant difference between these variables and the control group.

Conclusions: In the light of our data, the substrate binding site of the enzyme in cases with Gd-Med mutation may have undergone post-transcriptional or post-translational modifications, and therefore gene expression might be changed. As a further study, the decrease in gene expressions of patients with hemolytic anemia with Gd-Med mutation can be clarified by evaluating the promoter side of the gene.

Keywords: Enzyme Kinetics, Gene Expression, Glucose-6-phosphate Dehydrogenase, Hemolytic Anemia

S013

DEVELOPMENT OF LC-MS/MS BASED METHODS FOR 2ND TIER ANALYSIS OF SUAC, MMA AND BIOMARKERS OF MSUD

Murat Emrah Mavis, Gokce Goksu Gursu, Sule Yalcin

Altium International Lab. Cih. A.ş., Ar-ge Merkezi

Objectives: The main objective of this study was to develop LC-MS/MS based 2nd tier tests or *i)* Tyrosinemia type I *(primary targeted analyte: SUAC)*

ii) Methylmalonic Acidurias *(primary targeted analyte: MMA)*

iii) Maple Syrup Urine Disease *(primary targeted analyte: allo-isoleucine)* using DBS specimen.

Methods: According to Jasem methodology, 3 mm punches of DBS were extracted using 100 µL extraction reagent specific for both SUAC and MSUD biomarkers at room temperature for 15 min. Following the extraction step, extract was subjected to Agilent 6465B (Ultivo) system. The total run times for SUAC and MSUD biomarkers were 5.5 min and 10.5 min respectively. For the measurement of MMA, 3 mm DBS punch was extracted by 100 µL extraction reagent at room temperature for 15 min. After that, the extract was injected Ultivo system. The analysis time was 10.0 min.

Results: The linearity and accuracy of the methods were evaluated using four DBS-based calibrators and two DBS-based internal quality controls for all works in this paper. For three methods linearity, RSD % (inter-intraday) and accuracy were within the analytical acceptable ranges.

Conclusions: The measurement of biomarkers in the context of 2nd tier using original DBS sample provides reducing number of false positives (increasing positive predictive value) thereby avoiding unnecessary parental anxiety, follow-up efforts and costs. *The proposed* LC-MS/MS methods are centred on quick sample preparation (without derivatization, extraction, and injection) contributing reliable results. Besides, the methodologies developed are in conjunction with the NBS screening kit and confirmation kits for amino acidurias and organic acidemias.

Keywords: Mass spectrometry, liquid chromatography, metabolic disorder, dried blood spot, second tier test

S014

THE EFFECT OF EDTA ON FLOW CYTOMETRY CROSSMATCH OUTCOMES

Rasime Derya Güleç[1], Fatma Demet Arslan[2]

[1] Health Sciences University, Tepecik Training and Research Hospital, Tissue Typing Lab. İzmir, Turkiye

[2] Bakırçay University, School of Medicine, Department of Medical Biochemistry, İzmir, Turkiye

Objectives: Flow cytometry crossmatch (FCXM) assesses immunological risk before solid organ transplantation. When ethylenediaminetetraacetic acid (EDTA) is applied to serum, it eliminates high antibody titers, prozone effects, or interacting substances that induce a blocking effect. Our aim is to reveal the possible interference preventive effect of EDTA on outcomes within patient sera during routine FCXM studies.

Methods: Lymphocytes of 21 different kidney transplant candidate-donor pairs were separated and standard tricolor FCXM protocol was applied. For each patient, FCXM tests with EDTA-treated and untreated serums were conducted using donor cells against negative-positive controls and patients' serum, and patient cells (autologous) against patient serum. CD3, CD19, and anti-IgG staining data in cells were acquired using the DxFlex flow cytometer. The ratio of the median fluorescent values of positive control, patient sample, or autologous sample to negative control was calculated. The ratio of EDTA-treated and untreated studies of 21 patients was compared with each other.

Results: While T-cell reactivity in autologous samples after the EDTA-treated significantly decrease (p=0.009), the increase in the positive control (p=0.807), and the decrease in the patient samples (p=0.578) was not significant. On the other hand,

B cell reactivity after the EDTA-treated significantly decreased in positive controls (p=0.049), in patient samples (p=0.024), and in autologous (p=0.008) samples.

Conclusions: A significant reduction in the reactivity of both T and B cells was seen in EDTA-treated autologous samples in the FCXM assay. Our study showed that EDTA treatment can reduce non-specific binding, especially in B-cells.

Keywords: Flow cytometry, crossmatch, ethylenediaminetetraacetic acid, interference

S015

REVIEW OF THE DIAGNOSIS OF DIABETES ACCORDING TO GLUCOSE MEASUREMENT UNCERTAINTY

Öznur Asil[2], Kaan Kuzu[2], Giray Bozkaya[1]

[1] Health Science University, Izmir Faculty of Medicine, Bozyaka Education and Research Hospital, Department of Medical Biochemistry, Izmir, Türkiye

[2] Health Science University, Izmir Bozyaka Education and Research Hospital, Department of Medical Biochemistry, Izmir, Türkiye

Objectives: Measurement uncertainty is the expression of the statistical dispersion of the values attributed to a measured quantitythat is reported together with the patient result. When the measurement uncertainty is calculated and given with the test result; it indicates the limits within which the measurement can be found. In our study, our aim was to investigate the effect of glucose measurement uncertainty in the diagnosis of diabetes mellitus.

Methods: The glucose and HbA1c measurement uncertainty was calculated according to the Eurachem/CITAC Guide CG4 using a five-stage measurement uncertainty calculation model. According to the obtained glucose measurement uncertainty, patient results were examined between March-April-May 2023, retrospectively. The patients who were affected from the glucose measurement uncertainty in the diagnosis of diabetes mellitus were also evaluated by HbA1c results and HbA1c uncertainty.

Results: The measurement uncertainty value of glucose was calculated as ±5.6 mg/dL at a 95% confidence interval. According to this result which effects the diagnosis of Diabetes Mellitus, 681 of 77131 (0.88%) glucose results were close to the threshold value (126±5.6 mg/dL). According to the obtained glucose measurement uncertainty, the number of people with glucose results below 126 mg/dL and HbA1c values above 6.4% was 34 (0.04%).

Conclusions: Since measurement uncertainty is highly important in the diagnosis and clinical decisions of diabetes, it should be to further reduce measurement uncertainty with stricter analytical performance requirements.

Keywords: Glucose, Uncertainty, Quality Control, Standardization

S016

COMPARISON OF CREATININE LEVELS MEASURED BY JAFFE AND LC/MS/MS METHOD

Saadet İbiş[1], Turan Akdağ[2], Ali Ünlü[3], Hüseyin Dost[4], Menekşe Kuzu[5], Firdevs Sak[3]

[1] 29 Mayıs State Hospital, Ankara, Turkey

[2] Meram Vocational School, Necmettin Erbakan University, Konya, Turkey

[3] Department of Biochemistry, Faculty of Medicine, Selcuk University, Konya, Turkey

[4] Medical Supervisor, Ankara, Turkey

[5] Department of Biochemistry, Faculty of Pharmacy, Biruni University, İstanbul, Turkey

Objectives: As one of the most requested analyzes in clinical laboratories, creatinine is an important analyte for the prediction of kidney and muscle damage. Many methods are used to determine creatine levels. In the study, we aimed to compare creatine levels which measured by Jaffe and LC/MS/MS method.

Methods:The serum samples were taken from 102 healthy subjects who came to the 29 Mayıs state hospital for control purposes between January and April 2021. The creatinine levels were measured and compared in Mindray BS-800 device

using the Jaffe method using the archem kit and API 3200 tandem mass device measuring with the Liquid Chromatography Tandem Mass/Mass Spectrometer method. The samples were stored at -80 °C until analysis and were dissolved during analysis following the establishment of the measurement method and measured in API 3200 Mass Spectrometer and Mindray BS-800 device in our routine biochemistry laboratory.

Results: Correlation coefficient of all data (n=102) was measured as 0.9958. Mindray BS-800M-Archem Crea-J/LC/MS/MS for creatinine' s reference levels under 0.5 (n=32) was determined as 0.4938. Creatinine' s reference levels between 0.5-1.3 mg/dL (n=39) was determined as 0.9689 for jaffe an LC/MS/MS method. Creatinine' s reference levels above 1.4 mg/dL (n=31) was determined as 0.9924.

Conclusions: The correlation coefficients of the all data, between 0.5-1.3 mg/dL levels and above 1.4 mg/dL levels show a good correlation. However, the level of under reference levels 0.5 mg/dL did not show a well correlation.

Keywords: Jaffe method, LCMSMS, creatinine

S017

INTERCHANGEABILITY OF K3-EDTA AND SODIUM CITRATE TUBES FOR HBA1C MEASUREMENT BY CAPILLARY ELECTROPHORESIS

Semih Tek, Kamil Taha Uçar

Istanbul Başakşehir Çam and Sakura City Hospital, Department of Medical Biochemistry, İstanbul, Türkiye

Objectives: Glycated hemoglobin (HbA1c) is a vital parameter for Diabetes Mellitus diagnosis and monitoring. Traditionally, HbA1c measurements are performed using K3-EDTA tubes. This study aims to compare HbA1c results obtained from K3-EDTA and Sodium Citrate tubes using capillary electrophoresis.

Methods: Blood samples from 67 patients were collected in Greiner VACUETTE 2 ml K3-EDTA and Greiner VACUETTE 2 ml Sodium Citrate tubes (Greiner Bio-One, Kremsmünster, Austria). Measurements were conducted with a Sebia Capillarys 3 Tera analyzer (Sebia, Lisses, France). For data presentation, conformity to normal distribution was evaluated by Kolmogorov-Smirnov test. Since the data did not fit the normal distribution, the results were presented as median (interquartile range). Passing-Bablok regression analysis and Bland-Altman plots were used for comparison. Statistical significance was set at $p<0.05$ with a 95% confidence interval.

Results: Median HbA1c levels were 4.7% (5.6% - 9.4%) for K3-EDTA and 4.6% (5.5% - 9.5%) for Sodium Citrate. Passing-Bablok regression yielded the equation y (Sodium Citrate) = 0.000 (95% CI: -0.147-0.000) + 1.000 (95% CI: 1.000-1.019) x (K3-EDTA). The Bland-Altman plot showed a mean difference of 0.115%.

Conclusions: Results indicate that K3-EDTA and Sodium Citrate tubes are interchangeable for HbA1c measurement by capillary electrophoresis, ensuring flexibility in blood collection tube selection.

Keywords: Diabetes mellitus, Hemoglobin A1c, capillary electrophoresis, blood collection tube, method comparison.

S018

THE PERFORMANCE ASSESSMENT OF HEMOGRAM PARAMETERS: SIGMA VALUES WITH DIFFERENT TOTAL ALLOWABLE ERROR GOALS

Mehmet Akif Bildirici

Kastamonu University, Faculty of Medicine, Department of Medical Biochemistry, Kastamonu, Türkiye

Objectives: Six sigma process analysis is widely used to evaluate the performance of tests in clinical laboratories. This study aimed to compare the sigma values determined by using the total allowable error (TEa) goals of The Clinical Laboratory Improvement Amendments (CLIA) and The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) to evaluate the performance of some hemogram parameters.

Methods: The study was conducted in Kastamonu Teaching and Research Hospi

January-June 2022. Leukocyte, erythrocyte, hemoglobin, hematocrit, and platelet parameters studied on the Sysmex XN-1000 (Kobe, Japan) hematology autoanalyzer were included in the study. The 6-month mean bias and coefficient of variation (CV) were obtained retrospectively from proficiency test and internal quality control results, respectively. Sigma values were determined with the formula '(TEa-bias)/CV'. The acceptable sigma level was considered as 3.

Results: All tests had sigma values above 3 when CLIA TEa goals were used (i.e., process performance was acceptable). Six-month mean sigma values according to CLIA and EFLM TEa goals were 9.3 and 2.5 for leukocyte, 4.7 and 2.4 for erythrocyte, 6.2 and 2.8 for hemoglobin, 3.9 and 2.2 for hematocrit, and 6.1 and 2.2 for platelet, respectively.

Conclusions: The TEa goals set by CLIA and EFLM are different, and this causes significant differences in the sigma values of hemogram parameters. Tests that perform well according to CLIA goals have unacceptable performance according to EFLM. Consequently, a consensus should be achieved in the guidelines for standardizing hematology performance specifications.

Keywords: six sigma, performance specifications, hemogram, total allowable error

S019

EVALUATION OF PERFORMANCE CHARACTERISTICS OF ROCHE COBAS T511 COAGULATION DEVICE

Şükran Bıçakcı, Semih Fazlı Kayahan, Ayşe Betül Demir, Mehmet Şeneş

Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Turkey

Objectives: The aim of this study was to evaluate the analytical performance characteristics of prothrombin time(PT), activated partial thromboplastin time(aPTT) and fibrinogen tests performed on the Cobas t511(Roche Diagnostics, Germany) coagulation device and to verify the reference intervals determined by the manufacturer.

Methods: In the study, precision and bias validation was performed using CLSI EP15 A3 and method comparison was performed using CLSI EP9 A3 standards. Method comparison study was performed with STA-R Max-2(Diagnostica Stago SAS).In the method comparison,160 plasma samples for PT and aPTT and 44 plasma samples for fibrinogen were used.Indirect reference interval determination was performed for reference interval assessment.For this purpose, data of 435 patients aged <18years and 4700 patients aged >18years for PT and aPTT tests and 1033 patients for fibrinogen, which were studied on Cobas t511 device, were used.Statistical analyses were performed with SPSS ver.21 and Excel Analyze-it package programs.

Results: Intra-study,total %CV values for normal and high control samples used in the study were PT: 0.57-0.69,1.58-0.79 aPTT: 0.32-0.4,1.7-2.88 and fibrinogen: 2.06-1.84,2.65-2.35. Mean %bias values were PT: 2.45%,aPTT: 3.45%,fibrinogen: 5.54%. Regression equations for PT, aPTT and fibrinogen in device comparison were cobas t511=-0.56+0.68 STA-R max,cobas t511= -9.67+1.21 STA-R max,-cobas t511=-4.18+STA-R max and r was 0.872, 0.870, 0.995, respectively. In the reference interval study performed using the indirect method for Cobas t511; PT <18 years: 8.2-10.5s, >18 years: 7.9-10.6s, aPTT:<18 years: 23.4-34.6s, >18 years:22.4-32.8s, and fibrinogen:193-580 mg/dL.

Conclusions: The results of our validation studies for PT,APTT,fibrinogen on the Cobas t511 analyzer show that the device performs adequately for routine use in our laboratory.

Keywords: Coagulation, Method comparison, Verification, Prothrombin time, Activated partial thromboplastin time

S020

ANALYTICAL VERIFICATION OF THE HEMATOLOGY ANALYZER DYMIND DH615

Raziye Yıldız, Muammer Yücel

İzmir Atatürk Training and Research Hospital, Medical Biochemistry Laboratory, İzmir, Türkiye

Objectives: The Dymind DH615 is a new automated hematology analyzer that provides a complete blood count (CBC) including a 5-part WBC differential count. The purpose of this study was to test the Dymind DH615 automated analyzer and compare it to the Sysmex XN1000.

Methods:Analytical verification included estimation of repeatability, within-run precision, within-laboratory precision, and bias for low, normal, and high control samples. Acceptance criteria for analytical verification were defined using the European Federation of Laboratory Medicine (EFLM) 2019 Biological Variation Database. Method comparison between the Dymind DH615 and the Sysmex XN1000 with respect to hematologic parameters was performed on 31 patient samples.

Results: The Dymind DH615 demonstrated excellent repeatability and within-run precision for all CBC and WBC differential parameters for all level controls. Within-laboratory precision was within acceptable ranges, with the exception of eosinophils (11.9%, 12.10%), red blood cells (2.11%, 3.30%), hemoglobin (3.6%), mean cell volume (0.7%, 0.9%,1.0%), and platelets (6.8%). The Dymind DH615 and Sysmex XN1000 analyzers showed very good correlation coefficients (R2) except for monocytes, basophils, and MPV (0.7758, 0.8222, and 0.6639, respectively).

Conclusions: We conclude that the Dymind DH615 is a competent analyzer to provide reliable and accurate diagnostic results. In our opinion, the clinical performance of the Dymind DH615 Hematology Analyzer compares favorably with the analyzer Sysmex XN1000 and meets the requirements for clinical use in medium to large-sized hematology laboratories.

Keywords: blood cell count, hematology, verification

S021

CALCULATION OF MEASUREMENT UNCERTAINTY OF SERUM IMMUNOGLOBULINS ACCORDING TO ISO/TS 20914 GUIDELINE

Emine Feyza Yurt, Medine Alpdemir

Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: The aim was to calculate the measurement uncertainty (MU) of serum immunoglobulins (Ig) analyzed in Ankara Training and Research Hospital laboratory and to compare MU values with the maximum allowable measurement uncertainty (MAU) values published by European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) in 2022.

Methods: Serum IgG, IgA and IgM were analyzed by immunoturbidimetric method and serum Ig E by sandwich immunoassay method on Roche Cobas 8000 analyzer. Relative expanded MU ($\%U_{rel}$) values was calculated separately for each analyte based on two levels of internal quality control results according to the model in the ISO/TS 20914 document. The MU values obtained were compared with MAU values.

Results: The bias calculated from external quality control (EQQ) data for serum IgG, IgA and IgM (0.95%, 4.67% and 9.18%, respectively) was found to be lower than the desirable bias (4.4, 5.1 and 12.2, respectively) and was therefore neglected in the MU calculation according to the ISO/TS 20914. For the first and second level control levels, U_{rel} % was 4.0 and 4.1 for Ig G, 4.91 and 7.4 for IgA, 8.3 and 7.1 for IgM, respectively. EFLM MAU values for IgG, A and M are 5.3, 8.6, 8.9 respectively. The bias calculated from EQQ data for Ig E was 8.7%, $\%U_{rel}$ were 9.01% and 8.79%, however no comparison could be made because there is no MAU value in EFLM.

Conclusions: The measurement uncertainity for serum immunoglobulins were found to be within acceptable limits.

Keywords: Measurement uncertainty, ISOTS 20914, Serum immunglobulins

S022

EVALUATION OF THE DIAGNOSTIC USEFULNESS OF TRIGLYCERIDE GLUCOSE INDEX AS A GLYCEMIC CONTROL MARKER

Muzaffer Katar[1], Osman Demir[2]

[1] Tokat Gaziosmanpaşa Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

[2] Tokat Gaziosmanpaşa Üniversitesi, Tıp Fakültesi, Biyoistatistik Anabilim Dalı

Objectives: Diabetes Mellitus (DM) continues to be the most common endocrine disease today. Easily accessible, accurate and reproducible markers are needed in addition to the accepted markers to evaluate insulin resistance (IR) and glycemic control. Therefore, our study aimed to evaluate the use of triglyceride glucose index (TyG Index) as an indicator for insulin resistance and glycemic control.

Methods: Tg, HDL-c, HbA1c, fasting blood sugar (FBG), and insulin values of 7518 samples studied simultaneously in our TOGU Faculty of Medicine Hospital Laboratory between March 2023 and August 2023 were retrospectively examined. The patients were divided into two groups according to their HbA1c values: good glycemic control (<7%) and poor glycemic control (≥7%). ROC analysis was performed to evaluate the ability of the TyG Index to discriminate between insulin resistance and glycemic control. Statistical significance level was accepted as $p<0.05$.

Results: TyG Index ($p<0.001$) was found to be significantly higher in individuals with insulin resistance. When we looked at the areas under the ROC curve (AUC) to evaluate the ability of this parameter to distinguish insulin resistance, TyG Index was found to be 0.720 ($p<0.001$). In case of poor glycemic control, Tg/HDL-c ($p<0.001$) was also significantly higher and AUC value was 0.810 ($p<0.001$).

Conclusions: Acording to the current results, we think that the TyG Index is a useful marker of glycemic control and insulin resistance, and it may be a usefull parameter to detect metabolic changes due to T2DM in the early stages.

Keywords: Diabetes Mellitus, Insulin Resistance, Glycemic Control, Triglyceride, Glucose

S023

DETERMINATION OF MEASUREMENT UNCERTAINTY FOR ESTIMATED SERUM OSMOLALITY BASED ON DATA OBTAINED FROM DIFFERENT ANALYSERS (ISO/TS 20914)

Mehmet Fatih Alpdemir, Sezen Tutar

Ankara City Hospital, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: We aim to determine of the measurement uncertainty (MU) for estimated serum osmolality using glucose, urea and sodium (Na) analytes measured on different devices in the Medical Biochemistry Laboratory of Ankara Bilkent City Hospital.

Methods: Serum glucose, urea and Na were analyzed on Siemens Atellica CH (Siemens Healthcare GmbH) (9 analyzers) and Advia Chemistry XPT (Siemens Healthcare GmbH) (1 analyzer) analyzers. Relative expanded MU (U_{rel} %) values were calculated separately for each analyte based on two lot and two-level internal quality control (IQC) results between 01.03.2023 and 01.09.2023, according to the model in the ISO/TS 20914 document. From the obtained u_{rel}% values for tests, (u_{rel} OSM %) was estimated for osmolality according to the formula ($\sqrt{(2^2(\%u_{rel}\text{-Na})^2+(\%u_{rel}\text{Üre})^2+(\%u_{rel}\text{Glikoz})^2}$).

Results: The u_{rel} % values for Na, urea and glucose for Atellica and Advia Chemistry were 1.05, 4.84 and 3.22 for level 1 and 0.88, 3.80 and 2.82 level 2, and were 1.55, 2.48 and 2.70 for level 1, L2. 0.89, 2.84 and 2.67 for level 2, respectively. The estimated serum osmolality combined U_{rel} % (K=2, 95 % confident interval) was determined as 11.29 (Atellica), 9.12 (Advia Chemistry).

Conclusions: Since there was no MAU value for estimated osmolality in EFLM, no comparison could be made. We think that our study will contribute to the literature in this sense.

Keywords: measurement uncertainty, ISO, serum osmolality, estimated

S024

METHOD VERIFICATION AND METHOD COMPARISON FOR ELECTROCHEMILUMINESCENCE AND LC-MS/MS METHODS FOR SALIVARY CORTISOL MEASUREMENT

Fatih Serin, Medine Alpdemir, Mehmet Şeneş

University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: This study aimed to perform method verification for the measurement of salivary cortisol with the CORTII reagent based on the principle of electrochemiluminescence in the RocheCobas8000e801 module and Obikrom brand salivary free cortisol determination reagents in the LC-MS/MS device.A method comparison study was also conducted.

Methods: The study followed the CLSI guidelines for the evaluation of limit of blank (LOB),limit of detection(LOD),limit of quantitation(LOQ),linearity,precision and reference interval.The method comparison study was performed based on the CLSI guidelines with a total of 433 samples,including 393 morning salivary cortisol samples collected from 41 healthy adults and 40 different high concentrations obtained by adding RocheElecsysPCU1(lot.no:558012)quality control material to the saliva pool.

Results: TheLOB,LOD,and LOQ values of theLC-MS/MS method were 0.065nmol/L, 0.087nmol/L,and 0.276nmol/L,respectively.The method was considered linear between 0.690-41.4nmol/L.The within-run imprecision for 0.276nmol/L and 27.6nmol/L levels were 4.82% and 4.57%,respectively.TheLOB of theECLIA method was not evaluated;LOD and LOQ values of 1.5nmol/L and 3nmol/L were confirmed,respectively.Between 2.13-68nmol/L,the method was considered linear.At 5.16nmol/L,the within-and between-run imprecision were 3.85% and 5.5%,respectively;at 32.8nmol/L, the within-and between-run imprecision were1.37% and 2.54%, respectively.For both methods,all samples from 41 reference subjects were below the threshold reported in the kit insert. The regression equation was y=-0.179+1.244x and and r=0.988 by thePassing and Bablok regression analysis.The bias of the ECLIA method was 0.975nmol/L in concentration(Q1:0.031nmol/L,Q2:2.33nmol/L)and 17.9%in percentage based on Bland-Altman analysis.

Conclusions: Under the laboratory conditions of the study,the claimed performance values for both methods were acceptable.The bias of theECLIA method appears to increase with higher concentration;however,the minimum bias target of 19.3% for cortisol based on biological variation was achieved.

Keywords: method verification, method comparison, salivary cortisol, electrochemiluminescence, lc-msms

S025

EFFECT OF ANTIRERETROVIRAL THERAPY ON LYMPHOCYTE SUBGROUP AND CD38 AND HLADR IN HIV PATIENTS

Gamze Keşre[1], Hamdi Oğuzman[2], Mehmet Çabalak[3], Oğuzhan Özcan[2]

[1] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Molecular Biochemistry and Genetics, Hatay, Turkey

[2] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Medical Biochemistry, Hatay, Turkey

[3] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Infection Disease and Clinical Microbiology, Hatay, Turkey

Objectives: We aimed to investigate the effect of antiretroviral therapy on lymphocyte subgroups and T cell activation markers, CD38 and HLADR, in patients with HIV.

Methods: Thirty HIV patients, 30 follow-up patients who received 3 months of antiretroviral therapy, and 20 controls were included in the study. Whole blood samples were collected in EDTA containing tubes. The percentages of T and T lymphocyte subgroups and CD38 and HLADR expressions were estimated by flow cytometric method. Absolute counts were calculated by double platform method. IL-2 levels were studied by ELISA and viral load analyzed by PCR method.

Results: CD4+/CD8+T cell ratio and CD4+/CD38+T cell percentage were significantly lower (respectively, p=0.022, p=0.026), CD8+/CD38+T and CD8+/HLADR+T cell percentage were higher (p<0.001) in the newly diagnosed group compared with controls. CD4+/CD8+T cell ratio and CD4+/CD38+T cell percentage were significantly increased (respectively, p<0.001, p=0.006) but CD8+/CD38+T and CD8+/HLADR+T cell percentage were decreased (p<0.001, p=0.003, respectively), in the post-treatment group compared with newly diagnosed group. There was no change in the absolute number of CD8+T cells after treatment. There was a negative correlation between viral load and CD4+/CD38+T cell percentage and a positive correlation with CD8+/CD38+T cell percentage. Serum IL-2 values were significantly lower only in the treatment group.

Conclusions: CD38 expression on CD4+T and CD8+T cells has a potential as an additional marker together with CD4+T cell count in the evaluation of the efficacy of ART in patients with HIV.

This study was supported by Hatay Mustafa Kemal University Scientific Research Projects (projectnumber:21.YL.050).

Keywords: HIV, AIDS, CD38, HLA-DR, immune activation

S026

IDENTIFICATION OF THERAPEUTIC MOLECULES THAT WILL INTERACT COVALENTLY AGAINST SARS-COV2 IN SILICO APPROACHES

Murat Serilmez, Serdar Durdağı

Bahcesehir University,faculty of Medicine Durdagı Laboratory

Objectives: In the COVID19 pandemic period, which affects the whole world and new solutions for the disease are being investigated in the world.It is aimed to determine the molecules to be covalently bound in drug screening for important target proteins

Methods: It was prepared at neutral pH by downloading FDA approved 2360 molecules from Drugbank.

Covalent reactions were performed for each target protein.The molecules with the best docking score for each covalent reaction are ranked. The structures with the best docking score were taken into molecular simulation and Molecular mechanics with generalised born and surface area solvation (MMGBSA) values are calculated.

Results: The molecules with the best docking score for each covalent reaction are ranked. 2D and 3D ligand interaction diagrams of target proteins of molecules with high docking scores were analyzed.The structures with the best docking score were taken into molecular simulation and MMGBA values are calculated.Mainproteaz in the target molecules with a high docking score Octreotide (-9.710kcal/mol) for TMPRSS2 in the target Abarelix (-8.753) for Spike ACE2 in the target Leucovorin (-7.021) for RdRp in the target Candicidin (-7.817)

Conclusions: The method known as drug repurposing in different indications is remarkable as it becomes less costly in terms of both time and required resources.Non-covalent reversible binding in different target proteins is difficult and challenging and their therapeutic effects are limited.Molecules found to be active by in vitro testing will be recommended for further in vitro testing.

Keywords: Main Protease, TMPRSS2, SpikeAce2, RDRP, SarsCov2

S027

ADENOSINE DEAMINASE: AS AN INFLAMMATORY AND PROGNOSTIC MARKER IN THEEARLY PHASE OF THE DISEASE IN COVID-19 PATIENTS?

Senay Eren[1], Gulcin Alp Avcı[3], Mustafa Eren[2], Emre Avcı[4]

[1] Hitit University, Department of Molecular Biology and Genetics, Corum, Türkiye

[2] Corum Chest Diseases Hospital, Corum, Türkiye

[3] University of Health Sciences,gulhane Faculty of Dentistry, Department of Basic Medical Sciences, Microbiology, Ankara,türkiye

[4] University of Health Sciences, Gulhane Faculty of Pharmacy, Department of Biochemistry, Ankara,türkiye

Objectives: This study was made to investigate the usability of serum adenosine deaminase level as a biochemical and prognostic marker in COVID-19 patients and to compare it with some other hematological and biochemical parameters routinely used for this purpose.

Methods: Our study consisted of patients admitted to the Chest Diseases Hospital and diagnosed with COVID19, and a control group consisting of healthy volunteers. In serum samples taken within 48 hours after the first RT-PCR test that was found positive, serum ADA levels were studied in all groups and subgroups (the uncomplicated patient group (UPG), the mild-moderate pneumonia patient group (MMPPG), and the severe pneumonia patient group (SPPG)).Lymphocyte count in hemogram, neutrophil/lymphocyteratio (NLR),C-reactive protein (CRP),D-dimer and ferritin level were measured with serum ADA level simultaneously.ADA levels were determined by modifying the colorimetric method developed.

Results: A total of 61 individuals were included in our study, and no significant difference was found between all groups and subgroups in terms of ADA levels. On the other hand, lymphocyte count,NLR, CRP, D-dimer and ferritin values were found to be significantly different between COVID19 and the control group ($p<0.001$). This difference was also observed among the COVID19 subgroups. Again, a significant correlation was found between comorbidity, vaccination status hematological and biochemical values expressing risk in COVID19 patients and the UPG, MMPPG,SPPG patient subgroups (Sig.<0.001).

Conclusions: Through the data we've collected by our study, it was concluded that serum ADA levels can not be used as an inflammatory and prognostic marker in COVID-19 patients during the initial period.

Keywords: COVID-19, Adenosine deaminase, immunity, SARS-CoV-2

S028

THE EFFECT OF TAURINE ON PRESEPSIN AND INTERLEUKIN-6 LEVELS IN A LIPOPOLYSACCHARIDE-INDUCED SEPSIS MODEL

Yasemin Atıcı[1], Nida Aslan Karakelle[2], Safiye Göçer[3]

[1] Lokman Hekim University, Faculty of Medicine, Department of Medical Biochemistry Ankara, Turkey

[2] Lokman Hekim University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

[3] Lokman Hekim University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey

Objectives: Lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, plays an important role in the pathogenesis of sepsis. Taurine, a metabolic product of L-cysteine, inhibits the secretion of proinflammatory cytokines. The aim of this study was to investigate the effect of taurine on presepsin and interleukin-6 levels in a lipopolysaccharide-induced sepsis model.

Methods: In this study, 24 Wistar albino female rats, weighing 250-300 gr were used. Rats were divided into 4 groups as control, taurine, LPS, taurine+LPS. The rats were given 300 mg/kg/day saline or taurine by gavage for 10 days. After the 10th day, saline or LPS was injected intraperitoneally at a dose of 1 mg/kg/day for once. Presepsin and interleukin-6 (IL-6) levels in liver, kidney and serum samples were measured by ELISA method. Kruskal-Wallis test was used for statistical analysis. $p<0.05$ was considered statistically significant.

Results: Presepsin levels in serum, liver and kidney tissues increased in the LPS group compared to the control and taurine groups ($p<0.05$). In kidney tissue, taurine significantly decreased the increase in presepsin levels caused by LPS ($p<0.05$). When IL-6 levels were evaluated, a decrease was observed in the taurine+LPS group compared to the LPS group in serum, while an increase was observed in the LPS group compared to the control and taurine groups in the liver ($p<0.05$). No significant difference was observed in renal IL-6 levels ($p<0.05$).

Conclusions: Taurine may be effective in proinflammatory response by decreasing tissue damage, presepsin and IL-6 levels in LPS-induced sepsis model.

Ethics committee no: G.Ü.ET-23.045

Keywords: presepsin, sepsis, Interleukin-6, lipopolysaccaharide, taurine

S029

RETURNED ASSESSMENT OF SERUM VITAMIN B12 LEVELS OF PATIENTS DIAGNOSED WITH COVID-19

Gökçe Güven Açık[1], Burcu Gürer Giray[2]

[1] Ankara İl Sağlık Müdürlüğü Ankara Halk Sağlığı

[2] Yalova Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji Ad

Objectives: It is known that vitamins have effects such as disrupting the viral replication of the Coronavirus disease (COVID-19), developing an anti-inflammatory response and increasing immunity. We aimed to see the relationship between Vitamin B12 (Vit B12) serum levels and the prognosis of the disease in COVID-19 patients in this study.

Methods::The study included 9600 participants, aged between 18-65 years, who applied to Ankara Provincial Health Directorate Laboratory for SARS-CoV-2 diagnosis between March 2020 and December 2022. Sociodemographic information, serum Vit B12 levels of the patients were analyzed retrospectively from the Laboratory System. Serum vitamin B12 level was grouped in which 200-2000 pg/mL is normal, 150-200 pg/mL is mild deficiency, 100-150 pg/mL is severe deficiency and less than 100 pg/mL is very severe deficiency.

Results: The median age of the participants was 46.51(18-65) and the median Vit B12 level was 179.50 ng/L (75-641). 5834 (60.78%) patients had Vit B12 deficiency. Cough, loss of taste and smell, headache and pneumonia rates were higher in patients with severe and very severe Vit B12 deficiency than other groups ($p<0.01$). Vit B12 levels were higher than the platelet count of patients with mild and severe levels compared to normal rates ($p<0.01$).

Conclusions: It was determined that the clinical progression of COVID-19 patients with vitamin B12 deficiency was worse than those without vitamin B12 deficiency. Therefore, we think that Vit B12 supplemens may have a positive effect in COVID-19 patients. However, more comprehensive and longer duration studies with more patients are required.

Keywords: covıd-19, pandemic, vitamin B12

S030

CHANGES OF PLATELET-RELATED BIOMARKERS IN PULMONARY TUBERCULOSIS

Muammer Özdemir[1], Aslı Şule Tıpırdamaz Yurteri[2], Sedat Abuşoğlu[1]

[1] Selcuk University Medical Faculty, Department of Medical Biochemistry

[2] Necmettin Erbakan University Medical Faculty, Department of Medical Microbiology

Objectives: Tuberculosis (TB) is the second leading cause of death from infectious diseases in the world, after COVID-19. Studies have shown that people with TB or a history of TB have an increased risk of myocardial infarction, ischemic stroke, and peripheral arterial disease. This study suggests that these diseases may be associated with TB.

Methods: The Sysmex Blood Counting Device can measure specific parameters related to platelets, such as platelet large cell ratio (P-LCR), mean platelet volume (MPV), platelet large cell count (P-LCC), and plateletcrit (PCT). We plan to investigate the changes in these parameters in TB patients. 50 patients with TB and having first-line treatment were taken for the patient group. 50 patients without any chronic disease were selected as the control group. CBC parameters were analyzed retrospectively with SPSS. Parametric-nonparametric differences were compared using the One Sample KS test and 2 independent sample t-tests.

Results: The mean age was 57.36 years for the patient group and 54.78 years for the control group, $p=0.610$. PLT and MPV were 313.50/mm3 (118-711) and 252.50/mm3 (100-639); 9.15 fL (7.20-13.10) and 9.80 fL (7.60-11.9); $p=0.001$ and $p=0.005$ in the

patient and control groups, respectively. P-LCR and P-LCC were 20.85% (7.50-48.50) and 24.7% (11.30-40); 69/µL (19-155) and 61.5/µL (5-130); p=0.013 and p=0.206, respectively. PCT was 27% (13-66) for patients and 25% (2-59) for controls, p=0.093.

Conclusions: MPV, PLT and P-LCR, which can be easily measured together with CBC, may help predict vascular events that may develop in TB patients. Further studies in this field may shed light on how the disease leads to acute vascular events.

Keywords: MPV, PLT, P-LCC, P-LCR, Complete Blood Count

S031

DISCOVERY OF FIRST-IN-CLASS PLASMODIUM OTU INHIBITORS AND UNVEILING NOVEL PATHWAYS

Pınar Siyah[1], Serdar Durdağı[2], Fatih Kocabaş[3]

[1] Department of Biochemistry, School of Pharmacy, Bahcesehir University, İstanbul, Turkey

[2] Department of Pharmaceutical Chemistry, School of Pharmacy, Bahcesehir University, İstanbul, Turkey

[3] Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey

Objectives: Malaria, a deadly infectious disease, is a global concern, especially due to the rise of drug-resistant parasites, necessitating the development of innovative treatments.This study offers a groundbreaking perspective by comparing it to Crimean Congo Hemorrhagic Fever Virus, another intracellular parasite. Both rely on the viral OTU protease to disrupt NF-κB signaling pathways for host cell invasion. Notably, our research revealed shared amino acid sequences between malaria and Neospora parasites, similar to CCHFV's OTU domain. This discovery suggests that malaria and Neospora parasites may use OTU proteins to manipulate the NF-κB pathway. Our research suggests targeting OTU proteins is crucial for malaria treatment. We conducted comprehensive in silico, in vitro, and in vivo analyses.

Methods: We created 3D models of novel OTU deubiquitinases from malaria and Neospora parasites, showing robust activity in vitro and in vivo. Our research reveals how these OTU proteins affect immune pathways and ubiquitination. We meticulously screened a library containing approximately 5.000.000 compounds, subjecting them to stringent selection criteria encompassing docking scores, druggability, ADME-tox attributes, IC50 values, DUB inhibitory characteristics, and their impact on gene and protein expression profiles.

Results: We have identified four small molecules with potent anti-malarial. This study is a milestone in malaria treatment, extending to viral OTU-like proteins in malaria types.

Conclusions: Our study, which includes structural modeling, small molecule screening, deconjugation assays, and subsequent inhibitor development, paves the way for revolutionary anti-malarial therapies targeting these malarial OTU-like proteases.

Keywords: Malaria, deubiquitinases, anti-malarial, drug discovery, small molecules

S033

THE ROLE OF MICROBIOTA AND OXIDATIVE STRESS IN FOOD ALLERGIES

Gulcin Alp Avcı[1], Ulku Irem Yılmaz[2], Emre Avcı[3], Cumhur Bılgı[4]

[1] University of Health Sciences, Gulhane Faculty of Dentistry, Department of Basic Medical Sciences, Microbiology, Ankara, Türkiye

[2] University of Health Sciences, Gulhane Vocational School of Health Services, Ankara, Türkiye

[3] University of Health Sciences, Gulhane Faculty of Pharmacy, Department of Biochemistry, Ankara, Türkiye

[4] University of Yuksek Ihtisas, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: Food allergies appear with an increasing picture in the world, especially due to changes in environmental conditions. It is known that many factors play a role in food allergies starting from infancy, from genetic factors to environmental factors, from

the mother's nutrition during pregnancy to the packaged foods that children consume more and more every day. Microbiota plays an important role in the fulfillment of many different body functions. It is known that oxidative stress plays a role in the pathogenesis of many diseases, especially allergic diseases. Especially in food allergy, the process becomes more complicated with the effect of agents such as inflammation and oxidative stress. Therefore, in our study, it was aimed to reveal the effect of intestinal microbiota and oxidative stress on the process in individuals with food allergy caused by various factors.

Methods: Twenty patients diagnosed and followed up with food allergy and 20 healthy volunteers as the control group were included in the study. Live intestinal microbiota in the stools of individuals and MDA and GSH levels as markers of oxidative stress in serum were determined spectrophotometrically.

Results: When the study group was evaluated, it was determined that the oxidant balance was impaired in individuals diagnosed with food allergy, and a decrease in the number and variety of probiotics, a permanent flora element in the intestinal microbiota.

Conclusions: As a result, the importance of antioxidant supplements and especially personalized probiotic supplements is gradually increasing in eliminating the effect of allergic diseases on metabolism, which has increased in recent years.

Keywords: Oxidative stress, Probiotics, Microbiota, Food Allergies

S035

PLASMA LIPID PROFILE IS ALTERED IN CHRONIC MIGRAINE PATIENTS AND CORRELATED WITH MIGRAINE RELATED DISABILITY

Hale Gök Dağıdır[1], Merve Hilal Ceren Akgör[2], Doğa Vurallı[2], Hayrunnisa Bolay Belen[2]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey, Gazi University Neuropsychiatry of Education Research and Application Center (npm) Neuroscience and Neurotechnology Center of Excellence (nörom)

[2] Gazi University Hospital, Department of Neurology and Algology, Ankara, Turkey, Gazi University Neuropsychiatry of Education Research and Application Center (npm), Neuroscience and Neurotechnology Center of Excellence (nörom)

Objectives: Chronic migraine (CM) is a disabling common neurologic disorder predominantly affecting women and its treatment is challenging. Understanding the confounding factors in chronic migraine will provide alternative approaches for the management. Lipids have important functions in energy storage and cell membrane structure and they are the source of inflammatory and pain signaling molecules. However, its relationship with chronic migraine has not yet been clearly explained.

Methods: This study enrolled 30 woman subjects (15 CM patients and 15 age matched healthy controls). Clinical features, disease duration, body mass index (BMI) were recorded and migraine related cognitive symptoms scale (MigScog), headache ımpact test 6 (HIT-6), hospital anxiety and depression scale (HAD) and biochemistry laboratory tests describing the lipid profile were evaluated.

Results: Serum VLDL, TG, levels were significantly higher and HDL levels were significantly lower in CM patients than controls while BMI values were comparable in 2 groups. Migraine duration was positively correlated with VLDL (r=0,499), TG (r=0,609), HbA1c (r=0,435) and negatively correlated with HDL (r=-0,387). MigScog scores were correlated with VLDL (r=0,489) and TG (r=0,536) levels. HIT-6 showed correlateion with VLDL (r=0,435) and TG (r=0,518). HAD depresssion was correlated with VLDL (r=0,605) and TG (r=0,636). HAD anxiety was correlated with VLDL (r=0,444) , TG (r=0, 555) and negatively correlated with HDL (r=0,381).

Conclusions: We demonstrated abnormal serum lipid levels in CM patients and serum levels showed correlation with migraine related disability. Management strategies to regulate and control lipid parameters may have an important role in the treatment and prevention of CM.

Keywords: Chronic migraine, serum lipids, Migscog, HIT-6, HAD

S036

INVESTIGATION OF THE EFFECTS OF WHITE TEA ON THE DEVELOPMENT OF ATEROSCLEROSIS IN MICE FEED WITH ATEROGENIC DIET

Merve Hüner Yiğit[1], Mehtap Atak[1], Ertuğrul Yiğit[2], Tolga Mercantepe[3], Mehmet Kıvrak[4], Hüseyin Avni Uydu[5]

[1] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Medical Biochemistry, Rize, Turkey.

[2] Karadeniz Techninal University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Turkey.

[3] Recep Tayyip Erdogan University, Faculty of Medicine Department of Histology and Embryology, Rize, Turkey.

[4] Recep Tayyip Erdogan University, Faculty of Medicine Department of Biostatistics, Rize, Turkey.

[5] Samsun University, Faculty of Medicine Department of Medical Biochemistry, Samsun, Turkey.

Objectives: The aim of this study is to present comprehensive and up-to-date evidence on the possible antiatherosclerotic activity of white tea, which is known for its antidiabetic, neuroprotective, anticancer, antimicrobial and antiobesity properties.

Methods: In our study, C57BL-6J mice (n=8) were used in the control group, while ApoE $^{-/-}$ mice (n=32) were used in the sham, case, white tea (WT100) and white tea (WT500) groups. Case and sham groups were fed with control diet and all other groups were fed with atherogenic diet for 16 weeks. In the last 4 weeks of the 16-week feeding period, white tea was given by gavage to the WT100 (100mg/kg/day) and WT500 (500mg/kg/day) groups as a single daily dose. Animals were sacrificed, blood and aortic tissues were collected. Total cholesterol (TC), triglyceride (TG) and glucose (Glu) in serum were measured by enzymatic colorimetric method, while paraoxanase-1(PON-1) were measured by ELISA. Tumor necrosis factor-α (TNF-α), myeloperoxidase (MPO) andinterleukin-10 (IL-10) levels were measured in aortic arch tissue by ELISA. In addition, aortic root was examined histopathologically.

Results: Compared with the case group, in the WT100 and WT500 groups; It was determined that TC, TG, plaque burden, atherogenic cytokine TNF-α and oxidative stress marker MPO levels decreased ($p<0.05$), while the levels of anti-inflammatory cytokines IL-10 and PON-1 increased ($p<0.05$).

Conclusions: In our study, it was concluded that white tea reduced inflammation and dyslipidemia in atherosclerosis.

Keywords: Apo E--, Atherosclerosis, Dyslipidemia, Inflammation, White tea

S037

INVESTIGATION OF BCL11A (rs1427407) and HMIP (rs9399137) SNPs ASSOCIATED WITH HIGH HbF LEVELS IN β-THALASSEMIA

Yasemin Özküçük[1], Ebru Dündar Yenilmez[1], Göksel Leblebisatan[2], Abdullah Tuli[1]

[1] Cukurova University, Faculty of Medicine, Departmant of Medical Biochemistry, Adana, Türkiye

[2] Cukurova University, Faculty of Medicine, Departmant of Pediatric Hematology, Adana, Türkiye

Objectives: To evaluate the effect of rs1427407 and rs9399137 single nucleotide polymorphisms (SNP), which improve the phenotype by increasing HbF synthesis in β-thalassemia major or sickle cell anemia, on fetal hemoglobin (HbF) levels.

Methods: 44 pediatric patients (<18) who applied to Çukurova University Balcalı Hospital Pediatric Hematology Department with the complaint of β-thalassemia major or sickle cell anemia and received blood transfusion were included in our study. rs1427407 SNP were evaluated by tetra primer ARMS method and rs9399137 SNP was evaluated by RT-PCR method.

Results: According to the results of rs1427407 SNP of BCL11A locus of patients, 23 of them (52%) have ‘’C/C’’, 9 (20,45%) carries ‘’T/C’’ and 12 (27,27 %) carries “T/T” allel. SNP results obtained for rs9399137 in β-thalassemia and sickle cell anemia patients show 44 (100%) “T/T” allels.

Conclusions: As a result, "C" allel for rs1427407 polymorphism and "T" allele for rs9399137 polymorphism were found at high frequency. Significant differences were found related to HbF level at all two polymorphic points. When the rs1427407 and rs9399137 polymorphisms were examined together, it was determined that HbF synthesis was high ($p<0.001$) in individuals carrying the rs1427407 "C/T" and rs9399137 "T/T" alleles together. It was found that the rs1427407 polymorphism affected HbF levels more in patients with β-thalassemia with the "C/T" genotype compared to individuals with sickle cell anemia ($p<0.001$).

Keywords: SNP analyse, HbF levels, haemoglobinopathy

S039

THE IMPORTANCE OF PANEL DESING FOR FLOW CYTOMETRY

Cigdem Sonmez[1], Barıs Boral[2], Nuran Ahu Baysal[3]

[1] University of Health Sciences, Dr. Abdurrahman Yurtarslan Oncology Research and Training Hospital, Clinical Chemistry

[2] University of Health Sciences, Dr. Abdurrahman Yurtarslan Oncology Research and Training Hospital, Immunology

[3] University of Health Sciences, Dr. Abdurrahman Yurtarslan Oncology Research and Training Hospital, Hematology and Bone Marrow Transplantation

Objectives: Flow-cytometry immunophenotyping is crucial, especially for the diagnosis of hematological disease. Mixed-phenotype acute leukemia(MPAL) consists of a heterogeneous group of leukemias that are genetically, immunophenotypically, and clinically, diverse. Since the rarity of the disease, the diagnosis and treatment of MPAL is extremely challenging. Besides morphologic criteria, setting the correct diagnosis depends on the identification of immunophenotype by flow cytometry, and well-designed panels are crucial. The purpose is to demonstrate the importance of the panel design for the diagnosis of MPAL.

Methods: A 48-year-old male's diagnosed with T-Lymphoblastic Lymphoma(T-ALL)in another hospital bone marrow sample was evaluated with immunophenotyping. The cell count findings were bicytopenia, borderline (white-cell-count:29,29*109cell/L)leukocytosis. His bone marrow aspiration showed 53%of blasts. Immunophenotyping showed a population of blasts exhibiting positivity of two lineages, T-lymphoid lineage, and B-lymphoid lineage with strong positivity of CD34-cCD3-CD7-CD2-CD4-CD5-CD1a-CD3- cCD79a-CD10-CD19- terminal deoxynucleotidyl transferase(TdT)-Human Leukocyte Antigen–DR(HLADR), moderate positive of CD22 and negativity ofCD8-CD13-CD33-CD117-Myleperoxidase(MPO).

Results: Flow-cytometry is unique in its ability to measure, and analyze the cells with a gating strategy and well-designed panel. Acute leukemia orientation tube(ALOT)is the first step for evaluating leukemias. The ability of the panel to distinguish normal from pathological and the give information about the lineage may be established with the antibodies used in the panel. Although the patient was diagnosed with T-ALL, our ALOT tube findings showed two lineages. We performed multiple tubes for further information about the blasts and the patient's diagnosis switched from T-ALL to MPAL.

Conclusions:A systematic approach to designing multicolor flow cytometry panels is crucial for accurate diagnosis of acute leukemia diagnosis.

Keywords: flow cytomety, Mixed-phenotype acute leukemia

S040

FLOW CYTOMETRIC DIAGNOSIS OF MULTIPLE MYELOMA: A CASE REPORT

Alpaslan Öztürk, Lale Aydın Kaynar

Etlik City Hospital

Objectives: Multiple myeloma is a plasma cell neoplasm characterised by lytic bone lesions, hypercalcaemia, renal failure and immunodeficiency. This disease is especially seen in men aged 65-70 years. In multiple myeloma, tumour cells express CD38 and CD138 as detected by flow cytometry.

Methods: A 69-year-old male patient was admitted to the Hematology Outpatient Clinic of Etlik City Hospital with complaints of extreme fatigue, frequent infections and bone pain, especially in the back region. Laboratory tests revealed anemia (9.4 g/dL), leukocytosis (15.200/mm3), elevated creatinine (2.2 mg/dL) and elevated C-reactive protein (36.7 mg/L). The bone marrow sample of the patient who was ordered flow cytometry with a prediagnosis of multiple myeloma was received to our laboratory and multiple myeloma panel was studied from this sample after preliminary procedures.

Results: After analysing the dot graphs in flow cytometry, 0.12% of the cells were found to be CD38 and CD138 bright positive and located in the CD45 negative area. Monoclonal lambda light chain expression was observed in these cells after capturing. CD117, CD81, CD200, CD27 and CD28 positivity was observed in neoplastic cells.

Conclusions: The development of effective treatment modalities such as Daratumumab, a monoclonal antibody against CD38, will directly affect treatment planning and thus prognosis. In addition, the fact that multiple myeloma cases not expressing CD56 are associated with poor prognosis makes flow cytometry more important in the clinical evaluation of this neoplasm. In conclusion, this study once again emphasises the importance of flow cytometry in all stages of hematological malignancies.

Keywords: Flow cytometry, Multiple myeloma, CD38, CD138

S041

EVALUATION OF HEMOGLOBINOPATHIES WITH CAPILLARY ZONE ELECTROPHORESIS

Nazife Doğan

Istanbul Cam and Sakura City Hospital, The Department of Medical Biochemistry

Objectives: Hemoglobinopathies are genetic disorders that cause abnormalities in the structure of the globin chains in the hemoglobin molecule. Capillary Zone Electrophoresis (CZE) is widely used to diagnose hemoglobinopathies. Hemoglobinopathies are observed as quantitative abnormalities (thalassemia) and qualitative abnormalities (hemoglobin variants).

Methods: Hemoglobin electrophoresis was performed with the CZE method (Sebia Minicap) on 3238 whole blood samples at Istanbul Başakşehir Çam and Sakura Hospital between September 1, 2022 and August 31, 2023.

Results: Hemoglobin electrophoresis was evaluated according to electrophoretic patterns. Hemoglobinopathies (thalassemias and Hb variants) were observed in 12.66% of reports (410 samples). In the evaluation, 7.85% (254 samples) had beta thalassemia, 2.22% (72 samples) had alpha thalassemia, 1.76% (57 samples) had Hemoglobin S (Hb S) variant, 0.46% had Hemoglobin D (Hb D) variant in 15 samples, Hemoglobin F (Hb F) variant in 0.25% (8 samples), Hemoglobin C (Hb C) variant in 0.06% (2 samples), % Hemoglobin E (Hb E) variant was detected in 0.03% (1 sample) and Hemoglobin 0-Arab (Hb0-Arab) variant was detected in 0.03% (1 sample).

Conclusions: With CZE, both common (thalassemias and Hb S variant) and rare (Hb D, F, C, E, O-Arab variants) hemoglobinopathies are detected in a shorter time and at a lower cost. Advanced genetic analyses are recommended in electrophoresis reports to diagnose detected hemoglobinopathies definitively.

Keywords: Hemoglobinopathy, electrophoresis, thalassemia, hemoglobin variant

S042

THE IMPACT OF TRANSPORTING TUBES WITH A PNEUMATIC SYSTEM ON ROUTINE COAGULATION TESTS

Nur Benil Yabacı, Feyza Yağmur Tekeli, Seçkin Özgür Tekeli

Antalya Education and Research Hospital, Department of Medical Biochemistry, Antalya, Türkiye

Objectives: In the field of Clinical Biochemistry laboratories, there are many preanalytical variables that need to be controlled to obtain

accurate results in coagulation tests. In 2008, CLSI published a document that addressed the preanalytical stages of coagulation testing. In this document, it was emphasized that coagulation samples transported in pneumatic tubes should be protected from sudden agitation, as it could lead to protein denaturation and platelet activation.

Methods: Our study included 30 participants, from whom blood was collected into two different citrated coagulation tubes each. One of the samples was transported to the laboratory using a pneumatic tube, while the other was transported to the laboratory without using a pneumatic tube. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen tests were performed on both coagulation tubes simultaneously. The results were compared using paired sample t-tests, and the compatibility of the results was demonstrated using correlation analysis, Passing-Bablok regression analysis, and Bland-Altman analysis.

Results: In the coagulation tubes transported with a pneumatic tube, PT results were significantly lower compared to those not transported with a pneumatic tube ($p=0.006$), while fibrinogen results were significantly higher ($p<0.0001$). There was no significant difference in aPTT results between the two tubes ($p=0.719$). The correlation coefficients (r) for PT, aPTT, and Fibrinogen were determined as 0.776, 0.883, and 0.870, respectively.

Conclusions: Based on the obtained results, it is suggested that coagulation factors particularly associated with the extrinsic pathway may undergo changes in their activation during transportation with pneumatic tubes.

Keywords: coagulation tests, pneumatic system

S043

THE EFFECTS OF THE TRANSPORT CONDITION ON STEM CELLS

Oğuzhan Zengi

Istanbul Cam and Sakura City Hospital, Medical Biochemistry

Objectives: In the context of stem cell transplantation, hematopoietic progenitor cells (HPCs) are a critical component. Flow cytometric quantitation of CD34+ cells should provide a rapid, reliable, and reproducible assay. The best conditions for storing and transporting HPCs in a liquid state are not clearly defined or established in the current standards applied in stem cell transplantation. Our objective was to evaluate the optimal transportation conditions through courier and pneumatic tube systems (PTS) for peripheral blood and apheresis products, aiming to enhance CD34 recovery and thereby potentially improve the results of transplant procedures.
Methods: Samples were collected from the same group of patients, resulting in paired data. The collected samples were divided into two separate tubes - one tube was sent to our laboratory with PTS, while a courier brought the other. Samples were simultaneously tested on the Beckman flow cytometer and Sysmex hematology analyzer.
Results: We tested parameters such as CD34+, CD45+live, and leukocyte counts and found no clinically significant difference between the two transportation methods. For absolute CD34 counting, the mean bias was -1.7%, 0.001% for CD45live, and 1% for WBC count. There were no proportional and systematic differences in the Passing-Bablok analysis.
Conclusions: Based on our findings, it can be concluded that PTS can be selected as a transportation method for fast delivery of samples to the laboratory without affecting the flow cytometry results in the CD34 enumeration process.

Keywords: flow cytometry, CD34, Stem cell

S045

LINEAR REGRESSION MODELS CAN PREDICT THE DIRECT LDL-C TEST LEVELS WITH 74% ACCURACY: MACHINE LEARNING-BASED COMPUTING OF CHOLESTEROL

Süheyl Uçucu

Mugla Publıc Health Laboratory

Objectives: At levels below 400 mg/dl of triglyceride levels, LDL levels can be calculated computationally with equations such as Friedwald's equation and Martin Hopkins equation, but not at

levels above 400 mg/dl.This study aims to develop a model to differentiate LDL cholesterol measurement using machine learning methods in cases where LDL levels cannot be measured computationally at levels above 400 mg/dl but can be measured by direct LDL-c test.

Methods: This research includes data from a cohort of 24,000 data points and 2,000 cases. Cholesterol, LDL, HDL, and Glucose levels were evaluated within the scope of this study. Including Artificial Neural Network (ANN), K-Nearest Neighbor (KNN), Linear Regression (LR), Stochastic Variational Bayesian (SVR), Random Forest(RF), (XGB), Decision Tree (DT), Extreme Gradient Boosting Regressor Models were created using various machine learning (ML) methods. The models with the highest diagnostic accuracy were compared and then evaluated.

Results: The study found that linear regression models could predict LDL-C levels with 74% accuracy. The machine learning model was able to predict laboratory test results with 74% accuracy.

Conclusions: The algorithm, created using data from 2,000 cases, can directly predict LDL-C test results with 74% accuracy. The results of this study may make it easier for clinicians to measure LDL cholesterol levels in individuals with hypertriglyceridemia.

Keywords: Machine learning, Linear regression, Direct LDL-c, Artificial Neural Network ANN, K-Nearest Neighbor

S046

EVALUATION OF KNOWLEDGE LEVELS, ATTITUDES AND BEHAVIOURS OF MEDICAL BIOCHEMISTRY SPECIALISTS ABOUT GREEN AND SUSTAINABLE MEDICAL LABORATORY

Emine Feyza Yurt, Medine Alpdemir, Mehmet Şeneş

Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye)

Objectives: Our aim in this study is to analyze the level of knowledge, awareness, attitudes, and behaviors of medical biochemistry specialists in Turkey under green and sustainable medical laboratories guideline (GSMLG).

Methods: This research is a cross-sectional survey study. Medical Biochemistry specialists between 23 and 65 years, participated. The GSMLG was used as a reference. The survey questions are organized under five sections using the Google form (

https://docs.google.com/forms/d/e/1FAIpQLSedLGF9FHVWkYkN27unXguIAmOeBknBGYyyYp-_uVSDLkm6pA/viewform?vc=0&c=0&w=1&flr=0&pli=1). Excel was used for analysis of the responses.

Results: A total of 130 people responded to the questionnaire. According to section 1, 28.5% of the participants were academicians, 48.1% were specialists and 23.1% were assistants. The average age of the participants was 39.2±10.8 years and the gender distribution was 60% female and 40% male. 76.1% of the participants stated that they had heard of the concept of sustainability before in section 2. In section 3, it was observed that the participants realized sustainable green laboratory practices (energy and water consumption, waste and chemical use) that can be done individually. In the 4th section, it was determined that 83.1% of the laboratories where the participants work have chemical substance management, 74.6% have waste management, 44.6% have energy consumption and 43.7% have water consumption practices. In section 5, 60% of the participants stated that they did not have information about GSMLG.

Conclusions: Individual laboratory professionals have high sensitivity towards the environment. However, there are deficiencies in energy and water consumption management as managerial infrastructure. Most participants are not familiar with GSMLG.

Keywords: Sustainability, Green Laboratory

S047

BLOOD COLLECTION TRAINING STATUS OF LABORATORY TECHNICIANS

Pınar Eker

Maltepe University Faculty of Medicine

Objectives: The majority of laboratory errors occur at the phlebotomy stage during the preanalytical process. Phlebotomist is not defined as a profession in Turkey. This process is mostly carried out by laboratory technicians The aim of our survey is to determine the educational status of those who perform this process by separating practical and theoretical aspects.

Methods: The survey was administered via Google forms. 262 valid responses were evaluated.

Results: It was determined that 7.2% performed phlebotomy without any theoretical training during school or post-school, and 6.1% did it without any practical training. Those who performed phlebotomy without any training during school or after school were 3.4%. Of those who performed phlebotomy without any training at school or after school 66.7% graduated before 2014, 22.2% graduated between 2014 and 2020, and 11.1% graduated after 2020.In Turkey blood collection was included in the job description of laboratory technicians with the regulation published in 2014. Technicians who graduated before 2014 constitute the majority of the untrained group. It can be thought that it will be supportive about the group that should be prioritized in terms of training requirements.

Keywords: phlebotomy, laboratory technician, training

S048

MICROBIOLOGICAL EVALUATION OF DISPOSABLE HOLDERS USED IN THE BLOOD COLLECTION UNIT IN MULTIPLE USES

Nilgün Işıksaçan[1], Ramazan Korkusuz[2], Duygu Teksöz[1], Pınar Kasapoğlu[1]

[1] Sbu. Istanbul Bakırköy Dr. Sadi Konuk Research and Training Hospital, Department of Biochemistry, Istanbul, Turkey

[2] Sbu. Istanbul Bakırköy Dr. Sadi Konuk Research and Training Hospital, Departmant of Infectious Diseases Clinic, Istanbul, Turkey

Objectives: Some of healthcare facilities reuse blood tube holders in order to reduce costs associated with device purchase and waste removal. However, removing contaminated needles and reusing the holders can pose multiple potential hazards. We aimed to evaluate the disposable holders used in the blood collection unit microbiologically in case of multiple use.

Methods: The disposable holders (450201 VACUETTE® Standard Tube Holder, Greiner Bio-One, Kremsmuenster, Austria) were distributed to ten blood collection cabinets, one for each. The holders used for five weeks without any disinfection. On the first (40 swab), second (10 swab) and third (10 swab) days of the first week, a total of 60 swabs from the inner surface of the holder in each cabin, were taken. On the fifth day of the next four weeks, a total of 40 swabs were taken and, inoculated and evaluated for bacteria.

Results: At the end of the 1st, 2nd and 3rd day, it was observed that 10,40,10% of the holders grew Coagulase negative staphylococcus (CNS), 30,20,50% grew Staphylococcus epidermidis, 10% grew Staphylococcus aureus and 10% grew yeast. 2nd, 3rd, 4th, 5th week, 30.8, 28.6, 21.4, 33.3% had CNS, 46.2,42.9,57.1,46.7% had Staphylococcus epidermidis, 7.7% had Corynebacterium Spp, Also, 3rd week, 7.1% had Micrococcus Spp, 4th week, 7.1% had yeast, 5th week, 6.7% had Enterobacter spp and 6.7% had Enteroccus spp reproduction was observed.

Conclusions: It should be noted that non-disposable holders may be contaminated with bacteria or blood, which may pose a risk to the staff, patients and samples.

Keywords: holder, multiple use, contamination, bacteria

S049

INVESTIGATION OF THE RELATIONSHIP OF OXIDATIVE STRESS, INFLAMMATION, AND CELL ADHESION MARKERS WITH CLINICAL PARAMETERS IN FABRY DISEASE

Berna Kuş[1], Menderes Yusuf Terzi[2], Faruk Hilmi Turgut[3], Abdullah Arpacı[4]

[1] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Molecular Biochemistry and Genetics Hatay, Türkiye

[2] Hatay Mustafa Kemal University Faculty of Medicine Department of Medical Biology, Hatay, Türkiye

[3] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Nephrology, Hatay, Türkiye

[4] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Medical Biochemistry, Hatay, Türkiye

Objectives: The aim of the present study was to determine the relationship of growth differentiation factor 15 (GDF15), leucine-rich α-2-glycoprotein-1 (LRG1), galectin-3 (LGALS3) and oxidative stress parameters, *i.e.* total antioxidant level (TAS) and total oxidant level (TOS) with Fabry disease in blood serum samples.

Methods: 25 Fabry patients who applied to Hatay Mustafa Kemal University Medical Faculty Nephrology Clinic and 28 healthy volunteers were included in the current study. LGALS3, GDF15 and LRG1 protein levels in blood serum samples were measured by ELISA method, TAS and TOS levels were measured by colorimetric method and oxidative stress index (OSI) values were calculated. In addition, routine biochemistry parameters (glucose, ALT, AST, BUN, creatinine, sodium, potassium, calcium, phosphorus, uric acid) of the patient and control groups were measured. Receiver operating characteristic (ROS) test was performed to analyze the distinctiveness of some parameters in diagnosis of Fabry disease.

Results: GDF-15, LGALS3 and TAS values were significantly higher in the patient group than in the control group ($p<0.05$). Sodium was found to be significantly lower in the patient group compared to the control group ($p<0.001$). No significant difference was observed in other biochemical parameters between the groups ($p>0.05$). The area under the curve (AUC) values of the ROC analyses were 0.7821 ($p<0.05$) for LGALS-3, 0.6957 ($p<0.05$) for GDF15, 0.8879 ($p<0.05$) for sodium, 0.6714 in TAS ($p<0.05$), 0.5016 ($p>0.05$) for TOS and 0.5699 ($p>0.05$) for OSI.

Conclusions: As a result, LGALS3, GDF15, sodium and TAS can be promising parameters in differentiation of Fabry disease patients ($p<0.05$).

Keywords: Fabry disease, Alpha-Galactosidase A, GLA gene mutation, Biochemical parameters, Biomarkers

S050

THE IMPORTANT ROLE OF MULTIPLEX PCR IN THE DIAGNOSIS OF DUCHENNE MUSCULAR DYSTROPHY

Fatimazahra Smaili[1, 2], Khawla Zerrouki[1], Fatima Ezzahra Aouni[1], Ayad Ghanam[3, 4], Abddeladim Babakhouya[3, 4], Mariam Tajir[1, 2]

[1] Laboratory of Medical Genetics, Central Laboratory, Mohammed Vı University Hospital, Oujda, Morocco

[2] Epidemiology, Clinical Research and Public Health Laboratory, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

[3] Pediatrics Department, Mother and Child Hospital, Mohammed Vı University Hospital, Oujda, Morocco

[4] Laboratory of Immunohematology / Cellular Therapy For Adults and Children, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

Objectives: Duchenne muscular dystrophy (DMD) is a hereditary neuromuscular disease with X-linked recessive inheritance. It is caused by mutations in the gene *DMD* that codes for the dystrophin protein. Multiplex PCR allows the detection of 98% of rearrangements of the *DMD* gene in 70% of cases of dystrophinopathies. The objective of our work is to show the importance of this tool in the diagnosis of DMD.

Methods:**:** We report 14 patients referred to the Laboratory of Medical Genetics of the University Hospital Mohammed VI of Oujda for myopathy. Patients' DNA was extracted from peripheral venous blood. Its quality and quantity were

controlled by spectrophotometry. We performed a multiplex PCR for promoter and exons ranging from 3 to exon 60 of the *DMD* gene. We used three mixtures of primers.

Results: The results obtained are observed and interpreted according to the presence or absence of the expected bands under UV and based on a size marker as a reference. Multiplex PCR revealed deletions of the *DMD* gene in 4 patients (28.57%), which are grouped in exon regions 43 to 52.

Conclusions: Multiplex PCR plays a crucial role in the early identification of DMD gene mutations and therefore enables diagnosis and genetic counseling in this pathology and will be important for future therapeutic strategies.

Keywords: Multiplex PCR, Duchenne Muscular Dystrophy, genetic counseling

S051

THE INVESTIGATION OF EXPRESSION LEVEL DIFFERENCES OF CARDIAC AQUAPORINS IN H2O2 INDUCED H9c2 CARDIOMYOCYTES

Ayca Bostanoglu, Elif Merve Avcu, Beril Erdem Tuncdemir, Emel Saglar Ozer

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

Objectives: The aim of study is investigation of expression level differences of cardiac aquaporins (*AQP1* and *7*) in H9c2 cardiomyocytes after H_2O_2 treatment which can cause increased stress level in H9c2 cardiomyocytes.

Methods: By performing MTT assay, non-toxic H_2O_2 concentration for H9c2 cells was determined. After inducing H9c2 cells with H_2O_2 for 2 hours, RNA isolation and cDNA synthesis were performed. Expression levels of *AQP1* and *AQP7* were measured via qPCR on basis of *Beta actin* as a reference, DDCt values were calculated and statistically analyzed. This study was supported by Hacettepe University (Project number: FHD-2022-20252).

Results: MTT assay results showed that 200 mM H_2O_2 was non-toxic concentration. After treatment of cells with 200 mM H_2O_2, we found that *AQP1* expression level was not changed while *AQP7* expression level was significantly changed (p: 0,0312).

Conclusions: Since *AQP1* is known as aquaporin and *AQP7* is known as aquaglyceroporin, *AQP1* involves in water transportation and *AQP7* mainly involves in glucose uptake. Expression level of *AQP1* might not be changed because cells were always in aqueous environment during experiments. It is assumed that in an aqueous system, cells might stabilize their osmotic pressure and *AQP1* expression level might remain as same as before. On the other hand, increased expression level of *AQP7* can be related with the fact that *AQP7* coordinates glucose uptake that affects regulation of contracting and water balance in cardiomyocytes.

Keywords: AQP1, AQP7, H9c2, H2O2

S052

INVESTIGATION OF HNF1A GENE POLYMORPHISMS AND THEIR METABOLIC EFFECTS ON MODY AND TYPE 2 DIABETES

Deniz Kanca Demirci[1], Nurdan Gul[2], Bengu Tokat[3], Ilhan Satman[2], Oguz Ozturk[3], Hulya Yilmaz Aydogan[3]

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Haliç University, Istanbul, Turkey.

[2] Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey.

[3] Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey.

Objectives: We aimed to investigate the distribution of common hepatoctye-nuclear-factor-1A-(HNF1A)-gene variations and their effects on clinical features in patients with type 2 diabetes-(T2DM) and clinically-diagnosed maturity-onset-diabetes-of-the-young (MODY).

Methods: HNF1A-gene was sequenced in genomic-DNA samples of 41-patients with T2DM and 53-patients with MODY by Next Generation Sequencing-(NGS). Statistical analyses were performed by SPSS-software package-(version-20.0-SPSS Inc.,IL,USA). This study was supported by the Research Fund of Istanbul University (Project-No.TOA-2017-24194).

Results: The distribution of the HNF1A rs1169288-(A>C), rs1169289-(G>C), rs1800574-(C>T), rs56348580-(G>C) and rs55834942-(G>A) polymorphisms was similar in the study groups ($p>0.05$). T2DM patients with the rs1169289 and rs56348580 GG genotypes had lower C-peptide levels than non-carriers, while those with the rs1169288 rare-C allele had higher waist-circumference ($p<0.05$). In the MODY group, rs1169289 minor-C allele carriers (GC+CC genotypes) had higher ALT than GG genotype ($p<0.01$). In addition, serum triglyceride levels were significantly higher in MODY subjects with rs56348580 GG genotype than those with the C allele ($p<0.05$).

Conclusions: The association of HNF1A-gene polymorphisms with the decrease in C-peptide levels indicates that they affect endocrine-pancreas function in T2DM-patients. Also, the effects of these gene variations on ALT and triglyceride levels suggest that they affect liver function in MODY-patients. In conclusion, our findings show that HNF1A-gene polymorphisms may have different metabolic effects in T2DM&MODY patients. However, it cannot be excluded that the difference may be due to the onset of the two forms of diabetes at different ages, as in this study. Therefore, further studies with larger sample sizes are required for stronger findings.

Keywords: MODY, T2DM, NGS, HNF1A, single nucleotide polymorphism SNP

S053

IMMUNOGOLD LABELING OF MUTANT AVP PRECURSORS AT ELECTRON MICROSCOPY

R. Dilara Vaizoglu[1], Beril Erdem Tuncdemir[1], Emel Saglar Ozer[1], Ceren Acar[2], Mehmet Gül[3], Hatice Mergen[1]

[1] Hacettepe University, Faculty of Science, Department of Biology, Moleculer Biology Section, Ankara, Türkiye

[2] Inonu University, Faculty of Arts and Science, Department of Moleculer Biology and Genetics, Malatya, Türkiye

[3] Inonu University, Medical Faculty, Basic Medical Sciences, Histology and Embryology Section, Malatya, Türkiye

Objectives: The aim of this study is visualizing the aggregate structures formed by mutant arginine vasopressin hormone (AVP) precursors with mutations in the neurophysin II region 207_209delGGC, G88V, C98X, E108D-1, E108D-2, and R122H which are responsible with a rare disease autosomal dominant neurohypophyseal diabetes insipidus (ADNDI) via transmission electron microscopy.

Methods: Mutant AVP precursors were expressed in COS-1 cells. They were fixed, permeabilized, blocked and incubated with primary antibodies. The cells were then stained with 15nm Gold secondary antibodies. The cells were dehydrated, embedded in resin and polymerized. Araldite cell blocks were shaved and placed on copper-coated formvar and visualized at transmission electron microscopy (TÜBİTAK SGAB Project No: 118S688, Hacettepe University BAP Project No: 19929).

Results: Electron-dense aggregate structures were visualized within the intracytoplasmic and exocytoplasmic vesicles.

Conclusions: Protein misfolding due to AVP mutations leads to the formation of aggregate structures, causing Endoplasmic reticulum (ER) conformational distortion and potentially increasing intracellular toxicity. According to the results, mutant protein precursors labeled with immunogold were accumulated within the ER lumen at different densities, with disruption ER architecture and causing the structure to expand in a vesicular manner. This condition might have the potential to impair ER function. Some mutations showed both intracellular and extracellular aggregate structures. In addition, this study has also some updates in the imaging protocol for transmissi

Keywords: Aggregate, AVP, Transmission Electron Microscopy, ADNDI

S054

COMPARATIVE EVALUATION OF SILICA MEMBRANE AND PHENOL-CHLOROFORM DNA EXTRACTION TECHNIQUES FOR ANCIENT SAMPLES

Hazal Tahravi[1], Yeşim Doğan[2], Hatice Mergen[1]

[1] Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

[2] Ankara University, Faculty of Language, History and Geography, Department of Anthropology, Ankara, Türkiye

Objectives: Ancient DNA (aDNA) refers to DNA in severely degraded substances like teeth and bones retrieved from museum specimens or archaeological excavations. A key challenge in aDNA research is the scarcity of intact DNA in these samples. Also, the decay of aDNA complicates its extraction, severely limiting the recoverable DNA length. Hence, it's vital to design custom extraction methods to maximize DNA yield from specimens. However, the complexities of handling damaged DNA have led to innovative isolation techniques. Several methods exist, all aiming to optimize DNA production while reducing co-extraction of PCR inhibitors. This study aims to compare two extraction techniques in terms of efficiency in aDNA.

Methods: 5 femur, 5 talus specimens from Stratonikeia Ancient City, were subjected to both silica membrane and phenol-chloroform DNA extraction methods. Mitochondrial DNA was amplified using three distinct primer pairs, generating amplicons of 162 bp, 177 bp, and 218 bp. The success of each extraction method on different sample types was comprehensively evaluated. The study was supported by Hacettepe University BAP project number with FYL-2021-19646.

Results: We found that the phenol-chloroform extraction method exclusively yielded successful results for the shortest amplicon (162 bp) in femur and talus specimens. In contrast, the silica membrane approach consistently yielded successful outcomes for all three amplicons in these samples.

Conclusions: This study underscores the significance of selecting appropriate DNA extraction methods for different sample types. The success of the silica membrane method in yielding amplifiable DNA fragments of varying lengths, even from severely damaged samples, signifies its utility for diverse research applications.

Keywords: Ancient DNA, DNA extraction, mtDNA

S055

AUTOPHAGIC CELL DEATH STUDIES LACK EVIDENCE OF CAUSALITY

Yasmin Ghaseminejad[1], Ali Burak Özkaya[2]

[1] Izmir University of Economics, Faculty of Medicine, Izmir, Turkey

[2] Izmir University of Economics, Faculty of Medicine, Department of Medical Biochemistry, Izmir, Turkey

Objectives: Autophagy has been a topic of extensive interest for the last two decades due to its critical role in health and many diseases. Autophagic cell death (or type II cell death) is a popular yet controversial concept, and how it is measured may vary greatly from study to study. Here we examined the methodology of publications with autophagic cell death claims and investigated the indisputability of the provided evidence.

Methods: We retrieved all autophagic cell death studies published in 2022 via Web of Science and analyzed their experimental design and methods. We classified the evidence provided for autophagic cell death in three groups:

Proof of increased autophagy and no data on cell death

Proof of increased autophagy and increased cell death (correlation)

Proof of increased autophagy causing cell death (causal relationship)

Results: We have analyzed 50 articles and determined that the majority (78%) failed to present adequate evidence to demonstrate a causal relationship between

autophagy and cell death. The most common typeof evidence was demonstrating a correlation between autophagy and cell death (66%). Surprisingly, some articles (20%) even failed to provide adequate evidence for increased autophagy itself.

Conclusions: Since it is a popular topic, the number of articles mentioning autophagic cell death is increasing exponentially. However, not all published work contains autophagic cell death evidence. Here we report that misinterpretation of the term and poor experimental design are common practices among autophagic cell death studies.

Keywords: Autophagic Cell Death, Experimental Design, Quality Control

S057

MEASURING THE INHIBITION ACTIVITY OF HYPERGLYCEMIC ENZYMES WITH ZnO NANOPARTICLES

Nilgün Güler

Kayseri University, Mustafa Çıkrıkçıoğlu Vocational School, Department of Chemistry Technology, Kayseri. Türkiye

Objectives: Agents are required to decrease the activity of protein kinase C (PKC) and glyceraldehyde three phosphatase (GADPH) enzymes, which increase their activity in hyperglycemic conditions. These agents are available in inorganic or organic form. However, the natural origin of these agents that do not harm human health and the environment should be preferred.

Methods: In this study, PKC and GADPH enzyme inhibitors were used as positive controls in agarose gel electrophoresis. In addition, ZnO nanoparticles obtained by the green synthesis method and their separation effects and inhibition activities on pBR322 DNA were evaluated through gel images. Enzymes were incubated with ZnO nanoparticles overnight at 37°C before electrophoresis. Then mixture is put into the wells containing 1% agarose gel. It runs for 50 minutes at 80 volts. UV image results obtained from the trans illuminator and evaluated.

Results: In the UV images, it was observed that the supercoiled DNA Form 1 structure separated into Form 2 in the enzymatic environment. The separation was not seen when Nano ZnO was present. Thus, nanoZnO can also be used as a drug to control hyperglycemia-related oxidative- stress.

Conclusions: In addition, the presence of zinc in Nano ZnO can prevent oxidative stress caused by zinc deficiency and increase its role in treating Type 2 Diabetes Mellitus by providing the external zinc requirement.

Keywords: hyperglycemia, pBR322 DNA, Nano ZnO, diabetes mellitus

S058

IMPLICATION OF CELL-FREE miRNAs in FABRY DISEASE

Berna Kuş[1], Menderes Yusuf Terzi[2]

[1] Hatay Mustafa Kemal University, Graduate School of Health Sciences, Department of Molecular Biochemistry and Genetics, Hatay, Türkiye

[2] Hatay Mustafa Kemal University, Faculty of Medicine, Department of Medical Biology, Hatay, Türkiye

Objectives: Fabry disease (FD) is a rare lysosomal storage disorder which exhibits X-linked recessive inheritance. FD is caused by a loss of function mutation in the galactosidase alpha (GLA) gene which leads to accumulation of its substrate called globotriaosylceramide. FD is a systemic disorder affecting multiple organs pertaining to mainly renal,cardiovascular, and nervous systems. Until now, variousFD-causing mutations have been identified which manifests several gender and age-dependent disease phenotypes. Unveiling novel potential biomarkers associated with the different FD phenotypes e.g., miRNAs in the bodily fluids would be beneficial in the sub-classification of the FD patients in order to determine the correct treatment strategy. The aim of the current study is to classify deregulated FD-related miRNAs in bodily fluids based on their potential involvement in several cellular pathways i.e., cellular adhesion, inflammation, cell differentiation, apoptosis, and oxidative stress.

Methods: FD-related miRNAs were ascertained and classified by using the experimentally verified miRNAs in FD patients in the literature (PUBMED) and Human microRNA Disease Database" (HMDD V3.2).

Results: In Fabry patients, 32 deregulated miRNAs were identified and classified based on several pathways, of which 9 miRNAs were associated with apoptosis, 8 with cellular proliferation and differentiation, 6 with oxidative stress, 12 with inflammation, 7 with cellular adhesion, and 2 with cell cycle, angiogenesis, hypoxia, and stem cell differentiation.

Conclusions: The miRNAs determined in the current study would be instructive for the future single- and multi-center clinical studies in order to discover and/or corroborate the associations of miRNAs with FD pathogenesis as potential biomarkers.

Keywords: Fabry disease, miRNA, Biomarker, Alpha-galactosidase

S059

IMPORTANCE OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN CHRONIC LUNG AND LIVER DISEASES

Fatma Sengul[1], Fikret Akyurek[2]

[1] Adiyaman University, Faculty of Pharmacy, Department of Biochemistry, Adiyaman, Türkiye

[2] Selçuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

Objectives: Alpha-1 antitrypsin (AAT) is the most abundant circulating serine protease inhibitor and an acute phase reactant. AAT deficiency can cause chronic lung and liver diseases The aim of this study is to raise awareness about AAT deficiency, which can cause complications with high morbidity and mortality.

Methods: This study included patients applied to Selçuk University Medical Faculty Hospital with a history of chronic lung and liver disease. All patients between the ages of aged 1-97 were included in the study. Patients were divided into three groups according to AAT levels: <100 mg/dl (low group), 100-200 mg/dl (reference range group) and >200 mg/dl (high group). AAT concentration was measured by photometric method in Roche Cobas c702.

Results: A total of 1950 patients were included in the study. The mean age of the patients in the low group (n=80) was 29.7 years (min:1 max:78), in the reference range group (n=1559) was 35.9 years (min:1 max:95) and in the high group (n=311) was 42.5 years (min:1 max:97). While AAT levels were not low in 95.9% of the patients, they were found to be low in 4.1%.

Conclusions: Considering the long-term mortality and morbidity of the disease, this rate (4.1%) is not low. In addition, considering that the mean age of the low patient group was 29.7 years, it is considered to be too late to start the treatment of the disease. Considering the family history and clinical symptoms, we think that AAT test, which is a non-invasive test, should be performed at an earlier age in these patients.

Keywords: Alpha-1 antitrypsin deficiency, Alpha-1 antitrypsin, Lung diseases, Liver diseases

S060

EVALUATION OF IMMUNOLOGICAL TESTS PERFORMED BEFORE KIDNEY TRANSPLANTATION

Basak Celtikci

Hacettepe University Medical Biochemistry

Objectives: T lymphocyte can recognize antigen through Major Histocompatibility Complex (MHC) molecules: Human Leukocyte Antigen (HLA) Class 1 (HLA A, B and C) in all nucleated cells, and 2 antigens (HLA DR, DQ and DP) in antigen-presenting cells, macrophages and dendritic cells. HLA compatibility between the recipient and donor, the presence of anti-HLA antibodies in the recipient must be performed to ensure kidney transplant's success.

Methods: Panel reactive Antigen (PRA), Donor specific Antigen (DSA) and Complement-dependent cytotoxicity (CDC) are performed on each patient before kidney transplantation in our Hacettepe University Tissue Genotyping Laboratory. Our number of tests this year is 265. CDC is repeated 24 hours before kidney transplantation. If PRA is greater than 13% (in men) and 20% (in women), the recipient is

donor's wife and her birth & miscarriage numbers are high, the patient's first transplant was unsuccessful, multiple mismatches in their DSAs, CDC positivity (more than 20% dead B and T lymphocytes) and blood transfusion history, results will be confirmed by flow cytometry (fc).

Results: HLA determination by PCR analysis using Sequence specific primers (SSO) and/or Sequence specific oligonucleotide probes (SSP), and anti-HLA determination by Luminex method (for PRA and single HLA antigen), CDC and fc results will be presented from our laboratory.

Conclusions: The methods we use, such as CDC and fc, SSO/SSP HLA analysis with PCR, and PRA and single HLA antigen determination with Luminex have been complementing each other in terms of specificity and sensitivity, and are vital in terms of survival and complications.

Keywords: kidney transplantation, sso, ssp, luminex, cross-match, flow cytometry

S061

PROTECTIVE EFFECT OF NEUROPEPTIDE-S ON A CELLULAR MODEL OF PARKINSON'S DISEASE

Aleyna Öztüzün[1], Fatma Gonca Koçancı[2], Ebral Çubukcu[3], İrem Akçalı[4], Tuğçe Çeker[1], Mutay Aslan[1], Aysel Ağar[4], Mehmet Bülbül[4]

[1] Akdeniz University, Faculty of Medicine, Department of Medical Biochemistry, Antalya, Türkiye

[2] Alanya Alaaddin Keykubat University, Vocational School of Health Services, Medical Laboratory Techniques Program, Antalya, Türkiye

[3] Akdeniz University, Institute of Health Sciences, Department of Medical Biotechnology, Antalya, Türkiye

[4] Akdeniz University, Faculty of Medicine, Department of Physiology, Antalya, Türkiye

Objectives: Oxidative stress, altered levels of monoamine oxidases (MAO) and catechol O-methyl transferase (COMT) enzymes involved in the catabolism of dopamine are held responsible for dopaminergic neuron damage in Parkinson's disease. We aimed to determine the effects of brain derived Neuropeptide-S (NPS) on lipid peroxidation, dopamine catabolizing enzymes and dopamine levels on a cellular model of Parkinson's Disease.

Methods: Cellular model of Parkinson's Disease was created by treating human neuroblastoma cells (SH-SY5Y) with paraquat and the effects of NPS and the NPS antagonist ML154 was evaluated in cells treated with or without paraquat. Six different experimental groups were formed, 1- Control; 2- NPS (cells treated with 0.5 µM NPS for 24 hours); 3- Paraquat (cells treated with 1mM paraquat for 24 hours); 4- Paraquat + NPS (cells treated with 1mM paraquat and 0.5 µM NPS for 24 hours); 5/6- Paraquat + NPS + ML154 (cells treated with 1mM paraquat, 0.5 µM NPS and 0.1 µM or 1 µM ML154 for 24 hours). MAOA, MAOB, COMT, and TBARS levels were determined by ELISA method, while dopamine levels were measured by LC-MS/MS.

Results: Paraquat significantly increased lipid peroxidation, MAOB and COMT levels, and significantly decreased MAOA and dopamine levels compared to other groups ($p<0.05$). NPS treatment decreased paraquat-induced lipid peroxidation, normalized MAOA, MAOB, COMT to control levels and significantly increased cellular dopamine content ($p<0.05$).

Conclusions: This study demonstrated that NPS increased dopamine levels in a cellular model of Parkinson's disease by regulating dopamine catabolism and protecting against lipid peroxidation. #220S745.

Keywords: Parkinsons Disease, Neuropeptide-S, Paraquat

S062

PROTECTIVE EFFECTS OF NEUROPEPTIDE-S IN A RAT MODEL OF PARKINSON'S DISEASE

Tuğçe Çeker[1], Aleyna Öztüzün[1], İrem Akçalı[2], Fatma Gonca Koçancı[4], Mutay Aslan[1], Aysel Ağar[2], Mehmet Bülbül[2]

[1] Akdeniz University, Faculty of Medicine, Department of Medical Biochemistry, Antalya, Türkiye

[2] Akdeniz University, Faculty of Medicine, Department of Physiology, Antalya, Türkiye

[3] Akdeniz University, Institute of Health Sciences, Department of Medical Biotechnology, Antalya, Türkiye

[4] Alanya Alaaddin Keykubat University, Vocational School of Health Services, Medical Laboratory Techniques Program, Antalya, Türkiye

Objectives: Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN). Dopaminergic neuron damage occurs due to oxidative stress and altered levels of monoamine oxidases (MAO) and catechol-O- methyl transferase (COMT) enzymes which are involved in the catabolism of dopamine. In this study, we aimed to investigate the effects of brain derived Neuropeptide-S (NPS) on lipid peroxidation, dopamine catabolizing enzymes and dopamine levels on an in-vivo experimental model of Parkinson's disease.

Methods: Parkinson's disease model was created in rats by administering paraquat (PK) (7 days; 10 mg/kg) via gavage. Intranasal NPS was applied at a daily concentration of 40 nmol/500 µl while an NPS antagonist ML154 (20 nmol/5 µl) was delivered via intracerebroventricular (icv) administration for 7 days. Six different experimental groups were formed, Control; NPS; PK; PK+NPS and PK+NPS+ML154. MAOA, MAOB, TBARS, and COMT levels were determined in SN by ELISA. Dopamine levels in SN were measured by LC-MS/MS.

Results: Paraquat significantly increased TBARS, MAOB and COMT levels, and significantly decreased MAOA and dopamine levels compared to NPS and PK+NPS groups ($p<0.05$). NPS treatment decreased MAOB and COMT levels and normalized TBARS and MAOA to control levels. NPS treatment also significantly increased dopamine concentrations ($p<0.05$).

Conclusions: This study demonstrated that NPS increased dopamine levels in an in-vivo experimental model of Parkinson's disease by regulating dopamine catabolism and protecting against lipid peroxidation. This study was supported by a grant from TUBİTAK #220S745.

Keywords: Parkinsons Disease, Neuropeptide-S, Paraquat

S063

INVESTIGATION OF THE EFFECTS OF SPARSTOLIN B ON APOPTOTIC PATH ACTIVATION IN COLORECTAL CANCER CELLS

Bürke Çırçırlı[1], Çağatay Yılmaz[2], Esma Kırımlıoğlu[3], Mutay Aslan[2]

[1] Akdeniz University, Department of Medical Biotechnology, Antalya, Türkiye

[2] Akdeniz University, Department of Medical Biochemistry, Antalya, Türkiye

[3] Akdeniz University, Department of Histology and Embriology, Antalya, Türkiye

Objectives: Colon cancer is an important oncological problem in developed countries. Sparstolonin B (SsnB) is a polyphenol derivative and has been reported to have anti-carcinogenic activity. This study investigated the outcome of SsnB treatment on apoptotic pathway activation in human colorectal cancer cells (HCT-116) and in human healthy fibroblasts (BJ).

Methods: Cell proliferation was stimulated in HCT-116 and BJ cells treated with 10 nM phorbol 12-myristate 13-acetate (PMA) for 12 hours, while SsnB (25 µM) was applied to both cell types to determine apoptotic activity. The optimal doses and incubation times of PMA and SsnB were determined by MTT cell viability assay. Activation of the apoptotic pathway was analyzed by evaluating quantitative protein levels of caspase-3 and cleaved caspase-3 via immunofluorescence method. The number of apoptotic cells were defined by TUNEL method.

Results: HCT-116 cells treated with SsnB showed a 2.7 fold increased in caspase 3 and a 1.5 fold increase in cleaved caspase 3 protein levels compared

to controls ($p<0.05$). There was also a 1.9 fold significant increase in the number of apoptotic cells in colon cancer cell lines treated with SsnB compared to controls. BJ cells treated with the same doses of SsnB showed no significant change in caspase 3, cleaved caspase 3 and apoptosis.

Conclusions: Data obtained show that SsnB treatment activated the apoptotic cascade in HCT-116 cells and caused an increase in the amount of apoptotic cells. This supports the development of SsnB as a potential therapeutic agent in colorectal cancer treatment.

Keywords: Caspase 3, Cleaved Caspase 3, Colorectal Cancer, Sparstolonin B, Tunel

S065

INVESTIGATION OF THE EFFECT OF MANGOSTIN FRUITS ON BLADDER CANCER CELL LINE

Ayşegül Dalmızrak[1], Esra Demir[2], Ayla Solmaz Avcıkurt[1]

[1] Department of Medical Biology, Faculty of Medicine, Balıkesir University, Balıkesir, Turkey

[2] Department of Medical Biology, Instıtute of Health Sciences, Balıkesir University, Balıkesir, Turkey

Objectives: Mangostin (Clusiaceae plant family, Garcinia mangostana L.) is a tropical tree native to Southeast Asian countries. The nutritional value of the fruit of the tree is quite high due to the high due to the fiber, vitamins, minerals, flavonoids, folate, tannin, saponin, anthocyanin and terpenes it contains. However, the main phytochemicals found in the species are xanthones, which have many biological effects such as antibacterial, antifungal, antimalarial, antioxidant, proapoptotic, antiproliferative and anti-inflammatory. The anticancer effects of different types of xanthones have been evaluated from different perspectives in many types of cancer. However, there is not enough information about bladder cancer. Therefore, the study aimed to determine the effect of α- and γ-mangostin on the 5637 cell line.

Methods: The cytotoxic effect of α-/γ-mangostin on cell line was determined by the MTT method, the effect on reproductive viability was determined by colony formation assays, and the effect on cell migration was determined by wound healing assays.

Results: IC50 values of α-/γ-mangostin were determined as 9.7 and 7.5 µM, respectively. Colony formation assays showed that both α-/γ-mangostin reduced colony formation in a dose-dependent manner. In wound healing assays, it was determined that α-mangostin decreased migration, while γ-mangostin had the opposite effect and increased migration.

Conclusions: The opposing effects of α- and γ-mangostin on cell migration suggest that the substances act through different molecular mechanisms. Therefore, it is necessary to elucidate the molecular mechanism by examining the gene expression differences responsible for migration and invasion processes.

Keywords: Bladder cancer, ±-mangostin, ³-mangostin, migration

S066

INVESTIGATION OF THE RELATIONSHIP BETWEEN TRYPTOPHAN METABOLISM AND PD1/PDL-1 SIGNALING PATHWAY IN NON-SMALL CELL LUNG CANCER TUMOR MICROENVIRONMENT

Mehmet Tolgahan Hakan[1], Dilara Sönmez Zor[1], Cem Horozoğlu[2], Özlem Küçükhüseyin[1], Akif Turna[3], İlhan Yaylım[1]

[1] Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University

[2] Departmant of Medical Biochemistry, Faculty of Medicine, Halic University

[3] Department of Thoracic Surgery, Faculty of Medicine, Istanbul University-cerrahpasa

Objectives: The immunosuppressive effect of tryptophan (TRP) metabolism has begun to be demonstrated in solid organ cancers, and while high IDO1 enzyme activity-related T cell regulation is disrupted, simultaneous high expression of PD-1 has been reported in the literature.

Methods: TRP metabolites plasma levels were determined by High Pressure Liquid Chromatography (HPLC), IDO1 and PDL-1 gene expressions qPCR method in 70 Non-Small Cell Lung Cancer (NSCLC) patients and 72 healthy controls. Statistical analyses were performed using chi-square for categorical variables and Mann Whitney U, t-test and Spearman Correlation for numerical variables in spss program (IBM; 28,0).

Results: TRP and kynurenine (KYN) levels were found to be lower in NSCLC group compared to control ($p<0.001$; $p<0.001$, respectively). In patients with NSCLC with advanced tumor stage, TRP levels were found to be lower than in patients with early tumor stage ($p=0.025$), and KYN/TRP ratio was found to be higher in patients with advanced tumor stage compared to early tumor stage ($p=0.025$; $p=0.042$, respectively). Moderate-good positive correlation was found between TRP/IDO1 ($r=0.559$; $p=0.02$), and moderate-good positive correlation between PDL1/KYN ($r=0.566$; $p=0.018$).

Conclusions: It was determined that TRP can distinguish NSCLC from control with high specificity/sensitivity in ROC curve analysis results ($p>0.001$). We think that TRP may be an independent risk factor for NSCLC.

Keywords: Tryptophan Metabolism, Tumor Microenvironment, IDO1, PDL1

S067

SALVIA CADMICA BOISS. VAR. CADMICA RESIN ON PROLIFERATION AND APOPTOSIS LEVELS IN HUMAN COLON CANCER CELL LINE

Eissa Almaghrebi[1], Fatma Akat[1], Hakan Vatansev[2], Hüsamettin Vatansev[3]

[1] Selcuk University Institute of Health Sciences Department of Medical Biochemistry

[2] Department of Food Processing, Meram Vocational High School, Necmettin Erbakan University,

[3] Department of Medical Biochemistry, Selcuk University Faculty of Medicine

Objectives: Colon cancer is the third most common cancer in the world. A particularly rich source of phenolic compounds is the family Lamiaceae and its largest genus Salvia L. This genus contains about 1000 species distributed worldwide, providing a range of herbal substances used as medicines, dietary supplements, food additives and even as cosmetic and perfume ingredients. The aim of this study was to investigate the in vitro anticancer activity of S. cadmica bois. var. cadmica ethanol extract on HT-29 and HEK-293 cell lines.

Methods: S. cadmica boiss. var. c., which we used in our study, was collected from Konya province, ethanol extract was obtained and applied to HT-29 and HEK-293 cells. 8 different doses in the concentration range of 500- 1200 µg/ml were applied and proliferation analysis was performed by MTT method. Apoptosis analysis was performed on Cytoflex device.

Results: IC_{50} dose of HT-29 cell line was calculated with the help of % cell viability/concentration graph and IC_{50}: 1188.77 µg/ml was found at 48 hours. The determined IC50 dose was applied to HEK-293 cells and the viabilities at 24 hours and 48 hours were 95.86% and 85.73%, respectively. The IC_{50} dose obtained for S. cadmica boiss. var. c. ethanol extract was applied to the cells for apoptosis analysis and HT 29 cells went to early apoptosis by 35.19% and late apoptosis by 22.27%.

Conclusions: Our study provides evidence that the ethanol extract of S. cadmica boiss. var. cadmica shows promise as a cytotoxic and pro-apoptotic agent against colon cancer cells.

Keywords: Colon cancer, Apoptosis, Proliferation, Salvia Cadmica Boiss Var Cadmica

S068

THE PROTECTIVE EFFECTS OF MORIN OR TARAXASTEROL AGAINST THE CYTOTOXIC EFFECTS OF CISPLATIN/DOXORUBICIN IN MOUSE GC-1 SPERMATOGONIA CELLS

Ecem Kaya Sezginer[1], Omer Faruk Kirlangic[2, 4], Taner Ozgurtas[3], Cengiz Karakaya[4]

[1] Ankara University, Faculty of Pharmacy, Department of Biochemistry, Ankara, Turkey

[2] Ankara University, Vocational School of Health, Ankara, Turkey

[3] University of Health Sciences, Gulhane School of Medicine, Department of Medical Biochemistry, Ankara, Turkey

[4] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

Objectives: Chemotherapeutic compounds which are used against several cancer types can affect male fertility. This study was designed to determine the protective effects of morin or taraxasterol on cisplatin/doxorubicin (DOX)-induced cytotoxicity in mouse GC-1 spermatogonia (spg) cells.

Methods: GC-1 spg cells were treated with different concentrations of morin (12.5–200μM) and taraxasterol (40–200μM), and then exposed to cisplatin (10μg/mL) and DOX (0.5μg/mL). Cell viability and cell cycle analysis were performed by MTT assay and flow cytometry using propidium iodide.

Results: Morin or taraxasterol at all concentrations had no cytotoxicity on GC-1 spg cells. Treatment with cisplatin between 0.1–10μg/mL or DOX between 0.075–0.5μg/mL decreased cell viability of GC-1 spg for 24h. Pretreatment with 200 μM morin followed by cisplatin or DOX-treatment for 24h offered significant increase in cell viability compared to cisplatin and DOX treatment alone. Based on these results, morin demonstrated a greater effect on inhibition of chemotherapy-induced cytotoxicity compared to taraxasterol. 10μg/mL cisplatin and 0.5μg/mL DOX alone led to a significant decrease in the G1 phase of the cell cycle compared to control cells, which was accompanied with the increase at sub-G1 population. Whereas GC-1 spg cells pretreated with morin or taraxasterol significantly reduced cisplatin or DOX-induced sub-G1 apoptosis.

Conclusions: Morin significantly decreased chemotherapy-induced cytotoxic effects. Cisplatin or DOX treatment caused an increase in sub-G1 cell population, which was reduced by morin or taraxasterol pretreatment. Morin or taraxasterol might protect GC-1 spg cells from the side effects of cisplatin/DOX.

Keywords: Morin, GC-1 spg cells, chemotherapeutic agents, taraxasterol

S069

THE IMPACT OF THE VITAMIN D AND RESVERATROL ADMINISTRATION T2DM RAT AORTA TRACE ELEMENT AND MINERAL LEVELS

Duygu Aydemir[1], Merve Anapalı[2], Fatma Kaya Dağıstanlı[3], Turgut Ulutin[3], N. Nuray Ulusu[1]

[1] Koç Üniversitesi

[2] Atatürk Üniversitesi

[3] Cerrahpaşa Üniversitesi

Objectives: In this study, our aim is to fill this critical gap in knowledge by investigating the potential synergistic and individual effects of Vit D and resveratrol on T2DM-related aortic health

Methods: Animals were divided into eight, each group having seven animals, as follows: the healthy control group, a healthy group treated with resveratrol, the healthy group treated with Vit D, healthy group treated with resveratrol and Vit D, diabetic group, diabetic group treated with resveratrol, diabetic group treated with Vit D and diabetic group treated with resveratrol and Vit D. After microwave digestion, aorta samples were diluted to 1/10 in the ultrapure water. Diabetic animals were administered with 10% fructose-supplied drinking water for three weeks and resveratrol (1 mg/kg/day, by i.p. injection) and VitD (170 IU/week, oral route) alone or in combination (resveratrol 1 mg/kg/day, i.p. + VitD 170 IU/week, oral) was administered to the rats

in the treatment groups for four weeks. At the end of 9 weeks, all rats were sacrificed. Then trace elements and minerals levels including Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, and Zn were investigated in all aorta samples via ICP-MS following acidic digestion.

Results and Conclusions**:** We have found that there are very important differences between the groups in Na, Se, Zn, Fe, Rb, Sr, and Cu levels compared to the untreated healthy control rats**.**

Keywords: trace elements, minerals, Vitamin D

S071

INVESTIGATION OF NEUROTROPHIN LEVELS IN MULTIPAROUS WOMEN WITH STRESS URINARY INCONTINENCE

Kübranur Ünal[1], Musa Latif Çöllüoğlu[2], Elif Erdem[3], Cansu Özbaş[4], Leyla İbrahimkhanlı[1], Özhan Özdemir[3]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

[2] Gazi University, Faculty of Medicine, Institute of Health Sciences, Ankara, Türkiye

[3] University of Health Sciences, Faculty of Medicine, Department of Obstetrics and Gynaecology, Ankara, Türkiye

[4] Gazi University, Faculty of Medicine, Department of Public Health, Ankara, Türkiye

Objectives: The aim of the study is to investigate serum neurotrophin (NT) levels to understand how those molecules were changed in premenopausal multiparous women who suffer from stress-related urinary incontinence namely stress urinary incontinence (SUI) and mixed urinary incontinence (MUI). Diagnostic and distinguishing capabilities of the NTs for those disorders is also evaluated in the study.

Methods:Pre-menopausal multiparous women underwent a urodynamic examination, stress cough test and ICIQ-SF questionnaire. Participants have divided into three groups: a control group of healthy women and two patient groups of women diagnosed with SUI and MUI. Their venous blood was taken, and serum NTs were measured by ELISA. The data obtained from patients were compared between groups. ROC analysis was performed to determine the role of NTs in diagnosing urinary incontinence.

Results: The result showed that the serum NGF and NT-3 levels in both incontinence subtypes are significantly low compared to the control. BMI score and number of VD are higher in incontinence subtypes compared to control, and ICIQ-SF score is more elevated in MUI compared to SUI.

Conclusions: The differences in serum NT levels were observed in pre-menopausal multiparous patients with urinary incontinence. Serum NGF levels in MUI patients and serum NT-3 levels in SUI patients tend to be decreased. Although the significance in serum levels change of NGF and NT-3, their discriminatory potential was weak or moderate.

Keywords: Mixed urinary incontinence, multiparous, neurotrophin, pre-menopause, stress urinary incontinence

S072

EFFECTS OF VANILLIC ACID ON ACETAMINOPHEN-INDUCED ENDOPLASMIC RETICULUM STRESS, AUTOPHAGY AND CYTOTOXICITY IN AML12 CELLS

Tugce Karabas[1], Yagmur Ucar Yagci[2], Mervenur Barakli Kocabas[1], Gulben Sayilan Ozgun[1]

[1] Trakya University, School of Medicine, Medical Biochemistry Dept., Edirne, Türkiye

[2] Edirne State Hospital, Medical Biochemistry Laboratory, Edirne, Türkiye

Objectives: This study aimed to vestigate the effects of vanillic acid on acetaminophen-induced endoplasmic reticulum stress, autophagy, and cytotoxicity in the AML12 mouse hepatocyte cell line.

Methods: Firstly, the effect of vanillic acid on cell viability in AML12 cells were investigated by treating cells with 0-500 µM vanillic acid concentrations for 24 hours. Secondly, cells were treated with 5, 50, and 500 µM vanillic acid together with 10 mM acetaminophen for 24 hours. Then cell viability was determined by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, endoplasmic stress marker activating transcription factor 4 (ATF4) and autophagy marker microtubule-associated proteins 1A/1B light chain 3B-II (LC3B-II) were measured by western blot.

Results: Vanillic acid concentrations treated up to 500 µM concentration had no significant effect on cell viability of AML12 cells. 10 mM acetaminophen caused a significant increase in ATF4 and LC3B-II levels and cytotoxicity. All vanillic acid concentrations caused a significant increase in cell viability in acetaminophen-treated cells. 50 and 500 µM vanillic acid caused a significant decrease in ATF4 and LC3B-II levels in acetaminophen-treated cells.

Conclusions: Our study showed that vanillic acid decreases acetaminophen-induced endoplasmic reticulum stress, autophagy and cytotoxicity in AML12 cells.

Keywords: Acetaminophen, Vanillic acid, Endoplasmic reticulum stress, Autophagy, Hepatotoxicity

S073

COMPOSITIONAL AND FUNCTIONAL DIVERSITY OF COLON MICROBIOTA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Sibel Kucukyildirim[1], H. Ozgur Ozdemirel[1], Beril Erdem Tuncdemir[1], Saadet Alan[2], Ceren Acar[3], Hatice Mergen[1]

[1] Faculty of Science, Department of Biology, Hacettepe University, Ankara

[2] Faculty of Medicine, Department of Medical Pathology, Inonu University, Malatya

[3] Faculty of Science and Literature, Department of Molecular Biology and Genetics, Inonu University, Malatya

Objectives: Although there is an uncertain relationship between inflammatory bowel disease (IBD) and colorectal cancer, previous studies have suggested that specific changes in the colon microbiota may be associated with these diseases. Therefore, we aimed to reveal the compositional and functional microbial diversity in IBD and evaluate the potential of specific changes in the microbiota as a potential disease biomarker.

Methods: Colon biopsies of 10 IBD patients and 18 healthy subjects were included in this work. The composition of colon microbiota was profiled by sequencing the V3-V4 region of the 16S rRNA gene and the V9 region of the 18S rRNA gene. Bioinformatic and statistical analyses were done by using R. Then, functional annotation was done using FAPROTAX dataset based on the 16S data.

Results: Our analysis revealed compositional diversity of colon microbiota in IBD biopsies which was different than in healthy tissues (P = 0.001 for prokaryotes and P = 0.001 for eukaryotes). We found the abundance of *Pseudomonas* genus was significantly high in IBD biopsies (13.2% vs. 0.6%). Additionally, Malasseziales was revealed as the most abundant fungal clade in IBD biopsies (68.1%), especially in ulcerative colitis (76.5%).

In addition, our analysis showed that only two functions were significantly different between IBD subtypes based on the predicted functions(P<0.001): acetoclastic methanogenesis and sulfur respiration.

Conclusions: By contributing to the known diversity of colon microbiota in patients with IBD, this work may provide a basis for future studies that evaluate the potential marker taxa for early diagnosis of IBD and colon cancer.

Keywords: Crohns disease, Ulcreative colitis, Colon cancer, Amplicon sequencing, Next-generation sequencing, Biomarker, Microbiome

S074

EVALUATING THE BACTERIAL DNA LOAD IN BLOOD AS A POTENTIAL COLORECTAL CANCER BIOMARKER

H. Özgür Özdemirel, Sibel Küçükyıldırım, Beril Erdem Tunçdemir, Hatice Mergen

Faculty of Science, Department of Biology, Hacettepe Universtiy, Ankara 06800, Turkiye

Objectives: Since colorectal cancer is a global threat with its high rates of incidence and mortality, it is a subject of special interest in cancer biomarker studies. Although there is several non-invasive biomarkers commonly applied for colorectal cancers (such as the fecal occult blood test), the search for better biomarkers with higher sensitivity and reliability is going on. Regarding this, our work aimed to compare the bacterial DNA load in blood in colon cancer patients versus healthy individuals and evaluate its potential as a tumor biomarker.

Methods: The peripheral blood samples obtained from 12 patients with colorectal cancer and 18 healthy individuals were included in this work. Quantitative Real-time polymerase chain reaction was performed using pan-bacterial primers which target the V3-V4 hypervariable regions of the bacterial 16S rRNA gene with high specificity and sensitivity. The analysis was performed in triplicate and normalized to the *E. coli* DNA standard. Results were calculated as the number of copies of the 16S rRNA gene per µL of blood.

Results: Based on the calculations for 16S rRNA gene copies, we found colorectal cancer patients have five times more bacterial load in their blood compared to healthy individuals (in average 778545 vs. 146270 copies per µL), and this result was statistically significant (p<0.1).

Conclusions: Our results support the relationship between the leaky gut and colorectal cancer and may suggest that bacterial DNA load in the bloodstream may serve as a non-invasive biomarker for dysbiosis and tumor diagnosis.

Keywords: Colorectal cancer, qPCR, 16S rRNA, biomarker, bacteria

S075

DETERMINATION THE EFFECT OF BIOCHANIN A ON T98G CELLS THROUGH THE CHANGE OF INTRACELLULAR CALCIUM LEVEL

Ezel Demir, Beril Erdem Tuncdemir, Emel Saglar Ozer

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

Objectives: Glioblastoma is one of the most aggressive tumor among the brain tumors. Due to the negative effects of classical chemo/radiotherapy, new studies have been focused on flavonoids which are natural dietary compounds. Flavonoids can show their anti-carcinogenic effects in many ways and some of them either reducing or inducing Endoplasmic reticulum (ER) stress in the cell. Change of intracellular calcium level can effect ER stress. We aimed to understand whether Biochanin A which is a flavonoid shows its effect on T98G cells via changing intracellular calcium level.

Methods: T98G cells were treated with 50 and 100 mM Biochanin A for 24 and 48 hours, separately. Then, intracellular calcium levels were measured with Fluo-8 Calcium Flux assay kit. Fluorescence data were analyzed to determine the change of intracellular calcium level. This study was supported by Hacettepe University (Project no: FHD-2022-19959).

Results: Agonist-induced maximum calcium response of T98G cells was determined by stimulation with carbachol. Intracellular calcium levels of T98G cells after treatment with Biochanin A were compared to maximum calcium level. T98G cells did not show any significant change in terms of intracellular calcium level.

Conclusions: Understanding effects of dietary compunds on ER stress in cancer cells is important since these compounds can be targeted in an appropriate way to develop a treatment. Biochanin A did not show its effects on T98G cells through inducing ER stress. However, it can somehow reduce carcinogenic effect of tumors which is already known information. Therefore, reasearch about this mechanism can provide valuable knowledge to developing new treatment strategies.

Keywords: Biochanin A, T98G, intracellular calcium level, ER stress

S076

PROTEOMIC INVESTIGATION OF THE EFFECTS OF DINUTUXIMAB BETA IN INSULINOMA INS-1 CELLS

Ayse Karatug Kacar

Istanbul University

Objectives: Insulinoma is a tumor originating from pancreatic beta cells. Dinutuximab beta (DB) is a monoclonal antibody used for the treatment of high-risk neuroblastoma. The study aimed to investigate the changing protein profiles after DB application to Insulinoma INS-1 cells.

Methods: 4 experimental groups were formed. DB for 1. group, DB solver for 2. group, STZ and DB for 3. Group, the solvers of 3. group for 4. group were applied. Protein profiles were analyzed using Thermo Scientific nano LC- Dionex Ultimate 3000 RSL & Q-Exactive quadrupole Orbitrap mass spectrometer (MS/MS). UBE2L6, PSMA4, TSMB10, ARF1, UBE2V2, ERP29, NUDCD2, and ARF3 proteins that showed fold increase or decrease as a result of evaluation were validated with Simple Wes.

Results: There is a high number of proteins that are common among proteins or specific only to that group. Only down-regulated proteins were observed when the 1st and 2nd Groups were compared. When groups 3 and 4 were compared, both up-and down-regulated proteins were observed. Many signaling pathways have been shown, in which the protein ubiquitination pathway is dominant. Proteins associated with cancer and gastrointestinal diseases, cell death and survival, cell-cell signaling and interactions, and tissue development have been identified.

Conclusions: In line with the identified and validated proteins, proteins that cause cell death or proliferation were found in the pancreatic tumor of DB. The administration of relevant proteins to pancreatic tumor cells may be important for tumor and cancer therapy. The study will be supported by in vivo experiments.

Keywords: Dinutuximab beta, Insulinoma INS-1 cells, Proteomic, Cancer therapy

S077

THE EFFECT OF TRANSURETHRAL RESECTION AND BCG THERAPY ON CYTOKINE LEVELS IN NON-MUSCLE INVASIVE BLADDER CANCER

Oktay Üçer[1], Gökhan Temeltaş[1], Talha Müezzinoğlu[1], Zeki Arı[2], Funda Kosova[3]

[1] Manisa Celal Bayar University Faculty of Medicine, Department of Urology, Manisa, Turkiye

[2] Manisa Celal Bayar University Faculty of Medicine, Department of Medical Biochemistry, Manisa, Turkiye

[3] Manisa Celal Bayar University School of Health Science, Medical Biochemistry, Manisa, Turkiye

Objectives: The present study investigated the effect of treatment on interleukin (IL)-1, IL-6, IL-8, and neopterin levels in patients with non-muscle invasive bladder cancer (NMIBC).

Methods:: Thirty patients with NMIBC and 30 age-matched controls were included in the study. Preoperative, postoperative first control [at two weeks after second transurethral resection of bladder tumor (TURBT)] and the second control (at the end of intravesical immunotherapy) blood samples were analyzed using ELISA to determine IL-1, IL-6, IL-8, and neopterin levels. The mean cytokine levels of the patients were statistically compared and comparing the patients' and controls' levels.

Results: There were no statistically significant differences between the mean IL-1, IL-6, IL-8, and neopterin levels of the patient and control groups before initial TURBT. In the patient group, there were no statistically significant differences in the IL-6 and IL-8 levels after both TURBT and intravesical Bacillus Calmette-Guérin (BCG) therapy. The mean of preoperative IL-1 and neopterin levels significantly decreased after TURBT ($p<0.05$). However, this reduction does not continue after intravesical BCG instillation.

Conclusions: The findings of this study showed that the IL-1, IL-6, IL-8, and neopterin levels of the patients with NMIBC were similar to the levels of healthy controls. IL-1 and neopterin levels significantly decreased after TURBT. But these reduction did not continue after intravesical BCG instillation. These findings demonstrate that IL-1 and neopterin levels decrease after TURBT due to the reduction in tumor weight or tumor removal.

Keywords: Bladder cancer, neoptrin, IL-8, IL-1, IL-6

S078

6-HYDROXY-L-NICOTINE ACTS AGAINST AMYLOID BETA 1-42 AGGREGATION IN HUMAN CANCER CELLS

Lucian Hritcu[1], Adrian Tiron[1, 2], Crina Elena Tiron[1, 2], Paula Alexandra Stache[1, 2], Marius Mihasan[1], Razvan Stefan Boiangiu[1]

[1] Alexandru Ioan Cuza University of Iasi, Department of Biology, Iasi, Romania

[2] Center For Fundamental Research and Experimental Development İn Translation Medicine-transcend, Regional Institute of Oncology, Iasi, Romania

Objectives: 6-hydroxy-L-nicotine (6HLN) is a nicotinic derivative from the nicotine metabolism within *Paenarthrobacter nicotinovorans* that possess cognitive-improving abilities and antioxidant properties, eluding the side-effects of nicotine, the parent molecule. This study aims to elucidate the capacity of 6HLN to fight against amyloid beta (Aβ) 1-42 aggregation and reduce its toxicity in human cancer cells.

Methods:: Lung cancer cell line A549 and breast cancer cells MCF-7 were used in this study. The effects of cell growth medium supplemented by cell growth factors on Aβ1-42 aggregation were investigated. Moreover, the effects of 6HLN on intracellular Aβ1-42 aggregation and mitochondrial activity of cell lines such as A549 (human lung cancer (adenocarcinomic alveolar basal epithelial tumor)) and MCF-7 (human breast cancer (invasive breast ductal carcinoma)) were investigated.

Results: 6HLN fight against aggregation of Aβ1-42 used in the monomeric state and the disaggregation process of Aβ1-42 used in the fibrillar state in human cancer cells and showed inhibitory effects on mitochondrial activity against Aβ1-42-induced decreased of human cancer cell viability.

Conclusions: 6HLN could be considered a promising protective compound that could reduce Aβ1-42-induced toxicity.

Acknowledgements: This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-III-P4-PCE-2021-1692, within PNCDI III.

Keywords: 6-hydroxy-L-nicotine, amyloid beta 1-42, cancer cells, aggregation, toxicity

S079

INDUCTION OF APOPTOTIC CELL DEATH IN HUMAN LUNG CANCER CELLS THROUGH ENHANCED OXIDATIVE STRESS CAUSED BY RUBUS TERETICAULIS LEAVES EXTRACTS

Gamze Nur Öter[1,2], Ezgi Durmuş[3, 5], Ali Şen[4], Abdürrahim Koçyiğit[5]

[1] Istanbul University, Institute of Health Sciences, Department of Medical Biochemistry, Istanbul, Turkey, gamze.oter@istinye.edu.tr; gmznrotr@outlook.com

[2] Istinye University, Faculty of Medicine, Department of Medical Pathology, Istanbul, Turkey, gamze.oter@istinye.edu.tr; gmznrotr@outlook.com

[3] Bezmialem Vakıf University, Institute of Health Sciences, Department of Medical Biochemistry, Istanbul, Turkey, ebalkan@bezmialem.edu.tr

[4] Marmara University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey, ali.sen@marmara.edu.tr ; alisenfb@yahoo.com

[5] Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Turkey, akocyigit@bezmialem.edu.tr

Objectives: Since the conventional methods used in lung cancer treatment are insufficient, the search for alternative treatments continues.The aim of this study was to investigate for the first time the antiproliferative and apoptotic effects of the active ethanol (RTE) and chloroform (RTC) extracts of *Rubus tereticaulis* leaves in A549 lung cancer cell line.

Methods: Non-small cell lung cancer cell line, A549, was treated with RTE and RTC individually. Cytotoxic effects of RTE and RTC were quantitatively detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cellular ROS production was analyzed by using the fluorescent signal indicator 2.7 dichlorodihydrofluorescein diacetate (H2DCF-DA). Apoptosis was evaluated by fluorescence microscope after AO/EB staining and flow cytometry after Anexin V and PI staining.

Results: Both RTE and RTC induced cytotoxicity in A549 cells in a dose-dependent manner, which was accompanied with induction of reactive oxygen species (ROS) accumulation. The dead cells and apoptotic cells showed typical apoptotic morphologies. Both early apoptotic and late apoptotic cells detected by flow cytometry were increased in RTE and RTC treated cells. In addition, results showed that RTC has higher anti-proliferative, ROS generation and apoptotic effect than RTE. Therefore, the polarity of the solvent used to exert the anti-cancer effect of *Rubus tereticaulis* leaves is crucial.

Conclusions: These results suggest that *Rubus tereticaulis* leaves has an anti-cancer effect to lung cancer cancer cells through ROS-mediated apoptosis,and RTC could be an effective therapeutic or adjuvant strategy in cancer treatment.

Keywords: Lung cancer, anti-cancer effect, oxidative stress, Rubus tereticaulis, apoptosis

S080

Pllans-II: A PROMISING PROTOTYPE FOR TARGETED THERAPY IN CERVICAL CANCER

Eliécer Jiménez-charris, Alejandro Montoya-gómez, María Jose Sevilla-sánchez, Mildrey Mosquera-escudero

Grupo de Nutrición, Facultad de Salud, Universidad Del Valle, Cali - Colombia

Since chemotherapy treatments against cervical cancer adversely affect the patient's life quality, new molecules with antitumor potential and few side effects are required.

Objectives: We proposed to evaluate the *Pllans-II* antitumoral activity, a phospholipase A_2 from *Porthidium lansbergii lansbergii* snake venom against cervical cancer cells, the protein›s safety on healthy mice and the cytotoxic effect of the recombinant version-*rPllans-II*.

Methods: We evaluated *Pllans-II* antitumoral and antimetastatic effect against two cervical cancer cell lines - CaSki and HeLa - and determined the biochemical pathways affected by transcriptomic analysis. Besides, we verified the *Pllans-II* innocuous in healthy mice by intraperitoneal injection. Finally, we produced *rPllans-II* using *E. coli* BL21 (DE3) and evaluated the cytotoxicity effect by *in vitro* test.

Results: *Pllans-II* displayed cytotoxic, antiproliferative, anti-migratory, and antiadhesive effects against both cancer cell lines. The *Pllans-II* apoptotic effect in CaSki cells was related to the inability to resolve endoplasmic reticulum stress induced by sub-expression of genes such as PERK, ERO1, PDIs, HSP70, and CHOP. HeLa cell death

was associated with the junction blockage between $\alpha_5\beta_1$ integrins and fibronectin of the extracellular matrix. Additionally, *Pllans-II* was harmless in mice, evidenced by no hemorrhagic effect nor liver and kidney histological alteration, including no change in pro-inflammatory and liver damage markers. Finally, *rPllans-II* showed cytotoxic activity against both tumoral cervical cell lines.

Conclusions: The selective *Pllans-II* antitumor effects against cervical carcinoma cells suggest that it could be considered a promising prototype for therapy against cervical cancer.

Keywords: Bioprospecting, Snake venom, PLA2, Recombinant protein, Transcriptomic analysis, Antitumoral effect

S081

CYTOTOXIC EFFECT OF NEWLY SYNTHESIZED BENZIMIDAZOLE DERIVATIVES ON LUNG AND BREAST CANCER CELL LINE

Taner İlker Gümrükçüoğlu[1], Hakan Akgün[2], Bahar Bilgin Sökmen[2], Hakan Bektaş[2], Emine Gülçeri Güleç Peker[3]

[1] Gümüşhane University, Kurtun Vocational School, Department of Property Protection and Security, Gümüşhane, Türkiye

[2] Giresun University, Faculty of Arts and Sciences, Department of Chemistry, Giresun, Türkiye

[3] Giresun University, Faculty of Engineering, Department of Basic Sciences, Giresun, Türkiye

Objectives: Compounds with benzimidazole structure are important heterocyclic compounds used as drug active ingredients. The benzimidazole ring has a kind of privileged skeletal structure from which many important drugs used in different therapeutic fields are obtained. Benzimidazole-based heterocyclic compounds have a very important place in chemistry due to their different biological activity properties such as anticancer, antihypertensive, antibacterial, anthelmintic, antiviral, antidiabetic, antifungal, antihistaminic, antidiabetic, antiulcer, antituberculosis, antiepileptic, analgesic, antioxidant and anti-inflammatory. In this study, it is aimed to investigate the cytotoxic effect of a newly synthesized series of benzimidazole derivatives on lung cancer cell line (A549) and breast cancer cell line (MCF-7). MTT test at 5 different concentrations was used to evaluate cytotoxic effects.

Methods: In MTT analysis, A549 and MCF-7 cells were seeded into 96-well plates. Increasing concentrations of benzimidazole derivative compounds were applied to the manipulated cells; It was treated for 24 and 48 hours. 10 uL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37°C in a CO_2 incubator. Cell proliferation values were calculated with an ELISA reader at a wavelength of 570 nm.

Results: Compound number 14 had an IC_{50} = 159.2 µg/mL. It was determined that it showed cytotoxic effects against MCF-7 cells with an IC_{50} >100 µg/mL.

Conclusions: This remarkable selective cytotoxic effect of compound number 14 on the MCF-7 cancer cell line prompts further studies to reveal the effects of this substance at the cellular level, especially the mechanisms responsible for the inhibition of cell viability.

Keywords: cancer, benzimidazole, cytotoxicity, A549, MCF-7

S082

METABOLOMIC PROFILING IN DISTINCT TYPES OF LEUKEMIA

Ayşe Zehra Gül[1], Şahabettin Selek[1], Somer Bekiroğlu[2], Metin Demirel[1], Fatma Betül Çakır[3], Bülent Uyanık[4]

[1] Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Türkiye

[2] Tübitak Marmara Research Center, National Biological and Chemical Test Center, Gebze Kocaeli, Türkiye

[3] Bezmialem Vakıf University, Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye

[4] Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkiye

Objectives: This study was conducted to reveal phenotypic footprints of acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (HL) patients identifying affected metabolites and their pathway-related implications.

Methods: For the global serum metabolome profiling, quantitative 1H nuclear magnetic resonance spectroscopy (NMR) and untargeted high-resolution liquid chromatography mass spectrometry (HRMS) methods were applied. 2D NMR analyses were performed to validate metabolite annotations. Leukocyte isolates were examined with the HRMS platform under equal conditions to identify differentially expressed metabolites.

Results: The metabonome results revealed significantly altered amino acids with their catabolism products, TCA cycle intermediates and phospholipids while the leukocyte isolates showed elevated levels of long- chain fatty acids and acylcarnitines with low biotin levels. Group-based changes indicated glucogenic and branched-chain amino acids with choline oxidation pathway metabolites were affected in AML while fumarate was found most differential in CLL and NHL. Commonly increased metabolites produced in protein degradation pathways as methyl guanidine, urea and creatinine were recorded. Serum LCMS analysis showed the purine metabolism as the the most affected pathway in all groups. Leukocyte isolates exhibited differential levels of spermine, long-chain fatty acids, acylcarnitines and coenzymes among groups.

Conclusions: Complementary use of NMR-MS methodologies expanding the metabolite spectrum in both matrixes allowed comprehensive profiling of leukemia patients. The current findings eliciting group-based changes provide essential tips about pathogenetic mechanisms commonly observed in distinct subclasses along with individual driving forces. Yet, emerging results need to be validated with wider cohorts and omics information for each patient within the frame of personalized medicine approach.

Keywords: acute leukemia, chronic lymphoma, metabolomics, nuclear magnetic resonance, mass spectrometry

S083

INVESTIGATION OF SERUM TRYPTOPHAN AND KYNURENINE METABOLITES IN BREAST CANCER PATIENTS

Mohammad Ahmad Bik[1], Karam Mazın Kamıl Gharab[2], Duygu Eryavuz Onmaz[1], Sedat Abuşoğlu[1], Ali Ünlü[1]

[1] Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

[2] Mustansiriyah University, National Diabetes Center For Treatment and Reaserch, Baghdad, Iraq

Objectives: Breast cancer is one of the most common cancers in women around the world. Tryptophan (Trp) and its metabolites play an important role in a variety of physiological processes, but also play an important role in cancer by promoting tumor progression. Trp degradation in cancer patients is primarily mediated by increased activities of tryptophan 2,3 dioxygenase (TDO) and indoleamine 2,3 dioxygenase 1 (IDO1). Stimulation of IDO1 and Trp degradation leads to the accumulation of Trp metabolites such as kynurenines. The aim of this study is investigating the differences in serum tryptophan levels and kynurenine metabolites between patients with breast cancer and the control group.

Methods:**:** 50 breast cancer patients and 50 control subjects were included in this study. Trp and its metabolites were analyzed by LC-MS/MS. Statistical analysis was performed using IBM SPSS Statistics 21.0.

Results: Our results showed that serum kynurenine levels [286.5 (139.5-695.0) ng/ml vs. 232.5 (109.5-418.0) ng/ml, p=0.019] were statistically significantly higher and tryptophan/kynurenine ratio [87.5 (13.2-207.1) vs. 114.7 (33.1-246.0), p=0.011] was statistically significantly lower in patients with breast cancer compared to the control group. Serum levels of tryptophan (p=0.751), kynurenic acid (p=0.677), 3-hydroxykynurenine (p=0.094), and 3-hydroxyanthranilic acid (p=0.254) in the control group were similar to those in the breast cancer group.

Conclusions: Our results show that there is an irregularity in the concentration of metabolites of the

kynurenine pathway in patients with breast cancer. Particularly elevated kynurenine levels were found in breast cancer patients. However, our results indicate that further studies with a larger patient population on this topic are needed.

Keywords: Tryptophan, Kynurenine, Breast cancer

S084

INVESTIGATION OF THE IN VITRO AND IN VIVO EFFECTS OF TELOMERE-TARGETED NEW DRUG CANDIDATE COMPOUNDS IN DIFFERENT CANCER CELL LINES

Merve Yilmaz[1], Sibel Goksen[2], Gunes Esendagli[3], Sefik Evren Erdener[4], Ates Kutay Tenekeci[1], Larisa L. Birichevskaya[5], Ilgen Mender[6], Sergei M. Gryaznov[6], Z. Gunnur Dikmen[1]

[1] Hecettepe University Medical Faculty, Department of Biochemistry, Ankara, Turkey

[2] Hacettepe University Institute of Health Sciences, Department of Medical and Surgical Research, Ankara, Turkey

[3] Hacettepe University, Cancer Institute, Department of Basic Oncology, Ankara, Turkey

[4] Hacettepe University Faculty of Medicine, Neurological and Psychiatric Research and Application Center, Ankara, Turkey

[5] Laboratory of Molecular Biotechnology, Institute of Microbiology, National Academy of Sciences, Minsk, Belarus

[6] Maıa Biotechnology, Inc., Chicago, Il, Usa

Objectives: Telomerase-inhibiting therapies may have side effects due to varied cancer telomere lengths. Hence, there is a need for different molecules targeting cancer telomeres regardless of their length. A new phosphatidyl nucleoside conjugated molecule (L6) demonstrated lower IC50 value in various cell lines compared to 6-thio-dG (THIO). Based on in vitro evaluations, we have selected HT29 colon cancer cell line for further mechanism of action and anticancer efficacy studies with L6.

Methods: For in vitro TIF assay, cells were plated in 24-well plates, treated with 1 µM of 6-thio-dG (THIO), L6, MAIA-2022-013 and Ribo-thio for 96 hours. Cells were blocked with 5% BSA, incubated with g-H2AX and telomere probe. For in vivo xenograft model, $2x10^6$ HT29 cells were injected into CD1 nude mice flanks. Mice received L6 treatment with 3mg/kg and 6mg/kg doses twice a week for three weeks. For in vivo syngeneic model, $2x10^6$ CT26 cells were injected into BALB/c mice flanks. Mice were treated with L6, 3mg/kg twice a week for two weeks, with a two-day interval between administrations.

Results: Telomere Damage Induced Foci (TIF) method revealed significant telomeric DNA damage differences induced by L6 molecule in comparison with both the PBS control group and 6-thio-dG. In xenograft model, L6 dose was 3mg/kg with a treatment frequency of 4 times per week. Syngeneic model findings have demonstrated that L6 induces tumor regression, enhances survival, and elicits altered Treg and $CD8^+$ cell counts.

Conclusions: Our promising findings may inform the development of enhanced and safer cancer therapeutics through clinical applications.

Keywords: Cancer, Telomere, Telomerase, TIF Assay, In vivo models

S085

THE EFFECT OF AUTOVERIFICATION ON TURNAROUND TIME FOR CLINICAL CHEMISTRY AND IMMUNOASSAY TESTS

Havva Yasemin Çinpolat

Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Medical Biochemistry, Çanakkale, Turkey

Objectives: Auto verification (AV) is an important tool for developing quality processes in clinical laboratories. In this study, it was aimed to evaluate the effect of the AV system used in our laboratory for clinical chemistry and immune analysis tests on turnaround time (TAT).

Methods: The study was conducted in Çanakkale Onsekiz Mart University Hospital Medical Biochemistry Laboratory. In our laboratory, where MIA-Med (MIA Technology, Turkey) laboratory information system was used, 35 biochemistry tests were analyzed on the cobas c702 autoanalyzer (Roche Diagnostics, Germany), and 29 immune analysis tests analyzed on the cobas e602 immune analyzer (Roche Diagnostics, Germany) were autoverified by the middleware program (cobas infinity, Roche Diagnostics, Germany). After validation lasted about six months, AV was first started on 06.02.2023 and completed on 18.05.2023 for all the indicated tests. The data of the tests performed for one month before and after AV were obtained from the laboratory information system. The rates of tests exceeding TAT were calculated, and a comparison was made between the groups before and after AV.

Results: 71% of tests and 21% of tubes based results were autoverified. TAT after AV was improved by 6 minutes for emergency tests and 12 minutes for routine tests. While 6.4% of the results before the AV exceeded the TAT, 5.8% of the results after the AV exceeded the TAT ($p<0.001$).

Conclusions: AV contributes positively to the total testing process. With well-defined and validated rules, AV makes tests more reliable and results faster.

Keywords: autoverification, turnaround time

S086

DETERMINATION OF SIX SIGMA LEVEL OF SAMPLE REJECTION REASONS IN PUBLIC HEALTH LABORATORY IN A SIX MOUNTH PERIOD IN ANKARA

Pınar Koyuncu[1], Gökçe Güven Açık[2]

[1] Ankara Training and Reserach Hospital, Department of Medical Biochemistry, Ankara, Türkiye

[2] Ankara Provincial Health Directorate ,public Health Laboratory, Ankara, Türkiye

Objectives: Ankara Provincial Health Directorate Public Health Laboratory, collects blood and urine samples from various departments of all across the city.This aboratory works with l an integrated system.Thousands of samples from 5 different tube groups are collected every day (biochemistry, complete blood count, hbA1c, thalassemia and urine analysis). Our study was carried out to evaluate the rate and reasons for such rejections by the quality indicators and six sigma process, to determine the acceptability of the rejection reasons by improving the accuracy of the results and lowering pre-analytical errors.

Methods: The data were obtained from the laboratory information management system (LIS). The rejection reasons were grouped according to the sample types. The defects per million opportunities (DPMO) and six sigma levels were calculated.

Results: This cross-sectional comparative study was performed on 2048403 laboratory sample in a six mounth period in terms of the percentage and type of errors that occurred in Public Health Laboratory,ANKARA in 2023. Out of 2048403 blood and urine samples 11280 samples were rejected (%0,55). The types of studied errors included insufficient sample size (%51), hemolysis (%32), sample clotting (%12), and mistakes in centrifuge(%3).

Conclusions: In this study, the frequency of sample rejection on every department was found to be acceptable. To increase the overall quality of laboratory management by increasing the Sigma values; we think that clinical and cause based analysis of error rates should be done regularly.

Keywords: preanalytical error, sample rejection reasons, six sigma

S087

EVALUATION OF 25-HYDROXY VITAMIN D3 TEST REQUESTS IN THE CONTEXT OF RATIONAL LABORATORY PRACTICES

Gülnur Tekin[1], Ferdi Bişkin[1], Gülsüm Abuşoğlu[2]

[1] Necmettin Erbakan University, Faculty of Tourism, Department of Tourism Management

[2] Selcuk University, Department of Medical Services and Techniques, Vocational School of Health Services

Objectives: Unnecessary laboratory test requests place a significant workload and financial burden on the health system. This study aims to retrospectively analyze the 25-OH vitamin D_3 test requests from various medical departments for redundancy and associated costs.

Methods: Data from the university hospital in Konya were analyzed. Since the hospital adopted rational laboratory practices in September 2018, 25-OH vitamin D_3 data were retrieved from the hospital database for the period from September 2016 to 2020. Data analysis was conducted using formulas within MS Office Excel. Frequency and percentage distributions were calculated based on the 25-OH vitamin D_3 test requests from different clinics. The costs of unnecessary tests were estimated based on the health services price list of the Social Security Institution Health Implementation Community.

Results: Between 2016-2018, there were 54,732 25-OH vitamin D_3 test requests, totaling 1,992,148 TL. Between 2018-2020, there were 83,901 25-OH vitamin D_3 test requests, amounting to 2,757,516 TL. Faulty test requests, which are the primary focus of this study, totaled 5,062 and cost 194,876 TL between 2016-2018. Between 2018-2020, they numbered 3,710 with a cost of 119,734 TL. Post-2018, there was a 27% reduction in the number of erroneous test requests and a 39% reduction in their associated costs.

Conclusions: Conducting more studies nationwide is crucial to mitigate the adverse impacts of unnecessary test requests on patients and to ascertain the genuine costs

Keywords: Unnecessary Test Requests Cost of Unnecessary Test Requests Retrospective Analysis

S089

CALCULATION OF MEASUREMENT UNCERTAINTY OF CARBAMAZEPIN AND VALPROIC ACID TESTS

Ayşenur Macun Ayan

Balıkesir Devlet Hastanesi

Objectives: The uncertainty of measurement is a statistical parameter which characterises the distribution of the values reasonably attributed to the measurand. Carbamazepine and valproic acid are antiepileptic drugs and monitoring of their blood levels are crucial in the clinical assesment of patients.In this study,we aimed to calculate the uncertainty of carbamazepine and valproic acid measurements in Balıkesir State Hospital.

Methods: Carbamazepine and valproic acid tests were measured in Roche Cobas 6000 autoanalyzer. The uncertainty of measurement was calculated according to the Nordtest guideline. Intra-laboratory reproducibility (uRw) was calculated from the internal quality control results of these tests at two different levels between 01.11.2022 and 01.04.2023. Uncertainty of measurement (Ubias) calculated from 6-month external quality control data.The combined standard uncertainty value was calculated using the standard uncertainty value.Expanded uncertainty was calculated at 95% confidence interval (CI), including all components of uncertainty.

Results: At the %95 CI, expanded uncertainty of measurement for carbamazepine and valproic acid were 11.08 and 8.08,respectively.Carbamazepine internal quality control CV% for level 1 and level 2 were 3.16% and 5.97%,respectively.Valproic acid internal quality control CV% for level 1 and level 2 were 4.48% and 5.30%,respectively.Carbamazepine uRw value was calculated as 2.39%, RMSbias 4.97%, uCref 0.56%, ubias 5.00%, uC 5.54%, U11.08%.Valproic acid uRw value was calculated as 2.46%, RMSbias 3.14%, uCref 0.60, ubias 3.20, uC 4.04%, U8.08%.

Conclusions: The uncertainty of measurement for both tests was lower than the total allowable error (20%) determined by CLIA and Rilibak.Reporting of measurements with uncertainty values are important for clinical decision, patient safety and reliability of our measurement results.

Keywords: Uncertainty of measurement, Carbamazepine, Valproic acid

S090

CAN REFERENCE CHANGE VALUES FOR BIOCHEMISTRY ANALYTES BE ESTIMATED FROM EXTERNAL QUALITY ASSESSMENT AND AVAILABLE BIOLOGICAL VARIATION DATA?

Gizem Yılmaz Çalık[1], Mehmet Şeneş[2]

[1] Zile State Hospital, Department of Medical Biochemistry, Tokat, Türkiye

[2] University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: Patients receive healthcare services from different hospitals for various reasons. This limits the medical evaluation of consecutive test results from different laboratories because the autoanalyzers, reagents or methods used by laboratories may differ. Based on this limitation, we aimed to estimate the interlaboratory reference change value (IL-RCV) for biochemistry analytes (albumin, ALT, AST, amylase, total bilirubin, calcium, chloride, total cholesterol, CK, glucose, HDL-cholesterol, iron, LDH, lipase, magnesium, phosphate, potassium, total protein, sodium, triglyceride, urea and urate) from external quality assessment (EQA) and current biological variation (BV) data.

Methods:Data from 3 different monthly biochemistry EQA samples submitted by the RANDOX (RIQAS, England) in 2022 were analyzed and median CV_A was calculated for each analyte.Within-subject BV (CV_I) values were obtained from the EFLM database.Positive and negative IL-RCV were calculated with these data.

Results: CV_A values for albumin, amylase, calcium, chloride, creatinine, iron, LDH, lipase, magnesium, total protein and sodium were higher than their CV_I values.CV_A values of the other analytes were smaller than their CV_I values.The number of methods used in EQA was at least 7(chloride) and at most 21(amylase).

Conclusions: In BV studies,CV_I and CV_A values are calculated from healthy individuals with a single method. CV_Avalues used in our study were taken from EQA and were obtained using more than one method.EQA samples differ from human serum and analyte concentrations in these samples may not be compatible with those in healthy individuals.Nevertheless,RCV values obtained from these data can guide the evaluation of consecutive interlaboratory test results.

Keywords: reference change value, external quality assessment, biological variation

S091

EVALUATION OF UNNECESSARY CK-MB(MASS) TEST REQUEST ACCORDING TO CHANGES IN CURRENT GUIDELINES

Hüseyin Yaman

Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Türkiye

Objectives: Unnecessary testing is a common preanalytical error. For diagnostic purposes of acute coroner syndrome(ACS), it is not recommended to routinely measure of CK-MB(mass) in 2020 ESC guidelines. The aim of this study was to evaluate test numbers of CK-MB(mass) and high-sensitivity cardiac troponinT (hs-cTnT) and the short- and long-term changes observed with corrective-preventive action (CPA).

Methods:**:** As part of CPA, the CK-MB(mass) test was replaced from under the heading of 'cardiac biomarkers' to under the heading 'markers' on the physician screen in July 2021, the relevant units were informed and the search button with the parameter name was active. The total and combined number of CK-MB(mass) and hs-cTnT tests requested by clinic-polyclinic distributions were determined for the short (6-months) and long (18-months) periods before and after CPA.

Results: The number and rates of CK-MB and hs-cTnT tests before CPA were 2449,16570,14.8 for the short term and 7210,40271,17.9 for the long term, respectively. After CPA, the numbers and rates were 475,16986,2.8 for the short term and 1310,54032,2.42 for the long term, respectively. These differences were statistically significant($p < 0.01$)

Conclusions: It was determined that CK-MB(mass) requests were reduced in a short and long time after CPA, and this was achieved by reduction of unnecessary request with hs-cTnT. This reduction is also important for economically. We believe that it is important for all units to follow current guidelines and order tests in accordance with these guidelines.

Keywords: Unnecessary Testing, CK-MBMASS

S092

CALCULATED TRANSFERRIN: IS IT NECESSARY TO CALCULATE NEW FORMULA AND NEW REFERENCE INTERVAL?

Kezban Çavdar Yetkin, Semih Fazlı Kayahan, Hasan Alp Turgut, Hacer Doğan, Mehmet Şeneş

University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: Transferrin is a parameter measured (TrfM) and calculated (TrfC) in medical laboratories, used in conjunction with other markers such as iron, total iron-binding capacity (TIBC), and ferritin to assess the body's iron status. The aim of this study is to develop a new formula for calculated transferrin and calculate a laboratory-specific reference interval.

Methods: A new transferrin formula was calculated using multiple linear regression for turbidimetrically measured transferrin (Roche c6000) (n=259). Data from individuals < 60 years (n=878) and > 60 years (n=273) were extracted from the Laboratory Information Management System (LIMS) based on our currently used formula ($TrfC_{Old}$). Reference intervals for $TrfC_{Old}$ and newly calculated ($TrfC_{New}$) values were determined using the indirect method with the nonparametric percentile estimation method. Outliers were excluded using the Tukey method. The TrfH values obtained with the old and new formulas were evaluated against the reference intervals we used and newly calculated. Furthermore, $TrfC_{Old}$ and $TrfC_{New}$ values were compared with TrfM values, and the concordance of the results was assessed.

Results: For $TrfC_{New}$, the formula obtained was 0.008 x Iron (µg/dL) +0.007 x Total Iron-Binding Capacity (µg/dL) + 0.071. Reference intervals for $TrfC_{Old}$ were <60 years: 1.95-3.15 g/L; >60 years: 1.68-3.32 g/L, while for $TrfC_{New}$, they were <60 years: 2.12-3.30 g/L; >60 years: 1.85-3.40 g/L.

Conclusions: It is important for each laboratory to calculate its own formula for calculated parameters and establish and use laboratory-specific reference intervals for calculated parameters, as is the case for all parameters.

Keywords: Measured Transferrin, Calculated Transferrin, Multiple linear regression, Indirect reference interval calculation

S093

USE OF ARTIFICIAL INTELLIGENCE FOR REFERENCE INTERVAL CALCULATION IN THYROID FUNCTION TESTS: FUZZIFICATION OF TRANSITIONS AND GROUPING

Semih Fazlı Kayahan[1], Muhammed Fatih Alaeddinoğlu[2], Mehmet Şeneş[1]

[1] University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Türkiye

[2] Atatürk University Faculty of Open and Distance Education, Erzurum, Türkiye

Objectives: In this study, it was aimed to determine the most optimal grouping in calculating the reference intervals(RIs) of TSH and fT4 tests and to fuzzify the transitions between groups with artificial intelligence applications.

Methods: In our study,TSH and fT4 test data from individuals aged≥18years,studied on the Roche Cobas 8000 e801 autoanalyzer(Roche Diagnostic,U.S.A.)between January2019 and December2021,were used.Patients with diagnoses thought to affect thyroid function tests were excluded from the study.After filtering, 9455 reference individuals(5958 women,3497 men)with no missing data were identified as the reference sample group. Groupings were made using age,gender,TSH and fT4 results as criteria in the Kmeans algorithm.

Accordingly, the groups obtained are:Group-I:18-26years,Group-II:27-34years,Group-III:35-42years,Group-IV:43-52years,Group-V:53-65years,Group-VI:66-94years.We used the Tukey's method to eliminate outliers.RIs were determined using the non-parametric percentile method.With the Fuzzy C Means algorithm,the membership degree of each individual in the reference sample group was calculated according to the above groups and personalized RI were obtained.Age-specific RIs were calculated by taking the average of the reference values of individuals of that age.The results were compared with the RIs currently used in our laboratory.

Results: In the data,generally higher limits were obtained for TSH compared to the currently used RIs(Group-I:0.82-5.11µIU/mL,Group-II:0.82-4.54µIU/mL,Group-III:0.69-4.55µIU/mL,Group-IV:0.69-4.87µIU/mL,Group-V:0.68-4.78µIU/mL,Group-VI:0.60-5.93µIU/mL). For fT4, while a single RI(0.93-1.7ng/dL)is currently used,6 different RIs were obtained(Group-I:0.98-1.60ng/dL,Group-II:0.96-1.57ng/dL,Group-III:0.93-1.52ng/dL,Group-IV:0.91-1.53ng/dL,Group-V:0.92-1.56ng/dL,Group-VI:0.91-1.58ng/dL).A 22-years-old individual's membership degrees in the 6 groups obtained by the FuzzyCMeans algorithm were 68%,16%,7%,4%,3% and 2%, respectively.

The RI for this individual was 0.80-4.98µIU/mL for TSH and 0.97-1.59ng/dL for fT4.

Conclusions: In our study, we concluded that with the fuzzification of transitions and grouping by machine learning applications,RIs could be more personalized and realistic calculated.

Keywords: machine learning, reference interval, thyroid function tests, fuzzification, artificial intelligence

S094

RELIABILITY OF PROTEIN ANALYSIS IN COMPLETE URINE ANALYSIS

Fikret Akyurek[1], Fatma Sengul[2]

[1] Selçuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

[2] Adiyaman University, Faculty of Pharmacy, Department of Biochemistry, Adiyaman, Türkiye

Objectives: Proteinuria is defined as excess urinary excretion of any type of protein. However, the most excreted protein fraction in urine is albumin. Albuminuria can also be seen in some physiological conditions. Albuminuria can also be an early sign of kidney dysfunction. Albumin is measured semiquantitatively in urine strips because it provides early information about renal function. However, if a loss of renal function is suspected, it should be confirmed by quantitative methods. In this study, we aimed to investigate whether the screening method for renal function is compatible with the gold standard method.

Methods: The study included the results of 3739 patients who complete urine analysis and protein analysis in 24-hour urine.

Results: According to the gold standard method, the positive predictive value of the screening test was 93% and the negative predictive value was 51%. The specificity and sensitivity of the screening test were 95.3% and 40.8%, respectively.

Conclusions: According to the study data, the results of the screening test can be considered successful in identifying patients. However, it could not exclude non-patient individuals sufficiently. The high rate of false positives may be due to physiologic protein-losing conditions or low sensitivity of the method used. Under these data, we think that patients with proteinuria detected as a result of complete urine analysis should be confirmed by protein and albumin analysis in 24-hour urine.

Keywords: Complete urine analysis, proteinuria, albumin

S095

THE EFFECT OF URINE PH VALUE ON URINE BIOCHEMICAL ANALYSES

Kübra Kılıç Kartal, Güzin Aykal, Hamit Yaşar Ellidağ

Antalya Education and Research Hospital, Department of Medical Biochemistry, Antalya, Türkiye

Objectives: Urine analyses provide information about renal and urinary system as well as systemic and metabolic disorders.Normally, urine pH is around 5-6. Urine pH increases as a result of urine storage and contamination by microorganisms. The exact impact of these pH changes on urine biochemical analyses is not fully understood. The aim of our study is to investigate whether pH changes have an effect on urine biochemical analyses.

Methods: A urine pool was created from 24-hour urine and spot urine samples in our laboratory.Urine pH was measured using pH indicator strips (mColorpHast). Urine pH was adjusted to 7, 8, 9, 10, and 11. Protein, albumin, creatinine, sodium, chloride, calcium(Ca), magnesium(Mg), phosphorus, glucose, urea, uric acid, and amylase levels in urine samples were analyzed using the Beckman Coulter AU5800 autoanalyzer.

Results: In the pH range of 7-11, there was no significant change in protein, albumin, creatinine, sodium, chloride, glucose, and uric acid levels. A pH variation ranging from 3% to 17% was observed for urea, Mg, and phosphorus. In the case of Ca there was a 26.4% decrease at pH 7, 8, and 9 compared to pH 6 and a 22.2% increase at pH 11 compared to pH 10.

Conclusions: Our study has shown that pH changes affect some urine biochemical analyses. Particularly, there was an average of 25% variation observed in urine calcium analysis, even when pH was not at extreme levels. To mitigate variability in urine calcium analyses, we recommend measuring pH before conducting the analysis.

Keywords: urine biochemistry, urine pH, urine calcium

S096

DETERMINATION OF POTENTIAL DRUG TYPES FOR NERVOUS SYSTEM DISEASES USING MULTI-TASK LEARNING TECHNIQUE

Aytun Onay

Türk Hava Kurumu Üniversitesi,yazılım Mühendisliği Bölümü

Objectives: Drug design is a complex and time-consuming process that often takes many years. The CORINA Symphony tool was utilized to calculate 760 molecular features for each drug molecule in the dataset. In this study, the Clus-Multi-Task Learning (MTL) algorithm was applied to the nervous system (NS) drug dataset to determine NS disease groups (from N02, N03, N04, N05, and N06 groups). AD and WD were collected from Drugbank, the KEGG DRUG, and the PubChem databases.

Methods: 213 drugs, including both AD and WD, from different disease groups were classified using the Clus-Multi-task Learning (MTL) algorithm. In the tabular form of the dataset, each row represents a drug molecule, and each column indicates one of the 760 molecular descriptors. The last two columns denoted the class label corresponding to the withdrawn (WD) or approved (AD) status and the disease groups (multi-target classes) of NS drugs. MATLAB was used for data preprocessing. MTL was used to classify drugs by means of molecular descriptors.

Results: According to the results, MTL represented higher performance for the dataset. These results demonstrate that the classification model is suitable for both separation of ADs and WDs and determination of the NS disease groups. Accuracy rate (AR) was 0.82 for the dataset to determine NS disease groups and 0.95 for the dataset to classify NS drugs into approved and withdrawn categories.

Conclusions: The developed model can truly classify the test sets into both NS disease groups and NS approved and withdrawn categories.

Keywords: Drug Discovery, Multi-Task Learning, ToxPrint Chemotypes, Nervous System Drugs, Clus

S099

DEVELOPMENT OF A DRUG TO PREVENT MASTITIS PATHOLOGY THAT REDUCES MILK YIELD IN DAIRY COWS

Fatma Akat[1], Ali Şahin[2], Huseyn Babayev[3], Eissa Almaghrebi[4], Hüsamettin Vatansev[5]

[1] Selçuk University Institute of Health Sciences Department of Medical Biochemistry, Konya, Turkey

[2] Selcuk University Faculty of Medicine

[3] Silicosome Biotechnology

[4] Institute of Health Sciences Department of Medical Biochemistry

[5] Department of Medical Biochemistry, Selcuk University Faculty of Medicine

Objectives:Mastitis is the most common disease of dairy cattle and is an inflammation of the mammary tissue and milk glands. Neutrophils entering the tissue cause DNA release by NETosis mechanism. The aggregates and thrombotic aggregates formed by NETosis prevent the blood supply to the glands and the milk secreted from reaching the ducts, significantly reducing milk quality and milk yield. The mechanism depends on the catalytic activity of the protein PAD4 enzyme. Our aim was to inhibit the NETosis mechanism of neutrophils to prevent inflammation of mammary tissue in cattle.

Methods: We obtained our chemical libraries of molecules from selleckchem database.Our library, which we created using the DSV tool, consists of a combination of FDA approved chemicals and phytotherapeutic chemicals.We obtained the structure file of the PAD4 enzyme using structure prediction and validation tools. We obtained the amino acid sequence of Bos taurus using the NCBI database. The similarity ratio was calculated by pairwise alignment of the amino acid sequence with the human PAD4 enzyme. We predicted the structure of the PAD4 enzyme from Bos taurus using SwissModel. The accuracy of the model was ensured by the refinement module of the GalaxyWEB tool. The DSV program was used to analyze the binding of molecules to the catalytic site in the correct conformation.

Results:We detected “Diperpamide E, Guaijaverin and Luteolin-3’-D-glucuronide” molecules with ΔG° values less than-9. These 3 molecules, which we think are potential inhibitors, were found to bind to amino acids with catalytic functions. Conclusions:The molecules were proven to inhibit the PAD4 enzyme using in-silico conditions.

Keywords: Bovine mastitis, NETosis, PAD4, In-silico, Milk yield

S100

INVESTIGATION OF RE1-SILENCING TRANSCRIPTION FACTOR-COREST REPRESSOR COMPLEX BY MOLECULAR MODELING

Fulya Çağlar Çirkin[1], Cenk Selçuki[1, 2]

[1] Ege University, Institute of Health Sciences, Health Bioinformatics Program, İzmir, Turkey

[2] Ege University, Faculty of Science, Department of Biochemistry, İzmir, Turkey

Objectives: The REST/NRSF repressor constrains the expression of a considerable array of genes to neuronal tissues, effectively dampening their expression within non-neural contexts. In gene repression, certain auxiliary repressors such as CoREST and MeCP2 can remain bound to methyl CpG regions following NRSF-DNA dissociation, aiming to maintain low neuronal gene expression levels in specific cell types. REST has been recognized as a pivotal transcriptional controller, and its deviant expression has the potential to precipitate neurodegenerative disorders (NDs).

Methods: This study aimed to investigate the protein-protein interactions associated with neurological disorders and potential isoform structures of REST through the application of molecular modeling techniques. The Protein Data Bank (PDB) and AlphaFold databases were utilized to screen for structural isoforms of REST. Molecular docking analyses were conducted employing AutoDock Vina 1.2.2, while Molecular Dynamics (MD) simulations were carried out using AMBER20. Data analysis was performed using the xmgrace and VMD 1.9 software programs.

Results: Our findings illustrate the role of hydrogen bonds and electrostatic interactions in the interaction between REST and CoREST. These results hold the potential to aid in the identification of

therapeutic target regions for the treatment of neurological diseases.

Conclusions: Consequently, unraveling the intricacies of REST's mechanism holds promise for enhancing the efficacy of contemporary ND therapies. The majority of the computational calculations were conducted utilizing the resources provided by TUBITAK-ULAKBIM Truba, with additional support for this study being granted through Ege University Research Funds (BAP, Project No. 24211).

Keywords: RE1-Silencing Transcription Factor, coREST, Molecular Modeling

S101

HOW MACHINE LEARNING READS CLINICAL FEATURES: A FOCUS ON A1c

Ferhat Demirci[1], Deniz İlhan Topcu[2]

[1] Health Sciences University İzmir Tepecik Education and Research Hospital, İzmir, Turkey

[2] Health Sciences University İzmir Tepecik Education and Research Hospital, Medical Biochemistry, İzmir, Turkey,

Objectives: In modern healthcare, machine learning (ML) is a potent tool for processing and interpreting vast clinical datasets, enhancing diagnostic and predictive capabilities. In this study, we aimed to optimize the performance of various ML algorithms by analyzing clinical laboratory data and assessing the algorithmic accuracy and the importance of feature selection for clinical predictions.

Methods: A total of 3.913 patient results, which include age, triglyceride, HDL-C, Vitamin B12, cholesterol, insulin, folate, urea, creatinine, CRP, hemoglobin, MCV, RDW, and glucose measurements, were included in the study. Two data sets were created with (WGDS) and without glucose (WOGDS) levels. Python programming language with Pycaret and Numpy libraries was used for ML model development. Fifteen different ML models were created for each of the two datasets. The best model was selected according to the accuracy of the cross-fold validation.

Results: Xgboost and LightGBM algorithms showed the highest accuracy for WGDS and WOGDS datasets, respectively. WGDS has slightly higher accuracy than WOGDS dataset for training and test sets (0.90 vs. 0.81 for training and 0.89 vs. 0.80 for test sets). Glucose had the highest feature importance, followed by age and MCV for WGDS. Age, triglyceride, and HDL-C were the most important features of WOGDS.

Conclusions: The study demonstrated the essential role of careful feature selection in affecting the performance of clinical laboratory-related predictive models. This highlights the necessity of careful feature selection and understanding dataset composition in clinical ML applications.

Keywords: machine learning, A1c, Feature importance, Model performance

S102

CAN CHATGPT BE A "RELIABLE FACILITATOR" IN THE PREPARATION OF A BIOCHEMISTRY LABORATORY TECHNICAL SPECIFICATION?

Saliha Uysal

Balıkesir University, Faculty of Medicine, Department of Medical Biochemistry, Balıkesir, Türkiye

Objectives: This study aims to assess whether ChatGPT can be a reliable facilitator in clinical biochemistry and specifically in tender preparation processes.

Methods: Questions about the "most commonly used measurement method," "analyzers capable of performing the measurement," and "evaluation of analyzers based on their speeds and features" for frequently analyzed tests in biochemistry laboratories, namely complete blood count(CBC), creatinine, TSH, and HbA1c, were asked to ChatGPT4.0 in Turkish. The responses received were evaluated based on whether they covered the most commonly included systems in tenders. Analyzers were queried for features like "cap piercing" for CBC,"sample loading capacity" for creatinine, "continuous random access" for TSH, and "Cation Exchange Chromatography" for HbA1c.

Results: The most commonly used measurement methods for all markers were entirely consistent with the literature.ChatGPT introduced the Sysmex, Beckman, Mindray, and Abbott systems for CBC, and the Roche, Abbott, Siemens, and Beckman systems for both creatinine and TSH among the top four.For HbA1c analysis, Bio-Rad, Tosoh, Arkray, and Menarini systems were at the forefront.When querying device speeds by brand and model,results consistent with the information provided by the manufacturers were obtained. Device features were evaluated, and the answers to the questions asked for creatinine, TSH, and HbA1c were consistent with the information provided by the manufacturers. However, even though the Cell-dyn Sapphire and Cell-dyn Emerald systems have the "cap piercing"feature, they were not mentioned in ChatGPT's response.

Conclusions: ChatGPT is poised to be a"reliable facilitator"in the initial phase of laboratory tender processes, which is the preparation of technical specifications,even if not perfectly so at present.

Keywords: ChatGPT, Laboratory tender processes, Technical specification

S103

INVESTIGATION OF THE EFFECT OF GEL USED IN SERUM SEPARATOR TUBES ON VITAMIN B12 ANALYSIS

Tuba Batur[1], Halil İbrahim Akbay[2], Serap Sezer[3]

[1] Beyhekim Training and Research Hospital, Department of Biochemistry, Konya, Turkey

[2] Yüzüncü Yıl University, Faculty of Medicine, Department of Biochemistry, Van, Turkey

[3] Beyhekim Training and Research Hospital, Department of Internal Medicine, Konya, Turkey

Objectives: It was aimed to examining, due to the fact that falsely high results were observed in some serum vitamin B12 analyses, the effect of separator gels on serum vitamin B12 values.

Methods: Blood samples were collected from 30 healthy volunteers into two different serum gel tubes (Pirmax, gel&clot activator, 5 mL; Vacusera, gel&clot activator, 5 mL) and a serum non-gel tube (Vacuette, clot activator, 6 mL). Serum samples were analyzed for vitamin B12 in the Advia Centaur XPT immunoassay system. Results of assays of serum B12 in two different serum separator gel tubes and a plain serum tube were compared. An investigation of the effect of mixing serum separator tubes was also carried out.

Results: There was no difference between the measured initial vitamin B12 values in the samples taken into three different tubes ($p>0.05$). By mixing the serum gel tube samples, serum vitamin B12 results increased compared to the initial analysis ($p<0.05$). The results of samples collected into tubes with separator gels obtained by re-centrifugation of the samples were similar to the initial vitamin B12 analysis ($p>0.05$).

Conclusions: Mixing serum separator gel tubes can cause spuriously elevated B12 levels on the Advia Centaur XPT immunoassay systems. We believe that it is important for all laboratories, especially those accepting samples from outside their own institutions, to report the B12 results after re-centrifugation just in case the serum samples in the gel tube are mixed with the gel during transport.

Keywords: serum separator tube, vitamin B12, mixing

S104

IS TOTAL CHOLESTEROL CAUSE THE DIFFERENCE BETWEEN THE PAINT BINDING METHOD AND THE ALBUMIN CONCENTRATIONS MEASURED BY CAPILLARY ELECTROPHORESIA?

Elif Nihal Başer, Semih Fazlı Kayahan, Mehmet Şeneş

Ankara Eğitim ve Araştırma Hastanesi

Objectives: Differences have been observed between albumin concentrations measured on autoanalyzers and serum protein electrophoresis (SPEP) in some patients.The aim of our study was to investigate whether the difference between albumin measured on an autoanalyzer and by capillary zone electrophoresis (CZE) is associated with the total cholesterol concentration in the sample.

Methods: The study is a retrospective study and included 292 patients for whom simultaneous albumin,SPEP and lipid panel tests were requested between September 2019 and June 2023.Albumin,triglyceride and total cholesterol measurements were performed on the Roche cobas 8000 analyzer,while SPEP was conducted using the Minicap Flex-Piercing(Sebia) CZE device.The percentage difference values (PDV) between albumin measured on an autoanalyzer (S.Alb) and CZE (PE.Alb) were calculated using the formula PDV=(PE.Alb-S.Alb)/S.Alb*100.Patient results were grouped as ≤150 mg/dL and ≥151 mg/dL for triglycerides,≤200 mg/dL and ≥201 mg/dL for total cholesterol and ≤130 mg/dL and ≥131 mg/dL for LDL cholesterol.Differences in PDV between groups were evaluated using the Mann-Whitney U test.

Results: There was no significant difference in S.Alb-PE.Alb PDV between the ≤150 mg/dL and ≥151 mg/dL triglyceride groups (p=0.164).However,there were significant differences in PDV between the ≤200 mg/dL and ≥201 mg/dL total cholesterol groups and between the ≤130 mg/dL and ≥131 mg/dL LDL cholesterol groups (p=0.011 and p=0.016, respectively).

Conclusions: Studies have shown that an increase in cholesterol concentration leads to the expansion of albumin peptide chains, resulting in an increase in albumin absorption spectrum.We believe that laboratory specialists should provide appropriate warnings in their report interpretations for clinicians,especially in patients with high cholesterol levels, when evaluating SPEP results.

Keywords: capillary zone electrophoresis, albumin, cholesterol, LDL, triglyceride

S105

A PRELIMINARY STUDY ON INTERFERENCE OF THE URINARY PROTEIN ASSAY BY POVIDONE IODINE SOLUTION CONTAMINATION

Halil İbrahim Büyükbayram, İlter İlhan, Fevziye Burcu Şirin, Duygu Kumbul Doğuç

Süleyman Demirel University, Faculty of Medicine, Department of Medical Biochemistry, Isparta, Türkiye

Objectives: Povidone iodine (PVP) solutions are commonly used before urinary catatherization and sample collection in order to prevent any infectious contagion. This study was conducted to investigate any interfering effect of commercially available %10 PVP containing solutions on one of the colorimetric urinary total protein assays, the Automated Pyrogallol Red-Molybdate (PRM) method.

Methods: Three urine pools were prepared containing different amounts of total protein (6,3 mg/dl, 45,7 mg/dl and 119,8 mg/dl). PVP solutions at six different concentrations (2,5%, 1,25%, 0,6%, 0,3%, 0,15% and 0,07%) were added to the aliquoted urine pool samples. Urinary protein levels of the PVP-added samples were measured on Beckman Coulter AU 5800 autoanalyzer (Beckman Coulter, USA) with PRM method. The biases from the baseline results were calculated.

Results: In the samples containing 6,3 mg/dl total protein, the biases of the results were greater than 10% at all of the different PVP concentrations. In the samples containing 45,7 mg/dl protein, the biases were all exceeded 10% except 0,07% PVP concentration. Finally, in the samples containing 119,8 mg/dl protein, the biases were greater than 10% at 0,3% and higher PVP concentrations.

Conclusions: We briefly showed the interfering effect of PVP solution on urinary protein assay with PRM method on Beckman Coulter AU 5800 autoanalyzer. Health professionals should keep in mind the PVP contamination in clinically inconsistent high urine total protein results. Also in this case, using a different antiseptic solution at sampling process or choosing a different urinary total protein assay method may be considered.

Keywords: analytical error, urinalysis, pyrogallol red

S106

EVALUATION OF LIPEMIA INTERFERENCE WITH NATURAL ULTRALIPEMIC MATERIAL AND INTRAVENOUS LIPID EMULSION IN HEMOGLOBIN VARIANT ANALYSIS

Elmas Öğüş[1], Gül Kırtıl[1], Medine Alpdemir[1], Emel Çolak Samsum[2], Mehmet Fatih Alpdemir[3], Mehmet Şeneş[1]

[1] University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Turkey

[2] Clinical Biochemistry Laboratory, Pursaklar State Hospital, Ankara, Turkey

[3] University of Health Sciences Ankara City Hospital, Department of Medical Biochemistry, Ankara, Turkey

Objectives: In the preanalytical stage, lipemia interference is a common problem that affects accurate results. In this study, we aimed to evaluate lipemia interference in high, normal, and low HbA2 results with natural ultralipemic material (NULM) and intravenous lipemic emulsion (IVLE).

Methods: NULM and IVLE solutions were used to investigate the effect of lipemia in Hb(hemoglobin) variant analysis. The study was carried out according to the EP7-A2 guideline published by the Clinical and Laboratory Standards Institute for interference studies. Whole blood pools with high (≥ 5%), normal (2.5-5 %), and low (≤ 2.5 %) HbA2 results were diluted with both NULM and IVLE stock solutions to create sub-pools containing five different concentrations of triglycerides. UniCap capillary electrophoresis device (Shenzen YHLO Biotech Co., Ltd., China) was used in this study. The repeated ANOVA test was used for statistical analysis (SPSS ver. 22 IBM).

Results: The values of high, normal, and low main pools were 5.1±0.0, 2.6±0.06 and 2.1±0.0 for HbA2 %, 91.4±0.0, 96.0 ±3.5, and 97.1±0.0 for HbA %, respectively. HbF % for the high pool was 1.90 ±0.0. No interference was observed for HbA2, HbA, and HbF in either DULM or IVLE, according to repeated ANOVA analysis in pools, depending on lipid concentration.

Conclusions: In this study, the effect of lipemia interference on Hb variant analysis was investigated. The interference effect was not detected in both DULM and IVLE pools.

Keywords: Hb variant analysis, lipemia, interference, natural ultralipemic material, intravenous lipemic emulsion

S107

DOES TURBIDITY CAUSE INTERFERENCE IN QUANTITATIVE URINE TOTAL PROTEIN MEASUREMENT?

Ayşe Betül Demir, Kezban Çavdar Yetkin, Mehmet Şeneş

University of Health Sciences Ankara Training and Research Hospital, Department of Medical Biochemistry, Ankara, Turkiye

Objectives: In this study,the effect of turbidity on quantitve urine total protein,albumin and creatinin concentrations was investigated.

Methods: Urine samples were used in the study,where total protein and albumin were analyzed on the RocheCobas 6000 analyzer and visually categorized as turbid(n=48) and clear(n=29).Total protein(<150mg/L and >150mg/L) and albumin results(<20mg/L and >20mg/L) were taken into consideration when selecting samples.Turbid and clear urines were centrifuged at different speeds and times and divided into four groups GroupI:Without centrifugation,GroupII:-Centrifugation at 500 g for 5 minutes,GroupIII:Centrifugation at 1000 g for 5 minutes and GroupIV:Centrifugation at 1500 g for 5 minutes.Whether there was a significant difference between the groups was determined by the Repeated Measured ANOVA test.Additionally, percentage difference from the non-centrifuged group was calculated for each group.

Results: In turbid urine,the median(min-max) values for albumin,total protein and creatinine were 32.7mg/L(2-1882mg/L),414mg/L(83-2954mg/L) and 122mg/dL(38-303mg/dL) while in clear urine they were 689mg/L(3-1700mg/L),749mg/L(108-1949mg/L) and 76mg/dL(36-215mg/dL),respectively.Significant differences among groups were observed in turbid urine in both total protein and creatinine values, as well as in total protein/creatinine ratios(group differences from GroupI,$p<0.001$,$p<0.05$ and $p<0.001$,respectively).In clear urine,there were no significant differences among any of the groups

(p>0.05 for all groups).The percentage difference the groups from GroupI for total protein in turbid urine was as follows:-44%,-54% and -54.89%, respectively.Similarly,the percentage differences for total protein/creatinine were -38%,-47% and -50%,respectively.

Conclusions: In turbid urine,centrifugation is essential for obtaining accurate quantitative results for total protein,creatinine and total protein/creatinine results. Our study has shown that centrifugation at 1000g for 5 minutes is sufficient for removing turbidity.

Keywords: urine, interference, total protein, centrifugation, albumin

S109

A CONDITION THAT CAUSES LOW HBA1C MEASUREMENT BY HPLC METHOD

Seydi Ali Peker, Yunus Emre Haskılıç, Abdulkadir Tekin, Cihan Demir

Kirikkale Yüksek İhtisas Hospital, Department of Medical Biochemistry, Kirikkale, Türkiye

Objectives: The HbA1c test is used for diagnosis and monitoring of diabetes mellitus. Many different analytical methods are used for HbA1c measurements. Immunometry, high-throughput liquid chromatography (HPLC) and boronate affinity chromatography are the most widely used methods. In this case, we aimed to present a patient who was clinically incompatible with the HbA1c level measured by the HPLC method.

Methods: A 72-year-old female patient applied to the family health center, Routine examination was performed and laboratory analyzes were requested. When the desired fasting blood glucose level was determined as 121 mg/dL among the routine tests, the HbA1c test was added to the patient's laboratory panel by the clinician. HbA1c measurement was made in HPLC device (Tosoh G8; Tokyo, Japan) in our laboratory. The patient's Hba1c result was 2%. This result was shared with the clinician. There was a discordance between clinical situation and test result. So it was decided that the patient should also be evaluated in terms of hemoglobin variant. As a result of the analysis, HbD was 83.6%, HbA 7.7% and HbA2 0.7%. The patient's HbA1c concentration was measured by the turbidimetric immuno-inhibition method (Beckman Coulter, AU480, USA) and HbA1c was found to be 5.7%.

Results: The presence of hemoglobin variants cause interference in HbA1c results. Low or high results may occur in HbA1c results due to abnormal peaks in measurements made with HPLC.

Conclusions: In the monitoring of diabetic patients, it should be known that hemoglobinopathies affect HbA1c measurement methods and cause false results.

Keywords: HPLC, HbA1c, Immunometry

S110

BENIGN TRANSIENT HYPERPHOSPHATASEMIA IN CHILDREN

Emir Matpan[1], Aysun Toker[2], Mustafa Serteser[3]

[1] Acibadem Univercity, School of Medicine, Department of Medical Biochemistry, İstanbul, Turkey

[2] Acibadem Labmed Clinical Laboratories, İstanbul, Turkey

[3] Acibadem Univercity, School of Medicine, Department of Medical Biochemistry; Acibadem Labmed Clinical Laboratories, İstanbul, Turkey

Objectives: Transient hyperphosphatasemia (TH) is benign condition in which serum alkaline phosphatase (ALP) is transiently elevated without underlying systemic diseases.Therefore, it is quite beneficial to detect cases of TH and report them correctly before any further interventions.

Methods: Total number of 28 patients' data of which serum ALP activity analyses and electrophoretic separation of ALP isoenzymes performed in between 01.06.2022–01.07.2023,were collected retrospectively. Patients with high intestinal isoenzyme activity(n=4) and normal liver isoenzyme activity(n=2) were excluded. Then, the rest of 22 patients' results with ALP electrophoretic gels having faster anodal migration of isoenzymes and serum

ALP>1000 U/L, were reported with a note defining the TH diagnosis.

Results: The study group comprised of 22 toddlers(10 M/12 F) aged from 4 months to 3 years having serum ALP activity of (2258 ; 1005-4609 IU/L), (Median ; IQR).The liver and bone isoenzyme activity levels were found (676 ; 175-1890 IU/L), (1581 ; 482-3718 IU/L), (Median ; IQR) respectively. A seasonal peak of the cases was observed in autumn-early winter, indicating an association with viral infections and TH; but also this might be a detection bias. Additionaly, the clinical history of study participants could not be collected and evaluated with laboratory results because of the disadvantage of being a central laboratory, approving biological samples from other facilities.

Conclusions: The recognition of this phenomenon with elevated levels of serum ALP activity and typical electrophoretic isoenzyme migration pattern in pediatric ages (<5 years of age) resolving spontaneously is important to avoid wasting of time and resources in clinical laboratories for further diagnostic evaluations.

Keywords: hyperphosphatasemia, alkaline phosphatase, isoenzymes, viral infections

S111

PSEUDOHYPERKALEMIA CASE RELATED TO TROMBOSITOSIS

Ayşen Caniklioğlu, Rumeysa Betül Kılınçer

Yozgat Bozok University, Faculty of Medicine, Department of Medical Biochemistry, Yozgat, Türkiye

Objectives: The primary aim of this case study is to underscore the pivotal significance of discriminating thrombocytosis-associated pseudo hyperkalemia from the hyperkalemia.

Methods: An 80-year-old male patient with a history of cerebrovascular disease, hypertension, and diabetes mellitus presented with weakness and was admitted to the Neurology department. During follow-up despite elevated potassium values in hemolysis-free serum, the blood gas analysis potassium level remained within the normal range. Urea and creatinine values were within the normal range, and there were no clinical or electrocardiogram (ECG) findings consistent with hyperkalemia. A progressively rising thrombocytosis was detected in the complete blood count (CBC). With the suspicion of thrombocytosis-associated pseudo hyperkalemia, potassium level was measured in the plasma from the lithium-heparinized tube.

Results: The patient had elevated platelet count (1101 x 103 / µl) serum potassium level (5.26 mEq/L), while blood gas analysis potassium level (4.46 mEq/L) was within the normal range. Meanwhile, the potassium level measured in heparinized plasma was found to be 4.68 mEq/L.

Conclusion: Elevated platelets lead to a greater release of intracellular potassium during clotting, resulting in higher serum potassium levels compared to plasma potassium levels. This condition is defined as thrombocytosis-associated pseudo hyperkalemia. Early recognition of this condition will prevent the patient from receiving inappropriate hyperkalemia treatment and reduce confusion among healthcare professionals.

S112

WHAT IS A NON-REFRIGERATED CENTRIFUGE CAPABLE OF?

Hatice Bozkurt Yavuz

Uşak Training and Research Hospital, Clinical Biochemistry Laboratory, Uşak, Turkey

Objectives: The preanalytical phase significantly impacts laboratory outcomes, especially in remote centers where control is challenging.

Methods: Our secondary state hospital laboratory also served the Family Health Center (FHC). A patient with elevated FT4, FT3, and suppressed TSH was referred from FHC for suspected primary hyperthyroidism to Internal Medicine clinic in our hospital. However, subsequent tests at our hospital revealed normal values. To ensure that blood is drawn from the correct patient, we contacted FHC and confirmed that the barcodes were double-checked. Since blood was drawn into two separate tubes for biochemistry and hormone analysis, FHC samples were analyzed on cross analyzers.

Results: FT4, FT3, and TSH results were normal upon reanalysis. Yet, significant discrepancies in ALP, ALT, amylase, LD, HDL-C, and UIBC were observed in biochemistry analyses. Lower enzyme values and higher FHC sample density indicated a temperature-related error. FHC staff revealed that the centrifuge worked without cooling, and some samples sent without centrifugation. In the afternoon, it was requested to run the centrifuge without samples under the same conditions and it was learned that the temperature reached 63°C. The patient's hyperthyroidism diagnosis was incorrect due to the high-temperature centrifugation, leading to specimen rejection.

Conclusions: Poor control of centrifuge temperatures can cause misdiagnosis with primary hyperthyroidism due to TSH and thyroid-binding globulin denaturation. Liver diseases and iron deficiencies may also be overlooked due to erroneously low ALT, ALP, and UIBC results.

Keywords: Centrifugation, Enzymes, Hormones, Preanalytical Phase, Temperature

S113

THE PLACE OF FLOW CYTOMETRY IN DIAGNOSIS OF MYELODYSPLASTIC SYNDROME IN A PATIENT WITH PANCYTOPENIA: A CASE REPORT

İlknur Alkan Kuşabbi

Ankara Etlik City Hospital

Objectives: Myelodysplastic syndrome(MDS) is diagnosed morphologically.In addition,flow cytometry is a valuable and rapid diagnostic method in evaluation of increase in myeloblasts which is the most important factor determining prognosis of MDS and in estimation of dysplastic changes in bone marrow.

Methods: A 50-year-old male patient was admitted to Internal Medicine clinic with weakness and fatigue for last 2 weeks.He was followed up by Hematology department because of pancytopenia in hemogram.In peripheral blood smear,erythrocytes were anisocytic,leukocyte formula was consistent with hemogram,no atypical cells were seen.Since pancytopenia persisted in follow-up hemograms,bone marrow aspiration was performed. In flow cytometry,approximately 6% of CD45 cells were observed in CD45 dim area,and when these cells were examined,approximately 3% CD34 negative,CD33 and CD117 positive myeloblasts were determined.It was observed that granulocytes were divided into two groups with low and high granularity.There was significant loss of granularity in granulocytes.It was observed that maturation properties of granulocytic series were also impaired.With these findings,pre-diagnosis of MDS was considered. Bone marrow aspiration pathology result was also evaluated as MDS,which was consistent with flow cytometry result.In pathological examination MDS was evaluated as RAEB-II according to WHO classification,since 11% blasts were found and dysplastic changes were observed in myeloid and erythroid series,and treatment was planned accordingly.

Results: It was observed that bone marrow aspiration flow cytometry result and pathology result were compatible in diagnosis of MDS.

Conclusions:Although flow cytometry does not provide clear classification information for morphologically diagnosed MDS,it is a valuable and rapid diagnostic method that predicts whether the diagnosis is MDS and at what risk level it is in the classification.

Keywords: flow cytometry, myelodysplastic syndromes

S114

CAN REFLEX TESTING UTILIZE THE HEMATOLOGY ANALYZER FLAG?

Gülsüm Feyza Türkeş[1], Ekin Kırcalı[2]

[1] Ankara University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

[2] Ankara Atatürk Sanatoryum Training and Research Hospital, Department of Hematology, Ankara, Türkiye

Objectives: Reflex testing is the automatic addition of tests by analyzers based on algorithms established by laboratory professionals. We aimed to demonstrate that preliminary diagnoses of patients can be made using the reflex test that can be utilized on the hematology analyzer in this case series.

Methods: Patient #1 is a 70-year-old female who has Alzheimer's, hypertension, and osteoporosis. She admitted to a hematology outpatient clinic with fatigue. Chronic anemia and mild thrombocytopenia were found to be present in the patient's history. During the physical examination, she appeared pale and tachycardic. Abnormal lymphocyte/lymphoblast?, neutropenia, and pancytopenia flags have been observed on the hematology analyzer (Mindray, BC 6800, China). On peripheral smear (Mindray, Slide Maker&Stainer, China), immature myeloblasts were present. The patient was diagnosed with acute myeloid leukemia after obtaining a bone marrow biopsy.

Patient #2, an 82-year-old female who has hypertension, atrial fibrillation, and mild dementia, has been admitted to the hematology outpatient clinic. She was referred from an internal medicine clinic due to anemia. She had difficulty walking, skin pallor, and hepatosplenomegaly during physical examination. On hematology analyzer, blast? abnor. lymphoblast? immature granulocyte, lymphocytosis and leukocytosis flags appeared. Peripheral smear revealed leukocytosis, mature lymphocytosis, and smudge cells in almost every high-power field. Flow cytometry showed B-cell chronic lymphocytic leukemia in peripheral blood.

Conclusions: The hematology analyzer flags should be considered when performing reflex testing in our case series to avoid delays in diagnosis and treatment, particularly in hematological diseases.

Keywords: Hematology, Reflex testing, B-cell chronic lymphocytic leukemia, Acute myeloid leukemia

S115

FALSELY HIGH FREE TRIIODOTHYRONINE VALUE DUE TO MONOCLONAL ANTIBODY THERAPY

Didem Barlak Keti[1], İlknur Uzun[2], Sabahattin Muhtaroğlu[1]

[1] Erciyes University, Faculty of Medicine, Department of Medical Biochemistry, Kayseri, Türkiye

[2] Erciyes University, Faculty of Medicine, Department of Endocrinology, Kayseri, Türkiye

Objectives: Compatibility of thyroid function test results with each other and with the patient's clinic is important in diagnosis and follow-up. We reported a woman who had a false high free triiodothyronine value (fT3) due to monoclonal antibody therapy. The aim was to emphasize that oncology patients may encounter inappropriate results due to the use of monoclonal antibodies and to share the laboratory process we experienced in this case.

Methods: In a 64-year-old woman; fT3, free thyroxine (fT4), and thyroid stimulating hormone (TSH) values were found 10.4pg/mL, 1.03ng/dL, and 10.8µIU/mL, respectively. The patient was receiving thyroid hormone and monoclonal antibody therapy. Free triiodothyronine measurement was repeated in alternative platforms. The serum of the patient was treated with a heterophile antibody blocking tube (HBT). In addition, the patient sample was transferred to two separate tubes. While PEG was added to one, distilled water was added to the other at the same rate. Free triiodothyronine values in serum with PEG were compared with serum which was added distilled water. The same procedures were applied to the control serum.

Results: In the alternative platform, the fT3 value was found 3.18ng/mL. When the patient serum treated with PEG was compared to the serum that was added distilled water, a decrease (from 6.94ng/mL to 3.55ng/mL) was detected in fT3. No decrease in fT3 was observed in the control serum to which the same procedure was applied. Incubation of the patient sample with HBT showed 97% of recovery for fT3.

Conclusions: Inappropriate results may occur in

patients using monoclonal antibodies.

Keywords: monoclonal antibody therapy, free triiodothyronine

S116

CAN GLUCOSE, AND ELECTROLYTES OBTAINED ON BLOOD GAS ANALYSER BE USED INSTEAD OF BIOCHEMISTRY ANALYSER RESULTS IN ADULT PATIENTS?

Ahmet Burak Gürpınar[1], Abdullah Üner[1], Tevfik Noyan[1], Murat Cihan[2]

[1] Ordu University, Medical Faculty, Department of Biochemistry

[2] Ministry of Health, Ordu University Training and Research Hospital

Objectives: A blood gas analysers are vital equipment frequently used in emergency departments and intensive care units. The aim of this study was to determine the agreement in glucose, sodium, potassium and chloride parameters that measured both biochemistry auto-analyser and blood gas analyser.

Methods: In the present study, 120 patient's test results of glucose, and electrolytes measurements simultaneously in blood gas and autoanalyzer were evaluated retrospectively for about 1-months period.

Results: While the potassium, and chloride values measured on the auto-analyser were significantly higher ($p<0.01$), sodium significantly lower than those on the blood gas analyser ($p<0.01$). There was no statistically significant difference for glucose between the two analysers ($p>0.05$). In the Spearman correlation analysis between the two measurements, correlation coefficients (r) wase found as 0.924, 0.942, 0.862, and 0.983 ($p<0.001$) for sodium, potassium, chloride, and glucose respectively. According to the Bland-Altman analysis, the mean differences between the two methods were not statistically significant for glucose (mean difference 0.01 mg/dL, 95% CI -1.254–1.635, p = 0.796), sodium (mean difference 1.891 mmol/L, 95% CI -0.729–4.512, p = 0.155), and potassium (mean difference -0.719 mmol/L, 95% CI -2.674–1.235, p =0.467), however, there was statistically significant for chloride (mean difference 1.356 mmol/L, 95% CI 0.059–2.652, p = 0.04).

Conclusions: The results of our study suggest that glucose results measured by both analysers can be used interchangeably, but care should be taken when interpreting electrolyte values.

Keywords: autoanalyser, blood gase analyser, electrolytes, glucose

S117

COMPARISON OF ADVANCED GLYCATION END PRODUCT (AGE) AND ZINC LEVELS IN PATIENTS WITH DIABETES MELLITUS AND DIABETIC NEPHROPATHY

Alev Kural[1], Nazlı Helvacı[2], Özgür Can[3], Kürşad Nuri Baydili[4]

[1] Health Sciences University Bakırköy Dr. Sadi Konuk Teaching and Research Hospital Medical Biochemistry Department

[2] Health Sciences University Hamidiye School of Medicine Medical Biochemistry Department

[3] Health Sciences University Haydarpaşa Numune Teaching and Research Hospital Nephrology Departmen

[4] Health Sciences University Hamidiye School of Medicine Biostatistics and Medical Informatics Department

Objectives: Diabetes is a metabolic disease characterized by hyperglycemia. Diabetic nephropathy (DN) is the leading cause of kidney disease. Zinc is required for the processing and storage of insulin in the pancreatic β-cell. Zinc metabolism has been shown to be associated with an increased risk of diabetes. Advanced glycation end products (AGEs) are proteins or lipids that become glycated. It is a biomarker that plays a role in the development of diabetes. The aim of this study is to evaluate serum Zn and AGE levels in patients with diabetes and diabetic nephropathy.

Methods: Our study included 37 diabetes mellitus (DM) patients, 46 DN patients

and 27 healthy individuals between the ages of 18-75. Serum AGE and serum zinc levels were measured from the blood taken from these individuals. The biochemical data obtained from the study were analyzed with the SPSS 26.0 statistical analysis package program.

Results: In our study, HbA1c values were found to be significantly higher in the DM and DN groups compared to the healthy group ($p<0.001$), while creatinine values were found to be significantly higher in the DN group compared to the other two groups ($p<0.001$). While serum AGE levels were found to be significantly higher in the DN group compared to the other two groups ($p=0.012$), zinc levels were found to be significantly higher in healthy individuals than in patients with disease ($p=0.039$).

Conclusions: These data we obtained should be supported by further studies with larger participant groups.

Keywords: AGE, Zinc, Diabetic nephropathy, Diabetes Mellitus

S118

INVESTIGATION OF APELIN, ELABELA, ENDOGLIN AND IMA BLOOD LEVELS IN ACUTE MYOCARDIAL INFARCTION

Irem Arslanturk[1], Huseyin Fatih Gul[2]

[1] Kafkas University, Institute of Health Sciences, Department of Medical Biochemistry, Kars, Türkiye

[2] Kafkas University, Faculty of Medicine, Deparment of Medical Biochemistry, Kars, Türkiye

Objectives: Acute myocardial infarction (AMI), one of the leading causes of mortality and morbidity, is a disease characterized by cardiac myocyte loss caused by long-term ischemia. The aim of this study was to determine the blood levels of apelin, elabela, endoglin and IMA in AMI and to investigate the relationship of these parameters with HsTn-I, which is the gold standard biomarker in the diagnosis of AMI.

Methods: The study population consisted of 45 patients with AMI and 45 healthy individuals. Plasma apelin, elabela, endoglin and serum IMA levels were measured by ELISA method. Serum levels of routine biochemical markers were withdrawn from the hospital automation system.

Results: In the study, HsTn-I and endoglin levels were higher in the group with AMI (904.44±875.92 ng/L and 22.14±5.24 ng/mL) compared to the control group (11.57±4.11 ng/L and 11.44±5.40 ng/mL) ($p<0.001$); apelin and elabela levels were found to be statistically significantly lower in the group with AMI (220.62±69.13 ng/L and 1036.51±139.31 ng/L) compared to the control group (279.28±109.67 ng/L and 1386.08±139.00 ng/L) ($p<0.001$). IMA levels were found to be higher in the patient group compared to the control group, but it was not significant. In addition, there was a significant negative correlation between serum HsTn-I and plasma apelin and elabela levels; a positive and significant correlation was determined between plasma endoglin levels.

Conclusions: Finally, while apelin and elabela from plasma adipokine levels were decreased in the group with AMI, endoglin was found to be high. Finally, these adipokines may have roles in the etiopathogenesis, diagnosis and treatment of AMI.

Keywords: acute myocardial infarction, HsTn-I, apelin, elabela, endoglin, IMA

S119

WNT SIGNALING INHIBITORS FOR INCREASING OSSEOINTEGRATION

Rabia Şemsi[1], Erdal Ergünol[2], Duygu Dayanır[3], Remzi Orkun Akgün[4], Okan Ekim[5], Oral Cenk Aktaş[6], Altay Uludamar[2], Ayhan Özkul[7], Aylin Sepici Dinçel[1]

[1] University of Gazi, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

[2] Private Sector, Ankara, Türkiye

[3] University of Gazi, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye

[4] University of Çankırı Karatekin, Faculty of Dentistry, Department of Anatomy, Çankırı, Türkiye

[5] University of Ankara, Faculty of Veterinary Science, Department of Anatomy, Ankara, Türkiye

[6] Institute of Materials Science, Christian-Albrechts Universitat zu Kiel, Kiel, Germany

[7] University of Ankara, Faculty of Veterinary Science, Department of Pathology, Emeritus, Ankara, Türkiye

Objectives: Sclerostin (Scl) is a primary inhibitor controlling the Wnt signaling pathway. It can directly bind to LRP5/6 and inhibit the activation of LRP5-6 related signaling. This study aimed to evaluate the effect of targeted inhibition of Scl and to create a balanced stimulation of bone formation in tooth extraction sockets by suppressing the bone resorption mechanism. Secondly, we explored the regulatory interaction of Scl and bone during local administration.

Methods: New Zealand male rabbits (3-month-old, weight 2.5-3 kg) were fully randomized to ensure bias. Experiments were done in 3 groups (control, graft, Scl-Ab). Calculated doses of Scl-Ab were applied together with the graft material to extraction sockets, and results were obtained at two and 4-week intervals. Volumetric tomography slice imaging, histomorphologic analysis, and radiologic measurements were performed.

Results: Volumetric tomography analysis of a defined mandibular region of interest demonstrated that experimental animals treated with Scl antibody had higher average mandibular bone volume than graft and control groups. The Scl antibody of Group 3 had increased trabecular thickness of alveolar bone, a clinically relevant measure of bone quality related to bone formation.

Conclusion: A therapeutic composition of Scl antibodies supports early bone formation around dental implants and graft materials in extraction sockets. The local inhibition of Scl secreted within the bone microenvironment targets Wnt/beta-catenin pathway modulation that enhances the potential bone formation.

This project was supported by the Gazi University Scientific Research Projects Coordination Unit (BAP) (project code: TCD-2021-7060).

Keywords: Bone healing, Sclerostin, Bone formation-resorption, Osseointegration

S120

GOJIBERRY POTENTIATES THE EFFECT OF L-CARNITINE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA IN VITRO

Mine Ergüven[1], Haniyeh Behrouzizad[2], Esin Çetin Aktaş[3], Ayhan Bilir[4]

[1] İstanbul Aydın University, Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Türkiye

[2] İstanbul Aydın University, Institute of Graduate Studies, Food Safety and Nutrition Program, İstanbul, Türkiye

[3] İstanbul University, As Detae, Department of Immunology, İstanbul, Türkiye

[4] Atlas University, Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye

Objectives: In our study, we targeted to investigate the effect of gojiberry (GB) and L-carnitine (LK) in combination on chronic myeloid leukemia (CML) with the underlying mechanism.

Methods: Antioxidant capacity-determined GB extracts were applied to K562 leukemia cells for 72 h. Groups were determined as control 1 (no treatment), control 2 (Gleevec), LK, GB, and GB combined with LK (GB+LK). Their effects were determined by cell number, cell viability and apoptotic cell rate (Flow cytometry), the levels of apoptotic (Caspases-3,8,9, Bax) and anti-apoptotic (Bcl-2) proteins (ELISA), and the ultrastructure (TEM). Anova test was applied, and $p<0.05$ was accepted as statistically significant.

Results: The highest cell number and viability decrease was found in the combination group (PGB+LK<0.05). The combination group led to the highest increase in apoptotic cell rate in concomitant with the highest increase in the levels of caspases-3,8 (PGB+LK<0.05). In addition, the highest number of apoptotic cells was also determined in the combination group during TEM evaluation.

Conclusions: In the current study, it's detected firstly that GB combined with LK shows a synergistic effect through the induction of extrinsic apoptosis.

Keywords: Gojiberry, L-Carnitine, Chronic Myeloid Leukemia, Phytotherapy, Apoptosis

S121

THE EFFECT OF ENRICHMENT OF BLACK TEA AND YOGHURT DRINK WITH VITAMIN D3 ON SERUM 25(OH)D3 LEVELS IN RATS

Eda Selçuk[1], Özgür Baykan[2], Hayrettin Kara[3], Saliha Uysal[2], Elif Aksöz[4]

[1] Muğla Training and Research Hospital, Department of Medical Biochemistry, Muğla, Turkey

[2] Balıkesir University School of Medicine, Department of Medical Biochemistry, Balıkesir, Turkey

[3] Balıkesir University Health Practice and Research Hospital

[4] Balıkesir University School of Medicine, Department of Medical Pharmacology, Balıkesir, Turkey

Objectives: Various measures are being taken by health authorities to prevent vitamin D deficiency in the world. One of these measures is to add vitamin D to some foods that are frequently consumed in the society which are easily available and economically appealing to the general public. We aimed to investigate the effect of adding vitamin D to yoghurt drink with the purpose of increasing serum vitamin D levels, thus causing less lactose intolerance compared to tea and yoghurt drink, which are the most frequently consumed drinks after water in the world.

Methods: The control (C) group was fed only with a purified diet. In addition to the purified diet, each of the other groups were fed with yoghurt drink(Y), yoghurt drink fortified with fat-soluble vitamin D (YF); yoghurt drink fortified with water-dispersible vitamin (YW), tea (T), tea fortified with fat-soluble vitamin D (TF), tea fortified with water-dispersible vitamin D (TW) for 40 days.

Results: Serum 25(OH)D_3 levels of the YW group were 161%(P<0.001),124%(P<0.001) higher than those of the control, yoghurt drink groups, respectively. The 25(OH)D_3 levels of the TF group were 71%(P=0.001), 80%(P=0.001) higher than those of the control, tea groups, respectively. The 25(OH)D_3 levels of the TW group were found to be 185%(P=0.001), 200%(P=0.001) higher than those of the control, tea groups, respectively.

Conclusions: Fortifying black tea with water-dispersible vitamin D_3, yoghurt drink with water-dispersible or fat-soluble vitamin D can be applied as an effective way to reduce vitamin D deficiency in the population.

Keywords: Yoghurt drink, Water-dispersble vitamin D, Fat Soluble vitamin D, Supplementation

S122

THE PROGNOSTIC NUTRITIONAL INDEX (PNI) USED TO PREDICT OUTCOMES IN ISCHEMIC STROKE PATIENTS BY ASSESSING THEIR NUTRITIONAL STATUS

Naile Mısırlıoğlu[1], Defne Ozen[2]

[1] Sbü Gaziosmanpaşa Eğitim Araştırma Hastanesi/ ıstanbul

[2] Mcgill University Montreal/kanada

Objectives: Malnutrition has been reported to be related to adverse prognosis in acute ischemic stroke (AIS) patients. Unfortunately, traditional nutritional assessment tools usually increase the workload of neurologists, which makes them unfeasible in clinic work. We aimed to elucidate the association between the prognostic nutritional index (PNI), an easily obtainable baseline nutritional marker, and outcomes in AIS patients.

Methods: The present study retrospectively included 1405 patients. PNI was calculated as 5*lymphocyte count (10^9 /L) + serum albumin concentration (g/L), and the good prognosis was defined as modified Rankin Scale score of 0-3. The relationship between PNI and clinical parameters was evaluated.

Results: We found that the patients in the low PNI group had a higher frequency of anemia (12.9 vs. 2.3%, $P < 0.001$) and a higher level of the Controlling nutritional status (CONUT) score ($P < 0.001$). The relationship between PNI and nutrition-related factors, such as body mass index ($r = 0.208$, $P = 0.001$), age ($r = -0.329$, $P < 0.001$), total cholesterol

($r = 0.268$, $P < 0.001$) and hemoglobin concentration ($r = 0.328$, $P < 0.001$), was significant. Low PNI value (adjusted odds ratio: 2.250, confidence interval: 1.192-4.249, $p = 0.012$) stayed as an independent predictor for the poor outcome.

Conclusions: The PNI was independently associated outcomes in AIS patients. As an easily obtainable nutritional marker, PNI may be a useful nutritional assessment tool in the clinic work.

Keywords: ischemic stroke malnutrition prognostic nutritional index

S123

EFFECT OF NUTRITIONAL DISORDER ON SERUM TRYPTOPHAN-KYNURENINE PATHWAY METABOLITE LEVELS

Asli Parlakcerez[1], Fatma Humeyra Yerlikaya Aydemir[1], Duygu Eryavuz Onmaz[1], Ali Kandeger[2], Sedat Abusoglu[1]

[1] Department of Biochemistry, Selcuk University Faculty of Medicine

[2] Department of Department of Psychiatry, Selcuk University Faculty of Medicine

Objectives: The Tryptophan-Kynurenine pathway (KY) plays a critical role in cellular energy production in the form of nicotinamide adenine dinucleotide. KY is a key regulator of the immune system as energy requirements increase significantly during the immune response. Nutritional disorders cover a broad spectrum, including malnutrition and overnutrition leading to obesity. Our aim in this study was to evaluate the effect of malnutrition on the tryptophan-kynurenine pathway, a novel pathway associated with inflammation.

Methods: Materials and Methods: In this study, serum quinurenine levels were analyzed by LC-MS/MS in 54 participants aged 18-65 years with BMI between 25-30 (Group 1), 31 participants with BMI below 18 (Group 2) and 74 participants with BMI above 30 (Group 3). The study procedure was based on protein precipitation followed by concentration of the samples under nitrogen and subsequent analysis by tandem mass spectrometry.

Results: Statistical analysis showed that serum tryptophan levels were lower in Group 2 compared to Group 1 and Group 3 ($p<0.05$). Serum quinurenine, quinurenic acid, quinolinic acid levels and quinurenine/tryptophan ratio were significantly higher in Group 3 compared to Group 1 ($p<0.05$). In Group 2, serum kynurenine, kynurenic acid levels and kynurenine/tryptophan ratio were statistically significantly higher and serum 3-hydroxyquinurenine levels were statistically significantly lower compared to Group 1 ($p<0.05$).

Conclusions: Our findings suggest that quinurenine pathway metabolite levels are altered in individuals with eating disorders. Further studies in a larger population are needed.

Keywords: Eating disorders, Tryptophan, Kinurenine pathway

S124

USING DIFFERENT BLOOD SAMPLES HAS A SIGNIFICANT EFFECT ON AMINO ACID CONCENTRATIONS OBTAINED BY LC-MS/MS

Ceylan Bal[1, 2], Esra Özyurt[1], Gülsen Yılmaz[1, 2]

[1] Ankara City Hospital, Department of Medical Biochemistry, Ankara, Turkey.

[2] Ankara Yıldırım Beyazıt University, Department of Medical Biochemistry, Ankara, Turkey.

Objectives: Newborn screening programs enable the detection of newborn errors by analyzing amino acids from a dry blood spot. Althought capillary blood obtained from a heel prick is the recommended sample, it has become a common occurrence for phlebotomists to drop blood from other tubes onto filter paper instead of heel blood because of the easy aplication. The aim of this study is to investigate amino acid levels from different sample types collected from the same individuals and compare the results with capillary blood samples.

Methods: 30 healthy individuals were included in this study. Blood samples collected to the citrate tubes (group 1), gel tubes (group 2),plain tubes (group 3), heparin tubes (group 4), ED-

TA tubes (group 5), and compared with fingertip samples (group 6). Amino acid levels (valine, leucine/isolosine, methionine, phenylalanine, tyrosine, aspartic acid, alanine, arginine, citrulline, glycine, glutamine, glutamic acid) were analyzed by using Liquid chromatography-mass spectrometry.

Results: Highest mean/median values were obtained from capillary blood samples. Most of the amino acids were found to be different ($p<0.05$) from peripheral blood results (except for valine in groups 3 and 5, leucine in groups 3, 4, and 5, and aspartic acid in group 5,methionine and glutamine in all the groups, citrulline in group3).

Conclusions: The significant difference obtained from the results can be explained by the low concentrations of major amino acids within cells. Therefore, a different sampling method should not be used in newborn screening, or the laboratory should be informed about the sample type.

Keywords: LC-MSMS, BLOOD, NBS, AMINO ACID

S125

TRIMETHYLAMINE-N-OXIDE AND UNCOUPLING PROTEIN-1 LEVELS IN GESTATIONAL DIABETES MELLITUS

Sara Çıbık[1, 2], Hüsamettin Vatansev[2], Muslu Kazım Körez[3], Esranur Turgut[2], Özlem Seçilmiş[4]

[1] Altınbaş University, Faculty of Medicine, Department of Medical Biochemistry

[2] Selçuk University, Faculty of Medicine, Department of Medical Biochemistry

[3] Selçuk University, Faculty of Medicine, Department of Biostatistics

[4] Selcuk University, Faculty of Medicine, Department of Obstetrics and Gynecology

Objectives: Gestational Diabetes Mellitus(GDM) is a disorder of glucose tolerance that begins or is first detected during pregnancy, leading to serious complications. Trimethylamine-N-oxide(TMAO) levels, a short-chain amine obtained by oxidizing Trimethylamine(TMA), have been reported to have a positive association with the risk of diabetes. Uncoupling Protein-1(UCP-1) is a protein recognized as a marker for the thermogenic activity of brown and beige adipose tissues. Activation of brown adipocytes increases UCP-1 levels and stimulates glucose uptake into brown adipose tissue. This study aimed to determine whether TMAO and UCP-1 levels in the first trimester are associated with the development of GDM.

Methods: 6-12 weeks pregnant women were followed up until the oral glucose tolerance test(OGTT) performed at 24-28 weeks and TMAO and UCP-1 were analyzed from their sera at these two stages. TMAO levels were measured by LC/MS-MS and UCP-1 levels were measured by sandwich ELISA. GDM group(n=30) and control group including healthy pregnant women(n=30) were compared according to gestational periods.

Results: The GDM group showed higher serum TMAO levels than the control group both at 6-12 weeks (118,73±14,09 vs. 98,89±31,23,p=0,003) and 24-28 weeks(206,73±90,69 vs. 79,33±37,01,p<0,001). At weeks 24-28, lower serum UCP-1 levels were found in the GDM group compared to the control group(8,32±1,71 vs. 9,73±1,77,p=0,047).

Conclusions: The analysis concluded that serum TMAO measurement in the first trimester can be used as a biomarker for the risk of developing GDM in the future, but UCP-1 cannot be used for this purpose. Further studies are needed to elucidate the relationship between GDM and TMAO and UCP-1.

Keywords: Gestational Diabetes Mellitus, Trimethylamine-N-Oxide, Uncoupling Protein-1, Brown Adipose Tissue

S126

EVALUATION OF TRISOMY 21 RISK WITH DOUBLE AND TRIPLE SCREEN TESTS: A CASE REPORT

Mine Busra Pehlivan, Berna Seyhan

Etlik City Hospital

Objectives: The double screen test is performed in the first trimester of pregnancy using a

computer programme to assess maternal age, gestational week, maternal weight, presence of nasal bone, nuchal translucency (NT), pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG). Triple screen test is performed in the second trimester of pregnancy with β-hCG, alpha-fetoprotein (AFP) and anconjugated oestriol-3 (uE3) tests.

Methods: A 23-year-old woman with no history of previous pregnancy and delivery was admitted to Etlik City Hospital at the 12th gestational week. The values obtained from the patient's blood and radiological results were entered into the Prisca package programme and risk assessments were made.

Results: The free β-hCG and PAPP-A values were found to be 63.1 ng/ml and 1.16 mIU/ml, respectively. Trisomy 13, 18 and 21 risks calculated using Prisca package programme based on these values and patient information were found to be below the cut-off value (<1:10000). AFP 15.9 IU/ml, uE3 0.14 ng/ml and 29335 β-hCG mIU/ml were determined after analysis of the sample received for triple screen. The risk for trisomy 21 was calculated as >1:50. This ratio was found to be above the cut-off value.

Conclusions: Implementation of prenatal testing reduces the incidence of interventional procedures that can lead to serious complications such as preterm birth, haemorrhage and fetal death. Therefore, it is of great importance to implement double, triple and quadruple screen tests, to improve their performance and to calculate the risks in the most accurate way.

Keywords: Trisomy 21, Down syndrome, Double screen test, Triple screen test

S127

OXIDATIVE DNA DAMAGE OF ACETHAMIPRID IN ZEBRAFISH (DANIO RERIO), VERTEBRATE MODEL ORGANISM

Gülsüm Koçak[1], Burcu Eser[2], Rabia Şemsi[2], Göktuğ Gül[3], Pınar Arslan[4], Aysel Çağlan Günal[5]

[1] Institute of Natural Sciences, Department of Environmental Sciences, Toxicology 100/2000 Scholar

[2] Department of Medical Biochemistry, Faculty of Medicine Gazi University, Ankara

[3] Environmental Health and Environmental Sciences Program, Health Services Vocational School, Gazi University, Ankara

[4] Biology Department, Faculty of Science, Çankırı Karatekin University, Çankırı

[5] Biology Education Department, Faculty of Gazi Education, Gazi University, Ankara Türkiye

Objectives: Pesticides are used globally to protect agricultural crops against damage by insects, diseases, or weeds. Acetamiprid (ACE) is one of the widely used commercial neonicotinoids in agricultural production and causes serious health problems of environmental pollution, aquatic organisms, and humans. DNA damage caused by sublethal concentrations of acetamiprid (24 and 72 hours) was studied in zebrafish (*Danio rerio*), which can be used as a model organism in ecotoxicological studies.

Methods: The fish used in the experiment were obtained from commercial suppliers in Ankara and experiments were carried out in Gazi University Department of Biology Education. Zebrafish have become an extensively used model organism for exploring the adverse impacts of pollutants in aquatic environments. The fish were placed in 10 L aquariums and adapted to laboratory conditions for 15 days. Zebrafish were exposed to 10 and 100 mg/L acetamiprid for 24 and 72 h. 8-hydroxydeoxyguanosine (8OHdG, ng/100 mg tissue) is a biomarker of oxidative DNA damage that was detected by LC-MS/MS.

Results: There was a significant difference between groups ACE-10-24h and ACE-10-72h exposure. The whole-body tissue 8-OHdG values were significantly increased in the ACE-24 h (10,00-13,17 ng/100 mg tissue) group compared to the control group (1,91-7,90 ng/100 mg tissue). These results suggest that ACE could lead to oxidative DNA damage by causing an increase in 8-OHdG levels.

Conclusions: The results of the study showed that exposure to acetamiprid had effects on the DNA damage of zebrafish. This study provided valuable in-

formation on the toxicity of ACE on zebrafish.

Keywords: Zebrafish, Acetamiprid, DNA damage, 8-OhdG, Toxicity

S128

EFFECT OF STORAGE PERIOD ON BLOOD ETHANOL LEVELS

Tuba Çakmak[1], Giray Bozkaya[2]

[1] University of Health Sciences, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

[2] University of Health Sciences, Izmir Faculty of Medicine, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

Objectives: In ethanol analysis, a back up sample is taken simultaneously in addition to the test sample and stored at -20 °C for 6 months according to the guideline of the Ministry of Health's 'Procedures and Principles of Ethanol Analysis Procedures in Blood Samples'. The first plasma sample studied with ethanol was also stored with the back up sample. If officially requested by legal authorities, the back up sample is sent to the Forensic Medicine Institute. Otherwise, samples are disposed of as medical waste after 6 months. Our study aimed to compare ethanol levels of 114 samples stored for 6 months with those initially measured.

Methods:: The plasma of the test sample was used because it was not possible to dissolve and separate the plasma of the back up sample stored as whole blood. Ethanol analysis was performed using the enzymatic method with alcohol dehydrogenase on Roche Cobas-6000. SPSS version V21.0 was used for statistical analysis. The distribution of variables was evaluated by Kolmogorov-Smirnov test and the data were compared by paired sample-t test. The significance level was determined as $p< 0.05$.

Results: Only 51 of the stored samples had an initial measured ethanol level of >10 mg/dL. The ethanol levels of these samples were statistically significantly lower than the first measured ethanol levels ($p<0.001$).

Conclusions: We confirmed that the ethanol verification test cannot be performed by storing the plasma of the initial sample and should be performed using a back up sample stored as whole blood in confirmation laboratories.

Keywords: Ethanol, analysis, Refrigeration

S129

EVALUATION OF MONOCYTE COUNT TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN AMPHETAMINE USERS

Kadriye Akpınar

Ministry of Health Burdur State Hospital, Medical Biochemistry Laboratory, Burdur, Türkiye

Objectives: Amphetamine is a widely abused psychotropic compound due to its stimulant effects. It damages peripheral organs by causing oxidative stress and the inflammatory process. Monocyte count to high-density lipoprotein cholesterol ratio (MHR) has been believed to be a new marker of inflammation and oxidative stress. The aim of the study is to investigate the relationship between MHR and amphetamine.

Methods:Amphetamine users on probation (n=31, male) and healthy individuals (n=31, male) were included in the study. Complete blood count was measured by Mindray BC-6800 hematology analyzer. HDL cholesterol and amphetamine were measured by Abbott C8000 automatic biochemical analyzer. Data were analyzed by SPSS for Windows version 22.0 for statistical analysis.

Results: The MHR was significantly higher in amphetamine users ($p<0.001$). There were no statistically significant differences in monocyte count, HDL cholesterol, other complete blood count parameters, and inflammatory indicators such as CRP, neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR).

Conclusions: The result of the study indicated that MHR can be an early indirect marker of oxidative stress and inflammation in amphetamine users.

Keywords: MHR, monocyte to HDL ratio, amphetamine

S130

METABOLIC EFFECTS OF ORAL TITANIUM DIOXIDE IN JUVENILE RATS: INSIGHTS FROM NMR-BASED METABOLOMICS ANALYSIS

Fatmanur Köktaşoğlu[1], Metin Demirel[1], Kübra İzler[2], Fatih Gültekin[3], Eray Metin Güler[4], Somer Bekiroğlu[5], Şahabettin Selek[1]

[1] Bezmialem Vakif University, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey

[2] University of Health Sciences, Hamidiye Institute of Health Sciences, Istanbul, Turkey

[3] Lokman Hekim University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

[4] University of Health Sciences, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey

[5] Tubıtak Marmara Research Center, Institute of Chemical Technology, Kocaeli, Turkey

Objectives: Nuclear magnetic resonance (NMR) spectroscopy in metabolomics allows comprehensive metabolite analysis. Titanium dioxide (TiO_2), commonly referred to as E171, is widely used as a food additive. We conducted NMR-based metabolomic analyses at the tissue level in juvenile rats after oral administration of E171-TiO_2.

Methods: Female Sprague–Dawley rats were divided into control and E171-TiO_2-treated groups (n=9). TiO_2 was orally administered at 100 mg/kg/day for 6 weeks. Metabolomic analysis was performed using a 500 MHz NMR instrument with homogenized kidney and liver tissue.

Results: 32 and 52 metabolites were detected in liver and kidney tissue samples, respectively. Alterations were found as 4 metabolites in the liver and 5 in the kidney, using the t-test. PLS-DA and pathway enrichment analyses were conducted. In the liver tissue samples from the E171-TiO_2-treated group, levels of N-acetylglucosamine, fumarate, serine were increased while uracil level was decreased compared to control group ($p<0.05$). Conversely, in the kidney tissue samples of E171-TiO_2-treated group, there was reduction in N-methylhydantoin, 1-methylhistidine, alanine, fumarate and methylamine levels comparing to controls ($p<0.05$). Pathway analysis revealed alterations in pyrimidine metabolism; alanine, aspartate, and glutamate metabolism in liver tissue. In kidney tissue, the affected pathways included the citrate cycle, pyruvate metabolism, glyoxylate and dicarboxylate metabolism.

Conclusions: Emerging evidence suggests a potential link between aberrant metabolite profiles in hepatic tissues and the development of insulin resistance and hepatotoxicity. Similarly, perturbed metabolite patterns in renal tissues have been associated with adverse impacts on renal function, resembling characteristics reminiscent of kidney cancer.

Keywords: Nuclear magnetic resonance spectroscopy, Metabolomics analysis, Titanium dioxide TiO2 exposure, Metabolic pathway alterations

S131

THE IMPORTANCE OF URINE INTEGRITY TESTS IN DRUG SUBSTANCE ANALYSIS USING LC/MS-MS:AN EVALUATION OF REJECTED SAMPLES

Ömer Faruk Çakmak[1], Özge Yakar[1, 2], Mehmet Şeneş[1]

[1] Ankara Training and Research Hospital Medical Biochemistry Dept. ,ankara

[2] Rotakim Analiz Hizmetleri, Ankara

Objectives: The aim of this study is to assess the necessity of rejecting initial samples from individuals who fail integrity tests(creatinine,density,pH,nitrite) before conducting drug substance analyses.This evaluation includes cases where new samples were collected from individuals who initially failed the integrity tests.

Methods: A total of 53patients were included in the study,which involved toxicology tests conducted using the SCIEXQTRAP-5500-Triple Quadrupole-LC-MS/MS system and integrity tests performed on the Beckman-Coulter-AU480 device. Results were categorized based on rejection reasons,and the values of analytes were evaluated before and after resampling.Negative results from both the initial and subsequent samples were excluded.Microsoft-Excel was used for data analysis.

Results:Among the 53patients,42 were rejected due to creatinine,27 due to density,and 1 due to pH issues. Out of the 53patients,20 were excluded because both the rejected and resampled samples yielded negative results.Of the remaining 33samples,1 was rejected due to pH,4 due to density,16 due to creatinine,and 12 due to both creatinine and density issues.When analyzing based on analyte values,52 out of 65negatives turned positive according to LOQ values.While 56positives remained unchanged,4results turned negative.Using cut-off values,60 out of 88negatives became positive,with 24positives remaining unchanged,and 2positives turning negative.Considering patient status,10 out of 12negative patients turned positive based on LOQ values,while 21positive patients remained unchanged.Using cut-off values,10 out of 14negative patients turned positive,and 2 out of 19positive results turned negative.

Conclusions:When comparing analyte values,it is observed that positives remained unchanged.It is suggested that,for LC/MS-MS analysis,evaluation based on the LOQ value may be more accurate than the threshold value.Additionally,re-sampling is recommended for false-negative patients,while re-sampling for patients with positive results may be unnecessary.

Keywords: Urine integrity test, toxicology, LC-MS-MS, drug analysis, urine

S133

INVESTIGATION OF THE EFFECTS OF KRILL OIL ON TESTICULAR TISSUE IN HIGH FAT DIET-INDUCED OBESE RATS

Eda Yılmaz Kutlu[1], Mehtap Atak[1], Zehra Topal Suzan[2]

[1] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Medical Biochemistry, Rize, Türkiye

[2] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Histology and Embryology, Rize, Türkiye

Objectives: New sources of dietary components continue to be identified for the amelioration of obesity nd obesity-related diseases. Antarctic krill is a recognized as a new alternative source of Ω-3PUFA. Krill oil is derived from krill and contains about 40% phospholipids and Ω-3 fatty acids. This study aimed to investigate the effects of krill oil on testicular tissue in high-fat diet-induced obese rats.

Methods: In this study, a total of 3 groups(n=24) were formed as control(control diet), case(High-fat-diet) and Krill Oil(HFT+Krill oil 600 mg/Kg/day). The groups were given a daily diet ad libitum for 12 weeks. Krill oil was given by oral gavage. Rats were weighed weekly. The animals were sacrificed when 20% weight gain was detected in the case group compared to the control group as an obesity criterion. TBARS and Total SH levels in testicular tissues and TNF-α levels in serum were analyzed. Testicular tissue was examined immunohistochemically was hematoxylin-eosin(H&E) staining.

Results: TBARS, Total SH and TNF- α levels were significantly increased in the control group ($p<0.01$) compared to the case and krill oil groups. As a result of H&E staining was observed that the difference between groups was not significant.

Conclusions: It is thought that the low TBARS, Total SH and TNF- α levels in the case group compared to the control group is due to the high-fat diet content (casein, vitamins and aromatic compounds). Therefore, although the given diet provided weight gain, it is thought to have a protective effect against inflammation and oxidative damage.

Keywords: Krill oil, obesity, testis

S134

INVESTIGATION OF THE TREATMENT EFFECTIVENESS OF SIMULTANEOUS INHIBITION OF TRPC3/6 ION CHANNELS IN THE MOUSE LIVER FIBROSIS MODEL

Ebru Onalan[1], Cemil Demir[2], Tugce Kaymaz[1], Ibrahim Hanifi Ozercan[3], Arzu Etem[4]

[1] Firat University, Faculty of Medicine, Department of Medical Biology, Elazig, Turkiye

[2] Mardin Artuklu University, Health Services Vocational School, Department of Medical Services and Technics, Mardin, Turkiye

[3] Firat University, Faculty of Medicine, Department of Medical Pathology, Elazig, Turkiye

[4] Usak Training and Research Hospital, Department of Medical Biochemistry, Usak, Turkiye

Objectives: Liver fibrosis is a pathological condition characterized by the formation of fibrotic tissue as a result of excessive accumulation of extracellular matrix (ESM) components such as collagen in the liver in response to inflammation or direct toxic damage. The aim of this study is to investigate the possible therapeutic effects of blockade of Transient Receptor Potential Cation Channels (TRPC3/6 ion channels) and the inflammatory pathways mediating this therapeutic effect at the biochemical, histological and molecular level in the liver Carbon Tetra Chloride (CCl_4) fibrosis model created in mice.

Methods: BALB/c strain mice were injected with CCl_4 to induce liver fibrosis. Expression levels of anti-inflammatory and inflammatory cytokine genes in liver and spleen tissues of all animal groups were investigated, histological and biochemical analyzes were performed in liver tissues.

Results: Our analysis data by qRT-PCR revealed that Peroxisome Proliferator Activated Receptor (PPAR) beta/NFATC1/NFATC3 (Nuclear factor of activated T-cells) expressions were significantly increased in mice exposed to CCl_4, and that both colchicine and TRPC3/6 inhibition suppressed this pathway. In terms of PPARs, it was observed that only PPARbeta expression increased significantly, but PPAR alpha and PPAR gamma expressions did not change significantly.

Conclusions: In the study, it was shown that TRPC3/6 inhibition plays an important therapeutic effect in alleviating hepatic toxicity in CCl_4-induced liver injury and can improve hepatic dysfunction and prevent histopathological changes. In addition, its mechanism is to reduce oxidative stress and inflammation.

Keywords: Trpc36, ppar, nfat, liver fibrosis, fibrosis

S135

ISCHEMIA MODIFIED ALBUMIN LEVELS IN MIGRAINE PATIENTS

Gözde Ülfer[1], Burcu Polat[2], Çağrı Çakıcı[1]

[1] İstanbul Medipol University, Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Türkiye)

[2] İstanbul Medipol University, Faculty of Medicine, Department of Neurology, İstanbul, Türkiye)

Objectives: The aim of the study is to investigate the serum Ischemia Modified Albumin (IMA) levels in migraine patients.Ischemia is caused by modification of albumin caused by reactive oxygen derivatives formed as a result of ischemia. It is also related to diseases associated with oxidative stress and inflammation. The pathophysiology of migraine bases on the effect of neurogenic inflammation and oxidative mechanisms.

Methods: The study population consisted of forty patients and forty control subjects.IMA measurements from serum were made with the albumin cobalt binding test described by Bar-Or et al.The difference between the absorbance values of the samples and the blanks at 470 nm were recorded as IMA values.

Results: There was no statistically significant difference between two groups for the age and the genders. ($p>0.05$).The IMA value of migraine group was found to be statistically significantly higher than control group ($p=0.001$; $p<0.01$). There wasn't any correlation between disease duration, attack duration, Visual Analogue Scale(VAS score)with IMA values ($p>0.05$).A weak correlation was found to be statistically significant ($r=0.358$; $p=0.023$; $p<0.05$) between the IMA values and the frequency of attacks.Receiver operator curve (ROC) analysis showed that the cut-off point for IMA was 0.389. Sensitivity was 70%, specificity was 80%, positive predictive value was 77.8% and negative predictive value was 72.7%. The area under the ROC curve obtained was determined as 80.4% with a standard error of 4.8%.

Conclusions: As a result of this study, elevated IMA levels in migraine patients support the presence

of ischemia, hypoxia and oxidative damage. The IMA level may be a predictive biomarker in migraine patients.

Keywords: migraine, IMA, Hypoxia, oksidative stress

S136

THE EFFECT OF EUGENOL ON PARAOXONASE-1 AND PARAOXONASE-3 LEVELS IN AML12 MOUSE HEPATOCYTES

Yagmur Ucar Yagci[1], Tugce Karabas[2], Mervenur Barakli Kocabas[2], Eray Ozgun[2]

[1] Edirne State Hospital, Medical Biochemistry Laboratory, Edirne, Türkiye

[2] Trakya University, School of Medicine, Medical Biochemistry Dept., Edirne, Türkiye

Objectives: Eugenol is a substance primarily extracted from clove plants and has medicinal use. Paraoxonase-1 and paraoxonase-3 are bound to high-density lipoprotein in circulation and have antioxidant and anti-atherogenic properties; the liver mainly synthesizes them. The effect of eugenol on paraoxonase enzymes is unknown. We aimed to investigate the effect of eugenol on paraoxonase-1 and paraoxonase-3 protein levels in AML12 mouse hepatocytes.

Methods: First, AML12 cells were treated with 25, 50, 100, 250, 500, and 1000 µM eugenol for 24 hours, and the effect of eugenol on cell viability in AML12 cells was evaluated by MTT assay. Then, cells were treated with 5, 50, and 500 µM eugenol for 24 hours, and paraoxonase-1 and paraoxonase-3 were measured by western blot.

Results: 25, 50, 100, 250, and 500 µM eugenol did not cause a significant change, but 1000 µM eugenol caused a significant decrease in the cell viability of AML12 cells. Eugenol caused a significant increase in paraoxonase-1 levels at 500 µM without affecting paraoxonase-1 levels at 5 and 50 µM concentrations. 5, 50, and 500 µM eugenol did not cause a significant change in paraoxonase-3 levels in AML12 cells.

Conclusions: Our study showed that eugenol increases paraoxonase-1 levels and does not change paraoxonase-3 levels in AML12 mouse hepatocytes.

Keywords: Eugenol, Paraoxonase-1, Paraoxonase-3, Liver, AML12 cells

S137

INVESTIGATION OF THE L-ARGININE-NITRIC OXIDE-ASIMETRIC DIMETHYL ARGININE PATHWAY IN AUTISM

Şükriye Yabancıün, Fatma Hümeyra Yerlikaya Aydemir, Sedat Abuşoğlu, Ali Ünlü

Selcukuniversityfaculty of Medicine, Department of Biochemistry, Konya, Turkey

Objectives: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by stereotypical behaviors that begin early in life and continue throughout life. Serum levels of oxidative molecules such as Asymmetric Dimethyl Arginine (ADMA) pathway metabolites and Nitric Oxide (NO) were measured to investigate the levels and pathophysiological role of some molecules involved in neurodegeneration in neurodegenerative diseases such as ASD.

Methods: Fifty newly diagnosed and followed-up patients, aged 0-6 years, who met the DSM-IV ASD classification criteria, who applied to the Child and Adolescent Psychiatry Outpatient Clinic of the Republic of Turkey Ministry of Health Konya City Hospital, were included in the study. A control group consisting of healthy children, 45 volunteers aged 0-6 years, who applied to the Ministry of Health Konya City Hospital Health Board and were not diagnosed with any autism or other medical disease, were selected. The levels of ADMA, SDMA, L-NMMA, L-arginine, homoarginine, citrulline and ornithine, which are metabolites in the ADMA pathway, in the serum samples of the participants were analyzed by LC-MS/MS method. Serum NO, NOS and DDAH1 enzyme levels were determined by ELISA method.

Results: Serum ADMA, L-NMMA, arginine and citrulline levels were statistically significantly higher in the patient group than in the control group, serum SDMA, homoarginine, ornithine, NO, NOS, DDAH1 levels were found to be statistically signi-

ficantly lower in the patient group compared to the control group.

Conclusions: İt is understood that ADMA pathway metabolites and NO may be biomarkers in the early diagnosis and treatment of children with ASD.

Keywords: Nitric oxide, Oxidative stress, Autism

S138

EVALUATION OF THE EFFECT OF LITHIUM USE ON THYROID HORMONES AND INFLAMMATORY MARKERS

Rabia Zülal Bilge[1], Aslıhan Bilge Bektaş[1], Giray Bozkaya[2]

[1] University of Health Sciences,izmir Bozyaka Education and Research Hospital,department of Medical Biochemistry,izmir,türkiye

[2] University of Health Sciences,izmir Faculty of Medicine,izmir Bozyaka Education and Research Hospital,department of Medical Biochemistry,izmir,türkiye

Objectives: Lithium theraphy is main prophylactic treatment for bipolar disorders. Lithium affects normal thyroid functioning through multiple mechanisms. Therapy with lithium often produces granulocytosis in psychiatric patients which creates inflammatory response. The aim of our study was to investigate the effect of lithium on thyroid hormone levels and systemic immune inflammation index.

Methods: Patient data who received lithium treatment for bipolar disorders were retrospectively collected from Hospital Information System. There were 41 patients who received lithium treatment for more than 3 months and with a therapeutic drug monitoring more than twice. Systemic immune inflammatory index is calculated using platelet, neutrophil and lymphocyte counts(P*N/L). The tests were performed on the Cobas 8000 Modular Analyzer System using serum samples. The data were evaluated by Kolmogorov-Smirnov, One-way ANOVA, Friedman, paired sample-t tests using Statistical Package for the Social Sciences V22.0. The significance level was determined as $p<0.05$.

Results: There was a slight TSH and systemic immune inflammatory index increase which was not significant statistically ($p>0.05$). At the 4th-8th months of the treatment, neutrophil, leukocyte and free T4 levels increased compared to those in 16th-20th months of the the treatment but again not statistically significant ($p>0.05$).

Conclusions:No statistically significant difference was obtained in short-term follow-up in our study. Since it is predicted that lithium may alter the tests by different mechanisms. longer-term follow-up should be performed for such patients.

Keywords: Lithium, Thyroid, Marker

S139

RELATIONSHIP BETWEEN SERUM EMPAGLIFLOZIN AND METFORMIN LEVELS AND HEMATOLOGICAL PARAMETERS

Menekşe Kuzu[1, 2], Duygu Eryavuz Onmaz[1], Firdevs Sak[1], Sedat Abuşoğlu[1], Ali Ünlü[1], Fatma Hümeyra Yerlikaya Aydemir[1], Gülsüm Abuşoğlu[3]

[1] Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Turkey

[2] Biruni University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkey

[3] Selcuk University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Konya, Turkey

Objectives: Metformin and empagliflozin are drugs used in the treatment of Type-II-Diabetes Mellitus (T2DM). Some hematological parameters are associated with endocrine stress, diabetic complications, and systemic inflammation. Studies show that neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) are new potential markers of inflammatory response. This study aims to investigate the relationships between empagliflozin-metformin drug levels and hematological parameters.

Methods:**:** Drug levels were measured with LC-MS/MS in serum taken from 43 T2DM patients using empagliflozin and 34 T2DM patients using metformin. The hemogram parameters of these patients were scanned retrospectively and statistical analysis was performed with the SPSS

Statistics 22.

Results: Metformin blood levels positively correlated with lymphocyte levels (r=0.484,p=0.004); and negatively correlated with NLR (r=-0.386,p=0.024), PLR (r=-0.466,p=0.005), MLR (r=-0.343,p=0.047), PCT (r=-0.378,p=0.028) levels. Empagliflozin blood levels positively correlated with lymphocyte levels (r=0.307,p=0.045); negatively correlated with hematocrit (r=-0.377,p=0.013), plateletcrit (r=-0.301,p=0.05), NLR (r=-0.31,p=0.043), PLR (r=-0.436,p=0.003) levels. When the hemogram findings of patients using empagliflozin and metformin were compared, the MPV value of patients using empagliflozin was statistically significantly higher than those using metformin (p=0.028). The two groups had no significant difference regarding other hemogram parameters (p>0.05).

Conclusions: In our study, serum empagliflozin and metformin levels were found to be associated with hematological parameters that may be indicative of diabetic complications, inflammation, endocrine stress. The limited number of patients and lack of clinician follow-up and evaluation of the patients are the main disadvantages of our study. Studies conducted in a larger population with clinicians are needed.

Keywords: Drug level, Hematological parameters, Type II Diabetes, Empagliflozin, Metformin

S140

DEVELOPMENT OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR OPIPRAMOL

Beyza Nur Ozkan[1], Firdevs Sak[1], Fatma Hümeyra Yerlikaya Aydemir[2], Sedat Abuşoğlu[2], Abdullah Sivrikaya[2], Ali Unlü[2]

[1] Selcuk University Institute of Health Sciences, Department of Medical Biochemistry

[2] Selcuk University Faculty of Medicine, Department of Medical Biochemistry

Objectives: Opipramol is a tricyclic antidepressant that does not inhibit neuronal uptake of norepinephrine and/or serotonin. It is considered a weak dopaminergic, histaminergic and serotoninergic antagonist and binds to sigma receptors in the brain. Recent data in the literature also reveal some of the dangers and side effects that may be associated with the use of opipramol. Therefore, our aim was to develop a rapid, specific and reproducible sensitive method for the quantitative determination of opipramol in serum.

Methods: 200 µL of sample was taken into eppendorf tubes and 800 µL methanol were added.The mixture was then vortexed for 30 seconds and centrifuged at 2000xg for 10 minutes. Supernatants were taken into clean glass tubes and dried at 40°C under nitrogen gas. The dried residues were dissolved in 150 µl of methanol:water (10:90% v/v) 30 µL was injected into LC-MS/MS system.

Results: Linearity for opipramol is in the range of 0.1-2000 ng/ml. Total working time is 5 minutes. The recision value was less than 7.5% and the accuracy results ranged from 95.3% to 103.4%. Extraction recovery ranged from 95.8% to 98.4%, and matrix effect values were less than 10% for all analytes.

Conclusions: A rapid, cost-effective, reproducible LC–MS/MS method for the quantitative determination of opipramol has been developed.

Keywords: Mass spectrometry, adverse effect, therapeutic drug monitoring

S141

BIOLOGICAL EFFECTS OF 6-HYDROXY-L-NICOTINE AND COTININE IN VARIOUS EXPERIMENTAL MODELS OF DEMENTIA: IN SILICO AND IN VIVO STUDY

Razvan Stefan Boiangiu, Ion Brinza, Marius Mihasan, Lucian Hritcu

Bioactive Research Group, Department of Biology, Alexandru Ioan Cuza University of Iasi, Romania

Objectives: The impact of two structurally related nicotinic derivatives, namely cotinine (COT) and 6-hydroxy-L-nicotine (6HLN), was evaluated on memory impairment, anxiety-like behavior and oxidative stress in two animal models of Alzheimer's disease (AD): a rat model induced by brain infusion of beta-amyloid peptide 25-35 ($A\beta_{25-35}$) and a zebrafish model induced by scopolamine (SCOP).

Methods: COT and 6HLN were administered to $A\beta_{25-35}$-treated rats and SCOP-treated zebrafish and their performances were evaluated using specific *in vivo* tasks. The oxidative stress parameters and acetylcholinesterase activity were measured from the brain samples of the animals. Using *in silico* tools, we attempted to associate behavioral outcomes with the theoretical binding potential of these compounds into two different allosteric binding sites of α4β2 nAChRs.

Results: $A\beta_{25-35}$ and SCOP decreased the memory performances and increased the anxiety-like behavior in the *in vivo* assays and increased the oxidative stress and acetylcholinesterase activity in the brain of rats and zebrafish, respectively. As compared to nicotine, COT and 6HLN ameliorated, more effectively, the memory deficits and anxiety caused by $A\beta_{25-35}$ or SCOP. Also, the nicotinic derivatives significantly reduced the oxidative stress and acetylcholinesterase activity in the brain of $A\beta_{25-35}$- or SCOP-treated animals. Furthermore, we showed that COT and 6HLN indeed bind to α4β2 nAChRs with similar or even higher energy than nicotine and that the α4-β2 binding site is preferred over the α4-α4 binding.

Conclusions: COT and 6HLN might improve memory and anxiety-like behavior by modulating cholinergic activity and thus might represent new neuropharmacological agents in AD.

Keywords: Alzheimers disease, cotinine, 6-hydroxy-L-nicotine, nicotine, animal model

S142

QUERCETIN REDUCES AXL LEVELS, AN IMPORTANT THERAPEUTIC TARGET, IN LX2 CELL LINE

Merve Özel, Gülden Başkol

Erciyes Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

Objectives: Liver fibrosis is a prevalent condition caused by various forms of chronic liver diseases. Recent studies have reported that AXL, a receptor belonging to the tyrosine kinase family and expressed in various tissues, is significantly released into the bloodstream during the development of liver fibrosis and hepatocellular carcinoma.Interleukin 6 (IL-6) is a versatile cytokine with a four-stranded structure that plays multiple roles within the body. In the liver, IL-6 serves as a crucial inducer of the acute phase response and contributes to the body's defense against infections. Quercetin is recognized for its diverse biological and pharmacological activities, encompassing antioxidant, antiviral, anti-inflammatory, antiproliferative, and antifibrotic effects. Indeed, the favorable impacts of quercetin on liver injury and fibrosis have been substantiated through numerous animal models. In this study, it was aimed to investigate the effect of quercetin on AXL and IL-6 levels in HSC line (LX2).

Methods:**:** Quercetin IC_{50} value was determined as 125 µM according to the literature. AXL and IL-6 levels were determined by ELISA method.

Results: We found that quercetin statistically decreased the levels of AXL and IL-6 in LX2 ($p<0.001$, $p<0.001$).

Conclusions: The reduction in AXL and IL-6 levels suggests a notable anti-fibrotic effect of quercetin. Several studies have demonstrated that AXL inhibition leads to a decrease in IL-6 levels. In our study, quercetin may have exerted its anti-fibrotic effect through the AXL-IL-6 mechanism. However, more research is needed to investigate the potential use of quercetin in the treatment of fibrosis.

Keywords: Liver fibrosis, quercetin, AXL, IL-6

S143

THE ROLE OF DOXORUBISINE-LOADED MESENCHYMAL STEM CELLS IN ANAPLASTIC THYROID CANCER TREATMENT

Ayşe Özlem Silistreli[1], Hilal Koçdor[1], Arzu Yıldırım[1], Ezel Bildik[1], Halil Ateş[2], Erdem Erinç Silistreli[3], Mehmet Ali Koçdor[4]

[1] Dokuz Eylül University, Oncology Institute, Department of Basic Oncology, İzmir, Turkey

[2] Dokuz Eylül University, Oncology Institute, Department of Clinical Oncology

[3] Dokuz Eylül University, Faculty of Medicine, Department of Cardiovascular Surgery

[4] Dokuz Eylül University, Faculty of Medicine, Department of General Surgery

Objectives: Mesenchymal stem cells(MSCs) are adult stem cells capable of self-renewal and multi-generation differentiation. It can be isolated from many tissues such as bone marrow and adipose tissue. It has been demonstrated by studies that isolated MSCs are promising drug delivery vectors for treating of cancer and other diseases.

Methods:**:** MSCs were obtained from the patient lipoaspyrate with approval.Isolated MSCs were confirmed with Flow cytometrics. In the cell line CAL-62(Anaplastic Thyroid Cancer-ATC), Doxorubisine (DOX) IC50 and IC25 concentrations were determined by the MTT method. After DOX was loaded into MSCs with these doses, MSCs were called Conditional Media(CM). Wound healing analysis was performed to evaluate the effect of CM on migration and metastasis. The oxidative stress index(OSI) was examined in cell homogenates.

Results: In the analysis performed by flow cytometric method, it was identified as MSC by confirming CD73+, CD90+ and CD105+.According to the time of closure in the Control and DOX50 groups in the Wound Healing Analysis, the percentage of clearance of CM50, and CM25 was 37.7%, and 23.1% respectively. When the OSI was evaluated; Significant statistical differences were detected between the control group and other groups($p<0,005$).

Conclusions: It was observed that CM groups obtained by loading DOX to MSCs reduced the migration rate in ATC cells according to DOX groups. When the effectiveness of CM50 and CM25 doses were compared between the groups, it was understood that there was no statistical difference. In the analyses carried out; It was observed that MSCs could be loaded with drugs and were as effective as standard chemotherapeutics at lower doses.

Keywords: Mesenchymal stem cells, Anaplastic Thyroid Cancer, Adipose tissue, Lipoaspyrate

S146

ANALYTICAL PERFORMANCE VALIDATION OF THE NEONATAL IRT FEIA KIT DEVELOPED FOR NEWBORN CYSTIC FIBROSIS SCREENING

Ceyhan Ceran Serdar[1], Kübra Almacıoğlu[2], Emrah Kömürcü[2]

[1] Ankara Medipol Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Abd, Ankara, Türkiye

[2] Bome Trivitron Sanayi Urunleri Dis Ticaret A.s, Ankara, Turkey

Objectives: The analytical performance properties of the Trimaris Neonatal IRT FEIA kit, which was developed to measure IRT levels in dried blood spots (DBS) quantitatively for use in newborn Cystic Fibrosis screenings, were examined via method comparison analyses.

Methods:: Two different methodologies were applied for the method comparison studies using DBS samples of IRT levels within the analytical limits. First, IRT measurements were performed in 130 DBS samples in parallel with Trimaris Newborn IRT FEIA and its equivalent kit, and the results were examined by regression analysis and Bland–Altman analysis. Additionally, IRT values of 52 external quality control samples obtained from CDC were measured by Trimaris Neonatal IRT FEIA, and the results were examined by regression and Bland–Altman analyses.

Results: In the first analysis, the correlation(R) value was found to be 0.96, with slope:1.044 (95%CI: 0.992 – 1.096) and intercept:5.497 (95%CI: 0.404 – 10.591); Bland–Altman analysis indicated that the bias was 9.004ng/ml(11%). In the second analysis, the correlation(R) value was found to be 0.99, with slope:1.097 (95%CI: 1.061 – 1.163), and intercept:0.37 (95%CI: -4.67 – 5.41); Bland–Altman analysis indicated that the bias was 10.15ng/ml (10%).

Conclusions: As the total allowable error in newborn screenings assays using DBS is 30%, the bias values obtained in method comparison studies conducted both using the equivalent kit, and external quality control samples lie within the acceptable range.

Slope and intercept values within acceptable limits, and high correlation coefficients show that Trimaris Neonatal IRT FEIA performs quantitative IRT measurements from DBS samples accurately.

Keywords: neonatal screening, Dried Blood Spots DBS, IRT, Cystic Fibrosis, FEIA

S147

EFFECT OF SILYMARIN ON ACRYLAMIDE-INDUCED APOPTOSIS AND CYTOTOXICITY IN MOUSE HEPATOCYTE CELL LINE

Mervenur Barakli Kocabas[1], Tugce Karabas[1], Yagmur Ucar Yagci[2], Gulben Sayilan Ozgun[1]

[1] Trakya University, School of Medicine, Medical Biochemistry Dept., Edirne, Türkiye

[2] Edirne State Hospital, Medical Biochemistry Laboratory, Edirne, Türkiye

Objectives: Although previous studies reported the hepatoprotective effects of silymarin, its effect on acrylamide-induced hepatotoxicity is unknown. The present study aimed to determine the effect of silymarin on acrylamide-induced apoptosis and cytotoxicity in AML12 cells.

Methods: We treated AML12 cells with 12.5, 25, 50, 100, 200, and 400 µg/ml silymarin for 24 hours to determine the effect of silymarin on cell viability. AML12 cells were treated with 12.5, 25, and 50 µg/ml silymarin simultaneously with 10 mM acrylamide for 24 hours to determine the effect of silymarin on acrylamide-induced cytotoxicity. Cell viability was evaluated by MTT assay. AML12 cells were treated with 12.5 and 25 µg/ml silymarin simultaneously with 10 mM acrylamide for 24 hours to determine the effect of silymarin on in acrylamide-induced apoptosis. Then, procaspase-3 and cleaved caspase-3 levels were measured by immunoblotting.

Results: 400 µg/ml silymarin caused a significant decrease in cell viability in AML12 cells, and 100 and 200 µg/ml silymarin-treated cells were below 90% of the control group. Acrylamide caused a significant increase in cleaved caspase-3 levels and a significant decrease in cell viability. 12.5 and 25 µg/ml silymarin caused a significant increase, but 50 µg/ml silymarin did not change cell viability in acrylamide-treated cells. Procaspase-3 levels were not significantly different between groups. 12.5 and 25 µg/ml silymarin caused a significant decrease in cleaved caspase-3 levels in acrylamide-treated cells.

Conclusions: This study showed that silymarin caused a significant decrease in acrylamide-induced apoptosis and cytotoxicity in mouse hepatocyte cells.

Keywords: Acrylamide, Silymarin, Apoptosis, Cytotoxicity, AML12 cells

S148

POTENTIAL BENEFICIAL EFFECTS OF BROMELAIN AGAINST OXIDATIVE DAMAGE CAUSED BY METHOTREXATE IN RAT TESTICULAR TISSUE

Kürşat Kaya

Pamukkale University, Faculty of Medicine, Department of Medical Biochemistry, , Denizli, Türkiye

Objectives: Methotrexate is an antiproliferative folic acid antagonist used in the treatment of various types of cancer. However, the clinical use of methotrexate in cancer treatment is limited due to some side effects. Bromelain, obtained from of the unripe pineapple plant, is a mixture of proteolytic enzymes. Antioxidant effects of bromelain have been shown in many studies. This study aimed to investigate the potential beneficial effects of bromelain against male reproductive system oxidative damage caused by methotrexate administration.

Methods: Twenty eight rats were randomly divided into 4 equal groups. The rats in the first group were kept as the control group. The rats in the second group were given bromelain by gavage at a dose of 200 mg/kg once a day for 14 days. The rats in the third group were administered a single dose of 20 mg/kg methotrexate intraperitoneally on the third day. Bromelain and methotrexate were administered together in the same dose and manner to the rats in the fourth group.

Results: Methotrexate administration did not cause any significant change in MDA and GSH levels

and SOD and GPx activities compared to the control group, but caused a significant decrease in CAT activity (p=0,005). Administration of bromelain along with methotrexate caused a significant decrease in GPx activity (p=0,015) and a significant increase in CAT activity (p<0,001) compared to the group administered methotrexate alone.

Conclusions: In this study, methotrexate administration caused a partial oxidative damage, depending on the number of doses and duration of administration probably. Co-administration of bromelain with methotrexate showed limited protective effect.

Keywords: Methotrexate, Bromelain, Reproductive Damage, Oxidative Stress, Testicular Tissue

POSTER PRESENTATION ABSTRACTS

P001

ULTRASENSITIVE HIV-ENVELOPE PROTEIN GP41 DETECTION WITH MACHINE LEARNING BY ANTIMICROBIAL BIOSENSOR

Ergin Taşkın[1], Zihni Onur Uygun[2]

[1] Ağrı İbrahim Çeçen University, Faculty of Medicine, Department of Medical Biochemistry, Ağrı, Türkiye

[2] Kafkas University, Faculty of Medicine, Department of Medical Biochemistry, Kars, Türkiye.

Objectives: In this novel investigation, we pioneered the development of an innovative approach in the area of scientific literature. Our focus centered on the engineering of label-free HIV-gp41 targeting antimicrobial peptide sequences, acting as a biorecognition receptor for the identification of the HIV envelope protein.

Methods:The methodology involved a remarkably straightforward and exquisitely sensitive immobilization technique utilizing polystyrene thiol-terminated (pS) compounds in conjunction with gold nanospheres, which formed a linkage through SH bonds with the peptides and gold nanospheres (GNs). Through this immobilization process, the robustness of the biosensor interface was significantly enhanced, thus ensuring the steadfastness of the gold nanospheres on the biosensor platform. A comprehensive analysis of the modified composite material and sensor surface was conducted utilizing electrochemical impedance spectroscopy (EIS). This analytical procedure not only facilitated the characterization of the modifications but also facilitated the realization of remarkably sensitive detection capabilities for gp41 at levels as pictograms.

Results:The biosensor exhibited exemplary performance metrics, with a linear calibration range between from 5 to 600 pg/mL, underscored by an impressive coefficient of determination (R^2). The limits of detection (LOD) and quantification (LOQ) were calculated as 1.75 and 5.3, respectively, further attesting to the biosensor's remarkable sensitivity. Conclusions: Moreover, the biosensor exhibited a strong correlation and successfully identified spiked samples with minimal matrix effects. Impedimetric signals were processed, fitted and calculated by using machine learning Python codes to find the concentrations.

Keywords: biosensor, HIV, impedance, machine learning

P003

EVALUATION OF PARAMETERS USED IN COMPLETE URINE EXAMINATION TO DIAGNOSE URINARY TRACT INFECTION

Duygu Öcal[1], Özlem Doğan[2], Beril Yazıcı[3], Haluk Furkan Hanedan[3], Melis Demir[3], Elif İrem Arslan[3], Atalay Mert Yüksel[3], Yade Sultan Kurt[3], İsmail Utku Bilge[3]

[1] Ankara University, Faculty of Medicine, Department of Microbiology, Ankara,türkiye

[2] Ankara University, Faculty of Medicine, Department of Biochemistry, Ankara, Türkiye

[3] Ankara University, Faculty of Medicine, Ankara, Türkiye

Objectives: Urinary tract infections (UTI), which affect approximately 150 million people annually, are both One of the most common infections in hospitalized patients and in the general population. In this study; in urine, morphological and the detection of the presence of genetically differentiated Atypical Cells with autoanalyzers is demonstrated in urinary tract infections.

Methods:The study included 203 patients who were sent to Cebeci Center laboratory and had atypical cell positive results and urine culture. Atypical cell amount, leukocyte esterase activity, leukocyte count,-nitrite positivity, bacterial count, age and gender results will be evaluated.

Results:Power of diagnosing Urinary tract infection (UTI) in ROC analysis; Leukocyte count AUC:0,781. Number of bacteria AUC: 0,826. Diagnosing UTI using leukocyte esterase activity sensitivity in the process; It was found to be 68.51% for 500 U (c/µL) value. The sensitivity of the presence of nitrite in urine to diagnose UTI is 36.8%; selectivity is 96.6%. ROC performed to determine the power of atypical cell level to diagnose UTI AUC was found to be 0.689 and the cut off value was determined using the Youden index calculated as 0.105 µl. At this cut-off value, the sensitivity of the test is 0.829; selectivity is 0.476 ($p<0.001$).

Conclusions: It has been observed that leukocyte

count, bacterial count and atypical cell level can help in diagnosing UTI.

Keywords: urine tract infection, urine culture, atypical cell

P004

COMPARISON OF SERUM FREE LIGHT CHAIN AND PARAPROTEIN LEVELS IN MONOCLONAL GAMMOPATHIES

Raneen Sliman, Sammar Nafafaa

Ziv Medical Center

Objectives: Multiple myeloma (MM) is the second most common hematological malignancy. Since 2001, the serum free light chain (FLC) test has been available and its clinical utility proven, but guidelines have not recommended it as a replacement for electrophoresis. There is no evidence about concordance between paraprotein levels and FLC measurements

Methods:serum FLCκ and FLCλ (sFLC) concentrations are routinely measured at the Ziv Medical center biochemistry labs by Freelite® (The Binding Site Group Ltd, UK) . Paraprotein levels are measured by Sebia Capillarys 2 Instrument from serum electrophoresis and immunofixation. Correlation between paraprotein value and light chain in serum to examine the relationship between FLC and paraprotein levels in order to determinate significance of this ratio for patient treatment and for obtaining more meaningful information from these test results.

Results:The preliminary results show us a correlation (R^2=91, R^2=85) between paraprotein and FLC ratio and between paraprotein and Kappa light chain in IgG kappa gammopathy. In case of IgA gammopathies these correlations are less significant.

Conclusions: We found good agreement between methods for MM response assessment, but the FLC test analysis was more sensitive than the paraprotein evaluation during follow-up in monoclonal gammopathy patients. We will report on patients that have different patterns of paraprotein secretion in different types of heavy and light chains MM. Assessment the concordance or discordance between FLCκ, FLCλ or FLCκ/FLCλ and Paraprotein levels can be useful tool in monoclonal gammopathies diagnosis, follow-up and treatment.

Keywords: multiple myeloma, free light chains, paraprotein

P005

ROTATIONAL THROMBOELASTOMETRY AND CONVENTIONAL COAGULATION TESTS IN PATIENTS UNDERGOING CARDIAC SURGERY

Isabel Rodriguez Martin

Hospital Universitario Virgen Del Rocío

Objectives: Viscoelastic tests (rotational thromboelastometry, ROTEM®), together with the implementation of a specific algorithm for coagulation management in cardiac surgery, enable perioperative coagulopathy to be better controlled.

Methods:Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of allogeneic blood transfusions and clinical postoperative complications were analyzed before and after ROTEM® implementation.

Results:Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative period. In the group monitored with ROTEM® , decreased incidence of transfusion was observed for packed red blood cells (31.3% vs 19.8%, p=0.002), fresh frozen plasma (9.8% vs 3.8%, p=0.008), prothrombin complex concentrate administration (0.9% vs 0.3%, p=0.599) and activated recombinant factor VII (0.3% vs 0.0%, p=0.603). Increased incidence was observed for platelet transfusion (4.8% vs 6.8%, p=0.530) and fibrinogen concentrate (0.9% vs 3.5%, p=0.066), tranexamic acid (0.0% vs 0.6%, p=0.370) and protamine administration (0.6% vs 0.9%, p=0.908). Similar results were observed in the postoperative period, but with a decreased incidence of platelet transfusion (4.8% vs 3.8%, p=0.813). In addition, statistically significant reductions were detected in the incidence of postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035), and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026).

Conclusions: The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with dec-

reased incidence of allogeneic blood transfusion, clinical hematologic postoperative complications and lengths of ICU stay.

Keywords: Rotational thromboelastometry, coagulation, cardiac surgery

P006

IMPORTANCE OF MEDICAL BIOCHEMISTRY SPECIALIST MANAGEMENT IN LABORATORY QUALITY : A BEFORE/AFTER STUDY

Hilmi Furkan Arslan

Giresun Maternity and Pediatrics Training and Research Hospital, Department of Medical Biochemistry, Giresun, Türkiye

Objectives:Internal quality controlvalues; We aimed to compare the sigma values of the tests studied in our laboratory as a result of transferring them to the laboratory information system and evaluating them in accordance with the Westgard rules under the supervision of a medical biochemist.

Methods:Giresun Maternity/Child Hospital Biochemistry Laboratory has been managed by an active medical biochemistry specialist since December 2022. Between June 2022 and June 2023, the total %CV, bias, %TEa (Total allowable error) and sigma values of four hormone tests routinely studied in our laboratory were examined monthly. The coefficient of variation (total %CV) was obtained from internal quality control data, bias from external quality control data, and %TEa from CLIA''2024'' data. Sigma values were calculated using the formula Sigma=(%TEa-%Bias)/%CV. The tests were performed on the RocheCobas e601 device.

Results:When the sigma values between 2022 July-December were averaged, (Prolactin, FSH, LH) sigma values (<3) were found to be unacceptable, (Estrodiol) sigma values (3-6) were acceptable. The sigma value of any hormone test wasn't found to be perfect (≥6). When the average between January-June 2023 is considered, only (FSH) has an unacceptable (<3) sigma value, while the performance of the other three tests (LH,Estrodiol,Prolactin) has increased and sigma values (3-6) are at acceptable levels.

Conclusions: It has been shown that the transfer of internal quality control data to the laboratory information system under the management of a medical biochemist, the application of westgard rules, and regular training to the employees will increase the laboratory quality.

Keywords: Laboratory management, Sigma, CLIA2024

P007

NOISE EXPOSURE OF MEDICAL LABORATORY EMPLOYEES

Metin Özdemir[1], Tülay Özdemir[2]

[1] Samsun Gazi State Hospital, Department of Medical Microbiology, Samsun, Türkiye

[2] Samsun Gazi State Hospital, Department of Medical Biochemistry, Samsun, Türkiye

Objectives: To investigate noise levels and employee exposure in the laboratory ambient using standardized methods.

Methods:Noise and exposure measurements were conducted by a specialized company (TETRA-Test) in various sections of our hospital, including the laboratory. The Delta Ohm HD-2110 portable noise measurement device was calibrated using the CESVA Model CB006-Acoustic Calibrator before ambient noise measurements were taken (TS-EN-ISO/9612). In each laboratory room, a Svantec SV104 noise dosimeter sensor was placed on an individual's collar to measure personal task-based noise exposure (TS-ISO/1996-2).

Results:Laboratory ambient measurements (Leq/Lpeak) and employee exposure (Lex, 8h/LcPk) were as follows:

Biochemistry: Ambient 70.3 dBA/122.4 dBC, Employee 72.9 dBA/113.9 dBC

Bacteriology laboratory: Ambient 67.6 dBA/118.5 dBC, Employee 72.2 dBA/106.5 dBC

Pathology macroscopy: Ambient 69.9 dBA/121.1 dBC, Employee 75.7 dBA/125 dBC

Nuclear Medicine: Ambient 72.9 dBA/117.8 dBC, Employee 68.7 dBA/126.8 dBC

Conclusions: The measurements were found to be be-

low the minimum exposure action values specified in the regulations, which do not require the use of personal protective equipment (80 dBA, Lex, 8h). Personal task-based noise exposure exceeded ambient measurement values in all laboratory sections except for nuclear medicine.

Our laboratories are not considered high-risk areas for health and safety issues related to noise exposure, especially hearing-related risks. However, there is noise present that may affect work performance, increase stress, and cause distraction among employees. Laboratories are not entirely noise-safe environments.

Architectural planning, equipment selection, and placement in laboratories should take noise factors into account. Employee rotation should be implemented.

Keywords: laboratory, noise level, exposure

P008

MANAGEMENT OF INTERNAL QUALITY CONTROL IN HAEMOSTASIAS AT HEMATOLOGY LABORATORY FARHAT HACHED HOSPITAL, SOUSSE

Khalil Atıg[1], Néjia Brahem[2]

[1] Monastir University, Faculty of Health, Department of Medical Biology, Monastir, Tunisia

[2] Monastir University, Faculty of Pharmacy Monastir Tunisia

Objectives: Internal quality control primarily checks the fidelity and accuracy of the results of medical analyses.The aim of our work is to identify the achievements and the insufficiencies of the hemostasis IQC in our laboratory.

Methods:For our study, we cited the characteristics of the Laboratory's automaton, its dosing principle and the IQC sample used. Then we made an inventory of the internal quality control program, inventoried the good practices already existing and noted the insufficiencies.

Results:This study made it possible to highlight obvious aspects regarding the management of the IQC at the level of the monitoring of hemostasis examinations such as the use of DM-DIV reagents, three levels of IQC, etc.) and shortcomings such as the absence of documentary procedures specific to the IQC, the absence of records describing the curative and corrective actions and poor precision for the high level of the IQC of Prothrombin with a systematic error by default. This work has made it possible to make improvements to the management of the IQC mainly: By setting up procedures specific to the IQC, the creation of a program on the Excel software for the exploitation of the results allowing the study of the accuracy and the fidelity of the results, the comparison of CVs with international acceptability limits.

Conclusions: Following this work, the laboratory will apply the main actions proposed, to all the examinations carried out on the various automatons. He will provide training to technical staff on the management of the CIQ quality.

Keywords: ISO 15189, IQC, Clinical Biology, Haemostasias, Quality Management

P009

OBJECTIVE: THIS IS THE FIRST TIME YOU HAVE HEARD THE TERM "CORRECTED FASTING PLASMA GLUCOSE".

Ahmet Coşkun, Atilla Ünal

Köşdere Aile Sağlığı Mrekezi, Family Medicine Unit

Objectives: In Family Health Centers and most second and third-level hospitals, blood is taken from venous veins and collected in yellow jelly tubes. Then, it undergoes centrifugation, and Serum Glucose is analyzed using autoanalyzers. Unfortunately, due to an error, the results are consistently reported as Plasma Glucose. In reality, international diagnostic criteria are based on Plasma Glucose levels, yet in Turkey, we erroneously employ Serum Glucose as a diagnostic criterion. Consequently, Diabetes and Gestational Diabetes often go undetected.

Methods:For instance, pregnant woman with an actual gestational diabete is normally diagnosed when the Fasting Plasma Glucose is 92mg/dl. A pregnant woman whose Serum Glucose is 88mg/dl is equivalent to 92mg/dl Plasma Glucose value. Formarly, it was known that Macrosomia in pregnant women starts at 85mg/dl and above, and the Fasting Plasma Glucose is in the level of 85mg/dl. A further example is a normal patient with 120mg/dl value of Serum Glucose actually has 126mg/dl value of Plasma Glucose which known as Diabetes.

Results:Certainly, the diagnosis of diabetes is not reliant on a single measurement but at least two se-

parate measurements, with Fasting Plasma Glucose exceeding 126 mg/dl on separate days. Consequently, laboratory results should lead us to accept 120 mg/dl and above as indicative of Diabetes. Shouldn't we, therefore, acknowledge 126 mg/dl and above as the Corrected Fasting Plasma Glucose based on laboratory findings? Thus, the problem resolves itself.

Conclusions: Given the critical role that numerical values play in diagnosing DM and Gestational Diabetes Mellitus, it is imperative to enter these numbers accurately.

Keywords: Glucose, Serum, Plasma, C-FPG, DM-GDM

P010

DEVELOPMENT OF 3-METHOXY-L-TYROSINE (3-OMD) LC-MS/MS METHOD IN DRIED BLOOD SPOT (DBS) SAMPLES

Hazal Yilmaz, Murat Emrah Mavis, Gokce Goksu Gursu

Altium International Lab. Cih. A.ş., Ar-ge Merkezi

Objectives: Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare neurotransmitter disorder. Genetic variants of the AADC-encoding Dopa Decarboxylase (DDC) gene lead to decrease in catecholamines while increase in 3-methoxy-L-tyrosine (3-OMD) concentration. The aim of the study is based on the quantitative analysis of 3-OMD as a biomarker in DBS sample using LC-MS/MS.

Methods:With respect to the method, 3 mm punch of DBS was extracted with 200 µL extraction reagent at room temperature for 15 min. Following the extraction step, the extract was pipetted to HPLC vial with glass insert. Finally, the extract was injected to Agilent HPLC system (consisting of flexible pump-G7104A, column compartment-G7116B and autosampler-G7129C) equipped with Agilent 6465B triple quadrupole mass spectrometer (Ultivo). The total run times from injection to injection was 10 min.

Results:The linearity and accuracy of the methods were evaluated using four DBS-based calibrators and two DBS-based internal quality controls. As a result of the validation study, LOQ-LOD values were of 0.018 and 0.005 µM, respectively with 0.998 as R^2 value. The values of RSD% are for low and high concentrations 2.15 and 1.89, respectively. Recovery values were calculated 109.5% for low concentration, 105.8% for high concentration.

Conclusions: Quantitative analysis of 3-OMD, which is a biomarker of AADC deficiency, has an important role in the diagnosis. The developed LC-MS/MS method focus on rapid sample preparation (without derivatization, extraction and injection), which contributes to reliable results. In addition, the developed methodology is linked to the NBS screening kit and validation kits.

Keywords: 3-OMD, Chromatography, LC-MSMS, Biomarker, Dried blood spot

P011

DEVELOPMENT OF A SIMPLE LC-MS/MS METHOD FOR MEASUREMENT OF URINARY 8-HYDROXY-2-DEOXYGUANOSINE (8-OHDG) AS OXIDATIVE STRESS MARKER

Hazal Yilmaz, Murat Emrah Mavis, Gokce Goksu Gursu

Altium International Lab. Cih. A.ş., Ar-ge Merkezi

Objectives: Oxidative stress reflects a disturbance in the balance between production and accumulation of reactive oxygen species (ROS). As ROS level is high, oxidative damage occurs in proteins, lipids and DNA. 8-hydroxy-2-deoxyguanosine (8-OHdG) is a product of oxidative modifications of DNA thereby, urinary 8-OHdG is a non-invasive biomarker of oxidative damage to DNA. In this context, we developed an analytical method for the quantification of urinary 8-OHdG based on LC-MS/MS.

Methods:Urine sample was pipetted (100 µL) to a vial. Then 25 µL of internal standard was added subsequent to adding 375 µL of Reagent-1. Finally, the vial was injected to Agilent HPLC system equipped with 6465B triple quadrupole mass spectrometer (Ultivo). The total run time was 9 min.

Results:The linearity and accuracy of the methods were evaluated using six urine-based calibrators and three urine-based internal quality controls for all works in this paper. As a result of the validation study, the R^2 value was 0.999; RSD% values are 4.97 for low concentration, 1.99 for medium concentration, 1.42 for high concentration; recovery values were determined 99.4% for low concentration, 91.4% for medium concentration, 101.7% for high concentration and LOQ-LOD values of 0.156 and 0.047, respectively.

Conclusions: DNA damage is usually repaired ma-

inly by base excision repair and oxidized products are excreted in the urine. 8-OhdG is one of the most studied oxidized metabolites and is considered a biomarker for oxidative damage of DNA. Jasem LC-MS/MS methods focus on rapid sample preparation (differentiation, without the use of SPE cartridges) contributing to reliable results.

Keywords: Oxidative Stress Marker, 8-OHdG, LC-MSMS

P012

PRECISION OF COBAS C311 ROCHE ANALYZER IN DETERMINING THE VALUES OF TOTAL CHOLESTEROL – VERIFICATION STUDY

Melda Emin, Hristina Ampova, Irena Kostovska, Julijana Brezovska - Kavrakova, Svetlana Cekovska, Sonja Topuzovska, Jasna Bogdanska, Danica Labudovic, Katerina Tosheska - Trajkovska

Institute of Medical and Experimental Biochemistry, Medical Faculty, University "ss Cyril and Methodius", Skopje

Objectives: The verification of examination procedures is essential for biochemical laboratories in order to evaluate the analytical characteristics of automated analyzers. This process supports laboratory quality service and clinical decision making.

The aim of our study was to perform verification process fortotal cholesterol assay for Cobas c311 following the CLSI EP15-2 guidelines.

Methods:The verification was performed using two levels of quality control (QC) materials (normal and pathological)provided by the manufacturer, which were run three times a day, as three replicates for 5 consecutive days using Roche/Hitachi Cobas c311 analyser. We compared the intra-assay coefficient of variation (CV), and the inter-assay coefficient of variation (CV) between replicates with the manufacturer's claim.

Results:The average of all results for QC material with normal values was 2.35. The intra-assay CV value was 0.5, compared with the manufacturer's claimed value which is 1.1. The inter- assay CV value was 0.5, compared with the value claimed from the manufacturer being 1.6. For the pathological QC pool, the average of all results was 4.20. The intra-assay CV value from our measurements was 0.6 in comparison to the manufacturer's intra-assay CV value of 0.9. As for the inter-assay CV value, our measurements showed 0.4 and the manufacturer claimed value is 1.6.

Conclusions: Our study has confirmes that calculated values have been consistent with the manufacturer's claimed values.This study allowed the initial training and familiarization with the instruments and the identification of operational issues. It also represented an opportunity to evaluate the QCs and to obtain analytical performance information for quality assurance.

Keywords: Analytical performance, precision, verification, laboratory standardization

P013

ESTIMATING INTRA- AND INTER- ASSAY COEFFICIENTS OF VARIABILITY FOR LACTATE DEHYDROGENASE ENZYME ON COBAS c311 ROCHE ANALYZER

Hristina Ampova, Melda Emin, Irena Kostovska, Katerina Tosheska-trajkovska, Svetlana Cekovska, Julijana Brezovska-kavrakova, Jasna Bogdanska, Danica Labudovic, Sonja Topuzovska

Institute of Medical and Experimental Biochemistry, Medical Faculty, University "ss Cyril and Methodius", Skopje

Objectives: Lactate dehydrogenase enzyme assay is used to aid identifying location and graveness of tissue damage, or monitor how far certain conditions have advanced. LDH enzyme, composed of four subunits, can form five isoenzymes with divergent tissue dispersal. An elevation can imply heart and skeletal muscle damage, or liver malady. Emerging studies corroborate essential requirement in Oncology, establishing interrelation between elevated levels with poor clinical outcome and resistance to therapy.

Methods:We used two pools of control serums, provided by the manufacturer, normal and pathological. Our protocol contained triplicate measurements, three times per day, five days in a row. The method was particle-enhanced immunoturbidimetric, on Roche Cobas c311 analyzer. We compared the intra-assay CV and the inter-assay CV between replicates with the manufacturer's claim.

Results:The average of all results for the normal

pool was 164.1. The intra-CV value was 0.3, compared with the manufacturer's claimed value 0.8. The inter-CV value 0.2, compared with the value claimed from the manufacturer being 1. For the pathological pool values, the average of all results was 298.0. Our intra-CV value was 0.3 in comparison to the manufacturer's intra-CV value of 0.7. As for the inter-CV value, our measurements showed 0.2 and the manufacturer claimed value is 0.9.

Conclusions: We concluded that the results for LDH enzyme assay on the ROCHE Cobas c311 platform demonstrate that it is a reliable immunoturbidimetric assay, with the measured and calculated values being consistent with the manufacturer's claimed values.The intra- and inter-assay coefficients of variation were in the target range of 5% and 10% respectively.

Keywords: assay-precision, inter-assay CV, intra-assay CV, lactate-dehydrogenase enzyme

P014

A PILOT PERFORMANCE EVALUATION OF CEREBROSPINAL FLUID BIOMARKERS USED FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE

Tuba Saadet Deveci Bulut[1], Nigar Esra Erkoç Ataoğlu[2], Ümit Özgür Akdemir[3], Lütfiye Özlem Atay[3], Özlem Gülbahar[1]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

[2] Gazi University, Faculty of Medicine, Department of Neurology, Ankara, Turkey

[3] Gazi University, Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey

Objectives: Alzheimer's Disease (AD) is the most common cause of dementia in the elderly population. Its pathophysiology is associated with formation of extracellular β amyloid plaques and accumulation of intraneuronal hyperphosphorylated tau proteins.The diagnosis of AD is mostly made clinically.Laboratory and neuroimaging can be used to support clinical diagnosis.In order to diagnose AD more clearly and earlier,developed National Institute on Aging and Alzheimer's Association(NIA-AA) criteria by including CSF-biomarkers(2011) and the ATN(β amyloid deposition(A),pathologic tau(T),neurodegeneration(N)) classification(2018) was defined.Thus,diagnostic use of CSF biomarkers,amyloid-PET and FDG-PET examinations has become widespread.Proof of amyloid positivity(with CSF or PET) is required for new treatment studies targeting amyloid.CSF-biomarkers for AD were defined as amyloid-β42,phosphorylated-tau and total-tau.Although invasiveness is a disadvantage,CSF analysis is less costly and more accessible in our country compared to neuroroimaging.

In this study,CSF-biomarkers and FDG-PET results were shown and their diagnostic values were compared in our patient group evaluated with prediagnosis of AD.

Methods:The study sample consists of 17 patients. CSF-analysis were performed using the ElectroChemiluminescentImmunoAssay(ECLIA) method. FDG-PETs of the patients were evaluated by expert radiologists.

Results:Although 17 patients had CSF-biomarker analysis,8 of them had FDG-PET results.Between the methods, 6 of 8 patients were concordant and 2 were inconsistent according to N(neurodegeneration) of ATN classification.

Conclusions: CSF-biomarkers have important advantages in clinical interpretation and treatment planning of AD.The lower cost and availability of CSF-biomarkers compared to neuroimaging may increase the popularity of CSF-biomarkers.In addition, the fact that the study was conducted in an immunoassay autoanalyzer may contribute to the laboratory studies of AD by increasing the sample size of our study.

Keywords: Alzheimers Disease AD, Cerebrospinal Fluid CSF biomarkers, 18-F fluorodeoxyglucose Positron Emission Tomography FDG-PET, Immunoassay

P015

EVALUATION OF THE QUALITY PERFORMANCE OF THE COBAS 8000 ANALYZER

Özge Cindemir

Balıkesir Atatürk City Hospital, Medical Biochemistry Department, Balıkesir, Türkiye

Objectives: Laboratory results influence 70%–75%

of medical diagnosis; as a result the quality of the laboratory service directly affects the quality of health care. Clinical laboratory professionals should reduce errors as much as possible in the analytical phase by periodically assessing them with quality control processes.

The aim of this study was to evaluate the analytical performance of the biochemistry tests in the Roche Cobas 8000 within the framework of the national criteria determined by the Ministry of Health.

Methods:Bias(%) was obtained from external quality control (RIQAS, Randox international quality assessment scheme) data and coefficient of variation (CV) was obtained from internal quality control data studied for 20 consecutive days with 2 levels. Total analytical error (TAE) was calculated for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), chloride, total cholesterol (TC), creatinine, glucose, high-density lipoprotein (HDL), lactate dehydrogenase (LDH), potassium, total protein, sodium, triglyceride and urea tests. TAE and CV values were compared with the national criteria determined by the Ministry of Health.

TAE was calculated using the formula TAE%= Bias% + (CV%×1.65)

Results:The TAE values determined by the ministry vary between 9% and 30% according to the parameters, the %CV values vary between 5% and 10%. The %CV and TAE levels calculated for all our parameters were below the specific values stated for them.

CONCLUSIONS: The quality performance of the COBAS 8000 analyzer in our laboratory is successful compared to the values allowed by the ministry.

Keywords: total allowable error, analytical performance, quality control

P016

DETERMINATION OF TAMOXIFEN METABOLITES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Abdullah Sivrikaya, Esma Menevse, Fatma Zehra Erbayram, Hamiyet Kose, Oguzhan Tok

Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye)

Objectives: Tamoxifen, a selective estrogen receptor modulator, is one of the endocrine therapeutic agents widely used in the treatment of breast cancer. Tamoxifen is converted to its active metabolites (endoxifen and 4-hydroxy tamoxifen) by cytochrome P450 enzymes. The aim of this study was to develop and validate a tandem mass spectrometric analysis method for serum concentration levels of metabolites.

Methods:Chromatographic seperation was performed using an ABSCIEX API 3200 mass spectrometer equipped with an electrospray ion source (ESI) operating in positive mode. Validation studies were performed according to CLSI protocols to prove the accuracy and validity of the developed method. The performance of the method was evaluated with a serum pool was created from patient samples using 20 mg oral dose tamoxifen.

Results:The standard curves for endoxifen and 4-hydroxy tamoxifen levels was linear within the range of 1.95-500 ng/ml. Total analysis time was 10 minutes. The LOD for endoxifen and 4-hydroxy tamoxifen was 1.95 ng/mL, while the LLOQ value was 3.95 ng/mL. Reproducibility and recovery studies were performed for endoxifen and 4-hydroxy tamoxifen at concentration levels of 500, 31.25 and 3.90 ng/mL. The %CV values obtained from intra-day and inter-day repeatability were 0.1- 0.6 and 0.1-0.5, respectively. Recovery was above 99.7%.

Conclusions: The findings of our study show that the developed method is accurate and precise according to CLSI criteria. We believe that this method can be used for routine analysis in clinical laboratories due to the short analysis time and requiring small amount of sample.

Keywords: Endoxifen, 4-hydroxy tamoxifen, LC-MSMS, Therapeutic drug monitoring

P017

DETERMINATION OF TOCOPHEROL PROFILE AND QUANTITIES IN EXTRACTS OF RESIN OF DICLE CRAB IN DIFFERENT SOLVENTS.

Eissa Almaghrebi[1], Hakan Vatansev[2], Engie Aydin Kamal Alabbas[3], Fatma Akat[1], Hüsamettin Vatansev[4]

[1] Selçuk Üniversitesi,sağlık Bilimler Enstitüsü, Tıbbi Biyokimya Anabilim Dalı

[2] Necmettin Erbakan Üniversites Meram Meslek Yüksekokulu, Gıda İşleme Bölümü

[3] Northern Technical University, College of Health and Medical Technical, Medical Laboratory Department,kirkuk,ıraq

[4] Selçuk Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

Objectives: Crabs are crustaceans (Crustacea) found all over the world. Crabs, which are seafood, rank third after shrimps and lobsters due to their flavour and importance in the fisheries sector. Crabs are ten-legged crustaceans and their bodies are covered with hard shells. In general, crabs are considered to be highly nutritious and have beneficial effects on human health as they contain essential amino acids, proteins, unsaturated fatty acids, carbohydrates, fats and minerals. In our study, it was aimed to determine the amounts of tocopherol profiles in resin extracts of Dicle crab in different solvents.

Methods:For tocopherol analysis, 0.25 g of the resin of the Dicle crab was weighed, 20 ml of hexane was added and kept for 1 night. The mixture with hexane was filtered and the filtrate was taken into the balloon and evaporated in the evaporator. The oil remaining in the balloon was taken and analysed by HPLC.

Results:According to the solvents used, the highest amount of α-tocopherol (49,91µ/g) was found in methanol + water extracts. The highest amount of γ-tocopherol (5,13µ/g) was found in water extract. In methanol solvent, the highest amounts of β-tocopherol (43,07µ/g), γ-tocopherol (11,72µ/g), δ-tocopherol (2,26µ/g) were found.

Conclusions: In our study, the highest amount of α-tocopherol (49.91 µ/g) in methanol+water solvent, β-tocopherol (43.07 µ/g) in methanol solvent and γ-tocopherol (5.13 µ/g) in water extract were determined. We believe that these analyses may be helpful in future studies to determine the active substance and to investigate the positive and negative effects of these active substances on diseases.

Keywords: Dicle crab, tocopherol, extraction, analysis

P018

MEASUREMENT UNCERTAINTY OF THE CALCULATED TRANSFERRIN PARAMETER

Elif Nihal Başer, İbrahim Yangın, Mehmet Şeneş

Ankara Eğitim ve Araştırma Hastanesi

Objectives: The aim of this study is to calculate the measurement uncertainty of the calculated transferrin parameter according to ISO 20914 guidelines.

Methods:Iron (Fe) and total iron-binding capacity (TIBC) used in the calculation of transferrin (Tf) were measured on the Roche Cobas c701 analyzer. Tf was calculated using the formula Tf=0.007(-Fe + TIBC).The standard uncertainty of transferrin was calculated using the formula uc (Tf)=0.007^2 *(uc (Fe)^2 +uc(TIBC)^2),where $uc(x)=\sqrt{(uRw^2 + ucal^2)}$ (uRw:laboratory repeatability,$ucal$:calibrator uncertainty,uc:combined standard uncertainty).The uRw value was obtained by calculating the SD values of 6 months of internal quality control (IQC) results for pathological and normal control levels separately. Calibrator uncertainty values were obtained from the manufacturer. The found standard uncertainty value was expanded by multiplying it by a coverage factor (k=2) for a 95% confidence level.The decision on which level of uncertainties to use for Fe and TIBC parameters was made by looking at the midpoint of the mean values of IQC samples.

Results:The midpoint of the mean values for Fe and TIBC was calculated as 178 mcg/dL and 245 mcg/dL,respectively.The expanded uncertainty of calculated transferrin for a 95% confidence level ranged from 0.21 g/L to 0.25 g/L for four different conditions based on Fe and TIBC levels.

Conclusions: Laboratories should determine measurement uncertainty not only for measured parameters but also for calculated parameters to improve the accuracy and reliability of results and should share it with clinicians when necessary.

Keywords: measurement uncertainity, transferrin

P019

ASSESSMENT OF MEASUREMENT UNCERTAINTY OF ROUTINE BIOCHEMICAL TESTS

Levent Deniz

Sorgun State Hospital, Department of Medical Biochemistry, Yozgat, Türkiye

Objectives: Uncertainty is an integral part of any measurement made in clinical laboratories. Accreditation standards require laboratory results to calculate measurement uncertainty (MU) routinely. We aimed to calculate the MU values of frequently requested biochemical tests in routine.

Methods:MU values of aspartate transaminase (AST), alanine transaminase (ALT), urea, glucose, total cholesterol, lactate dehydrogenase (LDH), uric acid, total protein tests were calculated on the AU5800 (Beckman Coulter Inc., Brea, California, US.) autoanalyser in Sorgun State Hospital Biochemistry Laboratory. MU calculations were made according to the Nordtest guide using the internal and external quality control results during the three months and the uncertainty values obtained from the calibrator. The results were compared with the maximum allowable measurement uncertainty (MAU) values in the EFLM biological variation database.

Results:Expanded uncertainty values (U) of AST, ALT, urea, glucose, total cholesterol, LDH, uric acid, and total protein tests were 4.88, 5.18, 4.74, 3.98, 3.74, 7.00, 5.82, 3.18, respectively. MAU limits of AST, ALT, urea, glucose, total cholesterol, LDH, uric acid, and total protein tests are 14.4, 15.1, 20.9, 7.5, 7.9, 7.8, 12.5, and 3.9, respectively.

Conclusions: The measurement uncertainty for all tests studied was lower than the MAU determined by EFLM. MU is a key quality indicator that describes the performance of the analytical measurement system and the laboratory. Laboratories should create measurement uncertainty calculation models and inform the clinician about MU for clinical benefit, such as diagnosis and treatment.

Keywords: Measurement uncertainty, analytical performance, standardization, quality control

P020

EVALUATION OF THE DIAGNOSTIC USEFULNESS OF TRIGLYCERIDE/ HDL-C RATIO AS A GLYCEMIC CONTROL MARKER

Muzaffer Katar[1], Osman Demir[2]

[1] Tokat Gaziosmanpaşa Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

[2] Tokat Gaziosmanpaşa Üniversitesi, Tıp Fakültesi, Biyoistatistik Anabilim Dalı

Objectives: Diabetes Mellitus (DM) continues to be the most common endocrine disease today. Easily accessible, accurate and reproducible markers are needed in addition to the accepted markers to evaluate insulin resistance (IR) and glycemic control. Therefore, our study aimed to evaluate the use of triglyceride (Tg)/HDL cholesterol (HDL-c) ratio as an indicator for insulin resistance and glycemic control.

Methods:Fasting blood sugar (FBG), Tg, HDL-c, HbA1c and insulin values of 7518 samples studied simultaneously in our TOGU Faculty of Medicine Hospital Laboratory between March 2023 and August 2023 were retrospectively examined. The patients were divided into two groups according to their HbA1c values: good glycemic control (<7%) and poor glycemic control (≥7%). ROC analysis was performed to evaluate the ability of the TG/HDL-C ratio to discriminate between insulin resistance and glycemic control. Statistical significance level was accepted as $p<0.05$.

Results:Tg/HDL-c ($p<0.001$) was found to be significantly higher in individuals with insulin resistance. When we looked at the areas under the ROC curve (AUC) to evaluate the ability of this parameter to distinguish insulin resistance, Tg/HDL-c was found to be 0.720 ($p<0.001$). In case of poor glycemic control, Tg/HDL-c ($p<0.001$) was also significantly higher and AUC value 0.620 ($p<0.001$).

CONCLUSIONS: Based on the current results, we think that the Tg/HDL-c ratio is a useful marker of glycemic control and insulin resistance. With this parameter, it may be easier to detect metabolic changes due to T2DM in the early stages.

Keywords: Diabetes Mellitus, Insulin Resistance, Glycemic Control, Triglyceride, HDL

P021

CALCULATION OF ALBUMIN TEST MEASUREMENT UNCERTAINTY VALUE ACCORDING TO ISO/TS 201914 GUIDELINE

Meltem Yardım[1], Nilüfer Çelik[2]

[1] Yerköy State Hospital, Department of Medical Biochemistry

[2] Dr. Behçet Uz Child Disease and Pediatric Surgery Teaching and Research Hospital, Department of Medical Biochemistry

Objectives: Measurement of albumin is widely used in the prognostic evaluation of various diseases. This study was planned to calculate the measurement uncertainty (MU) is of the albumin studied with the Bromocresol green colorimetric method on the Beckman Coulter-DXC700 device according to the ISO/TS 201914 guideline and compare it with the maximum allowable measurement uncertainty (MAU) values.

Methods:Long-term precision (uRw) values were calculated from the internal quality control (IQC) data of our laboratory between January 1 and March 31, 2023. Calibrator uncertainty (ucal) was obtained from Beckman Coulter company. The combined standard uncertainty u(y) calculation was made with the formula √(uRw2+ucal2). MU were calculated separately for both levels of IQC values. Since the bias obtained from retrospective external quality control data was determined within the allowable error limits, it was not included in the expanded measurement uncertainty (Uy) calculation. The Uy value was obtained by multiplying the combined standard uncertainty by the coverage factor (k=2, 95% CI). The formula Urel%=U(y)/IQC mean*100 was used in the relative expanded uncertainty calculation.

Results:The relative expanded uncertainty value for the albumin was calculated as 5.18% for the

IQC-1 and 2.64% for the IQC-2. When the calculated values were compared with the EFLM Min(-MAU<3.8%) value, it was seen that the limit was exceeded at IQC-1.

Conclusions: High measurement uncertainty may cause deviations in the measured analytical value and errors in clinical evaluation. Each laboratory should monitör MU values to ensure the accuracy and reliability of the results, especially for tests such as albümin, where clinical decisions may be affected.

Keywords: Measurement uncertainty, albumin, ISO-TS 201914

P022

A COMPARISON OF BIOINFORMATICS PIPELINES FOR MICROBIOME RESEARCH

Sibel Kucukyildirim

Faculty of Science, Department of Biology, Hacettepe University, Ankara

Objectives: The high-throughput sequencing technologies and related data analysis tools have drastically improved life sciences. However, several factors may influence the findings, including the experimental design, sequencing methods, and bioinformatic analysis, which causes difficulties in making comparisons across studies. Therefore, this work aimed to evaluate the impact of the different bioinformatic pipelines in human microbiota analysis.

Methods:Intestinal tissues obtained from 18 healthy subjects were analyzed using different pipelines (e.g., Bioconductor, LotuS, SHAMAN) with default parameters to test the effect of the bioinformatic analysis on the taxonomic classification of microbiome studies.

Results:In this preliminary work, taxa assignments were consistent at both phylum and genus levels across all the pipelines, although species diversity indices (e.g., alpha diversity) were significantly different.

Conclusions: The findings indicate that bioinformatic analysis tools, like experimental desgins, significantly influence microbiome studies. In addition, it suggests that the results can only be reliably comparable if obtained from the same experimental designs and bioinformatics pipelines.

Keywords: microbiome, data analysis, bioinformatics, amplicon sequencing

P023

CALCULATION OF THE MOLECULAR DESCRIPTORS FOR THE NERVOUS SYSTEM DRUG DATASET

Aytun Onay

Türk Hava Kurumu Üniversitesi, Yazılım Mühendisliği Bölümü

Objectives: Drugs can be defined as small chemical molecules that bind to a target protein and alter its behavior. Nervous system (NS) drug datasets containing NS approved (AD) and withdrawn drugs (WD) (from N02, N03, N04, N05, and N06 groups) were taken from KEGG DRUG, PubChem, and Drugbank databases. Drug molecules were presented in SDF format in the databases. The name of the drug molecule, its characteristics, the coordinates of the atoms, the types of bonds between the atoms, the atoms from which they are located and stored, and other relevant information involved in this molecule were stored in SDF files. The dataset consisted of 213 drug molecules. 181 of them are NSADs. The remaining ones are NSWDs.

Methods:CORINA Symphony program, from Molecular Networks Inc. takes the drug data in SDF format and transforms chemical information into a useful number for each drug molecule. 760 descriptors were calculated by the program. 760 molecular descriptors contained 22 global molecular descriptors, 8 size and shape descriptors, 729 toxprint chemotypes and 1 user property.

Results:Because of some unrelated information in the source databases, the NS drug dataset was cleaned, analyzed, and transformed, and then computationally useful data for each drug molecule was obtained. Therefore, data preprocessing was applied to the dataset. The program computed a total of 760 descriptors for each drug molecule.

CONCLUSIONS: Data mining extracts valuable information from large databases or datasets using advanced search techniques or algorithms. In this study, a NS drug dataset was generated to develop machine learning models for NS drugs.

Keywords: Nervous System Drugs, Molecular Descriptors, CORINA Symphony Program, Toxprint Chemotypes

P024

COMPUTATIONAL INVESTIGATION OF STRUCTURE AND DYNAMICS OF AMYLOID BETA FIBRILS FOR DISCOVERY OF PEPTIDES THAT PREVENT PLAQUE FORMATION

Fulya Çağlar Çirkin[1, 2], Sevgi Gülyüz[1], Özgür Yılmaz[1], Sefer Baday[3], Özge Şensoy[4]

[1] Tubıtak Mam 3b Centre of Excellence, Istanbul, Türkiye

[2] Ege University, Institute of Health Sciences, Health Bioinformatics Program, Izmir, Türkiye

[3] Istanbul Technical University Institute of Informatics, Istanbul, Türkiye

[4] İstanbul Medipol University, School of Engineering and Natural Sciences, Istanbul, Türkiye

Objectives: Alzheimer's disease (AD) is a neurological disorder that affects brain functions and is typically characterized by memory loss and cognitive impairment. The pathogenesis of AD involves the production and accumulation of amyloid-beta peptide (Aβ), which leads to the formation of protein plaques. Atomistic level examination of the fibrils is of great importance for holistic understanding of the disease, thus discovery of peptides that might prevent plaque formation.

Methods:The objective of this study is to examine the peptide-peptide interactions of Aβ (1-42), which is associated with AD, using molecular modeling techniques. To screen for structural isoforms of Aβ (1-42), the Protein Data Bank (PDB) database was employed. Molecular docking analyses were conducted using the HPEPDOCK web server, while Molecular Dynamics (MD) simulations were performed with Gromacs. Data analysis was carried out using the xmgrace and VMD 1.9 software programs.

Results:We found that in our results, electrostatic and hydrogen bond interactions between the protein-peptide were more effective. Our findings are expected to make a significant contribution to potential diagnostic techniques for Alzheimer's disease by elucidating the non-covalent interactions among peptides specifically binding to Aβ (1-42).

Conclusions: In summary, the identification of peptides specifically binding to amyloid-beta (1-42), particularly in the earliest clinical stages, holds para-

mount importance for the purpose of slowing down the progression of AD. The majority of the computational calculations were conducted utilizing the resources provided by TUBITAK-ULAKBIM Truba and UHEM, with additional support for this study being granted through TUBİTAK (TUBİTAK-1004, Project No. 23AG013).

Keywords: Alzheimers disease, Amyloid Beta, Molecular Modeling

P025

INVESTIGATION OF DNA APTAMER MODIFICATIONS ASSOCIATED WITH GLIOBLASTOMA THROUGH MOLECULAR MODELING

Hasip Cirkin

İstanbul Kent University, Vocational School of Health Services, Medical Laboratory Techniques Program

Objectives: In adults, Glioblastoma (GBM) is the deadliest primary brain tumor in the central nervous system. Aptamers, single-stranded oligonucleotides, interact with their target molecules through precise structural interactions. Aptamer modification involves intentionally introducing chemical or structural changes to enhance their specificity and effectiveness in binding with their intended targets. These deliberate modifications aim to amplify aptamers' binding affinities, overall stability, or target specificity.

Methods:The aim of our study is to investigate DNA aptamers known to be effective in diseases such as glioblastoma by subjecting them to various modifications and conjugations and determining their binding efficacies through molecular dynamics simulations. To achieve this goal, we obtained the structures of these aptamers from the Aptagen database and prepared each aptamer for chemical modifications and conjugations using the CHARMM-Gui. Subsequently, we conducted molecular dynamics simulations of each modified DNA aptamer and aptamer-protein interaction in GROMACS and examined the structural changes before and after modification using the VMD program. The Xmgrace program was utilized for data analysis.

Results:Our findings have revealed that modifications applied to specific aptamers lead to significant alterations within their respective binding regions, resulting in a reduction of binding efficacy for certain aptamers while enhancing it for others. These variations have exerted diverse effects on the interactions between aptamers and the target molecules, thereby exerting a nuanced influence on their selectivity characteristics.

Conclusions: In summary, our findings suggest that further research is warranted to explore the potential of DNA aptamer modification and conjugation as innovative diagnostic and therapeutic tools for diseases such as GBM.

Keywords: Aptamer Modification, Molecular Modelling, DNA aptamer, Glioblastoma

P026

INVESTIGATION OF PCSK9 AND CETP POLYMORPHISMS IN DIABETIC NEPHROPATHY

Nazlı Helvacı[1], Alev Kural[2], Sibel Kuraş[1], Özgür Can[3], Fatih Hacımustafaoğlu[1]

[1] Health Sciences University Hamidiye School of Medicine Medical Biochemistry Department

[2] Health Sciences University Bakırköy Dr. Sadi Konuk Teaching and Research Hospital Medical Biochemistry Department

[3] Health Sciences University Haydarpaşa Numune Teaching and Research Hospital Nephrology Department

Objectives: Diabetic nephropathy (DN) is a major cause of end-stage renal disease worldwide. Dyslipidemia is a major component to diabetes's elevated risk of cardiovascular disease (CVD). Two proteins that play key roles in cholesterol metabolism are proprotein convertase subtilisin/kexin type 9 (PCSK9) and cholesteryl ester transfer protein (CETP). Gain-of-function mutations of PCSK9 raise LDL cholesterol levels and CVD risk; while loss-of-function mutations reduce both LDL cholesterol and CVD risk. CETP activity is thought to support the development of CVD by lowering HDL levels. In our study, we aimed to find out the possible role of PCSK9 rs505151 and CETP rs708272/rs5852 polymorphisms in DN.

Methods:54 DN patients and 30 healthy individuals between the ages of 18-80 were included in the study. First, genomic DNA was isolated from EDTA blood samples taken from individuals, and then gene variations were examined by touchdown PCR method. Statistical analysis of the data was done with SPSS 26.0.

Results:There was no statistically significant diffe-

rence in genotype distribution between the patient and control groups in terms of rs505151 (p= 0,3053), rs708272 (p= 0,6567) and rs5882 (p= 0,3907) polymorphisms. In addition, PCSK (rs505151), CETP (rs708272) and (rs5882) allele frequency distributions did not show a statistically significant difference between the patient and control groups.

Conclusions: In our study, no statistical significance was observed in terms of polymorphisms of CETP and PCSK9 genes in DN patients. Given the strong link between diabetes and CVD, further expansion of the study population is needed.

Keywords: PCSK9, CETP, Polymorphism, Diabetic Nephropathy

P027

THE SANGER SEQUENCING IN THE DETECTION OF THE RECURRENT MUTATION IN PRIMARY HYPEROXALURIA TYPE I

Jihane Ahmıdı[1, 2], Khawla Zerrouki[1, 2], Fatima Ezzahra Aouni[1, 2], Fatimazahra Smaili[1, 2], Sara Meziane[1, 2], Intissar Haddiya[2, 3], Yassamine Bentata[2, 3], Mariam Tajir[1, 2]

[1] Medical Genetics Laboratory, Central Laboratory, Mohammed Vı University Hospital Oujda, Morocco.

[2] Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco.

[3] Department of Nephrology, Mohammed Vı University Hospital Oujda, Morocco.

Objectives: The primary hyperoxalurias (PH) are a group of autosomal recessive disorders involving the overproduction of oxalate. There are 3 types, the most common and most serious of which is PH type I. This is due to a recurrent mutation c.731T>C (lle244Thr) in exon 7 of the *AGXT* gene. The objective of this study is to demonstrate the contribution of Sanger sequencing in the diagnosis of PH.

Methods:We report the case of a male patient, hospitalized in the nephrology department for chronic renal failure and referred to our medical genetic laboratory for a suspicion of PH type I. We extracted the patient's DNA from peripheral blood collected in an EDTA tube. After conventional PCR amplification of the exon 7 of the *AGXT* gene, the DNA fragments were purified and sequenced by the Sanger method on ABI SeqStudio.

Results:The molecular study in our patient revealed the presence of the recurrent mutation c.731T>C (lle244Thr) in the homozygous state in exon 7 of the *AGXT* gene.

Conclusions: PH type I is a rare genetic disease, characterised by the presence of recurrent kidney stones, nephrocalcinosis or even renal failure and systemic oxalosis. In 84% of cases, PH type I results from a recurrent mutation c.731T>C (lle244Thr) in exon 7 of the *AGXT* gene, identifiable by targeted DNA sequencing using the Sanger method. Establishing the genotype of patients with PH is necessary in order to offer appropriate genetic counselling.

Through this work, Sanger sequencing remains the first method of choice in the diagnosis of PH type I.

Keywords: Primary hyperoxalurias type I, Sanger Sequencing, Genetic counselling

P028

COULD GENE REGION VARIANTS ENCODING HYDROLASE ACTIVITY OF THE SOLUBLE EPOXIDE HYDROLASE ENZIME BE ASSOCIATED WITH TYPE 2 DIABETES?

Esma Ozmen[1, 6], Dilara Fatma Akın[2], Durmuş Ayan[1], Çağatay Emir Onder[3], Burcu Kose[4], İsmail Sarı[5], Cevat Yazıcı[6]

[1] Department of Medical Biochemistry, Faculty of Medicine, Niğde Ömer Halisdemir University, Niğde, 51240turkey

[2] Department of Medical Biology Faculty of Medicine, Medical Biology, Nigde Omer Halisdemir University, Nigde, 51000turkey;

[3] Department of Endocrinology, Nigde Omer Halisdemir University Training and Research Hospital, Nigde,tr-51100turkey

[4] Department of Biotechnology, Faculty of Science Literature, Nigde Omer Halisdemir University, Nigde, Turkey

[5] Department of Medical Biochemistry, Faculty of Medicine, Kırklareli University, Kırklareli, 39100, Turkey

[6] Department of Biochemistry, Erciyes University Faculty of Medicine, 38039 Kayseri, Turkey

Objectives: Epoxyeicosanoids function as signal mediators in critical biological processes such as platelet aggregation, vasodilation, and anti-inflammation. With all these properties, Epoxyeicosanoids have been associated with many diseases. Metabolism of epoxyeicosanoids is carried out by soluble epoxide hydrolase enzymes, and as a result dihydroxyeicosatrienoic acids, which is a less active form than epoxyeicosanoids, are formed. In our study, SNP/mutation analysis was performed in the gene region responsible for the hydrolase activity of EPHX2, which encodes the soluble epoxide hydrolase enzyme.

Methods:It was formed from 100 healthy and 100 T2DM patient groups. SNP/mutation analysis in the gene region responsible for the hydrolase activity of EPHX2 in both groups was performed by sanger sequencing using appropriate primers.

Results:A total of 12 mutations were detected in both groups as a result of Sanger sequencing. Two of the 12 detected mutations were missense mutations (p.Asn359Thr and p.Ser412Arg). It was determined that the pathogenic scores of these mutations were close to 1 for Poly-Phen2 and 0-100 for SNAP. In addition, two (c.1058+165C>T and c.1058+146G>A) SNPs were detected in the intron we observed in the T2DM group, which has never been detected and defined before in our study.

Conclusions: The mutations detected in our study, especially those that cause amino acid changes, may cause T2DM susceptibility in healthy individuals. It may even cause progression of disease pathogenesis in the T2DM group. However, these mutations should be studied more extensively in a high population patient group.

Keywords: Mutation, sEH, EPHX2, T2DM

P029

THE INVESTIGATION OF OXIDATIVE STRESS IN H2O2 INDUCED H9c2 CARDIOMYOCYTES

Ayca Bostanoglu, Beril Erdem Tuncdemir, Emel Saglar Ozer

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

Objectives: The aim of this study is investigation the production of ROS in H9c2 cardiomyocytes after H_2O_2 treatment that can cause increased oxidative stress in H9c2 cardiomyocytes in terms of reactive oxygen species (ROS) production.

Methods:By performing MTT assay, 200 mM was found as non-toxic concentration for H9c2 cells. After treatment H9c2 cells with H_2O_2 for 2 hours, the fluorescence probe 2,7-dichlorodi-hydrofluorescein diacetate (DCFH-DA) was used to detect ROS in cell culture (E-BC-K138-F, ELabscience, Wuhan, China). The fluorescence detection was performed with the 500 nm excitation wavelength and 525 nm emission wavelength with microplate reader. This study was supported by Hacettepe University (Project number: FHD-2022-20252).

Results:According to the results, ROS production was increased in H9c2 cells which were treated with H_2O_2 but that increase was not significant. The ROS production in non-treated H9c2 cells was already found as high as positive control in vitro.

Conclusions: H9c2 cells can intrinsically show high levels of ROS and a slight increase in ROS production was observed in these cells when they were treated with H_2O_2. Therefore, even though this increase in ROS production was not significant, it is important to see that H_2O_2 can induce ROS production in cardiomyocyte cells.

Keywords: H9c2, H2O2, oxidative stress, ROS

P030

INTRACELLULAR LOCALIZATION AND STRUCTURE OF MUTANT AVP PRECURSOR AGGREGATES IN AUTOSOMOL DOMINANT NEUROHYPOPHYSEAL DIABETES INSIPIDUS

R. Dilara Vaizoglu[1], Beril Erdem Tuncdemir[1], Emel Saglar Ozer[1], Ceren Acar[2], Mehmet Gül[3], Hatice Mergen[1]

[1] Hacettepe University, Faculty of Science, Department of Biology, Moleculer Biology Section, Ankara, Türkiye

[2] Inonu University, Faculty of Arts and Science, Department of Moleculer Biology and Genetics, Malatya, Türkiye

[3] Inonu University, Medical Faculty, Basic Medical Sciences, Histology and Embryology Section, Malatya, Türkiye

Objectives: The aim of this study is investigating the intracellular aggregate formations of mutant arginine vasopressin hormone (AVP) precursors (G45C, 207_209delGGC, G88V, C98X, C104F, E108D-1, and E108D-2) and their effect on the development of autosomal dominant neurohypophyseal diabetes insipidus (ADNDI) to understand whether ADNDI can one of an amyloid diseases.

Methods:COS-1 cells were transiently co-transfected with both mutant AVPs and segrotagranin II MYC for visualizing the aggregate structure. Cells were fixed, blocked and incubated with primary antibodies then, they were stained with fluorescence secondary antibodies. After the incubations, coverslips were mounted and the images were taken at confocal microscopy. (TÜBİTAK SGAB Project No: 118S688, Hacettepe University BAP Project No: 19929).

Results:The aggregate structures formed by mutant protein precursors have been observed as fibrillar aggregate structures in the Endoplasmic reticulum (ER) lumen.

Conclusions: Mutations in the AVP are implicated in the development of a rare disease ADNDI. Amyloid diseases are characterized by the formation of fibrillar amyloid aggregate structures, and ADNDI can be classified within this group. Immunofluorescence results provided that, unlike amyloid diseases, mutant AVP precursors were accumulated within the ER lumen not in the cytosol or extracellular space. Additionally, granular structures were observed within the cells and appeared distinct from the aggregate structures. Consequently, ADNDI categorized as a neurodegenerative disease associated with the formation of fibrillar protein aggregation.

Keywords: ADNDI, Aggregate, AVP, Immunofluorescence

P031

ROLE OF VITAMIN D RECEPTOR GENE POLYMORPHISMS ON NORMAL AND OVERWEIGHT PATIENTS WITH POLYCYSTIC OVARY SYNDROME (PCOS)

Zümrüt Mine Işık Sağlam[1], Vuslat Lale Bakır[2], Merve Nur Ataş[1], İlhan Yaylım[1], H. Arzu Ergen[1]

[1] Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Türkiye

[2] Clinic of Gynecology and Obstetrics, Haseki Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye

Objectives: PCOS is one of the most prevalent endocrinological disorder that severely effects women of reproductive age. Vitamin D deficiency contributes to menstrual dysfunction in PCOS-affected women. This study aims to investigate the association between PCOS metabolic characteristics and VDR gene polymorphisms, ApaI (rs7975232), TaqI (rs731236), and Cdx2 (rs11568820) in Turkish women.

Methods:120 PCOS patients (18-45 years old) were divided into two groups (BMI<25, n=77 and BMI≥25, n=43). SNPs were analyzed by RealTime PCR Systems. Statistical differences were evaluated by SPSS 21.0 programme.

Results:Frequencies of ApaI AC genotype (p=0,027), C allele (p=0,011) and Cdx2 T allele (p=0,048) were increased in BMI≥25 group. According to the ApaI variant; cholesterol (p=0.01) and LDL-cholesterol (p=0.02) levels were higher in the BMI≥25 group in those with the AA genotype. Also, carrying C allele may be a risk factor for high cholesterol (p=0,041), LDL-cholesterol (p=0,044) and triglyceride levels (p=0,028) in BMI≥25 group.

According to TaqI variant; AA genotype (p=0,020) and A allele (p=0,041) were associated with elevated triglyceride in BMI≥25 group.

When we evaluate the Cdx2 variant, triglyceride was observed to be higher in BMI≥25 in CC genotype (p=0,048) and C allele (p=0,030). HDL-cholesterol is elevated in T allele in BMI≥25 (p=0,027).

Conclusions: Haplotype analysis revealed no evidence linking these SNPs responsible for PCOS in Turkish population, but VDR ApaI, TaqI and Cdx2 gene polymorphisms might be associated with the metabolic features of PCOS according to their genotype and allele.

Keywords: VDR polymorphisms, PCOS

P032

EXPLORING DIET-RELATED BIOMOLECULAR ALTERATIONS IN MEDULLA OBLONGATA WITH IR SPECTROSCOPY AND BIOCHEMICAL ANALYSIS

Göktuğ Kaçıra[1], Şebnem Garip Ustaoğlu[2], Ayça Doğan[3]

[1] Department of Biology, Faculty of Science, Marmara University, Istanbul, Turkey

[2] Department of Medical Biochemistry, Faculty of Medicine, Altinbas University, Istanbul, Turkey

[3] Department of Physiology, Faculty of Medicine, Altinbas University, Istanbul, Turkey

Objectives: High energy diet may have an influence on brain function, besides their effects on metabolic system and diseases. The brainstem's medulla oblongata plays a crucial role in regulating a number of involuntary physiological functions, including breathing, heart rate, and blood pressure. The type of diet can have an impact on how the medulla oblongata functions and interacts with other regions of the brain/body. The aim of the current study is to evaluate the effects of high-calorie diets on the biomolecular content and structure of medulla oblongata of mice by infrared spectroscopic and biochemical analysis.

Methods:Different diets were given to male inbreed C57BL/6J mice. Study groups include control (low fat and low carbohydrate diet), high fat diet (HFD), and high carbohydrate diet (HCD). The mice were slaughtered at the end of 6 months and their brain tissues were collected. Medulla oblongata was used for both ATR-Fourier Transform Infrared (FTIR) spectroscopic studies and the measurement of tissue total antioxidant capacity.

Results:Both high-carbohydrate and -fat diet significantly decrease total protein and nucleic acid content while they significantly increase total saturated and unsaturated content with the acyl chain length of lipids of the tissue. Total antioxidant capacity of the tissue was also altered significantly by diet. Particularly high carbohydrate diets cause more profound alterations in the molecular structure and overall antioxidant capacity within this crucial brainstem area.

Conclusions: High-calorie diets, whether high in carbs or fats, have noticeable effects on the biomolecular content and structure of medulla oblongata.

Keywords: High Fat Diet, High Carbohydrate Diet, Medulla Oblangata, IR Spectroscopy, Total Antioxidant Capacity

P033

CAN BLOOD GAS ANALYSER RESULTS BE USED INSTEAD OF BIOCHEMISTRY AUTO-ANALYSER RESULTS IN PATIENTS TREATED NEONATAL INTENSIVE CARE UNIT?

Ahmet Burak Gürpınar[1], Tevfik Noyan[1], Murat Cihan[2], Abdullah Üner[1]

[1] Ordu University, Medical Faculty, Department of Biochemistry

[2] Ministry of Health, Ordu University Training and Research Hospital

Objectives: A blood gas analysers are vital equipment frequently used in emergency departments and intensive care units. The aim of this study was to determine the agreement in glucose, sodium, and potassium parameters that measured both biochemistry auto-analyser and blood gas analyser.

Methods:We conducted a prospective analytical observational study in neonatal intensive care units in patients who had a glucose, sodium and potassium and a blood gas performed within 1 h of each other during the first 24 h of their neonatal intensive care unit admission.

Results:A total of 382 paired tests were available for glucose, 396 for sodium, and 391 for potassium. For all three parameters, the values measured on the auto-analyser were significantly higher than those on the blood gas analyser ($p<0.001$). In the Spearman correlation analysis between the two measurements, correlation coefficients (r) wase found as 0.729, 0.872, and 0.947 ($p<0.001$) for sodium, potassium, and glucose respectively The mean differences between the two methods were statistically significant for glucose (mean difference 4.43 mg/dL, 95% CI 1.59–7.26, $p < 0.0001$) sodium (mean difference 1.53 mmol/L, 95% CI 1.03–2.03, $p < 0.0001$) and potassium (mean difference 0.38 mmol/L, 95% CI 0.34–0.42, $p < 0.0001$) on the Bland–Altman plots.

Conclusions: We concluded that the glucose, sodium, and potassium results obtained from blood gas analyser cannot be used instead of those obtained from an auto-analyser in patients who treated neonatal intensive care unit.

Keywords: auto-analyser, blood gas analysis, electrolytes, glucose, neonatal intensive care

P034

CHANGES IN THE LEUKOGRAM OF COVID-19 PATIENTS

Lajla Halilović[1], Berina Hasanefendić[1, 2], Emir Šeherčehajić[2], Armina Dedić[2], Suzana Tihić Kapidžić[1], Ahmed Velić[3], Lejla Ibričević Balić[4]

[1] Clinical Biochemistry and Immunology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2] Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Clinic For Infectious Diseases, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[4] Clinic For Hematology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Objectives: The aim of this study is to examine the morphological changes of leukocytes in COVID-19 patients with mild and more severe clinical picture and to examine their association with the severity of the clinical picture.

Methods:This descriptive and analytical study included 160 COVID-19 patients, who were divided into two groups according to the clinical picture: mild and more severe. Patients' blood was collected in tubes with EDTA anticoagulant, after which peripheral blood smears were prepared. The peripheral blood smears were evaluated by a team of cytoscreeners (clinical biochemistry specialists and medical laboratory engineers).

Results:The most common morphological changes of leukocytes observed in COVID-19 patients are: the presence of reactive lymphocytes - covicytes, pleomorphism of leukocyte nuclei, reduced granulation of granulocytes, vacuolization and hypersegmentation of neutrophils. A significant difference was observed in the frequency of occurrence of the pathological number of neutrophils ($p = 0.005$) and lymphocytes ($p < 0.001$) according to the severity of the COVID-19 infection.

Conclusions: Peripheral blood smear is a simple, fast, reliable and inexpensive method that can help in the diagnosis and monitoring of COVID-19 patients. The main finding of this study is the presence of reactive lymphocytes (covicytes) in COVID-19 patients with a mild clinical picture.

Keywords: COVID-19, peripheral blood smear, reactive lymphocytes

P035

COMPARISON OF LEUKOCYTE SUB-GROUPS (LYMPHOCYTE, MONOCCYTE AND NEUTROPHIL) MEASURED IN THE MINDRAY DEVICE WITH FLOW CYTOMETRIC MEASUREMENTS

Tuğçe Acar, İbrahim Türk, Zahide Adıyaman, Hatice Topaç, Derya Sönmez

University of Health Sciences, Istanbul Training and Research Hospital, Department of Clinical Biochemistry, Istanbul, Turkey

Objectives: In our study, we aimed to evaluate the compatibility of the hematology analyzer and flow cytometry device that we routinely use.

Methods:In our study,hemogram analysis were performed on the Mindray BC6800plus (Shenzhen,China) hematology analyzer; flow cytometry analysis was performed on the BD FACSLyric Flow cytometer (BectonDickinson Company,USA). Retrospective analysis was conducted on patients who underwent complete blood count and flow cytometry tests on the same day between January-2021 and May-2023. 109 patients with a WBC count $>10x10^9$/L(Group 1) and 42 patients with a WBC count$<10x10^9$/L(Group 2) were included. Comparisons were made for lymphocyte, monocyte, and neutrophil percentages obtained from both devices. Analyzes were performed using SPSSv26.0 and MedCalc.

Results:For lymphocytes, monocytes and neutrophils in group 1,respectively,in the correlation analysis, r values were 0.929, 0.478,and 0.938($p<0.05$).In the Passing-Bablok analysis were y=3.559(2.32–4.94)+0.998(0.96–1.03)x, y=-0.114(-2.00–0.54)+1.371(1.19–1.90)x and y=1.047(-0.10–3.67)+0.921(0.87–0.97)x. For lymphocytes and neutrophils,Pearson correlation was r=0.897 and r=0.790($p<0.05$), while Spearman correlation for monocytes was r=0.539($p<0.05$). Equations and 95% confidence intervals in Passing-Bablok analysis for lymphocytes,monocytes and lymphocytes,respe-

ctively y= 5.633(-2.18–9.81)+0.933(0.82–1.04)x, y=1.20(0.092–2.38)+1.00(0.87–1.29)x and y=6.98(-1.01–14.53)+0.835(0.69–0.98)x found.

Conclusions: The Bland-Altman chart demonstrated that 95% of the data were within ±1.96SD for lymphocytes and monocytes in both groups and the two methods were statistically compatible for these parameters. There was a significant difference between the two methods for other parameters except group 2 Neutrophil%. Limitations of our study include a small sample size, variations in flow cytometry analyzes being performed by different individuals, and the study was retrospective and spanning an extended duration.

Keywords: lymphocytes, monocytes, neutrophil, flowcytometry, hemogram

P036

5-YEAR EXPERIENCE OF FLOW CYTOMETRY

Alev Lazoglu Ozkaya[1], İbrahim Yazıcı[1], Konca Altınkaynak[1], Kadriye Akpınar[2]

[1] Erzurum City Hospital, Medical Biochemistry Laboratory, Erzurum, Turkey

[2] Burdur State Hospital, Biochemistry Laboratory,-burdur, Turkey

Objectives: Flow cytometry makes quantitative measurements at the single cell level according to the size and granularity of cells in suspension. This technique is widely used in the detection and analysis of various diseases, including hematological malignancies. In this retrospective study, we aimed to evaluate leukemias diagnosed using flow cytometry.

Methods:We reviewed patients diagnosed with acute leukemia between August 2018 and August 2023 at University of Health Sciences Erzurum City Hospital. Analyzes were performed using NAVIOS-EX-Flow-Cytometer (Beckman Coulter) and BD FACSCanto.

Results:Flow cytometric analysis of a total of 9465 patients has been performed in the last 5 years. Acute leukemia panel study was requested by hematologists for 340 of them. Of the patient samples, 14.41% was peripheral blood and 85,59% was bone marrow. 55 patients (26 male) were diagnosed with acute leukemia, of these, 34 were Acute myeloid Leukemia (AML) (61.8%) and 5 patients (14.7%) are under the age of 18. The percentages of patients according to FAB classification were 14,7%, 11,7%, 17.6%, 20,5%, 14,7%, 17,6%, 0%, 2,9% for AML M0, M1, M2, M3, M4, M5, M6, M7, respectively. There were 21 patients with Acute Lymphoblastic Leukemia. Under the age of 18 was 90,5%. There were 15 patients diagnosed with B Cell Acute Lymphoblastic Leukemia and 6 patients with T-ALL.

Conclusions: Flow cytometry makes myeloid and lymphoid separation faster and easier in patients with suspected leukemia. It also makes classification in the myeloid series. In this respect, it continues to be a fast and reliable test.

Keywords: flow cytometry, leukemia

P037

MULTIPLE MYELOMA WITH TRICLONAL GAMMOPATHY: CASE REPORT

Nazife Doğan

Istanbul Cam and Sakura City Hospital, The Department of Medical Biochemistry

Objectives: Multiple myeloma (MM) is the bone marrow's primary malignant proliferation of B lymphocytes. In triclonal gammopathy (TG), a heterogeneous mixture of three monoclonal immunoglobulins is produced excessively and simultaneously due to the overgrowth of a group of cell clones, each from a different genetic origin. Biclonal gammopathy is rarely seen in 3-4% of patients with MM, while TG is even rarer.

Methods:Protein electrophoresis was studied by capillary electrophoresis in the serum of a 66-year-old male patient with MM. To evaluate the electrophoretic pattern, the serum of the same patient was also studied with immune fixation electrophoresis (IFE) as a confirmation method.

Results:Since peaks were observed in the gamma globulin region with the capillary electrophoresis method, it was recommended to study IFE from the same patient in his report. TG consisting of monoclonal bands of IgG lambda, IgG kappa, and IgM lambda was observed in IFE.

Conclusions: Studies show that TG is associated with malignancies and inflammation.TG's clinical and bi-

ological appearance in MM is similar to MM with biclonal or monoclonal gammopathy. There are difficulties in determining whether the TG observed in MM originates from one cell clone or three unrelated clones. Remissions and recurrences are also observed in the clinical follow-up of MM. It is anticipated that validation methods such as IFI, which will be used in future studies with MM patients with TG, will contribute to laboratory and clinical data.

Keywords: Multiple myeloma, triclonal gammopathy, electrophoresis

P038

cFGF-23, sKLOTHO, AND 1,25 (OH)2 D3 VITAMIN LEVELS IN KIDNEY TRANSPLANT PATIENTS

Hatice Paşaoğlu, Ayşe Şenelmiş, Özge Tuğçe Paşaoğlu, Ülver Derici, Özant Helvacı

Gazi Üniversity Medical Faculty

Objectives: In this study we investigated cFGF-23 , sKlotho and 1,25 $(OH)_2$ D_3 Vitamin and in kidney transplant patients.

Methods:We studied 60 transplant patients (40 males, 20 females) at Transplantation Polyclinic of Gazi University, Faculty of Medicine. Age and sex matched 40 healthy subjects (25 males, 15 females) were enrolled as control group in the study. We used SPSS version 20 for statistical analysis. Mann-Whitney U test was used to compare the parameters across the groups. Spearman correlation test was used to analyze the correlation between different parameters in the same group.

Results:We measured of the serum cFGF-23, sKlotho, and 1,25 $(OH)_2$ D_3 Vitamin levels as 166,30± 94,96 pg/ml 2,82± 1,76 ng/ml , and 49,56± 13,73 pg/ml respectively in transplant patients. In control group we measured the serum cFGF-23, sKlotho, and 1,25 $(OH)_2$ D_3 Vitamin levels as 122,60± 71,13 pg/ml , 3,72± 3,59 ng/ml and 48.42± 12,13 pg/ml respectively. We found that cFGF-23 levels were significantly higher ($p < 0.05$) in patients group compared control group. This study revealed that there is a positive correlation between cFGF-23 and sKlotho both in transplant patient group ($p<0.05$) and in control group ($p<0.05$).

Conclusions: We found that there is a significant difference in cFGF-23 levels between transplant patients and healthy individuals. On the other hand, we could not find a significant difference in 1,25 $(OH)_2$ D_3 Vitamin and sKlotho levels across groups. This study revealed a positive significant correlation between cFGF-23 and sKlotho in transplant patients.

Keywords: Kidney Transplantation, cFGF-23, sKlotho, 125 OH2 D3

P039

COMPARISON ROUTINE OF LABORATORY PARAMETERS IN PATIENTS WITH SCHIZOPHRENIA AND BIPOLAR DISORDER

Elif Menekşe[1], Muhammed Emin Düz[2], Burak Yasin Avcı[1], Ecem Avcı[1]

[1] Amasya Üniversitesi Sabuncuoğlu Şerefeddin Eğitim ve Araştırma Hastanesi

[2] Amasya Halk Sağlığı Laboratuvarı

Objectives: Schizophrenia(SCH) and bipolar disorder(BD) are psychiatric disorders with poorly understood complex etiologies and pathogenesis.Some studies have identified a correlation between red blood cell abnormalities and these diseases.A standard blood test is one of the most prevalent laboratory tests performed on psychiatric patients.The purpose of this paper is to cast more light on the relationship between laboratory parameters of blood, for which only a limited number of documents have been published.

Methods:Laboratory data of 60 SCH patients aged 38.7±8.7 years and 43 BD patients aged 39.8±9.6 years were retrospectively analyzed between 01.08.2022 and 06.01.2023.Complete blood count parameters, TSH, fT3, fT4, vitamin B12, folate, glucose, urea, creatinine, ALT, AST, LDH, GGT, uric acid levels were investigated.Independent samples T-test was used with SPSS 26 package program for statistical analysis.

Results:There was no difference between the groups regarding age and gender($p=0.533$ and $p=0.386$, respectively). Folate levels($p=0.046$) in BD patients and creatinine levels in SCH patients($p=0.044$) were significantly higher.The groups did not differ in terms of other parameters.

Conclusions: A growing body of clinical trial data consistently supports the potential benefits of folate supplements, particularly levomefolic acid or 5-methylfolate, in enhancing clinical outcomes for specific psychiatric disorders. Folate levels may be higher in BD patients than in SCHbut this does not prove that

BD patients receive adequate folate. A systematic review of 63 studies found low creatinine levels in patients with schizophrenia.Unlike the literature, our study results detected higher creatinine levels in SCH patients than in BD patients.Given these data, we believe laboratory parameters will help distinguish BD and SCH.

Keywords: Schizophrenia, bipolar disorder, laboratory

P040

PREVALENCE OF DIABETIC NEPHROPATHY IN TREATED DIABETICS: SINGLE-CENTER STUDY

Berina Hasanefendić[1, 2], Snežana Unčanin[2, 3], Emir Šeherčehajić[2], Suzana Tihić-kapidžić[1], Velda Smajlbegović[4], Lajla Halilović[1], Denis Hasković[2, 5]

[1] Clinical Biochemistry and Immunology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2] Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Clinic For Nephrology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[4] Clinic For Oncology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[5] Clinical Pathology, Cytology and Human Genetics, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Objectives: Diabetes mellitus type 2 is a chronic disease accompanied by constant hyperglycemia. If it is not properly treated, various complications can develop, such as diabetic nephropathy. The aim of this study is to examine the differences between laboratory and physical parameters between diabetics on oral and insulin therapy, as well as between diabetics with and without diabetic nephropathy.

Methods:This cross-sectional observational study included 100 patients who suffer from diabetes mellitus type 2 and who use oral antidiabetics or insulin therapy of varying duration. Biochemical and physical parameters were used to compare patients who used oral and insulin therapy and to compare patients with and without diabetic nephropathy.

Results:Out of a total of 100 patients, 69 were on insulin therapy and 31 patients used oral therapy. Statistical significance was only observed when comparing the presence of hypertension in these two groups of patients ($p = 0,02$). Comparing the biochemical and physical parameters between diabetics with and without diabetic nephropathy statistical significance was shown in total proteins ($p = 0,0004$), systolic blood pressure ($p = 0,005$) and glomerular filtration ($p < 0,05$).

Conclusions: Diabetics who used insulin therapy had higher values of HbA1c and glomerular filtration and a higher incidence of hypertension and hyperlipidemia than patients who used oral antidiabetics. Diabetics who developed diabetic nephropathy had significantly higher concentrations of proteins in urine and lower values of systolic and diastolic blood pressure, lipids, HbA1c, blood sugar and GF in contrast to patients without renal nephropathy.

Keywords: diabetic nephropathy, diabetes mellitus type 2, proteinuria

P041

EVALUATION OF ADROPIN AND PHOENIXIN-14 LEVELS IN WOMEN WITH EUTHYROID HASHIMOTO'S THYROIDITIS

Sadinaz Akdu[1], Ummugulsum Can[2], Ahmet Hamdi Aktan[3]

[1] Fethiye State Hospital, Department of Medical Biochemistry, Muğla, Türkiye

[2] Konya City Hospital, Department of Medical Biochemistry, Konya, Türkiye

[3] Fethiye State Hospital, Department of Internal Medicine, Muğla, Türkiye

Objectives: Hashimoto's thyroiditis (HT) is an autoimmune disease that is common worlwide, and may result in metabolic disorders. Adropin and phoenixin (PNX) are newly discovered peptides that play a role in the regulation of metabolic homeostasis in many tissues. The aim of the study was to investigate peripheral circulating levels of these two peptides in patients with euthyroid HT and their relationship with the clinical parameters of these patients.

Methods:This study performed in 40 euthyroid women with HT and 38 age-matched healthy controls. Serum adropin and PNX-14 levels were determined

by ELISA method.

Results:We found that there was no statistically significant difference between the patient and control groups regarding AST, ALT, fasting serum glucose, fasting insulin, trigliseride, HDL-C, HOMA-IR, free T3, free T4, TSH, adropin and PNX levels ($p>0.05$). LDL-C, total cholesterol, BMI values were higher than those of controls ($p<0.05$, $p<0.005$, $p<0.001$ respectively). AntiTPO, AntiTG levels were significantly higher than those of controls ($p<0.001$). There was no correlation between PNX-14 and adropin values and clinical parameters.

Conclusions: In this study, we found that no significant differences in PNX-14 and adropin concentrations between the euthyroid HT and control groups. Our study is important in that it is the first study to examine adropin and PNX-14 levels in euthyroid HT.

Keywords: Adropin, Phoenixin-14, Hashimotos thyroiditis

P042

THE INVESTIGATION of KISSPEPTIN, SPEXIN and GALANIN in EUTHYROID WOMEN with HASHIMOTO'S THYROIDITIS

Ümmügülsüm Can[1], Sadinaz Akdu[2], Ahmet Hamdi Aktan[2]

[1] Konya City Hospital

[2] Fethiye State Hospital

Objectives: Hashimoto's thyroiditis (HT) is characterized by thyroid cell destruction resulting in hypothyroidism, insulin resistance and changes in metabolism. Kisspeptin, spexin and galanin regulate metabolism by controling appetite and body weight. We aimed to investigate whether kisspeptin, spexin and galanin are associated with the pathogenesis of HT with euthyroid female individuals.

Methods:This study was conducted in 45 women with HT and 45 women with healthy controls of the same age. The serum levels of kisspeptin, spexin and galanin were measured by using a ELISA technique.

Results:Kisspeptin ($p<0.01$), and galanin ($p<0.01$), anti-TPO ($p<0.001$), anti-Tg ($p<0.001$) and BMI ($p<0.05$) were significantly higher in patients compared to controls. Spexin, free T3, free T4 and TSH levels and HOMA-IR were similar between the two groups. Kisspeptin was correlated positively with galanin ($p<0.01$; $r=0.786$).

Conclusions: The kisspeptin and galanin levels increased in women with euthyroid HT. These findings suggest that kisspeptin and galanin may be associated with the pathogenesis of HT and these markers may contribute to the early diagnosis and treatment of patients with HT.

Keywords: Hashimotos Thyroiditis, Infalammation, Kisspeptin, Spexin, Galanin

P043

TYPE 1 COLLAGEN C-TERMINAL TELOPEPTID, OSTEOCALCIN AND VITAMIN D LEVELS IN PATIENTS WITH OSTEOPOROSIS

Gülşen Yalçın, Mehmet Çağatay Taşkapan, Harun Şentürk, Cengizhan Katırcı

İnönü Üniversitesi Turgut Özal Tıp Merkezi

Objectives: In our country, as in many parts of the world, osteoporosis is an important public health problem. Vitamin D deficiency is an important known risk factor for osteoporosis. It provides calcium and phosphorus balance in the body, is necessary for the health of bones and muscles. Osteocalcin is a non-collagenous protein synthesized from osteoblasts. Osteocalcin reflects the age-related and after menopause osteblastic activity in bone turnover. Type 1 Collagen C-Telopeptide (CTx) helps in the follow-up of anti-bone resorption treatments such as hormone replacement therapy in menopausal women and people with low bone mass.The aim of this study was to examine the vitamin D, osteocalcin and CTx levels of postmenopausal patients followed up in the Endocrine and Metabolism Department of İnönü University Turgut Özal Medical Center and to compare these parameters with healthy individuals.

Methods:CTx levels were studied with the electrochemiluminescence method in the Roche brand e611 device, Osteocalcin levels were studied with the chemiluminescence method in the Siemens brand Immulite 2000 device, vitamin D levels were studied with the LC-MS/MS method in the Thermo Scientific brand TSQ Quantum Access MAX model device.

Results:The results of 40 patients and 45 control groups were evaluated in the study. Patients had high CTx (patient 680±210, control 320±150 pg/mL) and osteocalcin (patient 32.8±4.2 control 12.5±2.4 ng/mL) levels, and vitamin D levels (patient 8, 4±2.1 control 27.8±8.5 ng/mL) was observed

Conclusions: The results were evaluated together with previous studies. Appropriate recommendations were made to the patients for the postmenopausal period.

Keywords: osteoporosis, Type 1 Collagen C-Telopeptide, osteocalcine, vitamin D

P044

OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE BIOMARKERS IN PATIENTS WITH GRAVES' ORBITOPATHY

Marija Sarić Matutinović[1], Miloš Žarković[2, 3], Jasmina Ćirić[2, 3], Svetlana Ignjatović[1], Biljana Nedeljković Beleslin[2, 3]

[1] Faculty of Pharmacy, University of Belgrade, Serbia

[2] Clinic of Endocrinology, University Clinical Center of Serbia, Belgrade, Serbia

[3] Medical Faculty, University of Belgrade, Serbia

Objectives: Graves' orbitopathy (GO) is a specific autoimmune disorder of the orbit likely mediated by thyrotropin-receptor antibodies (TSH-R-Ab). A body of evidence suggests the important role of oxidative stress in the pathogenesis of GO. At the cellular level, TSH-R-Ab have been found to induce oxidative stress through increased synthesis of reactive oxygen species (ROS). Our aim was to evaluate the levels of total oxidant status (TOS) and total antioxidant status (TAS) in patients with GO, and to assess their association with the course of GO.

Methods:The study included 91 consecutive patients with variable GO phenotype. Serum levels of TOS and TAS were measured spectrophotometrically, using commercial assays (Rel Assay Diagnostics). Stimulatory activity of TSH-R-Ab was measured with cell-based bioassay (Thyretain, Quidel).

Results:The severity of GO was significantly associated with the level of TAS. Patients with a mild form of GO had significantly lower levels of TAS (1.91 ± 0.45 mmol/L) compared to patients with severe GO (2.47 ± 0.63 mmol/L), (p=0.026). Serum levels of TOS did not differ significantly according to disease severity (p=0.784). Neither the levels of TAS, nor of TOS were significantly associated with the activity of GO. Additionally, serum level of TAS correlated positively with the stimulatory activity of TSH-R-Ab (ρ=0.234, p=0.025).

Conclusions: The results of this study suggest that the imbalance of the cellular redox state is reflected in the clinical presentation of GO. Future studies are needed to elucidate this association, which could be used to improve laboratory diagnostics and management of this disease.

Keywords: Graves orbitopathy, oxidative stress, antioxidant defense, thyrotropin receptor antibodies

P045

EFFECT OF AEROBIC EXERCISE ON FRUCTOSE INDUCED METABOLIC SYNDROME COMPONENTS THROUGH LIVER FGF21 AND PGC1α EXPRESSION

Belkis Narli[1], Kardelen Kocaman Kalkan[1], Fatmanur Er[2], Erdal Zorba[3], Mehmet Gunay[3], Mitat Koz[4], Canan Yilmaz[1]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

[2] Atatürk University, Faculty of Sport Sciences, Department of Action and Training Sciences, Erzurum, Türkiye

[3] Gazi University, Faculty of Sport Sciences, Department of Physical Education and Sports, Ankara, Türkiye

[4] Eastern Mediterranean University, Faculty of Health Sciences, Department of Physioytherapy and Rehabilitation,kıbrıs

Objectives: FGF21 regulates energy metabolism, promotes the oxidation of fatty acids, increases insulin sensitivity and reduces inflammation.PGC1α is an important factor in the regulation of cellular energy metabolism and mitochondrial biogenesis. The aim of our study was to investigate the effects of exercise on liver FGF21 and PGC1α levels in rats fed a fructose diet, and therefore the protective and therapeutic role of these molecules on MetS(Metabolic Syndrome) components.

Methods:In our study, 24 adult male Sprague Dawley rats weighing 225±10 g were used. Animals were divided into 4 groups(n=6) as Control, Fructose, Exercise, Fructose+Exercise. In this study, high fructose diet was given to rats in 20% of drinking water. Treadmill exercise was applied for 10 weeks.Blood pressures and body weights were measured in all groups. At the end of the experiment, blood and liver tissue

samples were taken.In the obtained serums, insulin, glucose and lipid parameters were measured. Liver tissue PGC1α and FGF21 levels and mRNA expressions were determined.

Results:Criteria for metabolic syndrome were successfully established with fructose. It was observed that the administration of exercise alone and in combination with fructose exerted positive effects and improved MetS criteria. It was also seen to increase FGF21 and PGC1α levels and mRNA expressions.

Conclusions: According to this study, exercise and exercise-induced FGF21 and PGC1α show promising results in the treatment of adverse metabolic disorders and complications caused by MetS.In addition, clinical use of treatment approaches based on the use of FGF21 and PGC1α can be recommended with further experimental studies.

Keywords: Fructose, Exercise, Lİver, PGC1alfa, FGF21

P046

INCREASED SERUM GLUTAMINE/GLUTAMATE AND AMELIORATION OF NEUROPATHY OF MICE WITH STREPOZOTOCIN-INDUCED DIABETES BRED IN ENRICHED ENVIRONMENT

Pavlina Gateva[1], Natasha Ivanova[1, 2], Milen Hristov[1], Zafer Sabit[1]

[1] Medical University - Sofia, Bulgaria

[2] Institute of Neurobiology, Bas, Bulgaria

Objectives: Enriched environment (EE) relays on a psychoneurological paradigm that multiple non-stressful challenges in every-day living exert beneficial effect on neuronal functioning. Breeding experimental animals in EE is thought to be useful for treating different neuropathic conditions, including peripheral neuropathy. Diabetic neuropathy is one of the most common and debilitating complication of long-lasting diabetes. Disturbances of glutamine/glutamate levels are often discussed in diabetic neuropathy. We hypothesized that EE probably works by changing glutamine/glutamate levels.

Methods:Thirty male ICR mice (20-25 g) were assigned to either EE (n=15) or standard laboratory conditions breeding (n=15). EE was created with different wheels, corridors and stairs changed every other day stimulating mice exploratory activity. Diabetic neuropathy was induced with streptozotocine (i.p.) 200 mg/kg and verified by a fasting glucose level above 8.3 mmol/l and formalin test, performed with intraplantar injection of 0.05% formalin solution and observation of two phases of antinociceptive behavior, an early (0th -5th min) and late (20th -30th min). Glutamine/glutamate level in blood was evaluated spectrophotometrically.

Results:Statistically significant decreases of second phase of the antinociceptive behavior was observed in the EE-bred group ($p < 0.05$) at the 5th week of the experiment. Serum glutamine/glutamate also statistically increased ($p < 0.05$).

Conclusions: EE is a suitable complementary treatment of diabetic neuropathy. The role of glutamine/glutamate in diabetic neuropathy merits further elucidation.

Keywords: diabetic neuropathy, streptosotozin-mice model, formalin test, glutamine-glutamate

P047

EFFECT OF GROUND GRAPE SEED ON NONALCOHOLIC FATTY LIVER DISEASE

Hüsamettin Vatansev[1], Fatma Akat[1], Esranur Turgut[2], Eissa Almaghrebi[2], Ali Habacı[2], Muslu Kazım Körez[3]

[1] Department of Medical Biochemistry, Selcuk University Faculty of Medicine, Konya, Turkey

[2] Selçuk University Institute of Health Sciences Department of Medical Biochemistry, Konya, Turkey

[3] Selcuk University Faculty of Medicine Department of Biostatistics, Konya, Turkey

Objectives: Non-alcoholic fatty liver disease (NAFLD) is an abnormal accumulation of fat in the liver. Grape seed extract is a source of powerful antioxidants and anti-inflammatory agents with various therapeutic effects. Our study aimed to investigate the effect of ground grape seeds on the disease in individuals with NAFLD.

Methods:The study included 30 patients diagnosed with NAFLD. Individuals took approximately 5 g/day black grape seed meal in the morning for 25 days in 2 periods.

Results:There was no statistically significant change in ALT, AST, triglyceride, total cholesterol, LDH, GGT, direct and indirect bilirubin, LDL, HDL, gluco-

se, TAS and Thiol levels at baseline and after 2 cycles of black grape seed meal. However, TOS (Friedman $\chi 2$=27.299, p<.001), OSI (Friedman $\chi 2$=23.094, p<.001) and PON-1 (F=5.086, p=.009) levels showed a significant change over time. When pairwise comparisons were analyzed for TOS and OSI parameters according to time, it was observed that the lowest TOS and OSI levels were reached after the second cycle, and a significant decrease was observed in patients treated with black grape seed meal compared to the baseline. In addition, when the pairwise comparison results for PON-1 levels were analyzed, it was found that PON-1 levels decreased significantly after the second cycle compared to baseline (209.08 ± 45.69 vs. 253.29 ± 60.71, $p_{bonferroni}$=.010).

Conclusions: This study describes the beneficial effect of using ground grape seeds for two months in patients with non-alcoholic fatty liver disease. We anticipate that the results may improve with a longer follow-up.

Keywords: Grape seed, Non-alcoholic fatty liver disease NAFLD, Treatment, Oxidative stress

P048

THE EFFECT OF TWO-WEEK HIGH-INTENSITY INTERVAL TRAINING ON INSULIN RESISTANCE

Sabahattin Muhtaroğlu, Tuğba Sarıtaş, Didem Barlak Keti

Erciyes University, Faculty of Medicine, Department of Medical Biochemistry, Kayseri, Türkiye

Objectives: High-intensity interval training (HIIT training) is a type of training that provides intense effort with short rest intervals. HIIT training has become a popular training method recently because it is economical in terms of time and a high performance increase is achieved in a short time. The aim of this study is to examine the effect of 2-week HIIT training on insulin resistance levels.

Methods:20 healthy men (10 experimental/10 control) between the ages of 18-30 participated in the study voluntarily. The control group consisted of 10 men who did not participate in any sports activities and had no sports background, and the experimental group consisted of 10 men who had a sports background and actively engaged in sports. The study covered 8 sessions. Sessions were held at the same time and 3 days a week. Before and after the sessions, a 3-minute active warm-up and cool-down on the bike was applied. The total HIIT training protocol lasted 2 weeks. Blood samples were collected at 3 different time intervals, at rest before exercise and at 15 and 30 minutes after exercise. Insulin resistance was calculated using the Homeostasis Model Assessment index.

Results:There was no statistically significant difference between the groups and within each group in terms of insulin resistance before exercise and at the 15th and 30th minutes after exercise.

Conclusions: We can say that 2 weeks of HIIT training does not provide a significant improvement in insulin resistance.

This poster was produced from graduate student Tuğba Sarıtas's thesis work

Keywords: High intensity interval training, insulin resistance

P049

EVALUATION OF ADIPOCYTE FATTY ACID BINDING PROTEIN IN PREDIABETIC PATIENTS

Aydan Taka Kucuk[1], Murat Aksıt[2], Ayfer Colak[2]

[1] University of Health Sciences, Tepecik Training and Research Hospital Internal Medicine Department, Izmir, Turkey

[2] University of Health Sciences, Tepecik Training and Research Hospital, Medical Biochemistry Department, Izmir, Turkey

Objectives: Adipocyte fatty acid binding protein (a-FABP) is a fatty acid chaperone that has been described as a fat-derived hormone. It regulates lipid homeostasis and is an important mediator of inflammation. Circulating levels of a-FABP are closely associated with metabolic syndrome diseases with diagnostic and prognostic significance. The aim of this study was to evaluate the levels of a-FABP in patients diagnosed with prediabetes.

Methods:The study included 35 patients with prediabetes and 38 age- and sex-matched control group patients. Biochemical tests were analyzed using the AU 5800 (Beckman Coulter Inc., CA, USA) autoanalyzer.HbA1c levels were analyzed by ion exchange high-performance liquid chromatography method on the Variant II (BioRad, USA) device. a-FABP measu-

rements were made using a commercial kit (Sunred, China) that works with sandwich ELISA.

Results:Serum a-FABP level was statistically significantly higher in the prediabetes group ((3.19 ng/ml (2.65-4.22)) than in the control group ((1.58 ng/ml (1.38-2.27)) (p<0.001). Serum a-FABP levels were positively correlated with age, glucose and HbA1c levels (r=0.240 p=0.041, r=0.437 p=<0.001, r=0.635 p=<0.001, respectively).

Conclusions: It is known that up to 70% of patients with prediabetes develop diabetes over time. Therefore, it is extremely important to follow up and treat prediabetes patients in order to prevent diabetes and diabetes-related complications. Because we found α-FABP to be higher in patients with prediabetes, drugs targeting this molecule may be a potential therapeutic target for treatment.

Keywords: Prediabetes, a-FABP, HbA1c

P050

EVALUATION OF ADIPOCYTE FATTY ACID BINDING PROTEIN IN PREDIABETIC PATIENTS

Aydan Taka Kucuk[1], Murat Aksıt[2], Ayfer Colak[2]

[1] University of Health Sciences, Tepecik Training and Research Hospital Internal Medicine Department, Izmir, Turkey

[2] University of Health Sciences, Tepecik Training and Research Hospital, Medical Biochemistry Department, Izmir, Turkey

Objectives: Adipocyte fatty acid binding protein (a-FABP) is a fatty acid chaperone that has been described as a fat-derived hormone. It regulates lipid homeostasis and is an important mediator of inflammation. Circulating levels of a-FABP are closely associated with metabolic syndrome diseases with diagnostic and prognostic significance. The aim of this study was to evaluate the levels of a-FABP in patients diagnosed with prediabetes.

Methods:The study included 35 patients with prediabetes and 38 age- and sex-matched control group patients. Biochemical tests were analyzed using the AU 5800 (Beckman Coulter Inc., CA, USA) autoanalyzer. HbA1c levels were analyzed by ion exchange high-performance liquid chromatography method on the Variant II (BioRad, USA) device. a-FABP measurements were made using a commercial kit (Sunred, China) that works with sandwich ELISA.

Results:Serum a-FABP level was statistically significantly higher in the prediabetes group ((3.19 ng/ml (2.65-4.22)) than in the control group ((1.58 ng/ml (1.38-2.27)) (p<0.001). Serum a-FABP levels were positively correlated with age, glucose and HbA1c levels (r=0.240 p=0.041, r=0.437 p=<0.001, r=0.635 p=<0.001, respectively).

Conclusions: It is known that up to 70% of patients with prediabetes develop diabetes over time. Therefore, it is extremely important to follow up and treat prediabetes patients in order to prevent diabetes and diabetes-related complications. Because we found α-FABP to be higher in patients with prediabetes, drugs targeting this molecule may be a potential therapeutic target for treatment.

Keywords: Prediabetes, a-FABP, HbA1c

P051

PREDICTION OF MICROBIAL FUNCTIONAL DIFFERENCES IN COLON CANCER PATIENTS BY USING FAPROTAX

H. Özgür Özdemirel[1], Sibel Küçükyıldırım[1], Beril Erdem Tunçdemir[1], Ceren Acar[2], Hatice Mergen[1]

[1] Faculty of Science, Department of Biology, Hacettepe Universtiy, Ankara 06800, Turkiye

[2] Faculty of Science and Literature, Department of Molecular Biology and Genetics, Inonu University, Malatya 44280, Turkiye

Objectives: Recently, the microbiota under different healthy and pathological conditions have been extensively studied, and these works showed that there was a relationship between the presence and/or abundance of particular species and the disease conditions. The gut microbiota has been a subject of special interest since the beginning of these studies because the gut provides one of the largest habitats in the human body with its high diversity and functionality. Regarding this, our work aimed to use a functional prediction based on DNA metabarcoding data to show differences in functional diversity in colon cancer tissues. In addition, we tested the functional diversity in different stages of tumor progression.

Methods:The DNA metabarcoding data from 9 patients with colon cancer and 18 healthy subjects were included in this work. We used SHAMAN and FAPROTAX for bioinformatic analyses and functio-

nal annotation prediction, respectively. Then, statistical analyses were performed using R. This work was supported by a grant from Inonu University Research Fund (FCD-2020-2065).

Results:Our analysis predicted that the microbial functional diversity was significantly different between the colon cancer and healthy subjects in three functions: ureolysis, xylanolysis, and dark hydrogen oxidation ($p<0.001$). Colon cancer tissues showed lower levels of these predicted functions which is related to the low abundances of Firmicutes, Bacteroidota, Actinobacteria, and methane-producing archaea.

Conclusions: This work will help us to understand which functions are particularly affected during colon cancer progression in relation to changes in microbial diversity.

Keywords: metabarcoding, microbiota, functional diversity, 16S rRNA

P052

INCREASED EXPRESSION LEVEL OF ATF GENE IN T98 GLIOBLASTOMA CELLS AFTER TREATMENT WITH BIOCHANIN A

Ezel Demir, Elif Merve Avcu, Beril Erdem Tuncdemir, Emel Saglar Ozer

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

Objectives: Since traditional treatment strategies for glioblastoma is harmful for healthy cells, usage of flavonoids like Biochanin A which is a natural dietary compound may be promising dietary compounds as potential treatment options for glioblastoma via their anti-carcinogenic properties. Biochanin A can show its effects through the change of expression levels of Endoplasmic reticulum (ER) stress-related genes in the cell. In this study, expression level of ATF4 which is one of the ER stress-related gene was investigated in T98 glioblastoma cells after the treatment with Biochanin A.

Methods:T98 glioblastoma cells were incubated with different concentrations of Biochanin A. Total RNA isolation and cDNA synthesis were performed. The difference of expression level of ATF4 was analyzed on basis of EF1a as a reference gene between in normal and Biochanin A-treated cells. This study was supported by Hacettepe University (Project no: FHD-2022-19959).

Results:ΔΔCt value of ATF4 gene was calculated and acording to the results, Biochanin A treatment significantly increased ATF4 expression level in T98 glioblastoma cells (p: 0,0002).

Conclusions: Dietarty compounds like Biochanin A can show their effects on cancer cells through the ER stress-related pathways. They can induce or reduce ER stress and consequently apoptosis in the cell. We found that Biochanin A had an effect on expression level of ATF4 which is an ER stress-related gene. Therefore, these kind of studies about the effect of Biochanin A on glioblastoma cells may be valuable and may have a potential for developing new treatment strategies.

Keywords: Endoplasmic reticulum stress, ATF4, Biochanin A, T98G

P053

THE EFFECT OF HOGNA RADIATA VENOM IN HUMAN BREAST CANCER CELL LINE

Hazal Tahravi, Refika Dilara Vaizoğlu, Hatice Mergen

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Türkiye

Objectives: Spider venoms, are abundant in bioactive substances with potential medical uses. Recent studies have highlighted the potential of spider venoms as a notable source of bioactive compounds that could be utilized for anti-tumor therapies. Spider venom derivatives and components showcase diverse pharmacological impacts, sparking heightened interest in their potential for cancer treatment development. The aim of this study is to examine the effect of *Hogna radiata* venom on cell viability.

Methods:To determine the non-cytotoxic concentration for MDA-MB231 breast cancer cells, spider venom was applied at different concentrations (500, 50, 5 and 0.5 mg/ml). After 24 hours of incubation, cell viability was measured by MTT assay. Also, 50 mg venom was ran via 10% polyacrylamide gel under SDS-denatured conditions. Then, protein bands were visualized by Coomassie blue staining of the gel.

Results:We found that the effect of the spider venom was increased in breast cancer cells in a dose-dependent manner compared to control cells which were not treated with venom. According to MTT assay

results, the venom has a cytotoxic effect on the cell line. The protein bands in the venom were seperated by their molecular masses ranging from ~10 to ~120 kDa.

Conclusions: Our results revealed that *Hogna radiata* venom has a cytotoxic effect on MDA-MB231 cells. Further molecular examinations using this venom could provide a promising avenue for treating breast cancer. This study underscores the significant role that natural toxins can play in advancing therapeutic approaches.

Keywords: Hogna radiata venom, breast cancer cells, cytotoxicity

P054

NOVEL PHOSPHOLIPID DERIVATIVES OF TELOMERE TARGETED ANTICANCER NUCLEOSIDES

Merve Yilmaz[1], Ates Kutay Tenekeci[1], Z. Gunnur Dikmen[1], Larisa L. Birichevskaya[2], Sergei M. Gryaznov[3]

[1] Hecettepe University Medical Faculty, Department of Biochemistry, Ankara, Turkey

[2] Laboratory of Molecular Biotechnology, Institute of Microbiology, National Academy of Sciences, Minsk, Belarus

[3] Maia Biotechnology, Inc., Chicago, Il, Usa

Objectives: The relationship between telomerase and telomeres is a promising focus for cancer treatment, as telomerase is inactive in the majority of human somatic cells. Telomerase activity and TERT expression are elevated in approximately 90% of primary human cancers. Novel molecules targeting telomeres and telomerase with low toxicity, high tumor specificity, and minimal side effects are under investigation. Hence, strategies focused on specific targeting of telomeres using modified nucleoside substrates of telomerase hold significant potential. In this study we aimed to test a panel of phospholipid derivatives of 6-thioguanine-containing nucleosides and identify potential new telomere targeted compounds using in vitro screening.

Methods:HT-29, A549, and HeLa cells were cultured in 10% FBS, 1% antibiotic, and 1% L-glutamine supplemented DMEM at 37°C with 5% CO_2. Compounds were dissolved in DMSO/PBS (1:2, v/v) or 100% DMSO. Cells ($2x10^3$ cells/well) were plated in 96-well plates in DMEM. After 24 hours, compounds were added at 9 concentrations (30-0.005 µM) and incubated for 96 hours for the MTT assay.

Results:We found HT-29 and HeLa cells to be highly sensitive to compounds designated as L6 and L8 (IC50: HT-29 0.08 µM, 0.20 µM; HeLa 0.15 µM, 0.17-µM). In A549 cells IC50 were 1.06 µM and 10.76 M, respectively. For comparison, in HT-29, HeLa, and A549 cell lines, the IC50 for the unconjugated nucleoside 6-thio-dG (THIO) was 0.20 µM, 0.12µM, and 3.04 µM, respectively.

Conclusions: We have identified two promising compounds as potential anticancer agents. These findings warrant further evaluation of these molecules in vivo models.

Keywords: Cancer, Telomere, Telomerase, Phosphatidyl Nucleosides

P055

A RARE CASE WITH AN UNEXPECTEDLY HIGH CA125 LEVEL

Feyza Yağmur Tekeli[1], Işın Üreyen[2]

[1] Antalya Training and Research Hospital, Department of Medical Biochemistry

[2] Antalya Education and Research Hospital of Gynecological Oncologyy,antalya , Turkey

[3] Medical Biochemistry

[4] Gynecological Oncology

Objectives: CA 125 is an antigenic tumor marker that is carried out when suspecting ovarian or other tissue neoplasm and is used in monitoring patients diagnosed with epithelial ovarian cancer.

Methods:A 49-year-old woman presented to the private gynecological department with weakness, abdominal pain, and abnormal uterine bleeding over the last year. Our patient was referred to the gynecologic oncology of our hospital due to suspected malignancy with a CA-125 level: of 445 IU/mL (reference range 0-35 IU/mL). The test was confirmed in our laboratory using the immunoassay method, and it measured 450 and 900 U/mL with a two-day interval between them. In terms of the possibility of analytical

error for the CA 125 test, interference was initially ruled out by using a heterophilic antibody-blocking tube, and the test was conducted with a different immunoassay kit. However, no difference was detected in the CA 125 test results. No tumoral formation was detected, including the performed colonoscopy and endoscopic screenings. There is a mild thickening of the uterine wall and minimal accumulation of fluid In pelvic tomography and ultrasonographic findings. The endometrial biopsy results indicated endometrial thickening and were evaluated as a polyp. The patient who underwent surgery for pelvic examination underwent total hysterectomy and oophorectomy.

Results:The uterine material at 16 weeks gestational size was diagnosed with adenomyosis and the pre-operative high CA-125 level (2335 IU/ml) decreased to 19,7 IU/ml three weeks after surgery.

Conclusions: Unexpectedly high CA-125 over 2000 IU/ml can be seen in benign gynecologic conditions such as severe adenomyosis.

Keywords: Ca 125, Adenomyosis, Interference, Tumor marker, Oncology

P056

INVESTIGATION OF THE ANTITUMOREGENIC EFFECT OF CITRUS AURANTIUM ON PANCREAS, THYROID AND PROSTATE CANCER CELLS

Arzu Yıldırım[1], Ezel Bildik[1], Mehmet Ali Koçdor[2], Hilal Koçdor[1]

[1] Dokuz Eylül University, Oncology Institute, Department of Basic Oncology, İzmir, Turkey

[2] Dokuz Eylül University, Faculty of Medicine, Department of General Surgery

Objectives: Three different extracts(Methanol/water, Ethyl acetate/water; water phase, Ethyl acetate/water; Ethyl acetate phase)obtained from Citrus Aurantium(C.aurantium-CA); It was aimed to determine the antitumorigenic and cytotoxic effect of the drug in combination with standard chemotherapeutic agents used in the clinic on PANC-1(Pancreatic ductal adenocarcinoma), CAL-62(Thyroid anaplastic carcinoma), PC-3(Prostate adenocarcinoma) cell lines.

MATERIALS and METHODS:Three different extracts of CA and standard chemotherapeutic agents used in the clinic,IC25 and IC50 concentrations were determined by the MTT method.Wound Healing to examine the effect of determined concentrations on migration and metastasis;to evaluate tumorigenesis capacity; SoftAgar Colony Analysis(3D Spheroid Model) was performed.The Oxidative Stress Index(OSI) index was calculated.

Results:At the lowest dose in all cell lines; Ethyl acetate/Water;Water phase of CA was effective. In the comparative analysis made with reference to the complete closure time of the area in the control group in the WoundHealing Analysis;In PANC-1, CAL-62, PC-3 cell lines, respectively;The difference between the control group and the CA50 group was determined as 70.8%, 63.8%, 65.8%.In 3D Spheroid Model analysis, statistically significant differences were found in PANC-1 and PC-3 cell lines(respectively $p<0.004$, $p<0.003$)while no significant difference was observed in CAL-62 cell line.When OSI was evaluated;statistically significant difference was observed only in CAL-62 cell line($P<0.004$).

Conclusions: The effect of CA on thyroid and pancreatic cancer cell lines has been examined for the first time in the literature.Our findings showed that the CA50 group was effective in preventing invasion and migration in all cell lines.We think that the antitumorigenic activity of CA should be supported by advanced cell culture and *in vivo* studies.

Keywords: C aurantium, invasion, migration, metastasis

P057

EVALUATION OF ALTERATIONS IN THE HDL SUBFRACTION AND HDL-RELATED ENZYMES IN PATIENTS WITH LIVER FAILURE AND IN HEALTHY DONORS

Çağatay Yılmaz[1], Tuğçe Çeker[1], Aleyna Öztüzün[1], Bürke Çırçırlı[3], Bora Dinç[4], İlker Öngüç Aycan[4], Mutay Aslan[1, 2]

[1] Akdeniz University, Faculty of Medicine, Department of Medical Biochemistry, Antalya, Türkiye

[2] Akdeniz University, Faculty of Medicine, Department of Gene and Cell Therapy, Antalya, Türkiye

[3] Akdeniz University, Institute of Health Sciences, Department of Medical Biotechnology, Antalya, Türkiye

[4] Akdeniz University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Antalya,

Türkiye

Objectives: This study aimed to investigate high-density lipoprotein (HDL) subfractions and HDL-associated enzyme changes in liver failure patients and healthy donors.

Methods:Groups of twenty patients with liver failure and twenty healthy donors were chosen for the study. Before the transplant, serum samples from each patient were analyzed. HDL subfractions analysis was done by continuous disc polyacrylamide gel electrophoresis. Immunoassay was performed to determine the plasma levels of apolipoprotein A-1 (ApoA-I), cholesteryl ester transfer protein (CETP), and lecithin–cholesterol acyltransferase (LCAT).

Results:Liver failure patients had significantly higher levels of triglycerides and very low-density lipoprotein compared to healthy donors. Additionally, these patients showed significant increases in levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, and blood urea nitrogen (BUN), while albumin levels were significantly lower compared to healthy donors. Although there was no significant difference in HDL cholesterol levels between the two groups, liver failure patients had a marked increase in the HDL-large subfraction and a significant decrease in the HDL-small subfraction compared to healthy donors. Moreover, liver failure patients had significantly lower serum ApoA1 levels compared to healthy donors, but there was no significant difference in LCAT and CETP levels between the two groups.

Conclusions: The profiles of HDL subfractions can distinguish between healthy donors and liver failure patients. The results also indicate that the levels of ApoA-1, which plays a crucial role in HDL metabolism, are lower in patients with liver dysfunction. This decrease in HDL-small subfractions may be due to impaired anabolism resulting from hepatic failure. Grant no #TSA-2018-2785 and #TTU-2021-5605.

Keywords: Liver transplantation, HDL subfractions, ApoA1, CETP, LCAT

P058

EFFECT OF 4-PHENYLBUTYRATE ON COBALT CHLORIDE-INDUCED CYTOTOXICITY IN AML12 CELLS

Yagmur Ucar Yagci[1], Tugce Karabas[2], Mervenur Barakli Kocabas[2], Gulben Sayilan Ozgun[2]

[1] Edirne State Hospital, Medical Biochemistry Laboratory, Edirne, Türkiye

[2] Trakya University, School of Medicine, Medical Biochemistry Dept, Edirne, Türkiye

Objectives: Cobalt chloride is a hypoxia-mimetic agent which inhibits hypoxia-inducible factor-1 degradation and increases the expression of hypoxia-related genes. 4-phenylbutyrate is a chemical chaperone that inhibits endoplasmic reticulum stress. In the present study, we aimed to investigate the effects of 4-phenylbutyrate on cobalt chloride-induced cytotoxicity in the AML12 mouse hepatocyte cell line.

Methods:AML12 hepatocytes were treated with 4-phenylbutyrate concentrations (25, 50, 100, 250, 500 and 1000 µM) together with 300 µM cobalt chloride for 24 hours. Then, cell viability was determined by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results:300 µM cobalt caused a significant decrease in cell viability. 4-phenylbutyrate concentrations did not change the cell viability of cobalt chloride-treated cells.

Conclusions: Our study showed that 4-phenylbutyrate does not change the cobalt chloride-induced cytotoxicity in AML12 hepatocytes.

Keywords: Cobalt chloride, 4-phenylbutyrate, Cytotoxicity, Hepatocytes, AML12 cells

P059

THE EFFECT OF ACETYLSALICYLIC ACID ON ACRYLAMIDE-INDUCED CELLULAR HEPATOTOXICITY

Mervenur Barakli Kocabas[1], Tugce Karabas[1], Yagmur Ucar Yagci[2], Gulben Sayilan Ozgun[1]

[1] Trakya University, School of Medicine, Medical Biochemistry Dept., Edirne, Türkiye

[2] Edirne State Hospital, Medical Biochemistry Labo-

ratory, Edirne, Türkiye

Objectives: Acetylsalicylic acid (aspirin) is a frequently used non-steroidal anti-inflammatory drug. There is no study investigating the effect of acetylsalicylic acid on acrylamide-induced hepatotoxicity. The present study aimed to determine the effect of aspirin on acrylamide-induced cytotoxicity in AML12 mouse hepatocytes.

Methods:AML12 cells were treated with 25, 50, 100, 150 and 200 µM acetylsalicylic acid simultaneously with 10 mM acrylamide for 24 hours to determine the effect of aspirin on acrylamide-induced cytotoxicity. Cell viability was determined by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results:10 mM acrylamide caused a significant decrease in cell viability compared to the control group. Aspirin concentrations did not cause a significant change in cell viability in acrylamide-treated cells.

Conclusions: This study showed that aspirin did not significantly alter acrylamide-induced cytotoxicity in AML12 mouse hepatocytes.

Keywords: Acetylsalicylic acid, Acrylamide, Liver, Cytotoxicity, AML12 cells

P060

INTERCELLULAR COMMUNICATION IN ATHEROSCLEROSIS: EFFECT OF DIFFERENT ENDOTHELIAL EXOSOME DOSES ON UPTAKE-DRIVEN CHANGES IN VASCULAR SMOOTH MUSCLE CELLS

Joumana Al Akhdar, Melike Nur Yangın, Fatima Hacer Kurtoğlu Babayev, Kemal Baysal

Koç University, Graduate School of Health Science, Cellular and Molecular Medicine, Istanbul, Türkiye

Objectives: Atherosclerosis is an inflammatory disease initiated by oxidized lipid accumulation in the vessel wall, fatty streak formation, and wall thickening, eventually leading to plaque rupture. During this progressive disease, phenotypic changes occur in endothelial cells (EC) and vascular smooth muscle cells (VSMC). Exosomes, vesicles released from cells, are essential in intercellular communication.

Methods:In this study, we sought to explore the mechanism of exosome-mediated intercellular crosstalk between EC and VSMC in an *in vitro* model. We isolated exosomes from HUVEC and added them onto VSMC, allowing their uptake. We aimed to observe the dose-dependent effect of exosomes on the recipient cells.We treated HUVEC with TNF-α (10 ng/ml) or LPS (50 ng/ml), using these agents as a low-level inflammation model, while non-treated cells served as controls. The exosomes were isolated by ultracentrifugation and characterized by Western blotting and Nanoparticle tracking analysis. Exosomes from treated or control cells were added onto VSMC at 20µg (LOW EXO) or 60µg (HIGH EXO) exosomes per 100,000 cells. We followed gene expression in recipient VSMC by qPCR. We measured the expression of genes for the contractile and synthetic phenotypes.

Results:The effect of HUVEC exosomes on VSMC viability was measured by MTT assay. LOW EXO treatment showed an increase in cell viability, indicating an increase in proliferation which also suggests the shifting of VSMC to the synthetic phenotype.

Conclusions: The effect of LOW EXO or HIGH EXO concentrations on VSMC migration was measured. We detected no significant apoptosis using Annexin V Assay.

Keywords: Atherosclerosis, Endothelial cells, Vascular Smooth Muscle cells, Exosomes, Phenotypic changes, viability, migration, apoptosis

P061

MACROPHAGE GENE EXPRESSION CHANGES OBSERVED IN IN VITRO CO-CULTURES WITH ENDOTHELIAL OR VASCULAR SMOOTH MUSCLE CELLS

Melike Nur Yangın[1], Joumana Al Akhdar[1], Fatıma Hacer Kurtoğlu Babayev[1], Kemal Baysal[2, 3]

[1] Koç University, Graduate School of Health Sciences, Cellular and Molecular Medicine, Istanbul, Türkiye

[2] Koç University, Kuttam, Koç University, Research Center For Translational Medicine, Istanbul, Türkiye

[3] Koç University, School of Medicine, Department of Biochemistry, Istanbul, Türkiye

Objectives: Atherosclerosis is a chronic inflammatory vascular disease. During atherosclerosis, monocytes enter the vessel wall and become activated to form macrophages. Two phenotypes have been re-

ported for macrophages. M1 is a classically activated macrophage and is proinflammatory, whereas M2 is an alternatively activated macrophage and is anti-inflammatory. Vascular smooth muscle cells (VSMC) and endothelial cells (EC) affect macrophage phenotype differently. We aimed to investigate macrophage gene expression changes in *in vitro* co-culture models of atherosclerosis.

Methods:THP-1 monocyte cells were activated into macrophages with PMA (phorbol 12-myristate-13-acetate). The MTT results revealed that 200 ng/ml PMA treatment for 24 hours effectively activated THP-1 monocytes into macrophages. Macrophages were co-cultured with VSMCs, or HUVECs in separate experiments using inserts. To simulate atherosclerosis, low-grade inflammation was induced using TNF-α (10ng/ml) or LPS (50ng/ml). Following a 72-hour incubation, total RNA was isolated from macrophages, and gene expression was measured by qPCR.

Results:In EC-macrophage co-cultures, both TNF-α and LPS treatment caused an increase in IL1-β (observed in M1 phenotype) and CCL22 expression relative to controls. The presence of EC in the co-culture with macrophages affects M1 polarization in response to TNF-α and LPS. In macrophage co-culture with VSMCs, LPS increased IL1-β (M1 polarization) and Arg1 expression (indicating an M2 phenotype), whereas CCL22 expression decreased (observed in M1 phenotype).

Conclusions: Macrophage heterogeneity plays a vital role in atherosclerosis. This study demonstrated the effects of EC and VSMC co-cultures with macrophages, resulting in significant heterogeneity in these cells, which indicates novel results.

Keywords: Macrophages, Atherosclerosis, Vascular Smooth Muscle Cells, Endothelial Cells, In Vitro Co-culture

P062

RETROSPECTIVE COMPARISON OF CARDIAC TROPONIN-T VALUES IN PATIENTS WITH AND WITHOUT CHRONIC KIDNEY DISEASE UNDIAGNOSED OF ACUTE MYOCARDIAL INFARCTION

Zahide Adıyaman, İbrahim Türk, Tuğçe Acar, Büşra Üresin Yazlak, Mevlüde Ayyıldız, Berrin Berçik İnal

Health Sciences University, İstanbul Teaching and Research Hospital, Medical Biochemistry, İstanbul, Türkiye

Objectives: Cardiac troponins used in the diagnosis of acute coronary syndrome(ACS) are elevated due to cardiac muscle damage. However, in patients with chronic kidney disease(CKD), high troponin levels may complicate the diagnosis of ACS. In this study, we aimed to compare hs-Troponin-T values in patients with and without CKD who were not clinically diagnosed with acute myocardial infarction.

Methods:In our study, 123 individuals were included. Patients with known coronary artery disease, acute myocardial ischemia, sepsis, heart failure and malignancy were excluded. Patients were divided into groups based on eGFR levels calculated using the CKD-EPI formula: Group 1) eGFR <30; 2) eGFR 30-60; 3) eGFR 60-90; 4) Control group. Hs-Troponin-T(Roche Cobas8000) was measured in all patients. The difference between groups was compared with Kruskal-Wallis. Spearman correlation analysis was performed between hs-Troponin-T and eGFR values.

Results:According to the Mann-Whitney U-test, there was a statistically significant difference in hs-Troponin-T levels between the control and CKD patient groups ($p<0.05$). Kruskal-Wallis test also revealed a statistically significant difference in hs-Troponin-T levels among the groups ($p<0.05$). Spearman correlation analysis showed a negative correlation between hs-Troponin-T values and eGFR levels ($r=-0.86$, $p=0.001$).

Conclusions: Our study demonstrated an increase in hs-Troponin-T levels as eGFR levels decreased. Consequently, we believe that the use of hs-Troponin-T is limited in patients with CKD and low eGFR levels. The diagnosis of ACS should rely on observing the changes in hs-Troponin-T values over time (increases/decreases). The limited sample size in each group and the retrospective data collection from patient files are the main limitations of our study.

Keywords: Troponin-T, eGFR, Chronic kidney disease

P063

THE IMPORTANT ROLE OF SANGER SEQUENCING IN THE DETECTION OF THE MAGHREBIAN MUTATION C.525DELT IN THE SGCG GENE IN LIMB-GIRDLE MUSCULAR DYSTROPHY

Fatimazahra Smaili[1, 2], Khawla Zerrouki[1, 2], Fatima Ezzahra Aouni[1, 2], Ayad Ghanam[3, 4], Abddeladim Babakhouya[3, 4], Mariam Tajir[1, 2]

[1] Laboratory of Medical Genetics, Central Laboratory, Mohammed V1 University Hospital, Oujda, Morocco

[2] Epidemiology, Clinical Research and Public Health Laboratory, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

[3] Pediatrics Department, Mother and Child Hospital, Mohammed V1 University Hospital, Oujda, Morocco

[4] Laboratory of Immunohematology / Cellular Therapy For Adults and Children, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

Objectives: Limb-girdle muscular dystrophies (LGMD) represent a heterogeneous group of myopathies and include autosomal recessive gamma-sarcoglycanopathy, due to mutations in the *SGCG* gene. The c.525delT (p.Phe175Leufs) mutation of this gene is a recurrent mutation found in 65% of autosomal recessive LGMD. The objective of our work is to show the importance of the Sanger Sequencing method in the determination of this mutation and its contribution to health strategies.

Methods:We report a patient referred to the Medical Genetics Laboratory of the Mohammed VI University Hospital of Oujda for a myopathy. The patient's DNA was extracted from peripheral venous blood. DNA quality and quantity were controlled by spectrophotometry. We performed a PCR amplifying exon 6 of the *SGCG* gene. The amplified fragments were purified and sequenced by the Sanger method on ABI SeqStudio from Applied Biosystems.

RESULTS:Sequencing showed the presence of the c.525delT mutation in the patient in the homozygous state.

Conclusions: We highlight the importance of molecular analysis by Sanger sequencing in the targeted detection of genetic mutations.

Keywords: Limb-girdle muscular dystrophies, SGCG gene, Sanger sequencing

P064

LABORATORY PERSPECTIVE TO ELEVATED SERUM AMYLASE: A CASE REPORT

Irem Acilan, Sedat Abusoglu, Abdullah Sivrikaya, Ali Unlu

Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye)

Objectives: Several medical conditions can cause isolated amylase elevation, such as salivary diseases, macroamylasemia, idiopathic hyperamylasemia (Gullo's syndrome). The aim of this case was to identify the protocols of laboratory to determine macroamylasemia.

Methods:A 1-year-old male patient with serum C-reactive protein (C-RP) elevation (24 mg/L) and hyperamylasemia (186 U/L) (reference range: 8-79 U/L) was transferred to our pediatric emergency department with acute pancreatitis suspicion. Although serum amylase levels were gradually increasing, serum lipase levels were in reference range. Transabdominal ultrasound resulted with non-specific findings. In order to exclude macroamylasemia, firstly the serum was precipitated with twenty-five percent of polyethylene glycol (PEG). Furthermore, amylase / creatinine clearance ratio (ACCR) and urinary amylase / urinary creatinine ratio (uAm/uCr) were calculated.

Results:The serum amylase level was 369 U/L before the precipitation with PEG, thereafter it was 252 U/L [(Amylase recovery was 68% (recovery cut-off for macroenzymes <40%)]. Then, as a second procedure, ACCR was calculated. The formula parameters were as follows: urine amylase: 822 U/L, serum amylase: 269 U/L, urine creatinine: 39 mg/dL, and serum creatinine: 0.32 mg/dL. The ratio was found 2.5% (reference range: 1.8%- 3.2%). Thirdly; the uAm/uCr was 2107 U/g creatinine (reference range: 40-440 U/g creatinine). All of reference ranges were applied from MayoClinic. Macroamylasemia was excluded on the basis of these findings. In salivary gland ultrasound examination, the diagnosis was confirmed as sialadenitis.

Conclusions: As a clinical biochemistry specialist, it is significant to determine or exclude macroamyla-

semia in patients with isolated amylase elevation for avoiding misdiagnosis.

Keywords: ACCR, Hyperamylasemia, Polyethylene glycol, Urinary amylase

P065

HIGH B12 LEVEL AS ACUTE PHASE REACTANT IN CHOLESTATIC LIVER DISEASES

Sabahattin Muhtaroğlu, Naci Tektaş, Semanur Doğan, Didem Barlak Keti

Erciyes University, Faculty of Medicine, Department of Medical Biochemistry, Kayseri, Türkiye

Objectives: Vitamin B12 or cobalamin is a water-soluble vitamin that is stored in the liver and used in metabolic processes. We aimed to reveal the relationship between vitamin B12 and cholestatic liver disease through this case.

Methods:A 68-year-old woman patient with no known chronic disease applied to the emergency department with a complaint of abdominal pain that had been increasing for the last 5 days. Stones were detected in the bile duct during imaging performed at an external center. In the biochemical analysis, serum levels of direct bilirubin, Gamma Glutamyl Transferase and Alkaline Phosphatase, which are markers of cholestasis, were detected to be high. Apart from these tests, serum B12 level was measured as 1026 (Reference range 197-771 pg/mL). The patient was operated on.

Results:Biochemical markers, including B12, of the patient who underwent cholecystectomy and resection of liver segments 2-3 decreased to normal levels. It is noteworthy that B12 level returns to normal before other cholestatic markers.

Conclusions: In our case, the B12 level returned to the normal range after treatment, which brings up the use of B12 level as an acute phase reactant in cholestatic liver diseases. It is important to conduct research on large patient groups by measuring B12 levels in the acute phase of cholestatic liver diseases and to elucidate its relationship with the disease.

Keywords: Vitamin B12, cholestasis, acute phase reactant

P066

THE SIGNIFICANCE OF SERUM TOTAL BILE ACID AS A PREGNANCY BIOMARKER: A CRUCIAL INDICATOR FOR OBSTETERIC CHOLESTASIS

Zubair Ahmed Rahojo, Sibtain Ahmed, Lena Jafri, Hafsa Majid, Abdul Mateenö

Aga Khan University Hospital, Krachi, Sindh, Pakistan

Objectives: This study aimed to assess the significance and diagnostic accuracy of measuring TBA levels in identifying obstetric cholestasis. The research sought to establish the correlation between TBA measurements and established clinical diagnoses, which serve as the gold standard for diagnosing OC.TBA measurements have garnered attention as a potential biomarker for OC.

Methods:A cross-sectional study was carried out at the Section of Clinical Pathology, Aga Khan University Hospital, spanning the period from August 2022 to January 2023. During this timeframe, TBA measurements were collected and subsequently compared to clinically documented diagnoses.Data analysis was conducted using robust statistical tools, including SPSS for in-depth analysis and MS Excel.

Results:The final analysis encompassed 114 patients with complete medical records. Among them, 90 patients (79%) exhibited elevated TBA levels, indicating potential OC. The remaining 24 patients (21%) had TBA levels within the normal range.90 patients had clinical documentation confirming OC diagnoses during their pregnancies. there was complete agreement of 100% between TBA measurements and clinical diagnosis.

Conclusions: This research underscores the importance of monitoring TBA levels during pregnancy, emphasizing the utility of TBA in conjunction with other hepatic biomarkers such as ALT, AST and ALP as a valuable tool for the diagnosis of OC.

Keywords: Serum Total Bile Acid, Obstetric Cholestasis, Pregnancy Biomarker

P067

INDIRECT PANCREATIC FUNCTION TEST-UTILITY OF FECAL PANCREATIC ELASTASE FOR DIAGNOSIS OF EXOCRINE PANCREATIC INSUFFICIENCY IN CHILDREN

Shagufta Nisar Ali

Aga Khan University Hospital

Objectives: Testing of pancreatic function is a valuable tool in the assessment, diagnosis, overall management, and prognosis of pancreatic disease. This study was done to evaluate the frequency of EPI (Exocrine Pancreatic Insufficiency) in Pakistani children based on FPE values at a clinical laboratory in Pakistan.

Methods:This cross-sectional study was performed at the section of Chemical Pathology, Aga Khan University Karachi from 2018 to 2021. A data mining of FPE results of age birth to 16 years was done from the laboratory information system. Duplicates were removed and only the first sample per patient was included, comprising of both inpatient and outpatients. FPE was analyzed using an enzyme-linked immunosorbent assay (Immundiagnostik AG, Germany). Levels > 200 µg/ml are considered normal value, 100 - 200 µg/ml as slight to moderate EPI and < 100 µg/ml suggestive of severe EPI respectively. Data was analyzed using SPSS version 19.

Results:A total of 3324 FPE results were performed during the study period. After application of exclusion criteria, a total of 551 were included in the final analysis. There were 334 (60.6 %) males and 217 (39.4%) females. The median age was 206 (IQR0-730) days. The mean FPE levels were 167 (IQR 120-249) ug/ml. Slight to moderate and severe EPI was found in 85 (15.4%%) and 276 (50.1%) respectively.

Conclusions: A high frequency of EPI in children from a single center is alarming. There is need to advocate the utility of indirect pancreatic function tests for optimal screening and better health outcomes.

Keywords: Exocrine Pancreatic Insufficiency EPI, Enzyme Linked Immunosorbent Assay, Fecal Pancreatic Elastase

P068

BONE TURNOVER MARKERS IN SYSTEMIC SCLEROSIS: IS THERE AN INCREASED RISK FOR OSTEOPOROSIS?

Nazlı Ecem Dal Bekar[1, 2], Gamze Tuna[3], A. Merih Birlik[3, 4], Gül Güner Akdoğan[1], G. Hüray İşlekel[3, 5]

[1] Izmir University of Economics, School of Medicine, Department of Medical Biochemistry, Izmir, Türkiye

[2] Technical University of Munich, Faculty of Life Sciences, Department of Molecular Life Sciences, Chair of Proteomics and Bioanalytics, Freising, Germany

[3] Dokuz Eylul University, Institute of Health Sciences, Department of Molecular Medicine, Izmir, Turkiye

[4] Dokuz Eylul University, Faculty of Medicine, Department of Immunology and Rheumatology, Izmir, Turkiye

[5] Dokuz Eylul University, Faculty of Medicine, Department of Medical Biochemistry, Izmir, Turkiye

Objectives: Scleroderma is a rare autoimmune disease characterized by progressive fibrosis of the skin and internal organs. Impaired collagen metabolism, glucocorticoid treatment, nutrient deficiency due to malabsorption, and frequent and severe vitamin D deficiency are considered risk factors for the increased risk of osteoporosis in scleroderma.

Methods:26 scleroderma patients and 20 healthy subjects were included in this study. Serum type I collagen cross-linked C-telopeptide (CTX-1) and type I collagen amino-terminal propeptide (PINP) were quantified by ELISA. Gastrointestinal involvement severity scores, modified Rodnan skin scores, disease subtypes, disease onset, medications, autoantibody positivity, CRP and ESR levels of the patients were recorded in detail.

Results:Serum CTX-1 concentrations were significantly higher, whereas serum PINP levels were found to be lower in scleroderma patients compared to healthy subjects ($p<0.05$). Diffuse SSc patients had higher serum CTX-1 levels compared to patients with limited SSc ($p<0.05$). Furthermore, there was a significant positive correlation between serum CTX-1 levels and mRSS scores and ESR levels of the patients ($r=0.493$ and $r=0.431$, respectively; $p<0.05$).

Conclusions: There are conflicting results regarding the increased risk of osteoporosis in scleroderma. In this study, the bone resorption and formation markers CTX-1 and PINP were evaluated together and significant data were obtained indicating a possible increased risk for osteoporosis with regard to bone turnover biomarkers in systemic sclerosis.

Keywords: Systemic Sclerosis, Bone Turnover Markers

P069

STRUCTURAL AND COMPUTATIONAL-DRIVEN RECOGNITION OF PHOSPHOINOSITIDE DERIVATIVE FOR HIV ERADICATION

Halilibrahim Ciftci[1], Belgin Sever[2], Nurettin Tokay[1], Masami Otsuka[3], Hiroshi Tateishi[4], Mikako Fujita[4], Hasan Demirci[1]

[1] Koc University, Department of Molecular Biology and Genetics, Istanbul, Turkey

[2] Anadolu University, Department of Pharmaceutical Chemistry, Eskisehir, Turkey

[3] Science Farm Ltd., Department of Drug Discovery, Kumamoto, Japan

[4] Kumamoto University, Medicinal and Biological Chemistry Science Farm Joint Research Laboratory, Kumamoto, Japan

Objectives: HIV has a devastating impact on people's lives. Although administration of multiple anti-HIV drugs hold promise to some extent, latent HIV reservoirs restrict the success of the therapy. Therefore, new approaches for complete HIV elimination from body must be implemented in particular targeting MA domain of HIV-1 Gag protein, which regulates membrane binding through its interaction with PIP2.

Methods:We developed IP6 derivative, L-HIPPO, and showed its binding to the MA domain 70 fold more strongly than that of the PIP2. Moreover, we elucidated three high-resolution crystal structures of the MA-IP6 complex at cryo-temperature (2.40 A, and 2.72 A resolution), and ambient-temperature (3.5 A resolution). We confirmed that IP6 was able to interact with residues in the highly basic region of HIV-1 gag matrix. We also performed molecular docking studies for L-HIPPO and its derivatives in the MA domain (PDB IDs: 7E11, 7E1] and 7E1K) and reported that L-HIPPO derivatives established crucial hydrogen bonds and salt bridge formations with key basic residues. Furthermore, we express and purify full length of Gag protein.

Results:The crystallization of Pr55Gag and MA with L-HIPPO were performed by using the paraffin oil method at room temperature and +4°C degree. The MA-L-HIPPO crystals were obtained in the several conditions. The diffraction experiments of HIV-1 MA-L-HIPPO crystals at the XRD is still in progress.

Conclusions: The structural and computational data will be used for the development of next generation drug candidates for HIV eradication.

Acknowledgement: This study is supported by TUBITAK under 2236 CoCirculation2 program with grant number 121C063.

Keywords: HIV-1, MA protein, L-HIPPO, X-ray Crystallography, Molecular Modelling

P070

EVALUATION SERUM LEVELS OF NRF-2, HEMOXYGENASE-1 AND NEOPTERIN LEVELS IN BRUCELLOSIS PATIENTS

Nihayet Bayraktar[1], Büşra Öztürk[1], Mehmet Çelik[2], Mehmet Reşat Ceylan[2], Mehmet Bayraktar[3]

[1] Harran Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı

[2] Harran Üniversitesi, Tıp Fakültesi, Enfeksiyon ve Klinik Mikrobiyoloji Anabilim Dalı

[3] Harran Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı

Objectives: Brucellosis is an infectious disease caused by Brucella. It is transmitted to humans by direct contact with infected animals, inhalation or consumption of animal products such as infecte meat, unpateurized milk and dairy products. Nuclear factor E2-related factor (Nrf-2) is the cellular sensor of oxidative and electrophilic stress. Neopterin is a biochemical marker associated with cell-mediated immunity. Heme oxygenase-1 (HO-1) is considered to be the main protein in diseases resulting from oxidative and inflammatory damage. In our study, we aimed to investigate whether these biomarkers can be reliable markers in the diagnosis, treatment and follow-up of brucellosis,

Methods:Brucellosis patients who applied to Harran

University Faculty of Medicine Infectious Diseases Outpatient Clinic were included in the study. Thirty individuals each from these groups, which consisted of 4 groups according to antibrucella antibodies titreş: 1/160 titer group, 1/320 titer group, 1/640 titer group, Healthy control group was also included. Serum Nrf-2, HO-1, and neopterin levels were determined by ELISA (BT LAB, China).

Results:According to analysis finding, it was found that these biomarkers were changed significantly in respect to their titer ratios. Nrf-2, HO-1 and neopterin levels were the lowest in the healthy whereas they were the highest in the patient groupt having 1/640 or more titer group.

Conclusions: Finding the highest levels of Nrf-2, HO-1 and neopterin in brucellosis patients, one may conclude that measurement of these parameters may be helpful and important clinically in the follow up brucellosis course and response to treatment.

Keywords: Brucellosis Nrf-2 Neopterin Hemoxygenas-1

P071

NUMERICAL CHANGES IN COVID-19 CASES AND CORRELATION WITH AGE AND BLOOD GROUP : A CROSS SECTIONAL STUDY IN . HYDERABAD , PAKISTAN

Zubaır Ahmed Rahojo, Zulfiqar Lagari, Amna Khatoon, Hamza Hanif, Zuneria Imran

Aga Khan University Hospital, Krachi, Sindh, Pakistan

Objectives: By analyzing COVID-19 cases, recoveries, age demographics, and blood group distributions, this study strives to uncover underlying disease dynamics and identify possible risk factors.

Methods:Carried out at LUMS Hospital hydereabad in Pakistan, this cross-sectional study enrolled 99 subjects, comprising 51 recoveries and 48 COVID-19-positive cases. Blood samples were procured to assess numeric blood parameters, while ABO blood groups were typified.

Results:Rhe cohort of recovered individuals displayed elevated hemoglobin, red and white blood cell counts, and diminished platelet count compared to the hospitalized group. The distribution of blood types exhibited the following percentages: A (22.2%), B (27.3%), AB (27.3%), O (23.2%). In addition, 22.2% demonstrated the RH-negative factor, while 78.8% were RH-positive.

Conclusions: Variations in blood parameters and blood types correlate with COVID-19 outcomes. Higher hemoglobin and blood cell counts were associated with recovery, while differing blood types hint at susceptibility. These findings offer insights for managing and controlling COVID-19.

Keywords: CIVID-19, BLOOD GROUP, BLOOD NUMERICAL CHANGES, AGE, CBC

P072

INVESTIGATION OF FECAL CALPROTECTIN TEST USED FOR INFLAMMATORY BOWEL DISEASE

Kübranur Ünal, Leyla İbrahimkhanlı

Gazi University, Faculty of Medicine,department of Medical Biochemistry, Ankara, Türkiye

Objectives: Fecal calprotectin (FC) is an important biomarker used to evaluate intestinal mucosal inflammation in stool samples. FC is commonly used in the detection of newly active Inflammatory bowel disease (IBD) and in follow up of therapy success in IBD patients. In this study was investigated the purpose of FC test used for IBD in our hospital.

Methods:440 patients with symptoms of IBD and had FC test analysis were included in the study, test results were retrospectively analyzed.

Results:Considering the purpose of using the FC test in this study , the rate of FC use was 49.1% (n:216) in patients with suspected IBD who applied for the diagnosis. Also, this rate was 50.9% (n:224) for patients who followed up with the diagnosis of IBD among all patients. When the 216 patients who used FC to confirm the diagnosis were compared in terms of diagnosis, the number of patients who were later diagnosed with IBD (16%, n:35) was statistically significantly lower than the number of patients diagnosed with non-IBD (84%, n:181) ($p<0.05$).

Conclusions: FC test is a useful biomarker in clinical decision making in patients with suspected or confirmed IBD and reduces unnecessary procedures. Many studies use an algorithm based on FC concentration for clinical decision making in IBD. It was determined that the FC tests used for confirm the diagnosis also to be useful excluding IBD in this study. As a result, FC is effective in excluding IBD and in the

follow-up of the disease in our hospital.

Keywords: Fecal calprotectin, Inflamatory Bowel Disase

P073

THE ROLE OF INFLAMMATORY MARKERS DERIVED FROM HEMATOLOGICAL PARAMETERS IN FEMALE MIGRAINE PATIENTS

Yasemin Atıcı[1], Esra Eruyar[2], Nida Aslan Karakelle[3], Safiye Göçer[4]

[1] Lokman Hekim University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

[2] Lokman Hekim University, Faculty of Medicine, Department of Neurology, Ankara, Turkey

[3] Lokman Hekim University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

[4] Lokman Hekim University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey

Objectives: Migraine is a chronic neurovascular disease that is a disabling brain disorder that affects millions of women worldwide. Although migraine is a neurological disorder known for its long, physiopathology remains unclear. The aim of this study was to investigate the levels of hematological parameters, C reactive proteine (CRP) and systemic inflammation index (SII), systemic inflammation aggregate index (AISI), systemic inflammatory response index (SIRI), lymphocyte/monocyte ratio (LMR), platelet/lymphocyte ratio (PLR), neutrophil/lymphocyte ratio (NLR) and lymphocyte/CRP ratio (LCR) used as inflammatory markers in patients diagnosed with migraine.

Methods:Fifty-seven migraine patients and thirty age-matched control subjects who applied to Lokman Hekim Akay Hospital Neurology outpatient clinic were included. Patients diagnosed with migraine using the Classification of Headache Disorders diagnostic criteria were included. Haematological parameters were measured and AISI, SIRI, LMR, PLR and NLO values were calculated. The significance level was accepted as $p<0.05$.

Results:Migraineurs and healthy controls did not differ significantly in age, smoking and body mass ındex. All of the migraine and control group members were female. Patients with migraine and healthy controls showed significant differences in the inflammatory markers CRP and LCR. CRP levels increased in migraine group ($p=0.015$). LCR levels decreased migraine group ($p=0.004$). SII, AISI, SIRI, LMR, PLR and NLR parameters were not significantly different ($p>0.05$).

Conclusions: These results suggest the characteristics of a continuous inflammatory process in migraine patients. It was concluded that LCR, as a marker of inflammation, may be a useful marker for migraine patients.

Ethics committee no: 2022111

Keywords: C-reactive protein, inflammation, lymphocyteCRP, migraine

P074

INVESTIGATION OF THE EFFECT OF THYMOQUINONE ON CLAUDIN-5 PROTEIN EXPRESSION IN A CELL CULTURE INTESTINAL INFLAMMATION MODEL

Öznur Yaşar, Ebru Kanımdan, Abdurrahim Koçyiğit

Bezmialem Foundation University

Objectives: Inflammatory bowel disease (IBD) is a condition that has become more prevalent in recent times, lacking a specific remedy for which the treatment remains undiscovered. Within the pathogenesis of the disease, the involvement of tight junction proteins in inflammation plays a significant role. In this project, the impact of thymoquinone (TQ), a phytotherapeutic agent, on the expression of claudin-5, a tight junction protein, has been investigated.

Methods:Raw 264.7 macrophage cells and HT-29 colon cancer cells were utilized as cell lines. Initially, Raw 264.7 cells were treated with 1 µg/ml LPS onto them to induce inflammation, and then incubated for 24 hours. Following the incubation period, the conditioned medium from Raw 264.7 cells was collected and transferred onto HT-29 cells, followed by a 4-hour incubation. Post-incubation, TQ was added at appropriate doses as determined by MTT, and the cells were further incubated for 24 hours. Similar procedures were conducted for control purposes without inducing inflammation. Subsequent to the incubation, cells were harvested, and protein isolation was performed. The expression levels of claudin 5 and be-

ta-actin proteins were measured through Western blot analysis. The Image-J analysis program was employed for quantification.

Results:According to the analysis results, it was determined that in both the groups where inflammation was induced and those where it was not induced, the expression of claudin-5 increased compared to the control groups.

Conclusions: Due to its observed elevation of claudin-5 expression, TQ holds promise as a potential therapeutic agent in the treatment of IBD.

Keywords: inflammatory, thymoquinone, cell culture

P075

DETERMINATION OF THE FREQUENCY OF DISCORDANT C-REACTIVE PROTEIN AND ERYTHROCYTE SEDIMENTATION RATE RESULTS IN ADULTS

Caner Yılmaz[1], Giray Bozkaya[2]

[1] University of Health Sciences, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

[2] University of Health Sciences, Izmir Faculty of Medicine, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

Objectives: C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) are frequently used tests for inflammation and numerous clinical conditions. Although they are thought to be positively correlated, in some cases CRP and ESR results show differences. When these two tests give discordant result, they are met with some suspicion by some clinicians. Since there is limited literature about frequency of discordance of CRP and ESR results, our aim was to determine the frequency of discordant results.

Methods:Retrospective research of 4924 patients whose both CRP and ESR had been tested simultaneously between May-June 2023 was done. Only adult and non-pregnant patients were selected. CRP level was measured by immunoturbidimetric method on Cobas 8000 analyzer using CRP4 kit. CRP $\geq$ 0.5 mg/dL results considered as abnormal. ESR level was measured by Westergren method and $\geq$ 25 mm/h considered as abnormal.

Results:There were 1419 discordant results which are sorted by as follows: Rheumatology (29.3%), Hematology (23.5%), Endocrine (9.9%), Neurology and Neurosurgery (%8.2), Otorhinolaryngology (8%), General Internal Medicine (6%), Infection Disease (5.1%), Dermatology (3.4%), Others (6.6%).

Conclusions: The possible reason of high frequency rates was thought to be high number of patients included in the study. Particularly hematology and rheumatology clinics in our hospital are more intensive than other clinics and patients are especially referred to these clinics. It should be kept in mind that there may also be incompatible results against clinician's expectation of compliance.

Keywords: Erythrocyte Sedimentation Rate, C Reactive Protein, Inflammation

P076

INVESTIGATION OF TRYPTOPHAN AND KYNURENINE IN PATIENTS WITH RHEUMATOID ARTHRITIS

Karam Mazın Kamıl Gharab[2], Mohammad Ahmad Bik[1], Duygu Eryavuz Onmaz[1], Sedat Abuşoğlu[1], Ali Ünlü[1]

[1] Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

[2] Mustansiriyah University, National Diabetes Center For Treatment and Reaserch, Baghdad, Iraq

Objectives: Rheumatoid arthritis (RA) is a systemic, autoimmune, inflammatory disease characterized by inflammation and tissue destruction resulting from excessive release of proinflammatory cytokines and can affect organs. Proinflammatory cytokines such as tumor necrosis factor, and interleukins play a role in the expression of cell adhesion molecules and in the migration and adhesion of leukocytes in inflamed tissue. An important immunosuppressive effect is thought to be due to the breakdown of tryptophan in the kynurenine pathway. Our aim was to investigate the relationship between tryptophan, Kynurenine, and the Tryptophan/Kynurenine ratio in patients with rheumatoid arthritis and to correlate the results with those of the control group.

Methods:21 RA patients and 50 apparently healthy control were included in this study. Tryptophan and Kynurenine were measured by LC-MS /MS. Statistical analysis was performed using IBM SPSS Statis-

tics 26.0.

Results:Our results showed that serum Trp levels were lower in RA group than control group [8640 (2280-13960) ng/ml vs. 11760 (4020-19660) ng/ml, p=0.000]. Kynurenine levels were higher in RA group when compared to control [266.7 (99.4-1120) ng/ml vs. 179.5 (75.6-903) ng/ml, p=0.001]. Tryptophan/Kynurenine ratio were statistically significantly lower in patients with RA compared to the control group [31.02 (7.9-133) vs. 61.13 (17.3-194.7), p=0.000].

Conclusions: Our results show that there is an increase in Kynurenine levels in RA patients. Inflammation-induced stress may increase the synthesis of Kynurenine pathway metabolites in RA patients. However, one of the significant drawbacks of the study was the limited number of patients. In addition, our results indicate that further studies with a larger patient population are needed on this topic.

Keywords: Rheumatoid arthritis, Tryptophan, Kynurenine, inflammation

P077

ANTI-INFLAMMATORY EFFECTS OF KV1.3 CHANNEL BLOCKER BIOMOLECULE ON LIPOPOLYSACCHARIDE-INDUCED PULMONARY INJURY

Nesrine Ahras-sifi, Amina Ladjel-mendil, Fatima Laraba-djebari

Usthb, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, Bp 32 El-alia, Bab Ezzouar, 16111, Algiers, Algeria

Objectives: Pulmonary inflammation induced by bacterial lipopolysaccharide fragments (LPS) is a crucial research focus, offering insights into potential therapeutic interventions. The voltage-gated-potassium (Kv) 1.3 channel plays a important role in the immune responsiveness of T-lymphocytes. In this study, we evaluated the effect of kaliotoxin (KTx), a hight specific Kv1.3 potassium-channel blocker, purified from *Androctonus australis hector* venom, on induced pulmonary inflammation by lipopolysaccharide (LPS).

Methods:Adults NMRI mice weighing 25 ± 2 g were used for experiments according to the European Community rules of the Ethical Committee for animal Welfare. Kaliotoxin was systemic delivery (0,8 ug/25 g) by intraperitoneal (i.p) route, after the injection of LPS (5 mg/kg) to induce pulmonary inflammation in the murine model.

Results:Obtained results showed that the administration of KTx after LPS injection induced a significant decrease in lung wet weight/dry weight in inflammatory markers characterized by a myeloperoxydase activity decrease, a marker of neutrophiles cells activation. KTx seems to be able to reverse the observed oxidative damage induced in LPS-model characterized by a decrease of NO and lipid peroxidation (MDA) accompanied by an increase of anti-oxidant markers (catalase and GSH) in the lung. Histological examination of lung tissues confirmed a reduction in tissue lesions and inflammatory cell infiltration in the presence of Kaliotoxin compared to the LPS-only model.

Conclusions: The study highlights the remarkable pulmonary anti-inflammatory effects of Kaliotoxin, achieved through the inhibition of Kv1.3 potassium channel. These findings underscore the potential of Kaliotoxin as a targeted therapeutic strategy for pulmonary inflammatory diseases.

Keywords: Inflammation, Kv13 channel blocker, Lung injury, Oxidative stress

P078

IL33 GENE VARIANTS AND EXPRESSION IN CHILDHOOD ASTHMA

Rihab Charrad[1], Anissa Berraies[2], Jamel Ammar[2], Kamel Hamzaoui[1], Agnés Hamzaoui[2]

[1] Faculty of Medicine of Tunis

[2] Hospital Abderrahmen Mami

Objectives: Interleukin-33 (IL-33) is one of the last discovered members of the human IL-1 family. It is involved in the pathogenesis of many inflammatory diseases. This study investigates the relationship between *IL33* gene variants and serum protein levels with the development of childhood asthma.

Methods:We analyzed in this case-control study the distribution of two *IL33* polymorphisms, rs7044343 and rs1342326, within 200 Tunisian children, using predefined Taqman genotyping assays. IL-33 serum levels were assessed by commercial sandwich Enzyme-linked immunosorbent assay (ELISA).

Results:The presence of rs1342326 polymorphism was significantly associated with a lower risk of

asthma development. The CC [OR= 0.20, CI (0.08 – 0.50)] and AC [OR= 0.24, CI (0.11– 0.49)] genotypes were associated significantly with a decreased asthma risk. The C allele was protective [OR =0.40; CI: 0.26 – 0.61, *P* = 0.00001]. No association was found between rs7044343 variant and asthma. The rs1342326 C allele was protective against allergic asthma [OR=0.18, IC (0.05-0.42)]. The haplotype C-C was significantly associated with a lower asthma risk [*P*=0.004; OR =0.317, CI= 0.144 - 0.701]. The level of IL-33 in sera was significantly increased in asthmatic children [1.48 ± 0.47 pg/mL] compared to controls [0.70 ± 0.18 pg/mL; *P <0.001*].

Conclusions: Furthermore, *this increase of IL-33* was associated with the presence of rs1342326 C allele, and it simultaneously was to be protective from asthma. The *IL33* rs1342326 polymorphism was associated with a lower childhood asthma risk in the Tunisian population and a higher IL-33 protein expression.

Keywords: Interleukin-33 Childhood asthma Inflammatory cytokines PolymorphismsCase-control study

P079

CRP-ALBUMIN RATIO AND NEUTROPHIL-LYMPHOCYTE RATIO IN ACUTE ISCHEMIC STROKE

Veli İyilikçi, Eyyüp Kanat, Hande Turan, İnanç Karakoyun, Ayfer Çolak

Health Sciences University İzmir Tepecik Education and Research Hospital, Department of Medical Biochemistry , İzmir, Türkiye

Objectives: In recent years, the CRP-albumin ratio (CAR) and neutrophil-lymphocyte ratio (NLR) has become an important marker of inflammation and oxidative stress in many diseases and predicts the prognosis of the disease. Atherosclerosis plays a role in the etiology of ischemic events. Therefore, in this study, we aimed to determine the relationship between acute ischemic stroke (AIS) and CAR.

Methods:In our study, we retrospectively analyzed the patients hospitalized and treated for AIS in the Neurology Clinic of SBU Tepecik Training and Research Hospital. Forty patients with biochemistry and CBC measurements, within 48 hours of admission, were included in the patient group and 40 patients without ischemic disease were selected as control group. Biochemistry and hematology results between both groups were compared and ROC analysis performed using Jamovi 2.3.28 software.

Results:For serum leukocyte, lymphocyte count, and sodium, chloride, CRP, creatinine, albumin level and NLR, CARs were significantly different between patient and control groups (p<0.01). When effect sizes were evaluated, albumin(0.80), CAR(0.624), and NLR(0.519) were ranked in the top three. According to the ROC analysis AUC calculated as 0.90 for albumin, 0.81 for CAR and 0.760 for NLR.

Conclusions: In conclusion, we found that patients with AIS had a higher CAR and NLR within 48 hours of hospitalization compared to the control group. This was consistent with previous studies suggesting that CAR and NLR could be associated with systemic inflammation and oxidative stress. It also suggested they can be used as a marker for the diagnosis of AIS.

Keywords: ischemic stroke, CRP-albumin ratio, neutrophıl-lymphocyte ratio

P080

SPURIOUS CARDIAC TROPONIN-T ELEVATION BY ASSAY INTERFERENCE: CLINICIANS CONSULTING THE LABORATORY FOR PATIENT ADVICE

Sena Türkmen[1], Okşan Demiröz Güleryüz[2], Ayla Akca Çağlar[2], Ercan Demir[2], Özlem Gülbahar[1]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry

[2] Gazi University, Faculty of Medicine, Department of Pediatrics

Objectives: When assay interference is suspected, investigations help understand underlying causes. Communication between clinician and laboratory is essential for successfully managing such interferences. We present a case with positive cardiac troponin-T (cTnT) results without cardiac cause.

Methods:A male adolescent with known growth hormone (GH) deficiency and no family history of heart disease or early sudden cardiac death presented to the pediatric emergency department with chest pain. Chest radiography, electrocardiogram (ECG) and laboratory testing were performed, including cTnT (Elecsys® TnT-hs). Imaging and ECG was unremarkable, cTnT was positive. Symptoms resolved without intervention and control ECG disclosed no cardiac pathology. Second cTnT testing showed an elevation but no further increase. He was discharged

and referred to the outpatient department for a follow-up. No change in cTnT concentration was seen that day. The laboratory was consulted for possible assay interference.

Results:Other assays unveiled that the patient's cardiac Troponin-I levels were negative. The structural difference makes cTnT more eligible for interference caused by skeletal troponin-T. The increased plasma cTnT levels were interpreted as secondary to assay interference. Skeletal troponin-T may have caused false positive results. Ecartation of a myopathy was advised. Final diagnosis is yet to be made. Electromyography showed 'prominent myopathic activity in the upper extremity'.

Conclusions: Sensitivity and specivity in assays vary depending on the type of troponin and interferences like this emphasise the importance of communication between laboratory and clinician. Physicians should consult the laboratory for results that seem unexpected.

Keywords: Troponin, Assay Interference, Clinical Correlation, Consultation

P081

ENZYME-CATALIZED SYNTHESIS OF NEW PHOSPHOLIPID DERIVATIVES OF ANTI-CANCER NUCLEOSIDES

Larysa L. Birycheuskaya[1], Marharita A. Vinter[1], Anatoli I. Zinchenko[1], Z. Günnur Dikmen[2], Sergei M. Gryaznov[3]

[1] Laboratory of Molecular Biotechnology, Institute of Microbiology, National Academy of Sciences, Minsk, Belarus

[2] Hacettepe University, School of Medicine, Medical Biochemistry Dept., Türkiye

[3] Maıa Biotechnology Inc., Chicago, Il, Usa

Objectives: Conjugation of antitumor nucleosides, such as 6-thio-deoxyguanosine (6TdG), with phospholipids may provide for improved pharmacological properties. This study outlines an enzymatic approach to 5'-phosphatidyl derivatives of 6TdG and 6-thioguanosine (6TG) using microbial phospholipase D (PLD).

Methods:Syntheses of phosphatidyl nucleotides was conducted in stirring biphasic reaction mixtures (10−35 ml, 37 □C) consisting of dichloromethane and 0.2 M sodium acetate buffer containing 0.15 M $CaCl_2$ (pH 6.0), in the ratio of 6/4, v/v. The reaction mixture also contained (per 1 ml): 5−10 μmol of nucleoside, 6−12 μmol of respective phosphatidylcholine (the molar ratio of nucleoside to phosphatidylcholine 1:1.2 to 1:1.6), and approx. 0.2 mg of PLD. Products were isolated using Silica gel LiChroprep 60, 40-63 μm column. The dry PLD enzyme was obtained from the culture of the *Streptomyces netropsis* BIM B-428D strain.

Results:16 liponucleotides containing phosphatidyl groups with saturated and unsaturated fatty acid residues of various lengths (C4−C18) were obtained. The compounds purity was at least 90% by TLC. Conversion rate of the nucleosides into liponucleotides ranged from 33 to 83 mol.% depending on the nucleoside and the phospholipid fatty acid compositions. The structure of the obtained compounds was confirmed by UV-spectroscopy and LC mass-spectrometry.

Conclusions: Several 5'-phosphatidyl derivatives of 6TdG and 6TG were prepared for the first time. The developed one-pot enzymatic method for liponucleotides synthesis is featured by its simplicity and high yields of the products. The obtained conjugates are being investigated for their anticancer activity *in vitro* and *in vivo*.

Keywords: anticancer prodrug, 6-thio-2-deoxyguanosine, phosphatidyl nucleoside, enzymatic synthesis, phospholipase D

P082

COMPARISON OF BIOCHEMICAL MARKERS BEFORE AND AFTER BARIATRIC SURGERY

Kübranur Ünal[1], Leyla İbrahimkhanlı[1], Hüseyin Göbüt[2], Ahmet Çağrı Büyükkasap[2]

[1] Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

[2] Gazi University, Faculty of Medicine, Department of General Surgery, Ankara, Türkiye

Objectives: Bariatric surgery is one of the most effective strategies in the treatment of obesity and obesity-related diseases. It is known that there are significant changes in patients after surgery. The aim of this

study is to compare biochemical markers in patients before and after bariatric surgery.

Methods:Pre and postoperative (after 3 months) serum levels of biochemical markers of 40 (24 female / 16 male) bariatric surgery patients were reviewed retrospectively. The data were described using a median (25–75% IQR).

RESULTS: When the before and after surgery test results were compared, it was observed that the levels of fasting blood glucose decreased statistically from 95 (87,5-117,5) to 90 (81,25-99,75), insulin from 13,45 (8,72-23,07) to 6,60 (4,35-8,95), HOMA from 3,25 (2,5-5,75) to 1,6 (0,9-2,07), C peptide from 3,45 (2,62-4,77) to 2,4 (1,7-3,22), triglycerides from 141 (112,75-179,25) to 113 (93-149,25), LDL cholesterol from 123,8 (103,1-151) to 106,8 (84,45-125,9), HDL cholesterol from 49 (43-56,75) to 41 (38-46), total cholesterol from 205 (184,25-229,25) to 167 (148-190,75) and T3 from 3,7 (3,3-3,9) to 3,3(3,2-3,7) in the postoperative group.($p < .001$) While the levels of osteocalcin increased statistically from 3 (2-4) to 4 (2-5), cortisol from 11,82 (8,98 -14,13) to 13,24 (11,4-15,2), T4 from 0,88 (0,8-0,95) to 0,95 (0,89-1,05) and ferritin from 23(10,25-63) to 27,5(13,25-89,25) in the postoperative group.($p < .001$)

Conclusions: It is important to review the levels of routine biochemical markers in predicting the positive and negative effects of bariatric surgery in the early stages. More advanced studies are needed to determine the late-stage effects of bariatric surgery.

Keywords: Bariatric surgery, obesity, biochemical markers

P083

ANALYSIS OF SPHINGOSINE-1–PHOSPHATE BY LC−MS/MS

Burcu Eser[1, 2], Aylin Sepici Dincel[2]

[1] Research and Development Center, University of Health Sciences, Ankara / Turkey

[2] Department of Medical Biochemistry, Faculty of Medicine, Gazi University, Ankara / Turkey

Objectives: Sphingosine-1–Phosphate (S1P) is one of the most important metabolites of sphingolipids in membrane lipids. It is a signaling sphingolipid that functions as a ligand for 5 different families of G-protein-coupled receptors (GPCRs). Metabolic processes initiated by binding of S1P to its receptors include vascular system and central nervous system development, survival and reproduction, immune cell trafficking, cell adhesion, cell survival and mitogenesis, stress responses, tissue homeostasis, and angiogenesis. Today, S1P is recognized as a critical regulator of many physiological and pathophysiological processes, including cancer, atherosclerosis, diabetes and osteoporosis. We modified a new ultrahigh performance liquid chromatography/triple quadrupole mass spectrometry (UHPLCMS/MS) analyse method for S1P

Methods:S1P analysis was performed by Agilent 6420 LCMS/MS. The analysis was carried out on a ACE3-C18 (2.1mm×50 mm×5 µm) reversed phase analytical column using gradient elution mode. Detection was performed using multiple reaction monitoring in electrospray ionization mode at m/z 380.8→264.7 and 81.7 for S1P. Calibration curves were linear over a concentration range of 3–200 ng/mL.

Results:The method showed excellent linearity with regression coefficients 0,998 (R2=0.998). The limits of quantification (LOQ) were 0.419 ng/ml for S1P. %RSD for all analytes ranged from 0.4–4.9% for intra-day and 0.3– 9.6% for interday experiments.

Conclusions: A simple LCMS/MS method has been developed and validated for measuring for S1P. Acknowledgements: This work was supported by TUBITAK (project numbers: 221S131) Gazi University BAP (project numbers: THD-2022-7529). The authors would like to thank the TUBİTAK and Gazi University for funding this project.

Keywords: Sphingosine-1Phosphate, LCMSMS

P084

DETERMINATION OF ALPHA-AMYLASE/ TRYPSIN INHIBITORS IN TURKISH WHEATS BY TARGETED HPLC-MS/MS

Haydar Özpınar[1], Sorel Tchewonpı Sagu[2], Aysel Ozpınar[3], Harshadraı M. Rawel[2], Florıan Schweıgert[2]

[1] Department of Nutrition and Dietetics, İstanbul Aydın University, 34295, İstanbul, Turkey

[2] Institute of Nutritional Science, University of Potsdam, Potsdam, Germany

[3] Department of Medical Biochemistry, Medical School, Acıbadem University, İstanbul, Turkey

Objectives: Wheat contains about 8% to 15% of proteins divided into the four so-called Osborne fractions according to their solubility: albumins globulins gliadins and glutenins. The amylase/trypsin inhibitors (ATIs) appear to be the major group of non-gluten fractions consisting of albumins and globulins. ATIs are bifunctional proteins with theability to inhibit both amylase and proteases activities, describing their role in cereals.The alpha-amylase/trypsin inhibitors (ATIs) are discussed as being responsible for non-celiac wheat sensitivity (NCWS), besides being known as allergenic components for baker's asthma. Different approaches for characterization and quantification including proteomics-based methods for wheat ATIs have been documented. In these studies generally the major ATIs have been addressed wheats. The main purpose of this study was to determine alpha amylase trypsin inhibitors in Turkish wheat using an advanced technique, HPLC-MS/MS.

Methods:21 Turkish wheat samples were used. ATIs were measured by targeted tandem mass spectrometric analysis (HPLC-MS/MS) after exraction.

Results:Preliminary characterization with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) documented the purity of the extracted ATIs with CM mixture and the amylase (60–80%)/ trypsin (10–20%) inhibition demonstrated the bifunctional activity of ATIs. Thirteen (individual/ common) biomarkers were established. Major ATIs (7–34%) were differently represented in samples. In total, 11 individual biomarkers for proteins P01083, P01083,P17314, P16850, P15851, P16159, P16159, P93602, P83207, Q4U199, Q41540, Q41540 and two common biomarkers for proteins P01084/P01085 and P81496/Q43723/Q43691 were found.

Conclusions: Finally, to our knowledge, in the proposed study for the first time so far the analysis of all Turkish wheat ATI entries reported.

Keywords: alpha-amylasetrypsin inhibitors wheats peptides markers ass spectrometry LC-MRM-MS

P085

IRREGULAR NUTRITION HABITS EVALUATION OF THE EFFECT ON CARDIOVASCULAR RISK PARAMETERS

Demet Nikbay[1], Fatma Humeyra Yerlikaya Aydemir[1], Duygu Eryavuz Onmaz[1], Ali Kandeger[2], Sedat Abusoglu[1]

[1] Department of Biochemistry, Selcuk University Faculty of Medicine

[2] Department of Department of Psychiatry, Selcuk University Faculty of Medicine

Objectives: The causes of cardiovascular diseases are divided into genetic and environmental factors. Among environmental factors, diet and habits play a major role. Our aim was to investigate the effect of cardiovascular risk parameters in individuals with irregular eating habits.

Methods:The study was conducted among 71 women with BMI>30 (Group 3) and 30 women with BMI<18 (Group 2) with irregular eating behavior. The control group consisted of 54 women with a BMI between 18-25 (Group 1). The levels of methylarginine and its metabolites in serum samples from the groups were measured by tandem mass spectrometry after a pretreatment procedure based on protein precipitation followed by derivatization.

Results:Serum ADMA [0.31 (0.1-1.24) vs 0.23 (0.1-0.8)], LNMMA [0.02 (0.01-0.08) vs 0.01 (0.01-0.11)], SDMA [0.24 (0.18-0.79) vs 0.22 (0.13-0.44)] and citrulline [21.8 (4. 33-56.3) vs 11.8 (2.5-44.8)] levels were statistically significantly higher and arginine levels were lower [55.1 (15.2-156) vs 103.7 (20.1-249.1)] compared to Group 1 ($p<0.05$). In Group 3, serum ADMA [0.29 (0.1-0.92) vs 0.23 (0.1-0.8), $p<0.001$], LNMMA [0.02 (0.01-0.08) vs 0.01 (0.01-0.11)] levels were significantly higher and arginine levels [65.9 (14.4-318) vs 103.7 (20.1-249.1)] were lower than in Group 1 ($p<0.05$).

Conclusions: Methylarginine and its metabolites, which are cardiovascular risk parameters in individuals with irregular dietary habits, may play an important role in the monitoring of cardiovascular comorbidities.

Keywords: cardiovascular disease, inflammation, ischemic stroke

P086

BORON ESTERS AS FOOD SUPPLEMENTS ENHANCE IN VITRO OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STEM CELLS

Serap Topsoy Kolukısa[1], Sema Akbaba[1], Resul Biberoğlu[1], Dursun Ali Köse[2]

[1] Turkish Energy, Nuclear and Mineral Research Agency Boron Research Institute (tenmak Boren), 06520, Türkiye

[2] Department of Chemistry, Hitit University, 19030, Türkiye

Objectives: Bones enable locomotion, protect organs and maintain body shape. Boron is a trace element found in mineral structure of bones. In this study, osteogenic properties of three boron esters, boron ascorbate, fructoborate and glucosamine borate, were investigated *in vitro*.

Methods: In order to determine working concentration for further studies, *in vitro* cytotoxicity test was performed according to ISO 10993-5 standard, by using L929 fibroblasts. Cell viability and proliferation of human adipose derived stem cells (hADSCs) were assessed with alamar blue assay, for 14 days. Osteogenic effect of boron esters was investigated by alkaline phosphatase (ALP) activity on proteome level and by expression of Runx2, Col1a1 and osteopontin on transcriptome level.

Results:MTT assay revealed that IC_{50} concentrations of boron ascorbate diester, fructoborate diester and glucosamine borate diester were 212.71 µM, 0.25 mM and 19.09 mM respectively.

Conclusions: Boron esters enhance osteogenic differentiation of hADSCs. Boron esters need to be further evaluated *in vivo*, in order to determine their potential for supporting bone healing.

Keywords: food supplement, osteogenesis, boron ascorbate, fructoborate, glucosamine borate

P087

RBC FOLATE LEVELS IN CHILDREN WITH GROSS MOTOR FUNCTION CLASSIFICATION SYSTEM (GMFCS) IV-V CEREBRAL PALSY: A CASE-CONTROL STUDY

Özlem Tezol[1, 2], Belgin Bayram[3], Sıddıka Songül Yalçın[2], Oytun Portakal[3]

[1] Mersin University, Faculty of Medicine, Department of Pediatrics, Mersin, Türkiye

[2] Hacettepe University, Institude of Child Health, Department of Social Pediatrics, Ankara, Türkiye

[3] Hacettepe University, Department of Biochemistry, Hacettepe University Hospitals, Central and Emergency Laboratory, Ankara, Türkiye

Objectives: The folate status of children with cerebral palsy (CP) was investigated in this study.

Methods:Red blood cell (RBC)-folate levels in 43 children with Gross Motor Function Classification System(GMFCS) IV-V spastic CP and 43 healthy children aged 3-10 years sampled from a tertiary care hospital was measured by immunoassay using a competitive paramagnetic-particle chemiluminescent method (Beckman Coulter, Inc, USA).

Results:Children with CP had significantly lower weight- and height for age z-scores (p=0.024 and p=0.001, respectively). Median RBC-folate levels were 529 (IQR 453-751) ng/mL in CP group and 409 (IQR 357-499) ng/mL in control group (p=0.001). No RBC-folate deficiency was detected in neither CP nor control group. The frequency of RBC-folate deficiency was 4.7% in CP group, while all healthy controls had normal RBC level of folate (p=0.494). Nineteen (44.2%) children with CP were consuming enteral feeding formula (EFF) every day. Median RBC-folate levels were 649 (IQR 512-842) ng/mL in EFF-consuming CP group and 512 (IQR 355-713) ng/mL in CP group-not consuming EFF (p=0.045). The EFF-consuming group had higher RBC folate levels than those of both EFF-free group and the control group (p=0.001). It was not found a strong correlation between the frequency of EFF consumption daily and RBC-folate level in CP group (r=0.34, p=0.025).

Conclusions: Our results suggest that although non-ambulatory GMFCS level IV and V CP children have lower anthropometric z-scores indicating poorer nutritional status, they have adequate folate status.

Further studies should investigate whether consuming enteral feeding formula improves other micronutrient levels or not in children with CP.

Keywords: RBC folate, cerebral palsy, immunoassay, hemolysate

P088

THE INFLUENCE OF THE GENETIC POLYMORPHISM OF CATECHOL O METHYLTRANSFERASE ON POSTTRAUMATIC STRESS DISORDER APPEARANCE

Valdete Haxhibeqiri[1, 3], Shpend Haxhibeqiri[2], Mimoza Hoxha Muhaxhiri[4], Vlora Kutllovci[4], Ervehe Myrtaj Dalipi[4]

[1] University Clinical Center of Kosova, Clinic of Medical Biochemistry, University of Pristina

[2] Institute of Kosovo Forensic Psychiatry

[3] Biohit Medical Laboratory

[4] Clinic of Medical Biochemistry

Objectives: of this study was to investigate whether the genetic polymorphism of COMT rs4680 (Val-158Met) contributes to the onset and severity of PTSD symptoms. To investigate whether there is an association between COMT SNP and PTSD.

Methods:In this multicentric research, which was conducted in 4 different countries, 719 participants (487 - 67.7% male and 232 - 32.3% female), who experienced the war in the former Yugoslavia were included. COMT rs4680 (Val158Met) genotypes were determined by PCR in all participants, divided into three groups (lifetime PTSD, current PTSD and control group). For data collection, various questionnaires were used such as: the PTSD scale administered by the clinician (CAPS), the Brief Symptom Inventory (BSI) and other questionnaires. The diagnosis of PTSD was determined according to DSM 4.

Results:Regarding the genetic polymorphism Val-158Met of COMT, in the group of people with lifetime PTSD, the results of linear regression for BSI showed a significant difference in the dominant model (P=0.031) and the allelic model (P=0.047). We also found a difference between the AG and GG genotypes in anxiety, somatism and psychoticism.

Conclusions: Our results indicate that the genetic polymorphism of COMT (Val158Met) is important for the severity of certain PTSD symptoms, especially in individuals with lifetime PTSD.

Keywords: PTSD, COMT, genetic polimorphism, CAPS

P089

MORUS ALBA L. EVALUATION OF THE CYTOTOXIC ACTIVITY OF LEAF EXTRACTS ON CACO-2 CELLS TOGETHER WITH ANTIOXIDANT ACTIVITY

Elif Senturk[1], Gulcin Alp Avcı[2], Emre Avcı[3], Cumhur Bılgı[4]

[1] Hitit University, Department of Molecular Biology and Genetics, Corum, Türkiye

[2] University of Health Sciences, Gulhane Faculty of Dentistry, Department of Basic Medical Sciences, Microbiology, Ankara, Türkiye

[3] University of Health Sciences, Gulhane Faculty of Pharmacy, Department of Biochemistry, Ankara, Türkiye

[4] University of Yuksek Ihtisas, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

Objectives: Research on medicinal plants, derivative drugs, and treatment methods have an important place in terms of determining the use, area, and mechanism of action of plants. Medicinal plants are used in many societies for various reasons. At the same time, it has serious importance in the pharmaceutical industry. One of the plants known to be medicinally important is Morus alba L.

Methods:In our study, it was aimed to evaluate the cytotoxic activity on the colon cancer cell line (Caco-2) together with the antioxidant activity of Morus alba L. leaf extracts collected from Bolu and Çorum provinces. The cytotoxic activity of the extracts on the Caco-2 cell line was investigated by line 3-4,5-dimethyl-thiazolyl-2,5- diphenyltetrazolium bromide (MTT) cytotoxicity assays. In antioxidant activity studies, 1,1-diphenyl-2-picrylhydrazil (DPPH) and total antioxidant level (TAS) were evaluated by spectrophotometric methods.

Results:In line with the data we obtained, it was determined that Morus alba L. leaf extracts have proliferative activity and high antioxidant activity rather

than cytotoxic activity on Caco-2 cells at varying rates depending on concentration and time.

Conclusions: With our study results, it is emphasized that when using medicinal plants, the use of products that have been scientifically studied, whose data have been proven, and whose concentrations have been determined

Keywords: Morus alba L, Caco-2, Cytotoxicity, Antioxidant Activity

P090

THE EFFECT OF WHITE TEA ON LEPTIN AND ASPROSIN LEVELS IN OBESE RATS-INDUCED BY HIGH-FAT DIET

Merve Nur Toraman[1], Adnan Yılmaz[2], Sibel Karakaş[2]

[1] Recep Tayyip Erdogan University Training and Research Hospital, Department of Nutrition and Diet, Rize, Türkiye.

[2] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Medical Biochemistry, Rize, Türkiye.

Objectives: We aimed to reduce the weight-reducing effect of white tea with high antioxidant activity, as well as to reduce leptin and asprosin levels, which are reported to be in high concentration in obese individuals.

Methods:In this study, 72 Sprague-Dawley male rats, 6-8 weeks old, obtained from the Experimental Animals Unit of RTEU, were used. Rats were randomly selected and 9 different groups (n=8) were formed. These groups were divided into two to examine both the protective effect and the therapeutic effect of white tea on obesity. The case groups were given white tea with high-fat diet, and the positive control group was given orlistat with a high-fat diet by oral gavage. The experiment, in the group where the protective effect was investigated continued for 12 weeks, and in the treatment group, after the obesity criterion was established continued for another 4 weeks. After the experiment was terminated, leptin and asprosin hormon levels were analyzed by ELISA method in serum samples obtained from rats.

Results:At the end, it was determined that white tea has a preventive effect on obesity. In the group of which the protective effect was examined, white tea significantly decreased body weight gain, leptin and asprosin levels ($p<0.05$). White tea decreased body weight gain in the treatment group, but it was not statistically significant.

Conclusions: The anti-obesity effect of white tea may be effective through the regulation of circulating appetite hormones leptin and asprosin.

This work was supported by the Research Fund of Recep Tayyip Erdogan University. Project Number: TYL-2020-1175

Keywords: White Tea, Obesity, Asprosin, Leptin

P091

SYNTHESIS OF A SERIES OF BENZIMIDAZOLE COMPOUNDS, DETERMINATION OF ANTIUREASE AND ANTIOXIDANT ACTIVITIES

Taner İlker Gümrükçüoğlu[1], Hakan Akgün[2], Bahar Bilgin Sökmen[2], Hakan Bektaş[2]

[1] Gümüşhane University, Kurtun Vocational School, Department of Property Protection and Security, Gümüşhane, Türkiye

[2] Giresun University, Faculty of Arts and Sciences, Department of Chemistry, Giresun, Türkiye

Objectives: Antioxidants; it prevents the formation of reactive oxygen species (ROS), acts as a kind of defense mechanism in the body to prevent the damage caused by ROS. Antioxidants neutralize free radicals. Evidence of antioxidant activity clearly shows us that there is a defense mechanism against diseases that may occur in the body. We concluded that the synthesized benzimidazole compounds have good antiurease and antioxidant activity. In the light of these results, it is aimed to show that newly synthesized benzimidazole compounds can be used as antiurease and antioxidant sources in various industrial areas such as pharmaceuticals, cosmetics and agriculture by supporting in vivo studies.

Methods:In this study, antiurease activity determination of a series of benzimidazole derivatives synthesized, urease inhibition assay, antioxidant activities determination of DPPH radical scavenging activity, ABTS radical scavenging activity and iron reducing power.

Results:The most potent compound showing urease inhibition activity is number 2, and the least active compound is compound number 4. All compounds showed higher activity than thiourea. The compound

with the highest antioxidant activity was determined as compound number 5, and the compound with the lowest antioxidant activity was determined as compound number 4.

Conclusions: From the findings, it was concluded that these benzimidazole compounds have good antiurease and antioxidant activity. In the light of these results, it can be suggested that these newly synthesized benzimidazole compounds can be used as antiurease and antioxidant sources in various industrial areas such as medicine and agriculture, supported by in vivo studies.

Keywords: ABTS activity, antioxidant activity, antiurease activity, benzimidazole, DPPH activity

P092

EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON MEMBRANE PROTEINS RESPONSIBLE FOR NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE OXIDASE ACTIVITY

Berkay Saraymen[1], Esma Saraymen[2], Aysun Çetin[3], Recep Saraymen[4]

[1] Erciyes University, Ernam Nanotechnology Application and Research Center, Kayseri, Türkiye

[2] Abdullah Gül University, Central Research Facility Application and Research Center, Kayseri, Türkiye

[3] Erciyes University, Faculty of Medicine, Department of Medical Biochemistry, Kayseri, Türkiye

[4] Tekden Hospital, Department of Medical Biochemistry, Kayseri, Türkiye

Objectives: People are widely exposed to titanium dioxide (TiO_2) nanoparticles through medical and consumer products. TiO_2 nanoparticles have pro-inflammatory properties similar to other nanomaterials. p22 and gp91 phox are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme components located in the membrane. In this study, it was aimed to investigate the effects of TiO_2 nanoparticles on NADPH oxidase activity along with p22 and gp91 phox expression levels in relation to oxidative damage.

Methods:According to flow cytometry with intracellular staining method, neutrophils isolated from EDTA peripheral blood samples of 40 healthy individuals aged 18-65 were treated with TiO_2 nanoparticles at concentrations of 0, 5 and 100 µg/mL for 24 hours at 37°C. Mean fluorescence intensity values of p22 and gp91 phox expression levels were measured. The effect of PMA-stimulated neutrophils on the change in the activity of the relevant enzyme was monitored with the stimulation index.

Results:A significant increase in NADPH oxidase activation was observed in neutrophils interacting only with 100 µg/mL TiO_2 nanoparticles. It was observed that this increase was associated with significant increases in p22 phox expression levels, but was not associated with the change in gp91 phox expression levels ($p<0.05$).

Conclusions: It can be stated that in the presence of 100 µg/mL TiO_2 nanoparticles, p22 phox is upregulated and gp91 phox is downregulated, leading to an increase in NADPH oxidase activity in neutrophils and may cause oxidative damage. The decrease in gp91 phox expression levels can be interpreted as a defense mechanism against oxidative damage.

Keywords: TiO2 nanoparticles, NADPH oxidase, p22 and gp91 phox, This study was supported by grants from Erciyes University Scientific Research Projects BAP Unit as TKB-2021-10213 project and the studies were carried out at the Erciyes University Genome and Stem Cell Center GENKOK and Abdullah Gül University Central Research Facility Application and Research Center

P093

EFFECT OF CAFFEIC ACID ON HYDROGEN PEROXIDE-INDUCED CYTOTOXICITY IN AML12 CELLS

Tugce Karabas[1], Yagmur Ucar Yagci[2], Mervenur Barakli Kocabas[1], Gulben Sayilan Ozgun[1]

[1] Trakya University, School of Medicine, Medical Biochemistry Dept., Edirne, Türkiye

[2] Edirne State Hospital, Medical Biochemistry Laboratory, Edirne, Türkiye

Objectives: Oxidative stress plays a significant role in various human diseases. Hydrogen peroxide, an oxidant, has been reported to induce apoptotic changes, leading to death in various cells, including human and mouse liver cells. Caffeic acid is an organic compound classified as hydroxycinnamic acid, which has

antioxidant and anti-inflammatory effects. We did not encounter any study investigating the effect of caffeic acid on hydrogen peroxide-induced hepatotoxicity. This study aimed to investigate the effect of caffeic acid on hydrogen peroxide-induced cellular hepatotoxicity.

MATERIALS and METHODS:AML12 cells were treated with 500 µM hydrogen peroxide and caffeic acid concentrations (10, 25, 50, 100, 150 and 200 µM) for 24 hours. Cell viability was measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results:500 µM hydrogen peroxide significantly reduced the cell viability in AML12 cells. 10, 25 and 50 µM caffeic acid caused a significant increase, 100 and 150 µM caffeic acid did not significantly change and 200 µM caffeic acid caused a significant decrease in cell viability of the hydrogen peroxide-treated cells.

Conclusions: Our study showed that 10, 25, and 50 µM caffeic acid prevents 500 µM hydrogen peroxide-induced cytotoxicity in AML12 cells. According to our results, caffeic acid can be beneficial for hydrogen peroxide-induced hepatotoxicity.

Keywords: Hydrogen peroxide, Caffeic acid, Hepatotoxicity, MTT assay, AML12 cells

P094

RETROSPECTIVE INVESTIGATION OF THYROID HEALTH IN BREAST CANCER PATIENTS

Bengisu Dönmez[1], Muhittin Serdar[2], Aysel Özpınar[2]

[1] Biochemistry ve Molecular Biology Dept., Institute of Health Sciences, Acibadem Mehmet Ali Aydınlar University, İstanbul/ Türkiye

[2] Acıbadem Mehmet Ali Aydınlar University, School of Medicine, Medical Biochemistry Dept., İstanbul/ türkiye

Objectives: Although a potential link between thyroid health and breast cancer risk has been suggested in the literature, highly conflicting results have been obtained, and the relationship between them remains unclear. The aim of this study is to examine thyroid functions and autoimmunity in breast cancer patients and to define the possible relationship between breast cancer and thyroid health.

Methods:The data of 815 patients included in the study, including 40 patients diagnosed with breast cancer, 79 patients with benign breast disease, and 696 patients in the control group, who applied to the hospitals and medical centers within the Acıbadem Healthcare Group between 2002 and 2022 and had thyroid function tests, were included in this study. SPSS 26 program was used for comparing the thyroid hormones and autoantibody levels of breast cancer patients with those with benign breast disease and the control group, statistically.

Results:Anti-thyroglobulin antibody (anti-TG) levels were found to be higher in breast cancer patients compared to other groups, and the risk rate of breast cancer in anti-TG positive women was 3.57 times higher than in anti-TG negative patients. In addition, the anti-TG antibody diagnostic performance was better than an anti-TPO antibody. As a result of this study, it was determined that anti-TG antibody was positivity associated with a high risk of breast cancer.

Conclusions: Further research is needed to elucidate the mechanisms underlying this association and to explore the potential of the anti-TG antibody as a biomarker for breast cancer risk.

Keywords: bening breast cancer, breast cancer, thyroid antibody, thyroid hormones

P095

DETERMINATION OF TAMOXIFEN METABOLITES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Abdullah Sivrikaya, Esma Menevse, Fatma Zehra Erbayram, Hamiyet Kose, Oguzhan Tok

Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye)

Objectives: Tamoxifen, a selective estrogen receptor modulator, is one of the endocrine therapeutic agents widely used in the treatment of breast cancer. Tamoxifen is converted to its active metabolites (endoxifen and 4-hydroxy tamoxifen) by cytochrome P450 enzymes. The aim of this study was to develop and validate a tandem mass spectrometric analysis method for serum concentration levels of metabolites.

Methods:Chromatographic seperation was performed using an ABSCIEX API 3200 mass spectrometer equipped with an electrospray ion source (ESI) operating in positive mode. Validation studies were

performed according to CLSI protocols to prove the accuracy and validity of the developed method. The performance of the method was evaluated with a serum pool was created from patient samples using 20 mg oral dose tamoxifen.

Results:The standard curves for endoxifen and 4-hydroxy tamoxifen levels was linear within the range of 1.95-500 ng/ml. Total analysis time was 10 minutes. The LOD for endoxifen and 4-hydroxy tamoxifen was 1.95 ng/mL, while the LLOQ value was 3.95 ng/mL. Reproducibility and recovery studies were performed for endoxifen and 4-hydroxy tamoxifen at concentration levels of 500, 31.25 and 3.90 ng/mL. The %CV values obtained from intra-day and inter-day repeatability were 0.1- 0.6 and 0.1-0.5, respectively. Recovery was above 99.7%.

Conclusions: The findings of our study show that the developed method is accurate and precise according to CLSI criteria. We believe that this method can be used for routine analysis in clinical laboratories due to the short analysis time and requiring small amount of sample.

Keywords: Endoxifen, 4-hydroxy tamoxifen, LC-MSMS, Therapeutic drug monitoring

P096

DETERMINATION OF THE TOXIC EFFECTS OF MALATHION ON FRESHWATER MUSSELS

Pınar Arslan[1], Aysel Çağlan Günal[2]

[1] Çankırı Karatekin University, Faculty of Science, Department of Biology, Çankırı, Türkiye

[2] Gazi University, Faculty of Gazi Education, Department of Biology Education, Ankara, Türkiye

Objectives: Organophosphate-derived pesticides are prohibited or restricted in some countries due to their toxicity to non-target organisms and their persistence in the environment. One of these pesticides, malathion, is an insecticide used in agriculture, domestic, and public health areas due to its low toxicity in mammals and high neurotoxicity in insects. This study aimed to investigate the effects of malathion on freshwater mussels.

Methods:Freshwater mussels were obtained from fishermen and acclimated to laboratory conditions for two weeks. At the end of this period, mussels were exposed to 2.78 mg/L (1/100 of the 96-hour LC_{50}) and 22.8 mg/L (1/10 of 96-hour LC_{50}) sublethal concentrations of malathion for 96-h and 21-d. There were also two control groups: negative and solvent. At the end of the exposure periods, the gills and digestive glands of the mussels were taken and the superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were examined.

Results:Exposed to 22.8 mg/L malathion for 21-d, digestive gland SOD values increased significantly compared to the negative control, while CAT values decreased significantly ($p<0.05$). However, gill GPx values differed significantly between groups according to exposure times ($p<0.05$).

Conclusions: The results observed that exposure to malathion in freshwater mussels has an effect on SOD, GPx, and CAT activities. Thus, it has been shown that undesirable adverse effects on non-target aquatic organisms in aquatic ecosystems may occur due to malathion contamination.

Acknowledgments: This study is supported by Çankırı Karatekin University Scientific Research Projects Unit with code FF240223B10.

Keywords: malathion, freshwater mussels, oxidative stress parameters

P097

INVESTIGATION OF THE TOXIC EFFECTS OF EMAMECTIN BENZOATE ON FRESHWATER MUSSELS

Meryem Bayrak[1], Pınar Arslan[2], Aysel Çağlan Günal[3]

[1] Gazi University, Institute of Science, Department of Environmental Sciences, Ankara, Türkiye

[2] Çankırı Karatekin University, Faculty of Science, Department of Biology, Çankırı, Türkiye

[3] Gazi University, Faculty of Gazi Education, Department of Biology Education, Ankara, Türkiye

Objectives: Emamectin benzoate (4'-deoxy-4'-epimethylamino-4'deoxy-vermectin) is a macrocyclic lactone insecticide derived from avermectin molecules isolated by fermentation of the soil microorganism *Streptomyces avermitilis*. It is used to control pests in agricultural products as its broad-spectrum effectiveness. Accordingly, it mixes with aquatic ecosystems,

increasing its accumulation level in aquatic ecosystems and causing undesirable effects on aquatic organisms. The aim of this study was to investigate the toxic effects of emamectin benzoate (EB) on freshwater mussels, a non-target organism.

Methods:The model organism of this study was freshwater mussels *Unio delicatus* (average weight of 33.8±3.0 g; length of 6.08±0.70 cm). After determining the mean lethal concentration of EB using Finney's probit analysis method as 0.2191 mg/L (95% confidence interval limits 0.1267 mg/L -0.3824 mg/L), the mussels were exposed to 21.9 µg/L and 2.19 µg/L of sublethal EB concentrations for 48h and 7d. There were two control groups in this study: control and solvent control. The sublethal effect of the pesticide on mussels was evaluated by tissue malondialdehyde (MDA) parameter.

Results:An increase in MDA values in the digestive glands of freshwater mussels exposed to EB was observed at low concentration in 48h and a decrease in 7d (P<0.05). A decrease was observed in MDA values in gill tissues compared to the control in 48h and an increase compared to the control in 7d.

Conclusions: The results of this study show that EB is effective on malondialdehyde, one of the lipid peroxidation products, thus creating oxidative stress in freshwater mussels.

Keywords: Emamectin benzoate, freshwater mussels, malondialdehyde

P098

THE EFFECT OF GENDER, AGE AND SEASON ON VITAMIN D LEVELS

Ertuğrul Avcı[2], Giray Bozkaya[1]

[1] University of Health Sciences, Izmir Faculty of Medicine, Bozyaka Training and Research Hospital, Medical Biochemistry Laboratory, Izmir, Turkey

[2] University of Health Sciences, Bozyaka Training and Research Hospital, Medical Biochemistry Laboratory, Izmir, Turkey

Objectives: Vitamin D is a steroid hormone synthesized in the skin under the effect of sun light or it is taken with meals. In our study, we aimed to determine whether vitamin D levels of patients admitted to our hospital vary according to age, gender and seasons.

Methods:The study was conducted between August 2022 and 2023 with 25 OH-D vitamin levels of 51601 adult patients. Vitamin D levels are measured by electrochemiluminescence method with Roche Cobas E602 immunology analyzer in our laboratory. The subgroups were determined according to the age, gender and seasons. In this study, the data were analysed with IBM SPSS Statistics 29.0.1.0. Data was compared with Mann-Whitney U test. Significance level was determined as p<0.05.

Results:There was a statistically significant difference according to age, gender and seasons (p<0.05). The median vitamin D level was 20.69 µg/L in males and 19.46 µg/L in females. The median vitamin D level was found to be 17.15 µg/L in young people and 21.69 µg/L in old age. The median vitamin D level was found to be 22.38 µg/L in summer and 17.20 µg/L in winter.

Conclusions: Since vitamin D levels were lower in female patients and younger patients and in winter, vitamin D supplementation is recommended for these people, especially in winter.

Keywords: Epidemiology, Seasons, Vitamin D

P099

INVESTIGATION OF HEAVY METAL LEVELS IN BLOOD AND URINE SAMPLES OF YOUNG ADULTS (18-24 AGES)

Nilay Topal, Meltem Pak Demir, Ayşe Aydanur Kulaç Ersen, Bulut Yurtsever, Fehime Aksungar

Acibadem Mehmet Ali Aydınlar Üniversitesi

Objectives: The escalating industrial employment of heavy metals has led to heightened human exposure, emerging as a global concern for public health.Recent efforts have spotlighted studies evaluating heavy metal concentrations across different age brackets. These studies juxtapose these levels with dietary, demographic, and socio-behavioral traits to underscore environmental vulnerability.This investigation aimed to gauge heavy metal levels among young adults in Istanbul, delving into existing risks.

Methods:The study encompassed 65 volunteers aged 18 to 24 years. Utilizing ICP-MS technique, levels of 11 distinct heavy metals (Pb,As,Hg,Cd,Cr,Cu,Zn,Se,Mo,Mn,Co) were quantified in whole blood,serum and urine samples from participants.A comprehensive questionnaire covering dietary habits, health status, and demographics was administered.Based on smoking, alcohol usage, gender, and seafood con-

sumption,the cohort was segregated into four subsets.

Results:Significantly elevated blood cadmium levels were noted among smokers (p<0.0001). Alcohol consumers exhibited reduced urinary copper excretion (p=0.0371) and augmented zinc excretion (p=0.0182). Comparison of blood heavy metal concentrations between seafood consumers and non-consumers revealed higher mean levels of arsenic, mercury, copper, molybdenum, and manganese in the former.However, statistically significant divergence only manifested in copper levels (p=0.011). Serum copper (p=0.0073) and molybdenum (p=0.0044) were notably higher in seafood consumers. Gender-wise scrutiny disclosed higher blood lead levels in males (p=0.028),while copper (p=0.006), manganese (p=0.001), and cobalt (p<0.0001) levels were elevated in females.

Conclusions:Although the study's subject pool is modest, it remains adequate for robust statistical analysis. Remarkably, this study provides a pioneering insight into heavy metal concentrations within this specific age group in Istanbul and the larger Marmara region.

Keywords: heavy metals, trace elements, ICPMS

P100

CORRELATION OF BLOOD, SERUM, AND URINE HEAVY METAL LEVELS

Ayşe Aydanur Kulaç Ersen, Nilay Topal, Meltem Pak Demir, Bulut Yurtsever, Fehime Aksungar

Acıbadem Mehmet Ali Aydınlar Üniversitesi

Objectives: The increasing industrial utilization of heavy metals over the last century has led to heightened environmental exposure for individuals. Therefore, determining heavy metal levels in humans holds significant importance. This study aims to assess the interchangeability of whole blood, serum, and urine samples in measuring 6 toxic heavy metals (Pb,As,Hg,Cd,Cr,Co).

Methods:The study involved 43 healthy volunteers. Heavy metal levels in whole blood, serum, and urine samples collected from participants were measured via the ICP-MS technique. Correlation between measurements was analyzed through regression.

Results:Strong correlations were observed in Co measurements across all three sample types (r=0.84, p<0.0001 each). The highest correlation between matrices for Hg occurred between Hg-blood and Hg-serum (r=0.85, p<0.0001), followed by Hg-serum and Hg urine (r=0.65, p<0.0001), and lowest between Hg-blood and Hg urine (r=0.39, p=0.0115). The correlation between As-blood and As-urine (r=0.67, p<0.0001) was the highest compared to other sample matrices. No notable correlations were found among the matrices for lead, cadmium, and chromium. The weakest correlations between whole blood and serum occurred for As (r=0.11, p=0.44), Pb (r=0.15, p=0.36), and Cd (r=-0.18, p=0.26).

Conclusions: According to our study, for heavy metal measurements: 1. Whole blood samples are reliable matrices for Cd, Pb, and As. 2. Hg measurements can be interchangeably conducted using both whole blood and serum, while Co measurements can utilize any of the three matrices. 3.The correlation between whole blood and urine in As measurements is acceptable. Confirmation of our results is necessary with a larger sample group encompassing subjects with diverse and heightened heavy metal exposures.

Keywords: heavy metal, trace elements, ICP-MS

P101

EFFECTS OF KB-R7943 ON INSULINE/GLUCOSE METABOLISM AND MOTOR ACTIVITY IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS

Natasha Ivanova[1], Pavlina Gateva[2]

[1] Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; Medical University of Sofia, Faculty of Medicine, Sofia, Bulgaria

[2] Medical University of Sofia, Pharmacology and Toxicology, Sofia, Bulgaria

Objectives: Literature data present that the Na+/Ca2+ exchanger inhibitor (NCX) KB-R7943 can influence the endocrine system via regulation of the insulin secretion. With the present work we aimed to study the KB-R7943 effect on insulin/glucose control and home cage motor activity in a rat model of streptozotocin -induced diabetes.

Methods:Mature male Wistar rats were used for the investigations. A single injection of streptozotocin at a dose of 55 mg/kg was used to induce diabetes.

Upon development of diabetes, the Na+/Ca2+ exchanger KB-R7943 was given by oral gavage at two doses: 5 mg/kg and 10 mg/kg for a 10-day period. Home cage locomotor activity recordings were done for up to 24 h and were analyzed by a software. Rats were sacrificed and blood samples were taken for the investigation of insulin and glucose levels using kits according to the instructions of the manufacturer.

Results:The diabetic rats were characterized with lower insulin and higher glucose levels compared to the controls. The diabetes impaired the home cage locomotion indicated by decreased motor activity. Although the dose of 5 mg of KB-R7943 was not effective, the treatment with 10 mg of the medication improved the impaired insulin/glucose metabolism, as well as the home cage locomotor activity.

Conclusions: The data of the current study suggest that chronic treatment with KB-R7943 can improve home cage locomotion via positive effect on insulin/glucose metabolism in a rat model of streptozotocin-induced diabetes.

Keywords: NaCa2 exchanger inhibitor, insulin, glucose, motor activity

P102

COMPARISON OF HEMOGRAM PARAMETERS IN BLOOD SAMPLES TAKEN DURING PERIODIC EXAMINATION OF HEALTH WORKERS

Nilüfer Alpaslan[1], Fatma Akat[1], Hüsamettin Vatansev[2]

[1] Selcuk University Institute of Health Sciences Department of Medical Biochemistry

[2] Department of Medical Biochemistry, Selcuk University Faculty of Medicine

Objectives: Although important diagnostic clues can be obtained by history and physical examination, interpretation of blood count is necessary to help clinicians in the diagnosis, prognosis, patient management and control of hematologic diseases as a result of laboratory examinations. In this study, it was aimed to determine the presence of health problems that develop depending on whether healthcare workers come into contact with patients or not by periodic examination results.

Methods:The study included 152 individuals who were nurses, patient caregivers, secretaries, medical technicians and security guards recruited to Selcuk University Medical Faculty Hospital in 2023. Data were obtained from the Occupational Health and Safety automation system. The hemogram levels of these individuals were compared according to their marital status, number of children, chronic drug use, presence of chronic disease and smoking.

Results:When the hemogram levels of the individuals were compared according to gender, HBG, HCT, PLT, RBC, MCH, MCHC, RDW, PCT, EO and PLR levels and when compared according to smoking, HGB, HCT, RBC MCH, MCHC levels were found statistically significant ($p<0.001$). Marital status, number of children, and contact with the patient had no effect on blood cells.

Conclusions: It was observed that the precautions taken with the consideration of contact with the patient by healthcare professionals did not make a difference, and only the differences between genders were consistent with the literature.

Keywords: blood, health workers, periodic examination, hemogram

P103

KINETICS OF SARS-CoV-2 NEUTRALIZING ANTIBODIES AFTER TWO DOSES OF BNT-162b2(BioNTech/Pfizer)VACCINE

Katerina Tosheska-trajkovska, Melda Emin, Hristina Ampova, Irena Kostovska, Sonja Topuzovska

Institute of Medical and Experimental Biochemistry, Medical Faculty, University Ss Cyril and Methodius, Skopje, North Macedonia

Objectives: The neutralizing antibody is an antibody that can block the binding and infection process of the virus. MAGLUMI ® Neutralizing Antibody kit could detect all those antibodies that can block the RBD-ACE2 combination. Studies evaluating the long-term duration of neutralizing antibodies (NAbs) after SARS-CoV-2 vaccination are essential to develop vaccination strategies.

Methods:In this study, 131 healthcare workers (HCW) received the two-dose BNT162b2 regimen. Of the 131 HCW enrolled in the study, 85 (64.9%) were female and 46 (35.1%) were male, with a mean age (±SD) of 45.2 ± 10.31 (range 26-55) years. Of them, 91 were seronegative and 40 were seropositive at baseline. The samples were collected at different

time points. Neutralizing antibodies (NAbs) were measured by CLIA method using Maglumi 800 analyzer.

Results:The median days that neutralizing antibodies were positive were 96 and 201 days for rapid and slow attenuation, respectively. No age and gender differences were found in Nabs levels. The decline in Nabs was pronounced (-96.8%) and approximately 47% of those tested were negative at day 180.

Conclusions: Whether this decline correlates with a corresponding reduction in clinical efficacy against the virus would need to be investigated in appropriate clinical trials. BNT162b2 elicits strong NAb production, especially 28 days after initial vaccination.

Further investigations are urgently needed to improve both the comparability of data and our understanding of which levels should be considered predictive of immune protection.

Keywords: SARS CoV-2, NAbs, BNT162b2

P104

ADENOSINE DEAMINASE ACTIVITY IN CHILDREN WITH RESISTANT EPILEPSY

Nurdan Şener[1], Didem Barlak Keti[1], Ayten Güleç[2], Mehmet Canpolat[2], Sabahattin Muhtaroğlu[1]

[1] Erciyes University, Faculty of Medicine, Department of Medical Biochemistry, Kayseri, Türkiye

[2] Erciyes University, Faculty of Medicine, Department of Pediatric Neurology, Kayseri, Türkiye

Objectives: Epilepsy is one of the common neurological disorders that affects all age groups. Although epileptic seizures can usually be controlled using a single medication, some patients may require more than one medication. Although most patients can achieve “seizure-free” status with drug therapy, 30% of these patients may develop resistance to antiepileptic drugs. Adenosine, a neuromodulator of cellular metabolism, has been shown to have an anti-convulsive and neuroprotective role. Extracellular adenosine concentration is regulated mainly by adenosine deaminase (ADA), which catabolizes adenosine to inosine, and adenosine kinase (ADK), which phosphorylates it to AMP. We aimed to determine whether there is a difference in terms of ADA activity in children with resistant and non-resistant epilepsy.

Methods: The study included 32 children with resistant epilepsy, who were followed up and treated at the Pediatric Neurology outpatient clinic, and 35 child patients with non-drug-resistant epilepsy and 20 healthy controls. ADA activity was determined using the Berthelot reaction.

Results:ADA activity was determined as 24.73 ± 7.57, and 30.46 ± 8.95 in children with non-resistant and resistant epilepsy, respectively. ADA activity showed a significant difference between resistant and non-resistant patient groups ($p = 0.008$). ADA activity had 93% sensitivity and %37 specificity at a threshold value of 20 IU/L. AUC was calculated as 0.681 (%95 CI 0.556 to 0.789).

Conclusions: There may be a relationship between increased ADA activity and resistance to treatment in children with epilepsy.

This poster was produced from graduate student Nurdan Şener’s thesis work with TYL-2022-12373 project codes.

Keywords: Adenosine deaminase activity, resistant epilepsy

P105

EVALUATION OF ANXIETY AND DEPRESSION LEVELS IN MALE PARTNERS OF INFERTILE COUPLE

Sondos Meksi[1], Amira Sallem[1], Rim Kooli[1], Dhekra Chebil[2], Manel Boussabbeh[1], Mouna Zarred[1], Hayet Rebai[1], Hana Messadi[1], Fadhila Chebil[1], Boutheina Kilani[1], Meriam Mehdi[1]

[1] Laboratory of Cytogenetics and Reproductive Biologie, Fattouma Bourguiba Teaching Hospital, Monastir, Tunisia

[2] Faculty of Medicine, University of Sousse, Tunisia

Objectives: Depressive and anxious symptoms are common psychological reactions to infertility. However, psychological aspects of childlessness among men have always been less investigated compared to women. The objective of our study was to evaluate levels of anxiety and depression among infertile Tunisian men.

Methods:276 infertile patients consulting the laboratory of cytogenetics and Reproductive Biology, Fatouma Bourguiba Teaching hospital, Monastir, Tu-

nisia were evaluated for the level of depression and anxiety as assessed with HAD (Hospital Anxiety and Depression) scale. Clear consent was obtained from all the participants.

Results:The mean age of the studied population was of 37.25 ± 6.43 years old. Anxious symptoms were recorded in 21.60% of the population whereas 12.10% of patients exhibited depressive symptoms. We also concluded that patients aged under 20 years are more likely to be anxious ($p<0.02$) whereas patients with infertility history > 12 mouths were the most depressed ones ($p>0.011$)

Conclusions: Psychological assistance is beneficial to reduce distress in infertile men as it can impact semen quality and therefore assisted reproductive technologies outcomes. Psychological support should be in stored in IVF laboratories.

Keywords: anxiety, male, infertility

P106

IDENTIFICATION OF DICKKOPF-1/KREMEN ANTAGONIST CANDIDATES USING VIRTUAL SCREENING

Sahra Setenay Baran[1], Yusuf Şimşek[2], Aylin Sepici Dinçel[3]

[1] Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Biyokimya Anabilim Dalı

[2] Gazi Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Tıbbi Hizmetler ve Teknikleri Bölümü

[3] Gazi Üniversitesi, Tıp Fakültesi, Temel Tıp Bilimleri, Tıbbi Biyokimya

Objectives: Dickkopf-associated protein-1 (DKK-1) is one of the natural extracellular inhibitors of the Wnt pathway which have an important role in many biological processes. Kremen are known as a group of transmembrane proteins, and can interact with DKK-1, potentiating its inhibitory effect. This enhances DKK-1's ability to suppress the Wnt signaling pathway. The balance of these molecules is important in many biological processes, particularly in bone health, and therefore therapeutic strategies targeting these molecules are among research focuses. It was aimed to investigate potential of small molecules to be anti DKK-1-Kremen candidates.

Methods:Virtual screening module in Shrödinger software was used to identify hit compounds that can restrain DKK1-Kremen interactions among small molecules from Pubchem and DrugBank databases. The stability and molecular interactions of hit compounds were examined by Desmond molecular dynamics simulation program.

Results:Computations reveal that compounds (PubChemID: 10742027, 102478053, 10017376, 619842, 657042 and 1127756) have been identified as potential candidates for inhibition of DKK-Kremen interaction. We observed that pi-pi interactions between aromatic rings play a critical role in the formation of ligand and protein complex.

Conclusions: Compounds identified as hit generally have aromatic rings. Therefore, compounds with aromatic moieties have been considered potential candidates for inhibition of DKK1-Kremen. Therefore, effects of these compounds should be investigated by in-vitro and in-vivo studies.

Keywords: Dickkopf-1Kremen, Virtual Screening, bone metabolism

P107

EVALUATION OF BLOOD SAMPLE REJECTION RATE IN CLINICAL LABORATORY

Müjgan Ercan Karadağ[1], Ayşe Ertekin[2], Mehmet Özcan[3], Rumeysa Duman[1], Berika Şahin[4], Esra Tunç[4], İsmet Enis Çeri[4], Gürkan Aydın[4], Mehmet Ali Alkan[4], Yasemin Engin[4]

[1] Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Biyokimya Anabilim Dalı, Afyonkarahisar , Türkiye

[2] Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Acil Tıp Anabilim Dalı, Afyonkarahisar

[3] Zonguldak Bülent Ecevit Üniversitesi Tıp Fakültesi Biyokimya Anabilim Dalı, Zonguldak

[4] Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi, Afyonkarahisar

Objectives: Quality indicators (QI) are basic tools used to improve the quality of laboratory services by reducing error rates and maintaining patient safety. In our study, it was aimed to compare the numbers and rates of rejected samples from the emergency department and compared to the quality specifications reported by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) “La-

boratory Errors and Patient Safety" Working Group (WG-LEPS).

Methods:In order to evaluate the number of biochemistry tubes reaching the medical biochemistry laboratory from the emergency department, the data were obtained retrospectively from the Laboratory Information System (LBS) of 2022. In the preanalytical stage, the number of biochemistry tubes were grouped according to the reasons for rejection, and their monthly and annual percentages were calculated. It was evaluated within the criteria of the IFCC WG-LEPS, which includes the three target criteria (25% high performance, 50% optimal performance, and 75% low performance).

Results:Quality The percentage of total preanalytical rejection was found as 2.3. According to the rejection numbers, the % KI values of the 'hemolyzed sample', 'faulty sample cup', 'wrong level' and 'clotted samples' were determined as 0.72, 0.03, 0.80 and 0.77, respectively.

Conclusions: It was determined that our laboratory had poor performance for 'hemolyzed sample' and 'faulty sample container'. It was found to be in the unacceptable range for 'clotted sample' and 'false levels'. As a result, we think that the quality of the process will be improved by increasing the frequency of training on sampling and transfer periodically.

Keywords: Quality indicators, Preanalytical phase

P108

DISTINGUISHING BONE MARROW CELLS IN TISSUE SLIDE IMAGES WITH AUTOKERAS DEEP LEARNING SYSTEM

Hikmet Can Çubukçu[2], Onur Burak Dursun[3]

[1] Ankara University Stem Cell Institute, Ankara, Turkiye

[2] Ankara University Graduate School of Health Sciences, Ankara, Turkiye

[3] General Directorate of Health Services, Ministry of Health, Ankara, Turkiye

Objectives: This study aimed to assess the effectiveness of an automated deep learning system, Autokeras, in distinguishing cells within bone marrow tissue slide images.

Methods:We utilized openly accessible data, comprising 171,374 single-cell images from bone marrow smears collected from 945 de-identified patients, for both developing and evaluating the Autokeras deep learning model. Initially, the data were partitioned into training and testing datasets, accounting for 80% and 20% of the total data, respectively. The training data was further divided into five distinct subsets to facilitate a stepwise training process. The development of the model was conducted using Google Colaboratory, an integrated online development environment. Model performance, aimed at identifying 21 distinct cell types, was evaluated using a confusion matrix and metrics such as positive predictive value (PPV), sensitivity, F1-score, and accuracy.

Results:Among five trials, the Autokeras deep learning system selected the efficientnetb7 architecture, achieving an overall model accuracy of 85%. The highest discriminatory performance was observed for the following cell types: erythroblast (F1 score: 94%, PPV: 93%, sensitivity: 95%), eosinophil (F1 score: 96%, PPV: 96%, sensitivity: 96%), lymphocyte (F1 score: 90%, PPV: 89%, sensitivity: 90%), segmented neutrophil (F1 score: 90%, PPV: 91%, sensitivity: 89%), plasma cell (F1 score: 90%, PPV: 90%, sensitivity: 90%), and blast (F1 score: 82%, PPV: 81%, sensitivity: 83%).

Conclusions: This proof-of-concept automated deep learning system has exhibited significant promise by offering a less labor-intensive alternative for distinguishing bone marrow cells.

Keywords: deep learning, machine learning, stem cell, bone marrow, tissue slide, hematology

ORAL FULL TEXTS

S003

TRIMETHYLAMINE N-OXIDE AND LIPOPOLYSACCHARIDE BINDING PROTEIN AS POTENTIAL BIOMARKERS IN VITAMIN D, VITAMIN B12, AND IRON DEFICIENCY

Sadinaz Akdu[1], Ummugulsum Can[2], Serdar Şahinoğlu[3]

[1] Fethiye State Hospital, Department of Medical Biochemistry, Muğla, Türkiye

[2] Konya City Hospital, Department of Medical Biochemistry, Konya, Türkiye

[3] Fethiye State Hospital, Department of Internal Medicine, Muğla, Türkiye

Introduction: Micronutrient deficiencies are quite common worlwide and have a major impact on public health. Vitamin D deficiency is among the risk factors for many diseases. Vitamin B12 deficiency has been associated with a wide variety of neurological, hematological, and gastrointestinal abnormalities. Iron deficiency can affect many organ functions of the human body and is the most common cause of anemia [1]. Recent studies suggest that these micronutrient deficiencies may be related to disorders in the structure and functions of the gut microbiota [2-4]. The imbalance in the intestinal microbiota, can cause intestinal barrier disorder and bacterial translocation and trigger a permanent systemic inflammation state, affect the immune system and metabolism [5].

Gut microbiota metabolites are considered potential disease biomarkers. In adition, these metabolites are of great importance in identfying therapeutic drug targets. Trimethylamine-N-oxide (TMAO) is known as a metabolite derived from choline, L-carnitine, and betaine found in foods of animal origin. These dietary molecules are metabolized to trimethylamine (TMA) in the colon by the gut microbiota. TMA, which then comes to the liver by portal circulation, is oxidized to TMAO by flavin monooxygenase 3 (FMO3) [6]. Many factors such as age, gender, dietary patterns, intestinal microflora composition, renal function and liver FMO activity have been reported that affect the circulating level of TMAO. Studies to date have shown that circulating TMAO concentrations are closely associated with various cardiometabolic diseases [7]. It is thought that endothelial dysfunction, inflammation, platelet activation and lipid metabolism alterations triggered by high TMAO levels may impact cardiovascular function. TMAO activates multiple intracellular signaling pathways that promote cardiovascular pathological changes. Additionally, TMAO induces the release of inflammatory cytokines [8]. TMAO is also related to a variety of central nervous system diseases, cancer and progresion of kidney diseases. However, the mechanisms related to the role of TMAO in the etiopathogenesis of diseases have not been fully revealed [9]. Therefore, more data are needed to fully understand the role of TMAO concentrations in various pathophysiological conditions.

Lipopolysaccharide binding protein (LBP) is the first protein involved in the recognition of lipopolysaccharide (LPS) in the outer wall of gram negative bacteria. Increased intestinal permeability leads to leakage of LPS into the bloodstream and low grade inflammation. LBP is recommended as a biomarker for intestinal permeability [10]. The intestinal barrier system allows nutrients and fluids to be absorbed, preventing harmful molecules such as endotoxins from crossing the intestinal epithelium and reaching the organs [11,12].Emerging evidence links vitamin D with microbiota status. It has been reported that vitamin D may have a regulatory effect on the intestinal microbiota composition, and that administration of vitamin D therapy may be effective in improving intestinal permeability [4]. Studies have reported that vitamin B12 deficiency may be related to changes in the intestinal microbiota [2,13]. Because vitamin B12 is synthesized by bacteria in the human gutmicrobiome, altered gut microbiota may also be considered an critical factor in vitamin B12 deficiency. In addition, it is thought that there may be changes in gut microbiome composition and function due to vitamin B12 deficiency [13]. Recent research suggests that there is a

biface interaction between the gut microbiota and iron. The bioavailability and absorption of iron can be affected by microorganism activity in the gut, and microbiota imbalance may have a rol in iron deficiency, while iron deficiency or iron overload can also affect the gut microbiota [3].

Based on these data in the literature, we aim to investigate the role of TMAO and LBP in cases with vitamin D, vitamin B12, and iron deficiency.

Methods: This case-control study included the patients in 33 with iron deficiency, 30 with vitamin B12 deficiency, 33 with vitamin D deficiency, 32 with combined deficiency (subjects with concurrent deficiencies of vitamin D, vitamin B12 and iron), 24 taking vitamin D supplements, and 32 age- and body mass index (BMI)-matched healthy controls were in current. The patient and control groups consisted of volunteers who applied to the outpatient clinic for routine control. The cases with newly diagnosed iron deficiency (serum ferritin concentration <30 ng/ml, transferrin saturation <20%), vitamin D deficiency (vitamin D concentrations of <20 ng/ml) and vitamin B12 deficiency (vitamin B12concentrations of <200 pg/ml) were included in the patient group. Vitamin D deficient patients were treated with oral vitamin D at 50000 IU/week for 2 months. After vitamin D treatment, 24 patients with a vitamin D concencentrations of >30 ng/ml were included. Exclusion criteria were obesity, diabetes, cardiovascular and gastrointestinal disease, renal failure, liver or kidney disfunction, having a pregnancy, using additional medicine including antibiotics, corticosteroids, immunosupresif drugs. All volunteers provided informed consent before participating in the study. The study was approved by Ethics Committee (2021/3376).

Fasting venous blood specimens were taken between 9:00 a.m. and 11:00 a.m. The blood samples were centrifuged and serum was extracted and portioned into tubes. Serum biochemical variables were analyzed immediately by photometric methods on the Roche Cobas c501 device (Roche Diagnostics, Mannheim Germany). Vitamin D, B12 and ferritin levels were measured by electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas e601 biochemistry analyzer. The measurement of serum TMAO and LBP levels were performed using by Enzyme-Linked ImmunoSorbent Assay (ELISA) technique (BT Lab Bioassay Technology Laboratory Human Elisa Kits, Shanghai Korain Biotech, China). TMAO catalog number: E4733Hu, standard curve range: 0.2-60 ng/mL, intra-assay: CV<8%, inter-assay: CV<10%. LBP catalog number: E0360Hu, standard curve range: 0.2-60 ng/mL, sensitivity: 0.12 ng/mL, intra-assay: CV<8%, inter-assay: CV<10%. Absorbance measurements were performed at 450 nm.

Statistically analyses were carried out using SPSS v. 22.0 (SPSS Inc., IL, USA). The chi-squared test was used for categorical variables such as gender. Normality distribution was checked with the Kolmogorov-Smirnov test. Continuous variables were compared with the Student's t and Mann-Whitney U test. Furthermore, correlations were calculated by Spearman's correlation test. A value of $p<0.05$ was recognized as significant.

Results: Demographic and biochemical characteristics of the patients groups and control cases are presented in Table 1. Iron and ferritin values of the iron deficiency patients and combined deficiency patients were lower than those of controls ($p<0.001$). Unsaturated iron binding capacity values of the iron deficiency patients and combined deficiency patients were higher than those of controls ($p<0.001$). Vitamin B12 values of the vitamin B12 deficiency patients and combined deficiency patients were lower than those of controls ($p<0.001$). Vitamin D values of the vitamin D deficiency patients and combined deficiency patients were lower than those of controls ($p<0.001$). Serum TMAO values were significantly lower in the iron deficiency group and combined deficiency group than in the control group ($p<0.01$). No statistically differences were found in TMAO levels in those with vitamin B12 deficiency and those with vitamin D deficiency compared to the control group. We found lower serum TMAO levels after vitamin D supplementation in those with vitamin D deficiency. There was no significant difference in serum LBP concentrations between the patient groups and the control group except for the group receiving vitamin D therapy. Serum LBP concentrations were significantly lower in the vitamin D suplemented group compared to the vitamin D deficiency group and the control group (Table 2). Spearman's Rho correlation analysis was performed. There was a positive correlation between TMAO and creatinine ($p<0.05$), ferritin ($p<0.05$) and iron ($p<0.001$) levels. TMAO levels were negatively correlated with glucose

levels ($p<0.05$). There was a significant negative correlation between serum LBP concentrations and ferritin levels(Table 3)

Discussion: Recently, the functions of vitamin D other than to control of bone metabolism have attracted attention. It has been reported that vitamin D may have a regulatory effect on the gut microbiota, whose changes are associated with many dissease. Recent researchs show that vitamin D can directly affect the gut microbiome and alleviate dysbiosis [4]. In our study, we wanted to analyze the TMAO levels, one of the intestinal microbiota metabolites, before and after vitamin D suplementation in patients with vitamin D deficiency. In a research investigating the effect of cholecalciferol suplementation on biomarkers associated with carrdiovascular disease in obese people with vitamin D deficiency, a significant reduction in TMAO levels was observed after supplementation [14]. In another study, high TMAO levels were thought to be associated with vitamin D deficiency and non-alcoholic fatty liver disease (NAFLD). TMAO concentrations were highest in patients with both vitamin D deficiency and NAFLD. Vitamin D values showed important opposite associations with circulating TMAO values [15]. Obeid at al showed that TMAO plasma levels were reduced after vitamins B plus vitamin D supplementation [16]. A study in mice reported that with vitamin D supplementation, the intestinal microbiota was regulated, and TMA and TMAO levels were significantly reduced [17]. On the other hand, in a study conducted in HIV-1 infected individuals, it was found that dietary supplementation containing vitamin D had no effect on TMAO level and intestinal microbial composition. In this study, there was no important difference in TMAO concentrations in those with vitamin D deficiency compared to the healthy group [18]. Although not statisticaly significant, in our investigation, we observed a decrease in serum TMAO levels after vitamin D supplementation in cases with vitamin D deficiency.

There are few studies investigating the relationship between vitamin B12 and intestinal microbial metabolites TMAO. Obeid at al. observed that plasma levels of TMAO decreased after B vitamins plus vitamin D supplementation [15]. Another study in vegetarians found no effect of intervention with vitamin B12 on plasma TMAO levels [19]. According to the results of our research, there was no considerable difference between the TMAO levels of the vitamin B12 deficient group and the control group.

There is a complex relationship between iron and gut microbiota. Iron is of great importance for microbial growth in gut. It has been reported that gut microbial metabolites regulate the host's iron homeostasis by inhibiting iron transport and storage [20]. The presence of iron in the intestinal lumen can prevent or promote intestinal dysbiosis [21]. In a study in mice, it was reported that iron overload led to intestinal dysbiosis and increased TMAO levels [22]. In another study, an inverse relationship was observed between transferrin and TMAO [23]. It has been reported that paraferritin containing FMO functions as a ferrireductase and iron plays a role in cellular uptake and, abnormalities of FMO enzymes may be associated with sideroblastic anemia [24]. In our study, we found TMAO levels to be significantly lower than the control in cases with iron deficiency and combined deficiency. In addition, there was a positive correlation between TMAO values and ferritin and iron values.

Serum LBP concentrations can be measured to assess changes in the gut microbiome [11,12]. Circulating LBP has been found to be an innate immune related component, associated with pathophysiological conditions such as obesity, metabolic syndrome [25]. It has been reported that it may be an important factor in the emergence of the metabolic syndrome through low-grade endotoxemia, systemic chronic inflammation and insulin resistance [26]. It has also been reported that LBP is associated with fatty liver [27]. In a study of patients with liver cirrhosis, they found an inverse interaction between vitamin D concentrations and LBP [28]. We did not observe a statistically important difference in LBP values between our study groups, except for the group that received vitamin D supplementation. Serum LBP levels were significantly lower in the vitamin D suplemented group compared to the vitamin D deficiency group and the control group. In addition, we found a negative correlation between LBP values and ferritin values. Based on the limited information about the roles of LBP in micronutrient deficiencies, we are unable to explaine the present findings at this time.

The limitation of our study is that we did not evaluate the participant's dietary intake and fecal microbiome composition in this study and the sample size was limited.

In conclusion we measured the levels of TMAO, a compound produced by the gut microbiome and associated with various chronic diseases, and LBP, a biomarker for intestinal permeability, in vitamin D, vitamin B12 and iron deficiency. Our findings support that although TMAO and LBP are not strong predictors of vitamin D, Vitamin B12 and iron deficiency, they are associated with the pathogenesis of these micronutrient deficiencies.

References

1. Berger MM, Shenkin A, Schweinlin A, Amrein K, Augsburger M, Biesalski HK, et al. ESPEN micronutrient guideline. Clin Nutr. 2022;41(6):1357–1424.

2. Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a modulator of gut microbial ecology. Cell Metab. 2014;20:769–778.

3. Yilmaz B, Li H. Gut microbiota and iron: the crucial actors in health and disease. *Pharmaceuticals*. 2018 Oct 5;11:98.

4. Battistini C, Ballan R, Herkenhoff ME, Saad SMI, Sun J. Vitamin D modulates intestinal microbiota in inflammatory bowel diseases. *Int J Mol Sci*. 2020;22(1):362.

5. Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. Nat Rev Immunol. 2020;20:40–54.

6. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, et al. Trimethylamine-N-Oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation. Cell Metab. 2013;17:49–60.

7. Gatarek P, Kaluzna-Czaplinska J. Trimethylamine N-oxide (TMAO) in human health. EXCLI J. (2021) 20:301.

8. Chen X, Zhang H, Ren S, Ding Y, Remex NS, Bhuiyan MS, et al. Gut Microbiota and Microbiota-Derived Metabolites in Cardiovascular Diseases. Chin. Med. J. 2023;136:2269–2284.

9. Li D, Lu Y, Yuan S, Cai X, He Y, Chen J, et al. Gut microbiota-derived metabolite Trimethylamine-N-oxide (TMAO) and multiple health outcomes: an umbrella review and updated meta-analysis. Am J Clin Nutr. 2022;116(1):230–243.

10. Schumann R.R. Old and new findings on lipopolysaccharide-binding protein: A soluble pattern-recognition molecule. Biochem Soc Trans. 2011;39:989–993.

11. Schoultz I, Keita ÅV. The Intestinal Barrier and Current Techniques for the Assessment of Gut Permeability. Cells. 2020;9:1909.

12. Seethaler B, Basrai M, Neyrinck A M, Nazare JA, Walter J, Delzenne NM, et al. Biomarkers for assessment of intestinal permeability in clinical practice. Am J Physiol Gastrointest Liver Physiol. 2021; 321(1), G11–G17.

13. Al-Musharaf S, Aljuraiban GS, Al-Ajllan L, Al-Khaldi N, Aljazairy EA, Hussain SD, et al. Vitamin B12 Status and Gut Microbiota among Saudi Females with Obesity. Foods. 2022 Dec 11;11(24):4007.

14. Ozorowski M, Wiciński M, Wróbel Ł, Fajkiel-Madajczyk A. Cholecalciferol supplementation lowers leptin and TMAO but increases no and vegf-a levels in obese vitamin d deficient patients: Is it one of the potential cardioprotective mechanisms of vitamin d? Nutr Metab (Lond). 2022;19(1):31.

15. Barrea L, Muscogiuri G, Annunziata G, Laudisio D, de Alteriis G, Tenore GC, et al. A new light on vitamin D in obesity: a novel association with trimethylamine-N-oxide (TMAO). *Nutrients.* 2019;11:1310.

16. Obeid R, Awwad HM, Kirsch SH, Waldura C, Herrmann W, Graeber S, et al. Plasma trimethylamine-N-oxide following supplementation with vitamin D or D plus B vitamins. Mol. Nutr. Food Res. 2017;61 (2).

17. Wang X, Li X, Dong Y. Vitamin D decreases plasma trimethylamine-N-oxide level in mice by regulating gut microbiota. *Biomed Res Int.* 2020 Oct 5;2020:9896743.

18. Missailidis C, Sørensen N, Ashenafi S, Amogne W, Kassa E, Bekele A, et al. Vitamin D and Phenylbutyrate Supplementation Does Not Modulate Gut Derived Immune Activation in HIV-1. Nutrients. 2019;11:1675.

19. Obeid R, Awwad HM, Keller M, Geisel J. Trimethylamine-N-oxide and its biological variations in vegetarians. Eur J Nutr. 2017;56:2599–2609.

20. Das NK, Schwartz AJ, Barthel G, Inohara N, Liu Q, Sankar A, et al. Microbial Metabolite Signaling Is Required for Systemic Iron Homeostasis. Cell Metab. 2020;31:115–130.e116.

21. Chieppa M, Giannelli G. Immune cells and microbiota response to iron starvation. Front Med. (Lausanne). 2018 Apr 18;5:109.

22. Sriwichaiin S, Thiennimitr P, Thonusin C, Sarichai P, Buddhasiri S, Kumfu S, et al. Deferiprone has less benefits on gut microbiota and metabolites in high iron-diet induced iron overload thalassemic mice than in iron overload wild-type mice: A preclinical study. Life Sci. 2022;307:120871.

23. Bean LD, Wing JJ, Harris RE, Smart SM, Raman SV, Milks MW. Transferrin predicts trimethylamine-N-oxide levels and is a potential biomarker of cardiovascular disease. BMC Cardiovasc Disord. 2022;22(1):209.

24. Barber M, Conrad ME, Umbreit JN, Barton JC, Moore EG. Abnormalities of flavin monooxygenase as an etiology for sideroblastic anemia. Am J Hematol. 2000;65:149–153.

25. Molinaro A, Koh A, Wu H, Schoeler M, Faggi MI, Carreras A, et al. Hepatic expression of Lipopolysaccharide Binding Protein (Lbp) is induced by the gut microbiota through Myd88 and impairs glucose tolerance in mice independent of obesity. Mol. Metab. 2020;37:100997.

26. Tomooka S, Oishi E, Asada M, Sakata S, Hata J, Chen S, et al. Serum lipopolysaccharide-binding protein levels and the incidence of metabolic syndrome in a general Japanese population: the Hisayama Study. J Epidemiol. 2022 Dec 24.

27. Latorre J, Díaz-Trelles R, Comas F, Gavaldà-Navarro A, Milbank E, Dragano N, et al. Downregulation of hepatic lipopolysaccharide binding protein improves lipogenesis-induced liver lipid accumulation. Mol Ther Nucleic Acids. 2022 Aug 5;29:599-613.

28. Triantos C, Kalafateli M, Aggeletopoulou I, Diamantopoulou G, Spantidea PI, Michalaki M, et al. Vitamin D-related immunomodulation in patients with liver cirrhosis. Eur J Gastroenterol Hepatol. 2020;32:867–876.

Table 1: Biochemical and demographic characteristics of patients and control subjects.

	Control (n=32)	Combined deficiency (n=32)	Iron deficiency (n=32)	Vitamin B12 deficiency (n=31)	After treatment of Vitamin D (n=25)	Vitamin D deficiency (n=32)	P
Female/Male	16/16	17/15	18/14	16/15	12/13	15/17	0.182
Age (years)	47.4 ± 13.9	48.4 ± 14.7	40.8 ± 14.7	46.9 ±12.4	46.4 ± 10.6	44.3 ± 10.5	0.135
BMI, kg/m2	25.3 ± 1.9	25.6 ± 2.5	24.9 ± 1.4	25.7 ± 2.7	24.6 ± 1.7	25.9 ± 3.1	0.243
Glucose, mg/dL	97.7 ± 4.7	98.0 ± 3.9	96.2 ±3.9	96.9 ±4.7	97.3 ± 4.1	96.9 ±5.1	0.620
Urea, mg/dL	31.9 ± 6.1	31.0 ± 7.2	30.2 ± 6.1	31.1 ± 5.1	28.9 ± 5.7	31.1 ± 6.5	0.578
Creatinine, mg/dL	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.205
AST, U/L	20.3 ± 4.9	20.3 ± 5.8	21.5 ± 5.8	21.2 ± 5.6	21.6 ± 5.9	19.8 ±5.4	0.729
ALT, U/L	22.2 ± 7.6	23.1± 5.7	24.2 ± 5.4	25.6 ± 6.26	25.6 ± 5.4	24.4 ± 6.9	0.233
Triglycerides, mg/dL	129 (58-237)	136 (67-388)	117 (43-188)	154 (49-287)	138 (80-317)	128 (61-293)	0.252
HDL-C mg/dL	50.3 ± 10.1	45.5 ± 8.9	47.3 ± 8.8	46.0 ± 5.8	47.4 ± 4.6	46.3 ±7.9	0.196
LDL-C mg/dL	116 (89-156)	124 (90-172)	121 (92-178)	120 (85-171)	126 (75-186)	122 (63-190)	0.666
Cholesterol, mg/dL	215 (183-293)	214 (165-274)	208 (152-282)	212 (161-284)	233 (169-296)	221 (146-298)	0.102
Iron, mcg/dL	120.9 (59-164)	32.2 (20-86)	30.1 (18-60)	85.6 (42-215)	95.3 (65-181)	94.8 (54-166)	
Unsaturated iron binding capacity, mcg/dL	263 (200-380)	394 (315-537)	405 (326-587)	298 (208-433)	277 (215-363)	267 (194-386)	
Ferritin, ng/mL	125.2 (36-244)	14.8 (3-41)	7.4 (3-13)	82.9 (26-301)	104.8 (34-314)	87.8 (3-314)	
Vitamin B12, pg/mL		187 (127-225)	388 (269-803)	160 (132-192)	433 (313-671)	430 (275-712)	

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

Bold font p-values (p<0.001) indicate statistical significance between all groups.

Table 2: Serum biomarkers of patients and control individuals

	Control (n=32)	Combined deficiency (n=32)	Iron deficiency (n=33)	Vitamin B12 deficiency (n=30)	After treatment of Vitamin D (n=24)	Vitamin D deficiency (n=33)	P
TMAO, ng/mL	63.2 (2.7-353.4)	10.0 (2.4-99.0) *	10.5 (2.4-75.4) *	48.3 (2.4-339.9)	17.5 (2.4-113.7)	41.3 (2.7-146.2)	<0.005
LPB, ng/mL	470.2 (175.1-1343.8)	410.2 (17.2-2085.4)	599.9 (47.1-2534.7) **	338.7 (28.0-813.2)	198.8 (20.7-931.4) ¥ ***	359.0 (157.6-594.5)	<0.005

TMAO, trimethylamine-*N*-oxide; LBP, Lipopolysaccharide binding protein.

*p < 0.01, compared with control group

**p < 0.05, compared with Vitamin B12 deficiency group

***p < 0.001, compared with Iron deficiency

¥ p < 0.05, compared with control group

Table 3: The association of biomarkers levels according to Spearman's correlation analyses

		Glucose	Creatinine	Ferritin	Iron
TMAO	r	-0 ,174	0 ,188	0,153	0,296
	p	**<0,05**	**<0,05**	**<0,05**	**<0,001**
LBP	r	<0.001	-0,121	-0,178	0,012
	p	0,997	0,115	**<0,05**	0,878

TMAO, trimethylamine-*N*-oxide; LBP, Lipopolysaccharide binding protein.

Bold font p-Values indicate statistical significance.

S015

REVIEW OF THE DIAGNOSIS OF DIABETES MELLITUS ACCORDING TO GLUCOSE AND HBA1C MEASUREMENT UNCERTAINTY

Oznur Asil[1], Kaan Kuzu[1], Giray Bozkaya[2],

[1]University of Health Sciences, Izmir Bozyaka Health Practice and Research Hospital, Medical Biochemistry Laboratory, Izmir, Türkiye
[2]Izmir University Faculty of Medicine, Bozyaka Health Practice and Research Hospital, Medical Biochemistry Laboratory, Izmir, Türkiye

Introduction: Measurement uncertainty is a parameter that defines the possible deviations related to the result of a measurement or in other words is a statistical measure of the variation in the test result of a biochemical measurement [1]. It provides a numerical assessment of the laboratory test results and allows the calculation of the closeness of these findings to the true values. Basically, measurement uncertainty draws the boundaries of the area where the measurements are obtained. Therefore, it is a very important factor when interpreting the test results correctly while evaluating the reliability of the laboratory test results [2].

Recently, the calculation of measurement uncertainty has become necessary with the implementation of the guide published by the International Organization for Standardization (ISO) for medical laboratory accreditation. According to the ISO 15189 quality standard for medical laboratories; Laboratories should determine the uncertainty of analysis results, taking into account the uncertainty components. The guide states that laboratories should present their calculations related to measurement uncertainties when requested [3]. The accurate reporting of measurement uncertainty in medical reports requires careful evaluation and discussion by laboratory medicine specialists to support the physicians in diagnosis of diseases [4].

There are many sources that can cause measurement uncertainty in laboratories. These include; the form of sample collection, sample matrix, environmental and measurement conditions, internal and external quality results of the devices, calibration, errors in methods and procedures. According to ISO 15189:2022, it is important to select the most appropriate model based on the suitability of the tests for the purpose when calculating measurement uncertainty to support the interpretation of the results. Numerous guides have been published regarding this calculation [5–8]. Among these guides, the Nordest Guide aims to provide a common, understandable and practical application in the calculation of measurement uncertainty [8].

In 2019, diabetes mellitus (DM) had an estimated prevalence of 9.3% in the global adult population (463 million), and this prevalence is anticipated to grow to 578 million (10.2%) by 2030 and 700 million (10.9%) by 2045. These figures reveal that DM is a rising health issue and a significant problem [9]. The diagnosis of DM which is increasing day by day, is based on any of the following criteria according to the World Health Organization (WHO) 2011 guideline and the American Diabetes Association (ADA) 2023 guideline [10,11]:

- Fasting plasma glucose (at least 8 hours of fasting is required) ≥ 126 mg/dL
- Random plasma glucose (at any time of the day) ≥ 200 mg/dL and diabetes symptoms are present
- Plasma glucose ≥ 200 mg/dL at the 2nd hour after 75 grams of glucose intake with oral glucose tolerance test (OGTT)
- HbA1c ≥ 6.5%

The diagnosis of DM relies on above criteria, each of which has a certain degree of measurement uncertainty associated with the glucose and HbA1c levels. By accounting for this uncertainty, the reliability and validity of the diagnosis can be enhanced.

The HbA1c test can be more widely used in the clinic to assess glycemic stability. This test does not require fasting, more preanalytical stable and has various advantages compared to fasting glucose and OGTT. However, these advantages are balanced by the lower sensitivity of HbA1c to the threshold value, its higher cost, the difficulty of accessing the HbA1c test in developing countries and the lack of a complete agreement between HbA1c and average glucose in some individuals. When diagnosing diabetes with HbA1c, it is important to remember that HbA1c indirectly measures the average blood glucose and to consider other factors that do not affect glycemia but can alter hemoglobin glycation, such as hemodialysis, pregnancy, HIV treatment, age, race/ethnic origin, genetic background and anemia/hemoglobinopathies. For these reasons, measuring fasting blood glucose for DM diagnosis is a more practical and reliable method in the clinic [11].

Since fasting plasma glucose and HbA1c levels are the main criteria for diagnosing DM, our aim was to determine the effect of measurement uncertainty in the diagnosis of DM.

Methods: For our study, we used 63 low-level (mean: 105 mg/dL ± 2.5) and 63 high-level (mean: 246.5 mg/dL ± 8.7) internal quality control data for glucose once a day, and 63 low-level (mean: 5.1% ± 0.2) and 63 high-level (mean: 10.2% ± 0.3) internal quality control data for HbA1c once a day in the Biochemistry Laboratory of Health Sciences University Izmir Bozyaka Education and Research Hospital from March to May 2023. In the same period, we used three-month external quality control data obtained from the Randox International Quality Assesment Scheme (RIQAS, Randox®, County Crumlin, England) quality control program for glucose, and three-month external quality control data obtained from the External Quality Assurance Services (EQAS, QCNet, BioRad Laboratories Inc.® California, USA) quality control program for HbA1c.

For the glucose test, blood samples were taken into vacuum tubes containing a separating gel and a clot activator (Vacusera®, Izmir, Türkiye). Then, the blood samples were centrifuged and glucose was measured by the hexokinase method in the Cobas 8000 Modular Analyzer System (Cobas®, Mannheim, Germany). For the HbA1c test, blood samples were taken into vacuum tubes containing K_2EDTA (Vacusera®, Izmir, Türkiye) and analyzed by the cation exchange High-Performance Liquid Chromatography (HPLC) method in the BioRad VARIANT II TURBO device (BioRad Laboratories Inc.® California, USA). The HbA1c test was measured using a method that was certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized according to the Diabetes Control and Complications Trial (DCCT).

Our study was conducted retrospectively and data collection started after the approval of the Clinical Research Ethics Committee of Health Sciences University Izmir Bozyaka Education and Research Hospital dated 16/08/2023 and numbered 2023/118.

The data were transferred to Microsoft Excel®. The measurement uncertainty values of glucose and HbA1c analyses were calculated based on the Nordtest Guide [7,8].

For the calculation of the within-laboratory reproducibility (Rw), the 3-month coefficient of variation (%CV) of the control materials was used.

$$Rw=\sqrt{[(normal\ control\ material\ \%CV)^2+(pathological\ control\ material\ \%CV)^2]/2}$$

For the calculation of the u(bias) component of the uncertainty obtained from the external quality control results, RMS bias and u(Cref) values are used.

RMS bias was calculated from the 3-month data obtained from the external quality control programs.

RMS bias= $\sqrt{[(In-group\ bias)^2+(Intergroup\ bias)^2]/2}$

$In-group\ bias^2=\sum In-group\ bias^2/n$

$Intergroup\ bias^2=\sum Intergroup\ bias^2/n$

n= number of external quality control results

u(Cref), the uncertainty component calculated using the true or expected values obtained from external quality control results, is defined as. u(Cref) component can be calculated using the relative standard deviation (%SR) value instead of %CV.

%SR=(standard deviation of the measurement/mean of the measurement) x100 formula is used to calculate %SR, so %SR=%CV can be accepted.

u(Cref) is obtained by dividing the average %CV values calculated from external quality control results by the square root of the number of laboratories using the same method and device for 3 months.

u(Creft)=$\%CV/\sqrt{n}$

n= number of laboratories using the same methods and equipment

All components used in the calculation of measurement uncertainty using the following formulas were converted to standard uncertainty value.

uRw=$Rw/2$

u(bias)=$\sqrt{RMS\ bias^2+u(Cref)^2}$

The combined standard uncertainty value was created using the standard uncertainty value.

uc=$\sqrt{(uRw)^2+(u(bias))^2}$

Then the combined standard uncertainty value was multiplied by the k factor and the expanded uncertainty value (U) was found. The k value, which is equivalent to 1.96 in the 95% confidence interval, was taken as approximately 2.

U=$2.uc$

Over a three-month period, 77131 glucose and 13613 HbA1c results obtained in our laboratory were retrospectively analyzed. The results that fell below the anemia limits determined by the World Health Organization were not included in the study [12]. The hemoglobin results of the individuals whose HbA1c results were considered to be measured low according to the measurement uncertainty were retrospectively evaluated.

Results: In our laboratory, the measurement uncertainty value for the glucose test was calculated. The internal quality control uncertainty value (uRw) was 1.45% (Table 1), the RMS bias according to the external quality control data was 2.08, u(Cref) was 0.16, the uncertainty value u(bias) was 2.09% (Table 2), and the expanded uncertainty value (U) was 5.1% (Table 3). According to the expanded measurement uncertainty value, the maximum error rate for the glucose value affecting the DM diagnosis (126 mg/dL) was calculated as ±6.4 (119.6 – 132.4) mg/dL. According to this result, it was seen that 845 (1.1%) glucose results were affected by the uncertainty value. According to the obtained glucose measurement uncertainty value, it can be considered that 423 (0.55%) results were measured low and 422 (0.55%) results were measured high. It was determined that the other results (98.9%) were not affected by the measurement uncertainty value for making the DM diagnosis.

In order to evaluate diagnosis of DM, the measurement uncertainty value of the HbA1c test was also calculated. The internal quality control uncertainty value (uRw) was 1.56% (Table 1), the uncertainty value u(bias) was 1.82% (Table 2), and the expanded uncertainty value (U) was 4.79% (Table 3). According to the expanded measurement

uncertainty value, the maximum error rate for the HbA1c value affecting the DM diagnosis (6.5%) was found to be ±0.31% (6.19% – 6.81%). Based on the glucose measurement uncertainty, the HbA1c values used for DM diagnosis of individuals with glucose results between HbA1c and the number of those between 6.19% and 6.5% was determined as 56 (0.07%). Similarly, the number of individuals with glucose results between 126 – 132.4 mg/dL and HbA1c values between 6.5% and 6.81% was found to be 76 (0.1%) (Table 4).

Discussion: Measurement uncertainty is a parameter that expresses the reliability of the measurement result and determines the possible range of the measured quantity [13]. The interpretation of test results and the clinical decision-making process may change with the reporting of measurement uncertainty [4].

In our study to calculate the measurement uncertainty of glucose and investigate its impact on retrospective patient results, we found that the uncertainty value was 5.1% at 95% confidence interval. This result was seen to be below the total allowable error limit (TEa) (6.96%) determined by the Clinical Laboratory Improvement Amendments (CLIA), Federal Register and Westgard [14]. TEa is an analytical quality requirement that sets the tolerable limits of uncertainty for a single test result or a single measurement. CLIA criteria specify the maximum error limits allowed for the substance being measured. It can be said that the system has diagnostic adequacy if the measurement uncertainty or total error for an analyte is within the TEa limits for that analyte [4]. In the study conducted by Keutmann et al., the uncertainty value of the glucose test measured by the hexokinase method was found to be 5.7% (±7.2 mg/dL). This corresponded to a value between 118.8 and 133.2 mg/dL at the limit value of 126 mg/dL for DM diagnosis [15]. Similarly, in the study conducted by Petersmann et al., the uncertainty value of the glucose test was found to be 8% [16]. Based on the other studies above, it was seen that the measurement uncertainty value we calculated for the glucose test was lower. However, in the study conducted by Bercik Inal et al., the uncertainty value of the glucose test was calculated as 4.6% [17]. Similarly, in the study conducted by Arslan İnce et al., the measurement uncertainty of glucose was calculated as 3%. However, both studies did not mention how many of the glucose results close to the DM diagnosis limit were affected by the measurement uncertainty value [18].

When the measurement uncertainty of glucose was calculated in our laboratory, it was found to be ± 6.4 mg/dL. According to this uncertainty value, the threshold value of 126 mg/dL for DM diagnosis was readjusted. The glucose results between 119.6 – 132.4 mg/dL were re-evaluated according to the range caused by uncertainty value. Out of 77131 glucose results, 845 of them (1.1%) were affected by the measurement uncertainty. Although this ratio seems low, if DM diagnosis was made only by looking at the glucose result, wrong diagnosis and treatment decision would be given for 845 people. However, by taking into account the measurement uncertainty of HbA1c, the results of these people were re-evaluated and their number decreased to 282.

The measurement uncertainty of HbA1c in our laboratory was determined as 0.31%. According to this uncertainty value, the threshold value of 6.5% for DM diagnosis was readjusted and the HbA1c results between 6.19% – 6.81% were re-examined. When the new results were evaluated together, it was noticed that 89 of 282 people received impaired fasting glucose (119.6 – 126 mg/dL) diagnosis according to the glucose result, while they received DM diagnosis according to the HbA1c result. These individuals may have been deprived of access to diabetes treatment, despite their need for it. On the other hand, it was determined that 61 people were classified as prediabetes according to the HbA1c result, while they received DM diagnosis according to the glucose result. These individuals may have received unnecessary treatment for diabetes, even though they did not require it. 132 people were observed to receive the same diagnosis with both glucose and HbA1c results (56 people received prediabetes diagnosis, 76 people received DM diagnosis).

Our study has some limitations. The most important one is that we do not know whether the reported fasting glucose results were taken after a complete fasting, whether the person was taking any other medication for diabetes treatment, how well they adhered to their diet and when they stopped it, while evaluating their results. These unknowns may have increased the inconsistent results between HbA1c and fasting plasma glucose.

These results show that the measurement uncertainty of glucose in our laboratory is lower than the uncertainty values found in most studies. However, it is clear that the values close to the diagnostic limit in DM are affected by this uncertainty. Therefore, evaluating glucose with HbA1c in the diagnosis of DM will enable a much more accurate diagnosis. In addition, improving the analytical performance conditions and reducing the measurement uncertainty further is our main goal.

Research ethics: The local Institutional Review Board deemed the study exempt from review. Health Sciences University Izmir Bozyaka Education and Research Hospital dated 16/08/2023 and numbered 2023/118.

Informed consent: Not applicable

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Research funding: None declared.

References

1. Meyer VR. Measurement uncertainty. Journal of Chromatography A, 2007;1158:15–24.

2. Padoan A, Sciacovelli L, Aita A, Antonelli G, Plebani M. Measurement uncertainty in laboratory reports: A tool for improving the interpretation of test results. Clinical Biochemistry 2018;57:41–47.

3.ISO 15189, Medical laboratories-requirements for quality and competence. International Organization for Standardization 2022.

4.Bal C, Serdar MA, Gungor OT, Celik HT, Abusoglu S, Uguz N et al. Calculation of measurement uncertainty of biochemical parameters. Turkish Journal of Biochemistry 2014;39:538–43.

5.ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories. International Organization for Standardization/International Electrotechnical Committee 2017.

6.ISO/TS 20914. Medical laboratories-Practical guidance for the estimation of measurement uncertainty. International Organization for Standardization/Technical Specification 2019.

7.Eurachem/CITAC Guide CG 4. Quantifying uncertainty in analytical measurements, 2nd Edition. (Quam:2000. P1).

8.Magnusson B, Hovind H, Krysell M. Handbook for calculation of measurement uncertainty in environmental laboratories: Nordtest Report TR 537. Nordtest, Finland, 2004.

9.Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice 2019;157:10784

10.World Health Organization. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. WHO guidelines approved by the Guidelines Review Committee. Geneva: World Health Organization; 2011.

11.American Diabetes Association (ADA). Classification and diagnosis of diabetes: standards of care in diabetes – 2023. Diabetes care 2023;46:19–40.

12. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. WHO/NMH/NHD/MNM/11.1. Geneva: World Health Organization; 2011

13. Tietz NW. A model for a comprehensive measurement system in clinical chemistry. Clinical Biochemistry 1979;25:833–9.

14. CMS, HSS. Clinical Laboratory Improvement Amendments of 1988 (CLIA) Proficiency Testing Regulations Related to Analytes and Acceptable Performance. Federal Register 2019; 84:1536–67.

15. Keutmann S, Zylla S, Dahl M, Friedrich N, Landgraf R, Heinemann L et al. Measurement uncertainty impacts diagnosis of diabetes mellitus: reliable minimal difference of plasma glucose results. Diabetes Therapy 2020;11:293–303.

16. Petersmann A, Macdonald R, Nauck M. Disregarded measurement uncertainty contributions and their magnitude in measuring plasma glucose. Journal of Diabetes Science and Technology 2022;16:161–7.

17. Bercik Inal B, Koldas M, Inal H, Coskun C, Gumus A, Doventas Y. Evaluation of measurement uncertainty of glucose in clinical chemistry. Annals of the New York Academy of Sciences 2007;1100:223–6.

18. İnce FD, Şentürk BA, Kap S, Akgöl E, Üstüner F. Klinik biyokimya laboratuvarında glukoz parametresi için ölçüm belirsizliği değerlendirilmesi. Türk Klinik Biyokimya Dergisi 2007;5:1–5.

Tables

Table 1. Glucose and HbA1c Internal Quality Control Data

	Mean ± Standard Deviation		**uRw****
Glucose (mg/dL)	Level 1 (105 ± 2.5)	2.15	1.45
	Level 2 (246.5 ± 8.7)	3.49	
HbA1c (%) **(mmol/mol)**	Level 1 (5.01 ± 0.2) (32.8 ± 2.2)	3.57	1.56
	Level 2 (10.2 ± 0.3) (87.7 ± 3.2)	2.57	
*Coefficient of Variation **In-lab reproducibility (uRW=*Rw/2*)			

Table 2. Glucose and HbA1c External Quality Control, In-Group and Intergroup Bias Data

	In-Group Bias²	Intergroup Bias²	RMS bias²	n		u(creft)²	u(bias)
Glucose	5.35	3.34	4.34	149	1.97	0.03	2.09
HbA1c	2.19	4.40	3.29	185	2.09	0.02	1.82

RMS bias= $\sqrt{[In-group\ bias)^2 + (Intergroup\ bias)^2]/2}$

(In-group bias)²= (∑In-group bias)²/n

(Intergroup bias)²= (∑Intergroup bias)²/n

n= Mean number of laboratories using the same method and the same instrument

u(cref): $CV\%\sqrt{n}$ *Uncertainty component derived from the expected value of external quality control results*

CV%*: Coefficient of Variation*

u(bias)= $\sqrt{(RMS\ bias)^2 + (u(Cref))^2}$

Table 3. Calculation of Uncertainty Value

	Total Uncertainty Value (uc) (%)		Extended Uncertainty Value (U) (%)	
Glucose	$\sqrt{\left[(1.45)^2 + (2.09)^2\right]/2}$	2.5	2.5 * 2 (scope factor)	5.1
HbA1c	$\sqrt{\left[(1.56)^2 + (1.82)^2\right]/2}$	2.4	2.4 * 2 (scope factor)	4.8

uc= $\sqrt{[(uRw)^2 + (u(bias))^2]/2}$

U=*uc x k (scope factor)*

Table 4. Distribution of Results Affected by Measurement Uncertainty

		HbA1c (%)	
		6.19 – 6.5	6.5 – 6.81
Glucose (mg/dL)	119.6 – 126	56#	89*
	126 – 132.4	61**	76#

When all the results were re-evaluated taking into account the measurement uncertainty of glucose and HbA1c, it was seen that a total of 282 people were affected:
*89 people were diagnosed with impaired fasting glucose according to their glucose result, while they were diagnosed with diabetes mellitus according to their HbA1c result.
**61 people were diagnosed with diabetes mellitus according to their glucose result, while they were classified as prediabetic according to their HbA1c result.
#132 people received the same diagnosis with both their glucose and HbA1c results (56 people were diagnosed with prediabetes, 76 people were diagnosed with diabetes mellitus).

S016

COMPARISON OF CREATININE MEASUREMENTS BY A SPECTROPHOTOMETRIC METHOD AND LC/MS/MS METHOD

Saadet İBİŞ[1], Turan AKDAĞ[2], Ali ÜNLÜ[3], Firdevs SAK[3], Hüseyin DOST[4], Menekşe KUZU[5]

[1]29 Mayıs State Hospital, Ankara, Turkey

[2]Meram Vocational School, Necmettin Erbakan University, Konya, Turkey

[3]Department of Biochemistry, Faculty of Medicine, Selcuk University, Konya, Turkey

[4]Medical Supervisor, Ankara, Turkey

[5]Department of Biochemistry, Faculty of Pharmacy, Biruni University, İstanbul, Turkey

Introduction: Renal function tests are important for the management of kidney disease. Serum creatinine assay is important for determining kidney and muscle damage [1]. Creatine-phosphate is a source of energy for muscle tissues. It's dehydrogenated form, creatinine is the major form in blood. Serum creatinine ranged between 44-106 µM [2]. Higher than 1000 µM serum creatinine levels shows a pathological condition. Higher than 140 µM serum creatinine levels requires clinical investigation and higher than 530 µM serum creatinine levels indicates severe kidney damage [3]. Endogenous production of creatinine is related to age, gender, and muscle mass [4]. There are several methods for creatinine measurement.

Jaffe's reaction is one of the most common used method; briefly is performed as follows; serum was treated with alkaline picrate solution to form, a colored complex and its absorbance is detected at 510 nm. Mean serum creatinine levels were 0.9-1.5 mg/dL (80-133 µmol/L) in men and 0.7-1.3 mg/dL (62-115 µmol/L) in women [5].

Despite technical developments, the pitfalls of serum creatinine measurement by spectrophorometry have not been overcome yet. High performance liquid chromatography (HPLC) is noted as an alternative technique. Even so, this method has some disadvantages such as matrix-based interventions, labor intensive preanalytical processes, and being a time-consuming process [6]. In clinical laboratories, LC-MS/MS provides highly sensitive, selective, and efficient method for the measurement of molecules. Recently, creatinine analysis by LC MS/MS in serum [7] and urine [8] have been reported in the literature.

There are autoanalyzers belonging to many companies of different trademarks and models in clinical laboratories. Method comparisons are based on the results which obtained by analyzing patient samples with the new test method and the reference method. We aimed to compare creatinine levels between the Jaffe method (with Archem kit) and the LC MS/MS method in our laboratory.

Methods: The serum samples were obtained from the subjects (n=102) admitted to 29 Mayıs State Hospital for routine controls between January and April 2021. Blood samples were centrifuged at a 3500 rpm for 10 minute and kept at -80 C0 until the assay.

The Jaffe method commonly used in clinical laboratories for creatinine analysis is spectrophotometric method, that alkaline medium serum creatinine + and picric acid forms a yellow-orange compound. In the presence of peroxidase (POD), the hydrogen peroxide reacts with N-(3-sulfopropyl)-3-methoxy-5-methylaniline and 4-aminoantipyrine to form blue colour. The rate of change at 520/800 nm is directly proportional to the amount of creatinine in the sample.

In this study, the serum creatinine levels were measured by JAFFE method using Archem kit on Mindray system and compared with API 3200 Mass Spectrometer.

Ethical Committee

The study was approved by the Non-Invasive Clinical Research Ethics Committee of the Faculty of Medicine in Selcuk University (Decision No: 2021/367).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows 21.0 and analyses computer programs were used to perform the statistical analyses (SPSS Inc., Chicago, IL, USA). The Passing-Bablok regression analysis and Bland-Altman plots were used for the comparison of Jaffe and LC-MS/MS methods. A p-value as <0.05 were considered as statistically significant.

Results: Comparison of Mindray BS-800M and Archem Crea-J/LC/MS/M according to <0.5 mg/dL of serum creatinine, 0.5-1-3 mg/dLof serum creatinine, >1.4 mg/dLof serum creatinine were given in Figure 1a-1c.

Correlation coefficient (r) of all creatinine levels (n=102) was 0.9958. For <0.5 mg/dL (n=32) serum creatinine levels r was 0.4938. Also, at 0-1.3 mg/dL (n=39) and >1.4 mg/dL (n=31) serum creatinine levels r was determined as 0.9689 and 0.9924, respectively (Table 1).

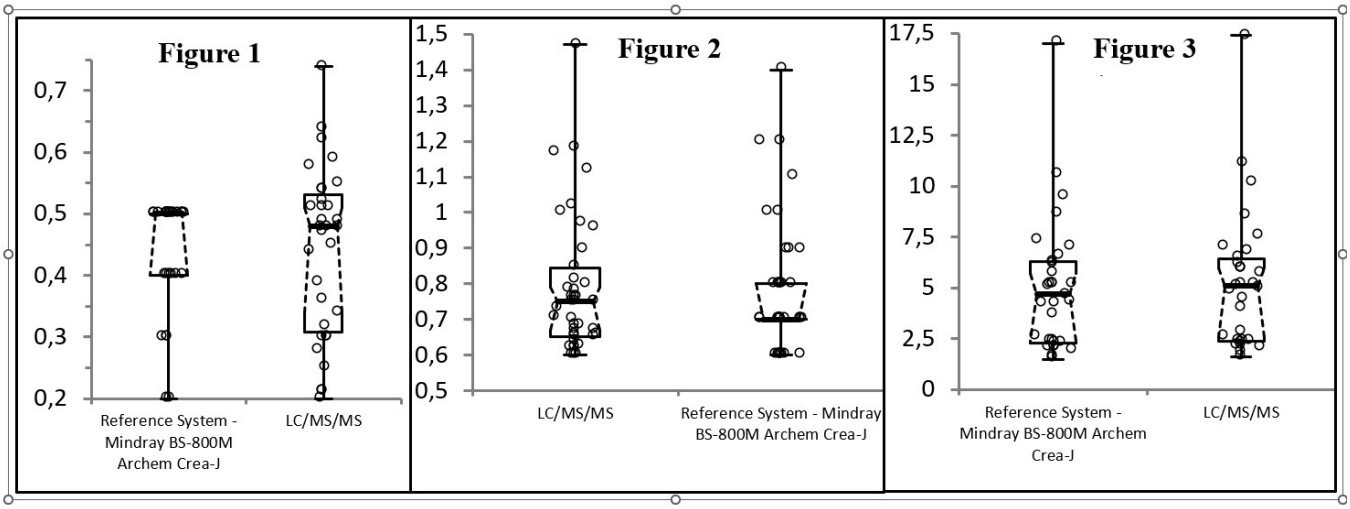


Figure 1: Comparison of Mindray BS-800M Archem Crea-J/LC/MS/M (<0.5 mg/dL of serum creatinine)

Figure 2: Comparison of Mindray BS-800M and Archem Crea-J/LC/MS/MS (0.5-1.3 mg/dL of serum creatinine)

Figure 3: Comparison of Mindray BS-800M and Archem Crea-J/LC/MS/MS (>1.4 mg/dL of serum creatinine)

Table 1. Analysis of Passing-Bablok regression of devices according to creatinine levels

Statistical analysis	n	Regression equation	Spearman's Correlation Coefficient r (R)	Linear model validity Cusum test for linearity
Mindray BS-800M - Archem Crea-J/LC/MS/MS (All creatinine levels)	102	y = 1.0424x – 0.0011	0.9958	0.0002
Mindray BS-800M - Archem Crea-J/LC/MS/MS (<0.5 mg/dL of serum creatinine)	32	y = 1.0572x – 0.0288	0.4938	0.8439
Mindray BS-800M - Archem Crea-J/LC/MS/MS (0.5-1.3 mg/dL of serum creatinine)	39	y = 1.0128x – 0.0038	0.9689	0.2707
Mindray BS-800M - Archem Crea-J/LC/MS/MS (>1.4 mg/dL of serum creatinine)	31	y = 1.0195x + 0.1679	0.9924	<0.0001

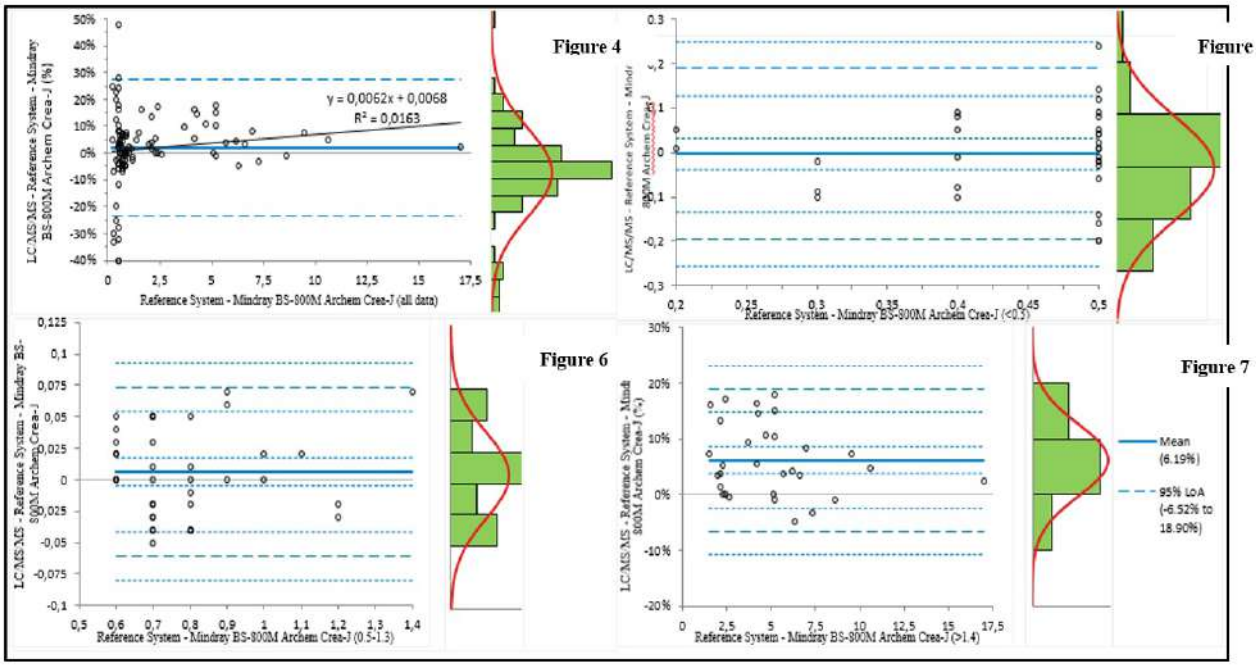


Figure 2a-2d: Comparison of Mindray BS-800M and Archem Crea-J/LC/MS/MS

Discussion: Chronic kidney disease affects millions of people worldwide and is associated with increased morbidity and mortality as a result of kidney failure and cardiovascular disease [9]. Creatinine, as the most commonly used endogenous glomerular filtration marker in clinical practice [10], consists predominantly of creatine and phosphocreatine in skeletal muscle [11]. With the early diagnosis of chronic kidney diseases, end-stage renal failure may be prevented with drug treatments. Since measurement of serum creatinine levels is diagnostic for renal failure, it is important to determine it's accurately and precisely in clinical evaluations [12].

Various methods are available for the measurement of creatinine. However, the most widely used method is the Jaffe reaction, which is based on color formation between picric acid and creatinine in alkaline medium [13]. As another method, liquid chromatography combined with mass spectrometry (LC/MS/MS) method was developed and valida-

ted for the quantification of creatinine [8]. The goal of the presented study was to validate the use of creatinine methods in the clinical measurements. As secondary, to measure and compare creatinine levels in healthy individuals between LC/MS/MS and Jaffe methods. So, total 102 healthy subject were enrolled in the study as; under 0.5 mg/dL (n=32), between 0.5 and 1.3 mg/dL (n=39) and above 1.4 mg/dL (n=31) serum creatinine levels.

A study reported that a correlation coefficient of 0.81 were measured between Roche enzymatic and Jaffe based creatinine measurement [14]. A good correlation (r=0.99) was also found for Beckman enzymatic and Jaffe based creatinine measurement in the study. In our study, a good correlation (r=0.9958) was detected for Jaffe and LC/MS/MS assays (Table 1). A study reported that Jaffe and enzymatic methods were not comparable with tandem MS for creatinine measurements, especially for serum creatinine levels under 0.5 mg/dL [15]. As similar, in our study the corelation coefficient between Mindray BS-800M-Archem Crea-J and LC/MS/MS under 0.5 mg/dL (n=32) serum creatinine levels was determined as 0.4938. As shown in Table 1, between 0.5 and 1.3 mg/dL serum creatinine levels and above 1.4 mg/dL (n=31) serum creatinine levels was determined as 0.9689 and 0.9924, respevtively.

Jaffe and enzymatic methods are common automated methods used to measure serum creatinine [16, 17]. These methods are indirect and highly dependent on the reaction mode. they are also susceptible to interference by endogenous or exogenous substances [18, 19]. As similar, Ou et al reported that method comparison results demonstrated that the enzymatic and Jaffe methods had good correlation (r=0.990 and 0.971) to LC-MS/MS method [20]. Küme et al. reported that the Jaffe method gave higher creatinine results than the enzymatic method, especially at low levels in both urine and serum [21]. It has been reported that patients with renal impairment have increased salivary creatinine levels [22, 23].

In a study, it was also found that whilst there was a negative correlation between serum and saliva creatinine levels in healthy subjects, whereas there was a significant positive correlation in patients with renal impairment [23]. Bernstone et al. proposed that creatinine may be measured from saliva using simplified rapid LC-MS/MS assay, and that salivary concentrations are on average 15% of those in serum [24]. At the study, it was found that whilst there was a negative correlation between serum and saliva creatinine levels in healthy subjects, whereas there was a significant positive correlation in patients with renal impairment. They also proposed that creatinine may be measured from saliva using simplified rapid LC-MS/MS assay, and that salivary concentrations are on average 15% of those in serum [24].

Conclusions: Correlation coefficients of all serum creatinine levels, between 0.5-1.3 mg/dL serum creatinine levels and above 1.4 mg/dL serum creatinine levels showed a good correlation. However, under 0.5 mg/dL did not show a correlation.

There are some limitations in the study; a few articles are available in the literature and there are no more examples with individuals of different ages and body masses. However, we proposed that this study may serve as an example for further studies.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Committee Approval: This study was approved by the non-invasive clinical research ethics committee of the Faculty of Medicine in Selcuk University (Decision No: 2021/367).

Financial Disclosure: No financial disclosure is declared by the authors.

Authorship Contributions: Concept: Sİ, AÜ, TA, HD, MK, FS, Design: Sİ, AÜ, TA, HD, Supervision: Sİ, AÜ, HD, MK Funding: None, Materials: SI, AU, Data collection and/or processing: Sİ, AÜ, TA, HD Analysis and/or interpretation: Sİ, AÜ, TA, HD, MK, Literature search: Sİ, AÜ, TA, Editing: Sİ, AÜ, TA, Critical review: Sİ, AÜ, TA, HD, MK, FS

Acknowledgments

We would like to thank the staff of the 29 Mayıs State Hospital.

References

1- Delanaye P, Cavalier E, Pottel H. Serum Creatinine: Not So Simple! Nephron. 2017;136(4):302-308. doi: 10.1159/000469669. Epub 2017 Apr 26. PMID: 28441651.

2- Tietz NW. In Textbook of Clinical Chemistry (1st ed.) 1986; p1810.

3- Mădăraş MB, Buck RP. Miniaturized biosensors employing electropolymerized permselective films and their use for creatine assays in human serum. Anal Chem. 1996 Nov 1;68(21):3832-9. doi: 10.1021/ac960239r. PMID: 8914485.

4- Tietz NW. Fundamentals of Clinical Chemistry 1987; p680.

5- Toora BD, Rajagopal G. Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. Indian J Exp Biol. 2002 Mar;40(3):352-4. PMID: 12635710.

6- Tsikas D, Wolf A, Frölich JC. Simplified HPLC method for urinary and circulating creatinine. Clin Chem. 2004 Jan;50(1):201-3. doi: 10.1373/clinchem.2003.024141. PMID: 14709648.

7- Fernández-Fernández M, Rodríguez-González P, Añón Álvarez ME, Rodríguez F, Menéndez FV, García Alonso JI. Simultaneous determination of creatinine and creatine in human serum by double-spike isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS). Anal Chem. 2015 Apr 7;87(7):3755-63. doi: 10.1021/acs.analchem.5b00769. Epub 2015 Mar 19. PMID: 25751287.

8- Hou H, Xiong W, Zhang X, Song D, Tang G, Hu Q. LC-MS-MS Measurements of Urinary Creatinine and the Application of Creatinine Normalization Technique on Cotinine in Smokers' 24 Hour Urine. J Anal Methods Chem. 2012;2012:245415. doi: 10.1155/2012/245415. Epub 2012 Nov 11. PMID: 23209947; PMCID: PMC3503330.

9- Sandilands EA, Dhaun N, Dear JW, Webb DJ. Measurement of renal function in patients with chronic kidney disease. Br J Clin Pharmacol. 2013 Oct;76(4):504-15. doi: 10.1111/bcp.12198. PMID: 23802624; PMCID: PMC3791974.

10- Traynor J, Mactier R, Geddes CC, Fox JG. How to measure renal function in clinical practice. BMJ. 2006 Oct 7;333(7571):733-7. doi: 10.1136/bmj.38975.390370.7C. PMID: 17023465; PMCID: PMC1592388.

11- Rahn KH, Heidenreich S, Brückner D. How to assess glomerular function and damage in humans. J Hypertens. 1999 Mar;17(3):309-17. doi: 10.1097/00004872-199917030-00002. PMID: 10100067.

12- Chen TK, Knicely DH, Grams ME. Chronic Kidney Disease Diagnosis and Management: A Review. JAMA. 2019 Oct 1;322(13):1294-1304. doi: 10.1001/jama.2019.14745. PMID: 31573641; PMCID: PMC7015670.

13- Syal, K., Banerjee, D., & Srinivasan, A. (2013). Creatinine estimation and interference. Indian journal of clinical biochemistry : IJCB, 28(2), 210–211. https://doi.org/10.1007/s12291-013-0299-y.

14- Cheuiche AV, Soares AA, Camargo EG, Weinert LS, Camargo JL, Silveiro SP. Comparison between IDMS-traceable Jaffe and enzymatic creatinine assays for estimation of glomerular filtration rate by the CKD-EPI equation in healthy and diabetic subjects. Clin Biochem 2013;46:1423-1429.

15- Yildirimel M, Atalar M, Abusoglu S, Eryavuz Onmaz D, Sivrikaya A, Abusoglu G et al. Measurement of serum creatinine levels with liquid chromatography-tandem mass spectrometry: comparison with Jaffe and enzymatic

methods. Turkish J Biochem 2021;46(3):263-271.

16- Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. Clin Chim Acta. 2004 Jun;344(1-2):137-48. doi: 10.1016/j.cccn.2004.02.007. PMID: 15149882.

17- Pauwels S, Cassiman D, Vermeersch P. Evaluation of the interference by homogentisic acid and other organic acids on the enzymatic and Jaffé method creatinine assay. Clin Chem Lab Med. 2012 Jan 6;50(4):749-50. doi: 10.1515/cclm.2011.836. PMID: 22505563.

18- Liu WS, Chung YT, Yang CY, Lin CC, Tsai KH, Yang WC, et al. Serum creatinine determined by Jaffe, enzymatic method, and isotope dilution-liquid chromatography-mass spectrometry in patients under hemodialysis. J Clin Lab Anal. 2012 May;26(3):206-14. doi: 10.1002/jcla.21495. PMID: 22628238; PMCID: PMC6807401.

19- Allegaert K, Kuppens M, Mekahli D, Levtchenko E, Vanstapel F, Vanhole C, et al. Creatinine reference values in ELBW infants: impact of quantification by Jaffe or enzymatic method. Creatinine reference values in ELBW infants: impact of quantification by Jaffe or enzymatic method. J Matern Fetal Neonatal Med. 2012 Sep;25(9):1678-81.

20- Ou M, Song Y, Li S, Liu G, Jia J, Zhang M, et al. LC-MS/MS Method for Serum Creatinine: Comparison with Enzymatic Method and Jaffe Method. PLoS One. 2015 Jul 24;10(7):e0133912. doi: 10.1371/journal.pone.0133912. PMID: 26207996; PMCID: PMC4514740.

21- Küme T, Sağlam B, Ergon C, Sisman AR. Evaluation and comparison of Abbott Jaffe and enzymatic creatinine methods: Could the old method meet the new requirements? J Clin Lab Anal. 2018 Jan;32(1):e22168. doi: 10.1002/jcla.22168. Epub 2017 Feb 15. PMID: 28205269; PMCID: PMC6816857.

22- Kovalčíková A, Janšáková K, Gyurászová M, Podracká Ľ, Šebeková K, Celec P, et al. Salivary creatinine and urea are higher in an experimental model of acute but not chronic renal disease. PLoS One. 2018 Jul 6;13(7):e0200391. doi: 10.1371/journal.pone.0200391. PMID: 29979784; PMCID: PMC6034877.

23- Venkatapathy R, Govindarajan V, Oza N, Parameswaran S, Pennagaram Dhanasekaran B, Prashad KV. Salivary creatinine estimation as an alternative to serum creatinine in chronic kidney disease patients. Int J Nephrol. 2014;2014:742724. doi: 10.1155/2014/742724. Epub 2014 Apr 10. PMID: 24818023; PMCID: PMC4003770.

24- Bernstone L, Jayanti A, Keevil B. A simplified, rapid LC-MS/MS assay for serum and salivary creatinine. Clin Mass Spectrom. 2018 Nov 29;11:21-26. doi: 10.1016/j.clinms.2018.11.004. PMID: 34841069; PMCID: PMC8620133.

S020

ANALYTICAL VERIFICATION OF THE HEMATOLOGY ANALYZER DYMIND DH615

Raziye Yıldız, Muammer Yücel

İzmir Atatürk Training and Research Hospital, Medical Biochemistry Laboratory, İzmir, Türkiye

Introduction: Manufacturers validate analyzer devices according to guidelines. However, the performance characteristics of each analytical analyzer need to be robustly evaluated according to the situation in which the device will be operated and by laboratory personnel responsible for the operation of the device targeting the specific population in the environment [1,2].

Complete blood count (CBC) is measured in almost all medical laboratories worldwide to screen for diagnosis, follow-up, and therapeutic monitoring of many hematological and non-hematological disorders. The Dymind DH615 is a new generation, multiparameter, automated hematology analyzer that provides CBC with a 6-part white blood

cell (WBC) differential count.

The purpose of this study was to assess the analytical performance and compare the new

hematology analyzer Dymind DH615 (Shenzhen, China) with Sysmex XN1000 (Kobe, Japan) whose usefulness in routine diagnostics was confirmed by earlier studies [3]. The repeatability, reproducibility, and bias for the Dymind DH615 were checked, and the results of CBC were compared with the Sysmex XN1000.

Methods: This study was conducted in May 2023 at the Department of Clinical Biochemistry Laboratory, Atatürk Training and Research Hospital (İzmir, Turkey).

Only Dymind-specified reagents, controls, and calibrators were used, according to the manufacturer's instructions. Random patient samples were selected from the routine workflow from abnormally low, reference, and abnormally high analytical ranges. All samples were collected in standard K2-EDTA collection tubes (Vacusera, İzmir, Turkey) and processed within 2 hours of venipuncture. Blood samples were stored at room temperature until the time of analysis. Clotted samples and samples with insufficient volume were excluded from the study.

Dymind DH-615 is an automatic 6-DIFF hematology analyzer with immature granulocytes and reticulocytes, which can provide screening information for abnormal blood samples. 35 reportable parameters and 29 researchable parameters are available. Dymind DH-615 testing speed can be up to 100 tests/hour and the minimum sample volume required is 20 µL. Sysmex XN analyzers were used for inter-instrument method comparison. Both analyzers determine CBC parameters using the impedance method, WBC DIFF using flow cytometry, and HGB using a colorimetric method.

Analytical verification included estimation of repeatability, within-run precision, within-laboratory precision, and bias for control samples. Control samples were analyzed at low, normal, and high concentrations. Repeatability values were obtained from a result of 20 repeated studies at all control levels. Within-run and within-laboratory precision values were obtained from three replicate studies for five days, according to the Clinical and Laboratory Standards Institute (CLSI) Document EP15-A3. Bias for each control sample was calculated as the deviation of the overall mean obtained during the study period from the target value declared by the manufacturer [4]. Precision and bias data were evaluated based on meeting the manufacturer's declared specifications and the Analytical Performance Specifications (APS) in the European Federation for Laboratory Medicine (EFLM) Database of Biological Variation [5].

Method Comparison was performed by measuring each sample for each parameter in duplicate on both hematology analyzers on 31 patient samples. Statistical analysis of the method comparison was performed with a Passing-Bablok regression analysis.

Results: Repeatability, within-run precision, within-laboratory precision, and bias for the tested parameters are listed in Table 1. Repeatability and within-run precision values for all CBC and WBC differential parameters for all level controls met APS in the EFLM Database of Biological Variation. The following parameters fully met the criteria for analytical verification at all three concentration levels (low, normal, and high): WBC, neutrophil count (NEU), lymphocyte count (LYM), monocyte count (MON), and basophil count (BAS). Eosinophil count (EOS), RBC, HGB, mean cell volume (MCV), platelets (PLT), and mean platelet volume (MPV) partially met the criteria.

The repeatability values of CBC parameters met the manufacturer's declared specifications, except for RBC, HGB, and PLT in low-level control and RBC in normal-level control.

The Dymind DH615 and Sysmex XN1000 analyzers showed very good correlation coefficients except for MON, BAS, and MPV (0.7758, 0.8222, and 0.6639, respectively). Passing-Bablok regression equations and their 95% confidence intervals are shown in Table 2.

The limitation of our study is that it was performed only on basic hematological parameters in whole blood. Retic-

ulocyte and body fluid cell count were not evaluated.

Discussion: In conclusion, the analytical verification of the Dymind DH615 automated analyzer showed adequate analytical characteristics. Based on the Passing-Bablok regression analyses, we conclude that the Dymind DH615 is interchangeable with the Sysmex XN-1000 for all parameters except for MON, BAS, and MPV. In our opinion, the Dymind DH615 Hematology Analyzer meets the requirements for clinical use in medium to large-sized hematology laboratories.

Keywords: blood cell count; hematology; verification; biological variation; automated analyzer

References:

1. Clinical Laboratory Standards Institute. H26–A2: Validation, Verification, and Quality Assurance of Automated Hematology Analyzers, 2nd ed. Wayne, PA: Clinical Laboratory Standards Institute; 2016. Available from: https://clsi.org/standards/products/hematology/documents/h26/

2. Clinical Laboratory Standards Institute. Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods, 2nd ed. Wayne, PA: 2016. Available from: https://clsi.org/standards/products/hematology/documents/h20/

3. Becker PH, Fenneteau O, Da Costa L. Performance evaluation of the Sysmex XN-1000 hematology analyzer in assessment of the white blood cell count differential in pediatric specimens. Int J Lab Hematol 2016;38(1):54-63.

4. CLSI. User verification of precision and estimation of bias; approved guideline. In: *CLSI document EP15-A3*, 3rd ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2014. Available from: https://webstore.ansi.org/search/find?in=1&st=CLSI+EP15-A3

5. Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, et al. The EFLM Biological Variation Database;2021. Available from: http://www.biologicalvariation.eu

Parameter	Control samples' target value	Repeatability (%)	Within- run precision (%)	Within-laboratory precision (%)	EFLM quality requirements for imprecision (%)	Bias (%)	EFLM quality requirements for bias (%)
WBC ($x10^9$/L)	4.56	2.1	1.6	2.8	8.1	1.3	7.4
	7.83	1.5	1.6	1.8	8.1	0.6	7.4
	22.51	0.8	0.9	1.2	8.1	0.5	7.4
NEU ($x10^9$/L)	3.27	2.4	2.2	3.4	10.5	1.8	10.3
	4.92	1.6	1.8	2.4	10.5	0.4	10.3
	16.15	0.8	0.7	1.2	10.5	0.1	10.3
LYM ($x10^9$/L)	0.72	3.3	4.2	4.6	8.1	2.8	9.4
	2.16	3.2	3.5	4.0	8.1	2.3	9.4
	4.72	1.8	1.6	1.6	8.1	0.4	9.4
MON ($x10^9$/L)	0.36	5.6	5.3	6.2	10.1	0.0	9.7
	0.46	5.7	7.5	7.8	10.1	2.2	9.7
	0.88	3.7	4.6	4.9	10.1	5.7	9.7
EOS ($x10^9$/L)	0.2	7.6	8.0	11.9	11.3	10	25.2
	0.29	6.2	6.1	12.1	11.3	3.4	25.2
	0.77	4.5	4.3	4.6	11.3	3.9	25.2
BAS ($x10^9$/L)	3.64	2.5	2.3	3.4	9.3	1.4	10.9
	5.71	1.8	1.9	2.3	9.3	1.2	10.9
	16.76	1.0	1.0	1.2	9.3	0.6	10.9

RBC ($x10^{12}$/L)	2.34	0.9	1.2	3.3	2.0	3	2.6
	4.62	0.7	2.0	2.1	2.0	1.3	2.6
	5.48	1.1	0.8	0.9	2.0	0.7	2.6
HGB (g/dL)	6.2	1.1	0.6	3.6	2.0	2.9	2.4
	13.5	0.9	1.0	1.4	2.0	0.2	2.4
	17.5	0.5	0.5	0.7	2.0	0.1	2.4
MCV (fL)	78.8	0.5	0.4	0.7	0.6	5.5	1.4
	88.5	0.3	0.3	0.9	0.6	1.5	1.4
	98.7	0.3	0.4	1.0	0.6	3.7	1.4
PLT ($x10^9$/L)	66	3.9	4.8	6.8	5.7	7.2	7.6
	292	2.5	2.3	3.5	5.7	0.8	7.6
	472	1.4	2.9	3.9	5.7	15.7	7.6
MPV (fL)	7.1	1.7	1.6	1.7	1.7	11.4	2.8
	7	1.2	1.2	1.2	1.7	13.9	2.8
	8.8	1.2	0.9	1.1	1.7	7.6	2.8

Table 1. Results for repeatability, within-run precision, within-laboratory precision, and BIAS using Dymind DH615 automated analyzer.

The values of control samples are stated by the manufacturer. WBC - white blood cell count. NEU - neutrophil count. LYM - lymphocyte count. MON - monocyte count. EOS - eosinophil count. BAS - basophil count. RBC – red blood cells. HGB – hemoglobin. MCV – mean cell volume. PLT – platelets. MPV - mean platelet volume.

Parameter	**Regression equation**		**Range tested**	**95% CI for slope**	**95% CI for intercept**
WBC ($x10^9$/L)	y = 1,0249x - 0,1174	R^2 = 0,9986	2.2—30.72	1.01 to 1.04	-0.30 to 0.06
NEU ($x10^9$/L)	y = 1,0133x - 0,0019	R^2 = 0,9989	1.72-28.46	1.00 to 1.03	-0.13 to 0.13
LYM ($x10^9$/L)	y = 1,0075x + 0,0395	R^2 = 0,9901	0.15-4.51	0.97 to 1.05	-0.04 to 0.11
MON ($x10^9$/L)	y = 0,927x + 0,0675	R^2 = 0,7758	0.09-1.51	0.74 to 1.11	-0.07 to 0.21

EOS (x10^9/L)	y = 0,971x + 0,0191	R^2 = 0,9437	0.00-0.45	0.88 to 1.06	0.00 to 0.04
BAS (x10^9/L)	y = 0,7212x + 0,0005	R^2 = 0,8222	0.01-0.33	0.60 to 0.84	-0.01 to 0.01
RBC (x10^{12}/L)	y = 0,9781x + 0,1437	R^2 = 0,9797	2.72-5.69	0.93 to 1.03	-0.08 to 0.37
HGB (g/dL)	y = 0,959x + 0,5484	R^2 = 0,9965	4.9-17.7	0.94 to 0.98	0.28 to 0.82
MCV (fL)	y = 1,0629x - 4,33	R^2 = 0,973	61.6-99.6	1.00 to 1.13	-9.93 to 1.27
PLT (x10^9/L)	y = 1,0856x - 4,0169	R^2 = 0,9908	69-1078	1.05 to 1.12	-18.33 to 10.30
MPV (fL)	y = 0,7734x + 3,0025	R^2 = 0,6639	9-13.8	0.56 to 0.98	0.81 to 5.20

Table 2. Passing-Bablok regression analysis for the method comparison between the Dymind DH615 and the Sysmex XN1000 for hematological parameters.

WBC - white blood cell count. NEU - neutrophil count. LYM - lymphocyte count. MON - monocyte count. EOS - eosinophil count. BAS - basophil count. RBC – red blood cells. HGB – hemoglobin. MCV – mean cell volume. PLT – platelets. MPV - mean platelet volume. 95% CI – 95% confidence intervals.

S052

INVESTIGATION OF HNF1A GENE POLYMORPHISMS AND THEIR METABOLIC EFFECTS ON MODY AND TYPE 2 DIABETES

Deniz Kanca Demirci[1], Nurdan Gul[2], Bengu Tokat[3], Ilhan Satman[2], Oguz Ozturk[3], Hulya Yilmaz Aydogan[3]

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Haliç University, Istanbul, Turkey.

[2] Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey.

[3] Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey.

Introduction: Maturity-onset diabetes of the young (MODY) is a monogenic diabetes form characterized by autosomal dominant inheritance, onset before 25 years of age, absence of β-cell autoimmunity, and sustained pancreatic β-cell function To date, mutations have been identified in at least 14 different genes [1]. Among these genes, HNF1A is a transcription factor and is mainly expressed in the liver, pancreas intestines and kidneys. It is responsible for liver development and growth, lipid metabolism, protein synthesis, insulin secretion and glucose reabsorption. HNF1A also regulates the expression of HNF4A and PDX1 for pancreatic development, differentiation and maintenance of beta cell function [2]. HNF1A is highly polymorphic and mutations can be found at any site of the gene [3]. However, location of the mutations leads to the clinical heterogeneity of MODY3 and associate the risk of type 2 diabetes [2]. Some HNF1A mutations are observed in both MODY3 and type 2 diabetes (T2DM), in which case the age of onset is used for differential diagnosis [3]. It is not fully explained why different HNF1A mutations cause different types of diabetes. However, it is suggested that common HNF1A gene mutations causing MODY3 are localized in exons 1, 2 and 4 encoding the DNA binding site, while those associated with T2DM are mostly found in exons 8 and 9 [3-5]. Also, MODY cases usually misdiagnosed as T2DM due to the common clinical features such as low risk of ketosis or obesity [4, 6]. Therefore, in our study, it was aimed to investigate the distribution of common HNF1A gene variations and their effects on clinical features in study groups consisting of patients with T2DM and clinically diagnosed MODY.

Methods: Genomic-DNA samples of 41 patients with T2DM and 53 patients with MODY were isolated with commercial DNA isolation kit (Epicentre MCD85201). HNF1A-gene was sequenced in by Next Generation Sequencing-(NGS) and genetic data is analysed with bioinformatics methods (NGS data analysis programs; Haploview 4.2; Mutation assessor; PolyPhen2). Statistical analyses were performed by SPSS-software package-(version-20.0-SPSS Inc.,IL,USA). This study was supported by the Research Fund of Istanbul University (Project-No. TOA-2017-24194).

Results: The main characteristics of the study population are given in Table 1. Accordingly, age ($p<0.001$), waist circumference ($p=0.021$), urea ($p<0.001$) and creatinine levels ($p=0.009$) ($p<0.05$) were found to be higher in T2DM group than in MODY patients. On the other hand, there was no significant difference between the study groups in the distribution of gender, serum levels of glucose, HbA1c (%), C peptide, alanine transaminase (ALT), aspartate transaminase (AST) and lipid profile ($p> 0.05$). A higher prevalence of diabetic complications, including retinopathy, nephropathy, and neuropathy, was observed in the diabetic group compared to the MODY group, but the difference was not statistically significant ($p <0.05$) (Table 1).

As shown in Table 2, the frequency of all *HNF1A* alleles and genotypes in the study groups were distributed according to Hardy-Weinberg equilibrium ($p>0.05$). The allelic and genotypic frequencies of *HNF1A* polymorphisms were not significantly different between study groups ($p>0.05$).

We found that *HNF1A* polymorphisms were associated with some clinical features in both study groups (Table 3).

In the T2DM group, the HNF1A rs1169289 (C>G) GG genotype was associated with lower C-peptide ($p<0.05$) and higher urea ($p<0.01$) levels compared to the C allele (CC+ GC genotypes). T2DM patients with minor C allele (CC+ AC genotypes) of the rs1169288 (A>C) have increased waist circumference values ($p<0.05$) compared to the patients with AA genotype. Lastly, lower C-peptide levels ($p<0.05$) were seen in the T2DM patients with GG genotype of rs56348580 (G>C) than minor C allele carriers (CC+ GC genotypes). However, HNF1A polymorphisms had no effect on C-peptide levels in MODY patients. In the MODY group, subjects with the HNF1A rs1169289 C allele showed higher ALT levels ($p<0.01$) than GG genotype, while patients with rs56348580 GG genotype had increased serum triglyceride levels ($p<0.05$) than those with the C allele. Moreover, MODY patients with rs55834942 rare A allele have high creatinine levels within reference values ($p<0.05$) (Table 3).

Discussion: The distribution of the HNF1A mutations throughout the gene cause MODY3 or T2DM and explain the clinical heterogeneity [5]. HNF1A is mainly expressed in the liver, gut, pancreas, and kidneys and functions as a transcription factor to regulate insulin secretion, glucose reabsorption and lipid metabolism that links to diabetes mellitus. HNF1A also regulates the genes involved in glucose and amino acid metabolism, including glycolysis, Krebs cycle, and oxidative phosphorylation. So, the mutations of HNF1A gene leads to the beta cell dysfunction by reducing glycolysis and ATP production as well as by causing impaired insulin exocytosis, glucose uptake and epigenetic changes [2].

HNF1A mutations are found associated with C-peptide, waist circumference and urea levels in the patients with T2D, while associated with ALT, creatinine and triglyceride levels in the patients with MODY in our study. The association of HNF1A gene polymorphisms with the decrease in C-peptide levels indicates that they affect endocrine-pancreas function in T2DM patients. However, MODY3 is characterized by reduced insulin secretion before the onset of diabetes and displays a distinct phenotype from type 1 and type 2 diabetes. Lipid metabolism plays a critical role in liver metabolism. Thus, the effects of HNF1A gene variations on ALT and triglyceride levels suggest that they affect liver function in MODY patients.

In conclusion, our findings reveal that HNF1A gene polymorphisms may have different metabolic effects in T2DM and MODY patients. However, it cannot be excluded that the difference may be due to the onset of the two forms of diabetes at different ages, as in this study. Therefore, further studies with larger sample sizes are required for stronger findings.

Table 1: Characteristics of the Study Groups

	Groups		
	Adult MODY (n=53)	**T2DM (n=41)**	***P* value**
Sex (M/F)	14/39	17/24	0.124
Age (year)	40.21 ± 11.69	59.42 ± 8.35	**<0,001**
Diabetes markers			
Glucose (mg/dl)	162.79 ± 66.12	162.61 ± 58.84	0.989
HbA1c (%)	7.77 ± 1.98	7.69 ± 0.82	0.807
C peptide (ng/mL)	2.37 ± 1.30	2.56 ± 1.40	0.508
Obesity markers			
BMI (kg/m^2)	29.36 ± 5.61	30.67 ± 5.25	0.263
Waist circumference (cm)	97.9 ± 11.75	104.97 ± 12.23	**0.021**
Lipid Profile			
Total-Cholesterol (mg/dL)	193.93 ±49.25	202.08 ± 53.91	0.458
Triglyceride (mg/dL)	177.66 ± 132.72	186.63 ± 131.25	0.748
HDL-Cholesterol (mg/dL)	44.98 ± 13.28	40.74 ± 12.81	4.233
LDL-Cholesterol (mg/dL)	119.16 ± 41.08	126.84 ± 42.41	0.382
VLDL-Cholesterol (mg/dL)	27.7 ± 11.86	34.29 ± 25.75	0,123
Liver function			
ALT (U/L)	24.78 ± 15.56	21.97 ± 8.13	0.284
AST (U/L)	19.37 ± 1.23	19.27 ± 6.68	0.955
Kidney function			
Urea (mg/dL)	24.32 ± 12.53	34.37 ± 18.46	**<0,001**
Creatinin (mg/dL)	0.72 ± 0.23	0.92 ± 0.48	**0.009**
Diabetic complications			
Nephropathy (%)	30.8	40.0	0.551
Retinopathy (%)	8.3	33.3	0.094
Neuropathy (%)	30.0	38.5	0.620

Table 2: Distribution of *HNF1A* polymorphisms in study groups

***HNF1A* polymorphisms**			**Adult MODY (n=53)**	**T2DM (n=41)**
rs1169289 (G>C) Exon 1	Genotypes, n (%)	**GG**	18 (34%)	13 (31,7%)
		CG	21 (39,6%)	21 (51,2%)
		CC	14 (26,4%)	7 (17,1%)
	Allelles, n (%)	**G**	57 (53.77%)	47 (57.32)
		C	49 (46.23%)	35 (42.68%)
rs1169288 (A>C) Exon 1	Genotypes, n (%)	**AA**	18 (34,0%)	9 (22,0%)
		AC	25 (47,2%)	23 (56,1%)
		CC	10 (18,9%)	9 (22,0%)
	Allelles, n (%)	**C**	61 (57.55%)	41 (50.0%)
		A	45 (42.45%)	41 (50.0%)
rs1800574 (C>T) Exon 1	Genotypes, n (%)	**CC**	44 (83,0%)	33 (80,5%)
		CT	9 (17,0%)	8 (19,5%)
		TT	0	0
	Allelles, n (%)	**C**	97 (91.51%)	74 (90.24%)
		T	9 (8.49%)	8 (9.76%)
rs56348580 (G>C) Exon 4	Genotypes, n (%)	**GG**	37 (69,8%)	27 (65,9%)
		GC	16 (30,2%)	12 (29,3%)
		CC	0	2 (4,9%)
	Allelles, n (%)	**G**	90 (84.91%)	66 (80.49%)
		C	16 (15.09%)	16 (19.51%)
rs55834942 (G>A) Exon 8	Genotypes, n (%)	**GG**	45 (84,9%)	29 (70,7%)
		GA	8 (15,1%)	10 (24,4%)
		AA	0	2 (4,9%)
	Allelles, n (%)	**G**	98 (92.45%)	68 (82.93%)
		A	8 (7.55%)	14 (17.07%)

Statistical analysis was performed using Chi square test Values are given as number of samples and percentage (%) in the table.

Table 3. Effects of *HNF1A* polymorphisms on metabolic parameters in study groups

T 2 D M	HNF1A polymorphisms									
	r s 1 1 6 9 2 8 9 (exon 1)		**r s 1 1 6 9 2 8 8 (exon 1)**		**r s 1 8 0 0 5 7 4 (exon 1)**		**rs56348580 (exon 4)**		**rs55834942 (exon 8)**	
	GG	**C**	**AA**	**C**	**CC**	**CT**	**GG**	**C**	**GG**	**A**
Glucose	156.4±9.3	163.6±12.5	147.6±9.0	165.1±11.2	166.6±10.9	138.8±7.9	165.4±12.8	153.2±9.2	167.0±12.0	147.1±9.4
HbA1c	8.0±0.2	7.5±0.2	7.3±0.4	7.7±1.3	7.6±0.2	7.7±0.3	7.7±0.2	7.6±0.3	7.7±0.2	7.5±0.3
C peptide	**1.7±0.2***	2.9±0.3	2.9±0.4	2.5±0.3	2.5±0.3	2.9±0.8	**2.2±0.3***	3.2±0.4	2.4±0.3	3.1±0.4
BMI	32.0±1.4	29.9±1.0	30.7±1.6	30.5±0.9	30.1±0.9	32.4±2.0	30.2±1.0	31.1±1.5	30.2±0.9	31.3±1.7
Waist circ.	107.0±4.5	104.0±2.3	98.5±2.9	**107.0±2.5***	105.1±2.3	104.5±5.6	104.3±2.6	106.3±3.5	104.6±2.5	105.9±3.9
Total-C	206.8±18.3	200.6±8.9	176.4±13.2	209.6±9.5	206.0±9.3	187.8±17.6	211.3±11.3	185.1±8.8	207.5±10.8	190.1±9.5
TG	159.8±16.5	194.3±28.3	169.0±22.7	187.6±25.1	182.9±24.5	186.6±22.5	193.0±29.3	164.9±16.5	187.8±27.5	173.2±18.2
HDL-C	45.3±3.3	38.9±2.4	38.8±3.3	41.5±2.3	41.1±2.3	39.9±3.8	40.9±2.7	41.0±2.3	41.3±2.6	40.0±2.3
LDL-C	132.0±57.3	125.1±6.3	106.8±8.7	132.8±7.7	130.3±7.5	114.1±12.0	133.7±8.8	114.2±7.5	130.4±8.5	119.1±7.9
VLDL-C	28.9±3.4	36.0±5.6	30.8±3.7	34.6±5.0	33.8±4.8	33.6±3.8	35.7±5.8	29.8±2.3	34.9±5.5	31.0±2.6
ALT	22.1±2.3	21.5±1.8	22.1±3.0	21.6±1.6	20.8±1.5	27.0±3.3	21.4±1.5	22.4±3.4	21.0±1.4	23.6±3.6
AST	19.3±1.5	19.0±1.6	16.8±0.9	26.1±6.4	18.2±1.0	23.8±4.5	18.5±0.9	21.2±3.5	18.2±0.9	22.4±3.8
Urea	**46.9±7.9****	32.9±2.4	32.1±1.7	38.5±3.8	34.5±2.3	46.5±11.0	38.5±4.3	34.1±2.7	38.7±4.0	32.8±2.7
Creatinin	1.1±0.2	0.8±0.1	0.9±0.1	0.9±0.1	0.9±0.1	1.2±0.3	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1
MODY										

Glucose	149.4±16.7	169.7±10.7	174.3±14.2	156.9±11.7	164.4±10.2	154.9±20.9	161.7±11.7	165.4±13.5	159.5±10.2	181.4±17.7
HbA1c	7.5±0.6	7.9±0.3	7.8±0.4	7.7±0.4	7.8±0.3	7.6±0.4	7.6±0.3	8.2±0.5	7.6±0.3	8.6±0.7
C peptide	2.3±0.5	2.4±0.2	2.3±0.2	2.4±0.3	2.4±0.2	2.3±0.3	2.5±0.3	2.0±0.2	2.4±0.2	2.1±0.3
BMI	29.7±1.4	29.2±1.0	31.2±1.3	28.5±1.0	29.3±0.9	29.4±1.6	30.0±0.9	28.1±1.6	29.5±0.9	28.8±2.5
Waist circ.	96.8±3.7	98.6±2.6	99.3±3.9	97.3±2.6	97.5±2.4	99.1±4.9	102.3±2.3	93.6±4.2	97.3±2.2	101.0±6.5
Total-C	198.2±12.3	191.8±8.8	191.6±12.6	170.7±24.7	197.0±7.8	175.9±16.2	195.4±9.1	191.0±11.5	198.8±7.9	169.6±14.0
TG	185.8±31.5	173.8±23.6	192.5±26.7	195.2±8.7	173.2±19.7	205.1±61.2	**205.8±25.8***	117.8±11.0	188.6±21.7	120.1±20.7
HDL-C	45.1±3.6	44.9±2.3	45.1±2.9	44.9±2.6	45.5±2.1	41.9±4.8	44.3±2.5	46.4±2.8	45.4±2.2	42.7±3.2
LDL-C	125.3±9.8	116.3±7.1	114.1±10.2	121.7±7.0	122.1±6.4	100.4±9.7	121.6±7.3	113.9±9.3	122.8±6.2	99.6±13.6
VLDL-C	27.1±2.7	28.0±2.2	32.3±3.5	25.5±1.9	28.3±1.9	22.5±3.6	30.0±2.3	23.3±2.3	28.6±1.9	23.5±4.3
ALT	19.4±3.1	**27.8±2.8****	25.7±3.2	24.3±2.9	26.1±2.5	19.0±2.8	27.1±3.1	19.9±1.3	25.7±2.5	20.0±2.1
AST	17.1±1.9	20.7±1.6	19.3±2.1	19.4±1.5	20.0±1.4	15.3±1.8	20.6±1.7	16.6±1.0	19.8±1.4	16.8±1.3
Urea	24.6±2.9	24.2±2.4	25.5±3.4	23.7±2.2	23.2±2.0	29.0±4.7	24.3±2.1	24.4±3.7	22.9±2.0	33.7±4.0
Creatinin	0.7±0.1	0.8±0.1	0.8±0.1	0.7±0.1	0.7±0.1	0.8±0.1	0.7±0.1	0.8±0.1	0.7±0.1	**0.9±0.1***

Statistical analysis was performed using Mann-Whitney U t test.

Values are given as mean± standart error (X ± SEM) in the table.

*, $p<0.05$; **, $p<0.01$

References

[1] Nkonge KM, Nkonge DK and Nkonge TN. The epidemiology, molecular pathogenesis, diagnosis, and treatment of maturity-onset diabetes of the young (MODY). Clinical Diabetes and Endocrinology; 2020; 6:20. https://doi.org/10.1186/s40842-020-00112-5

[6] Miyachi Y, Miyazawa T and Ogawa Y. HNF1A Mutations and Beta Cell Dysfunction in Diabetes. Int. J. Mol. Sci.; 2022; 23, 3222. https://doi.org/10.3390/ijms23063222

[2] Li L-M, Jiang B-G and Sun L-L. HNF1A：From Monogenic Diabetes to Type 2 Diabetes and Gestational Diabetes Mellitus. Front. Endocrinol.; 2022; 13:829565. doi: 10.3389/fendo.2022.829565

[3] Colclough K, Bellanne-Chantelot C, Saint-Martin C, Flanagan SE and Ellard S. Mutations in the Genes Encoding the Transcription Factors Hepatocyte Nuclear Factor 1 Alpha and 4 Alpha in Maturity-Onset Diabetes of the Young and Hyperinsulinemic Hypoglycemia. Hum Mutat; 2013; 34:669–685.

[4] Jafar-Mohammadi B, Groves CJ, Owen KR, Frayling TM, Hattersley AT, McCarthy MI and Gloyn AL. Low Frequency Variants in the Exons Only Encoding Isoform A of HNF1A Do Not Contribute to Susceptibility to Type 2 Diabetes. PLoS ONE; 2009; 4(8): e6615. https://doi.org/10.1371/journal.pone.0006615

[5] Firdous P, Nissar K, Masoodi SR and Ganai BA. Biomarkers: Tools for Discriminating MODY from Other Diabetic Subtypes. Indian J Endocrinol Metab.; 2022 May-Jun; 26(3): 223–231. doi: 10.4103/ijem.ijem_266_21

S059

IMPORTANCE OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN CHRONIC LUNG AND LIVER DISEASES

Fatma Sengul[1], Fikret Akyurek[2]

[1] Adiyaman University, Faculty of Pharmacy, Department of Biochemistry, Adiyaman, Türkiye

[2] Selçuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

Introduction: Alpha-1 antitrypsin (AAT) stands as the predominant serine protease inhibitor in circulation. It acts as a responder during acute phases. While hepatocytes primarily manufacture and release AAT, smaller quantities are also secreted by monocytes, macrophages, pulmonary alveolar cells, and the intestinal epithelium [1]. AAT is a crucial protein primarily produced in the liver, playing a pivotal role in protecting various organs, particularly the lungs, from damage caused by enzymes. This protein inhibits the activity of neutrophil elastase, an enzyme that can break down connective tissue, and its deficiency can lead to severe conditions such as emphysema and liver disease [2]. The plasma concentration of AAT in the bloodstream typically fall within the range of 0.9 to 1.75 grams per liter, and the protein undergoes clearance with a half-life spanning from 3 to 5 days. Beyond the blood, AAT is also found in saliva, milk, urine, tears, semen, and bile. However, its distribution throughout the body is not consistent. For instance, within the lower respiratory tract's epithelial lining fluid, the concentration of AAT is estimated to be approximately 10% of its levels in the bloodstream [3].

AAT is a crucial serine protease inhibitor that plays a significant role in maintaining the protease-antiprotease balance in the lungs and liver. Alpha-1 antitrypsin (AAT) deficiency is a hereditary condition that significantly increases the risk of serious lung and/or liver disease in children and adults [4]. The deficiency leads to decreased circulating AAT levels, predisposing individuals to liver cirrhosis and chronic obstructive pulmonary disease (COPD) [5, 6]. In the lungs, reduced levels of AAT result in an imbalance of protease and antiprotease, leading to lung disease [7]. Liver disease in AAT deficiency is attributed to the aggregation of AAT polymers within the endoplasmic reticulum of liver cells, leading to the formation of periodic acid-Schiff positive inclusions, a hallmark biopsy feature in AAT deficiency-related liver disease [8, 9]. Furthermore, AAT deficiency is a commonly overlooked cause of lung disease, particularly in the form of emphysema, and is associated with genetic predisposition to COPD [3, 7]. The deficiency is also linked to metabolic panniculitis, pancreatic panniculitis, and systemic vasculitis [6, 10]. Additionally, AAT deficiency is a rare genetic disorder that affects the lungs and liver, leading to lung and liver diseases during childhood and adulthood [11].

The standard of care for lung disease due to AAT deficiency is AAT augmentation therapy, while liver transplantation is used to treat AAT deficiency-related liver disease [12]. However, there are several new and emerging treatment options under investigation for both lung and liver manifestations [13]. Despite the availability of therapy, AAT deficiency remains underdiagnosed, emphasizing the need for increased awareness and early detection [14]. The underdiagnosis of AAT deficiency is attributed to various factors, including obstacles to early diagnosis and treatment, as well as the complexity of proving the efficacy of AAT replacement therapy [15].

As seen, AAT deficiency leads to chronic lung and liver diseases. The aim of this study is to raise awareness about AAT deficiency, which can cause complications with high morbidity and mortality rates.

Methods: This study included patients applied to Selçuk University Medical Faculty Hospital with a history of chronic lung and liver disease. All patients between the ages of aged 1-97 were included in the study. The demographic data of the patients and their AAT levels were obtained from the laboratory information system. Patients were divided into three groups according to AAT levels: <100 mg/dl (low group), 100-200 mg/dl (reference range group) and >200 mg/dl (high group).

AAT concentration was measured by photometric method in Roche Cobas c702.

Results: A total of 1950 patients were included in the study. The mean age of the patients in the low group (n=80) was 29.7 years (min:1 max:78), in the reference range group (n=1559) was 35.9 years (min:1 max:95) and in the high group (n=311) was 42.5 years (min:1 max:97) (Table 1). While AAT levels were not low in 95.9% of the patients, they were found to be low in 4.1%.

Table 1: Number of people in groups and mean age of groups

Groups	n	Age (mean)
Low (<100 mg/dl)	n=80	29,7 years (minimum:1 maximum:78)
Reference ranges (100-200 mg/dl)	n=1559	35,9 years (minimum:1 maximum:95)
High (>200 mg/dl)	n=311	42,5 years (minimum:1 maximum:97)

Discussion: AAT is a protein produced in the liver that plays a crucial role in protecting the body's tissues from enzymes released by inflammatory cells. Its primary function is to inhibit an enzyme called neutrophil elastase, which can damage healthy tissues if not properly controlled. AAT deficiency, a genetic disorder where the body doesn't produce enough of this protein, can lead to various lung and liver conditions [16]. Understanding AAT and its role in the body is vital in managing and treating conditions associated with its deficiency.

Liver impairment progressing to liver failure or cirrhosis has been attributed to the toxic accumulation of synthesized mutant alpha 1-antitrypsin peptide within the cytoplasm of the hepatocytes. Additionally, AAT deficiency accounts for a significant proportion of pediatric liver transplants due to metabolic disease in the western world. The condition has also been associated with autoimmune hepatitis and chronic obstructive pulmonary disease, and the level of alpha-1 antitrypsin may result in different reductions in lung function. Moreover, AAT deficiency has been reported in association with diverse conditions such as chronic pancreatitis, subcutaneous and systemic panniculitis [17-19]. In the study conducted by Silverman et al., it was reported that the risk of liver fibrosis and cirrhosis is considerably high in patients with AAT deficiency. Additionally, it was noted that the amount of accumulated polymerized molecules is associated with the stage of cirrhosis [20].

According to the findings of the conducted study, it was determined that 95.9% of patients did not exhibit low AAT levels, while 4.1% were found to have low levels. Considering the long-term mortality and morbidity associated with the disease, this percentage might not be considered low. Furthermore, given that the average age in the low-level patient group was 29.7, it is inferred that there is a delay in initiating treatment for the disease. Taking into account family history and clinical symptoms, we believe that the AAT test, a non-invasive and cost-effective test, should be performed at earlier ages in these patients. This proactive approach could potentially prevent the onset of complications.

Conclusions

In conclusion, AAT deficiency is a complex disorder that significantly impacts lung and liver health. It is crucial to increase awareness, improve diagnosis, and explore new treatment options to effectively manage the associated lung and liver diseases. The imbalance of protease and antiprotease due to reduced AAT levels leads to the development of lung diseases such as COPD, while liver disease is characterized by the aggregation of AAT polymers. Early diagnosis and appropriate management are crucial in addressing the impact of AAT deficiency on lung and liver health.

References

cytokines (4). It performs its functions through a receptor named JAK (8, 9). It has been shown in many recent studies that the development of many types of cancer can be stopped by the hypermethylation of this receptor of IL-6 (10). It has been emphasized that IL-6 is an important marker of experimental cancer (11), and it has been reported that IL-6 levels increase in some cancer patients (3, 4). Another cytokine, IL-1, can be produced in many types of cell where it occurs such as monocytes, B-cells, keratinocytes, mesangial cells and endothelin (12). First identified as leucocyte pyrogen in 1940, it is a molecule with a molecular weight of approximately 10 000 daltons. When given to experimental animals, it leads to fever, an increase in the secretion of colony stimulant factor (CSF), neutrophilia, loss of appetite, sleep and synthesis of acute phase proteins. At higher doses, it causes hypertension, leucopenia and an increase in cardiac output. The metabolic effects of IL-1 include secretion of adrenocorticotropic hormone (ACTH), formation of thromboxane A, an increase in the synthesis of prostaglandin, lipoprotein lipase inhibition, increase in sodium excretion, and reduction in albumin synthesis (13). Neopterin is a derivative of pteridine, and is produced from monocytes formed from macrophages. There are studies on the high concentration of neopterin in malign diseases, especially hematological, gynecological, gis?? and lung cancers (14). Metastasis in tumor cells is a complex process with many steps. Adhesion, migration and invasion of cancer cells are very important events in the metastatic process of cancer (15). IL-8 is a multi-functional inflammatory cytokine. It is produced by the cells of many cancers, such as melanoma, squamous cell carcinoma, cervical cancer, ovarian cancer, small cell lung cancer, colon cancer and stomach cancer (16, 17).

Studies have shown a correlation between the incidence of bladder cancer and the presence of cytokines. Our purpose in this study was also to examine the difference in IL-1, IL-6, IL-8 and neopterin in bladder cancer patients before and after treatment.

Methods: This is a sex-matched case-control study, conducted at the Department of Urology at Celal Bayar University Faculty of Medicine and the Department of Biochemistry at Celal Bayar University Faculty of Haealth Science. The study was approved by the hospital ethics committee. All patients and volunteers involved in the study gave their informed consent.

Patients: NMIBC patients who applied to the Department of Urology at Celal Bayar University Faculty of Medicine, and 30 age- and sex-matched controls (group 1) comprised of patients with urolithiasis, who had no malignancies, chronic diseases and infection, were included in the prospective clinical study. TURBT operations in all the patients were performed by urologists. The exclusion criteria were as follows: pTa (in low grade) and ≥pT2 UCB, another malignancy except bladder cancer and chronic diseases. Six patients with pTa and 5 patients with pT2 were excluded from the study. Only thirty patients with NMIBC, who received intravesical therapy, were enrolled in the study. Blood samples (n=30) were obtained from all the patients before TURBT (group 2), twenty days after TURBT (group 3) and at the end of intravesical immunotherapy (group 4). Eighteen of 30 patients had Ta (intermediate or high risk), while the remaining 12 patients had T1 transitional cell carcinoma (TCC) of the bladder. In these 12 patients, re-TURBT was performed before intravesical immunotherapy. None of the patients received early intravesical chemotherapy. Informed consent was obtained from all the patients and controls who participated in the study and the local ethics committee approved the study protocol.

Assay: IL-1β, IL-6, IL-8 and neopterin were evaluated in serum samples isolated from peripheral blood and banked at -80°C. All samples from each patient were run in the same assay. Patient plasma samples were analyzed by an enzyme-linked immunosorbent assay (ELISA) with commercial kits for IL-6 and IL-8 (Invitrogen), IL-1β (IBL-Hamburg) IBL International and neopterin (Shanghai Yehua Biological Technology Co., Ltd.). The values were measured in triplicate and the mean concentrations were determined from the standards provided.

Statistical analysis: The software SPSS for Windows, version 16.0 was used in the statistical analysis of the data. Associations between continuous variables were assessed by the Mann-whitney-u test. In all analyses, a p value

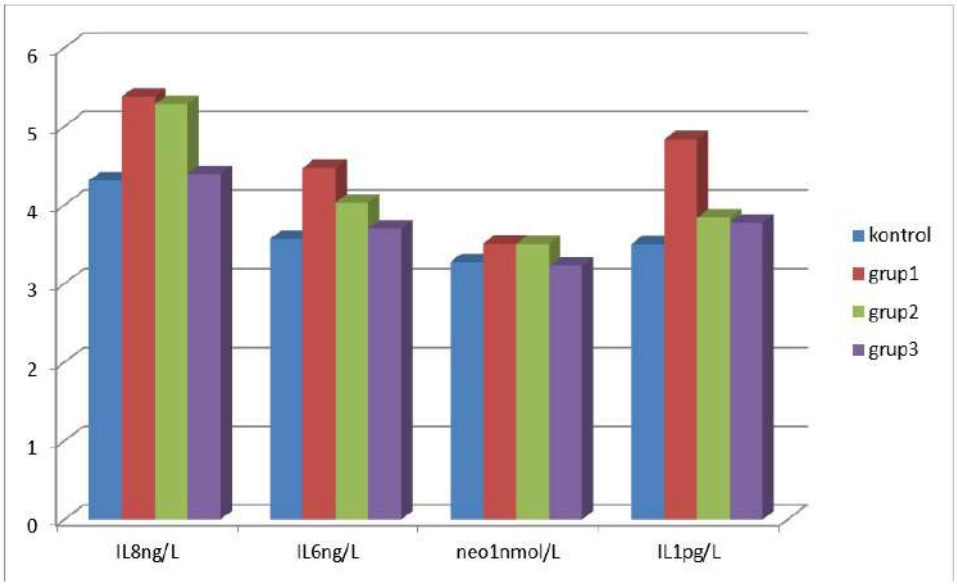
Results: Our purpose in this study was also to examine the difference in IL-1, IL-6, IL-8 and neopterin in bladder cancer patients before and after treatment. Our studuies were constructed from control and case (bladder cancer

patient group, 1st treatment group and 2 nd treatment group). We couldn't find any significant statistical difference between age, sex and weights of these group.

Table I: IL-1, IL-6, IL-8 and Neoptrinin levels before and after treatment in the bladder cancer patient

Gruplar	IL8ng/L	IL6ng/L	neo1n-mol/L	IL1pg/L
kontrol		3,568367	3,277648	3,505511
grup1		4,4725	3,514254	4,83554
grup2	5,28322	4,031444	3,510221	3,838707
grup3	4,3953	3,698443	3,227116	3,777437

We were made comparison between the control group and the case groups (Table-1) by Mann Whitney-U test. IL-1, IL-6, IL-8 levels were statistical ($p<0.05$) increased but Neoptrinin level was signifacntly increased in the group 1 and 2 than control. There was no statistically significant difference between control groups and group3. When we compared group 1 t0 group 2, IL-6, IL-8 and Neoptrinin levels were no statistically significant difference, however IL-1 level was signifacntly decreased ($p<0.05$). There was statistically significant decreased ($p<0.05$) between groups 1 and group3. Groups 2 compared to group3, IL-1, IL-6, IL-8 and Neoptrinin levels were significant decreased ($p<0.05$)



Discussion: Urinary bladder cancer is the most common urinary tract malignancy (18) Bladder cancer (BC) presents with an estimate of 72,570 new cases diagnosed and 15,210 deaths across the United States (19). A number of risk factors for bladder cancer have been established, including cigarette smoking, exposure to industrial aromatic amines and the uptake of drugs, such as phenacetin, cyclophosphamide and chlornaphazine (20). Smoking was found to be a principal independent risk factor for bladder cancer (21). The majority of bladder cancers are diagnosed at an early stage, i.e. confined to the urothelium and lamina propria. These patients are often managed successfully with local therapies. Intravesical therapy is used routinely after resection of these superficial tumors to reduce risk of recurrence of higher risk tumors. Intravesical BCG (bacillus calmette-guerin) is the most commonly used agent and is thought to act by generating immune response against the residual tumor (22). The gold standard for bladder cancer screening is still urine cytology, as it is non-invasive, safe and inexpensive. Although it is highly specific, the results are not reproducible and the interpretation is highly dependent on the skill of the cytologist. This calls for searching other markers for screening of bladder cancer which should be specific, sensitive, reproducible,

non-invasive and done at an acceptable cost (23). Due to the invasive nature of the procedure, but also for adding accuracy in the detection, biomarkers assessed in blood or urine are considered as beneficial for supporting clinical assessment. This is also relevant for disease prognosis as biomarkers measured at the DNA, RNA and/or protein levels provide the potential to choose best surveillance measures and treatment regimens for specific patient populations regarding halting the development of muscle invasive disease (19). Genetic polymorphisms in a number of metabolic enzymes were found to act as modulators of bladder cancer risk (18).

Inflammation is a physiological, protective process that the organism activates in response to tissue damage. The persistence of stimuli that induce an inflammatory response or the failure of the mechanism that ends it may result in chronic inflammation even among patients with cancer, as first proposed by Virchow in 1863(24). The concept that inflammation can cause cancer is further supported by the observation that polymorphisms in genes encoding pro-inflammatory cytokines are associated with an increased risk of some cancer types (25). Accordingly, the aim of the present study was to investigate the differences in IL-1, IL-6, IL-8 and neopterin in bladder cancer patients before and after treatment.

Cytokines are produced during the activation of innate and acquired immunity, and are the principal means for intercellular communication of a microbial invasion. Cytokines serve to initiate the inflammatory response and to define the magnitude and the nature of the acquired immune response. The response of critically ill patients to their injury and/or invading pathogens is dependent, in large part, on the pattern of cytokines which are produced. The immunologic response of critically ill patients can vary from a strongly proinflammatory response, characterized by increased production of tumor necrosis factor-[alpha], interleukin (IL)-1, interferon (IFN)-[gamma], and IL-12 to one predominantly of anergy, characterized by increased production of TH2 cytokines, like IL-10 and to IL-4. Therapeutic efforts to modify the host immune response in critical illness will require a more thorough understanding of the cytokine milieu and the factors that determine their production (26). Cytokines regulate growth, trafficking, signalling, and differentiation of both stromal and tumor cells. The cytokines produced by cancer cells function to create optimal growth conditions within the tumor microenvironment, while the cytokines secreted by stromal cells may influence the behavior of malignant cells. Cytokines induced by hypoxia, a hallmark feature present in progressive cancers, include vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF), IL-1, and IL-6 (27)

Several cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and interleulin-8 (IL-8), are proinflammatory cytokines, inducing a systemic inflammatory response reflected by increased levels of neopterin or tumor necrosis factor-alpha (TNF-α), while other cytokines such as the IL-1β receptor antagonist are secreted as a feedback control mechanism of the systemic inflammatory response. Proinflammatory cytokines produced at tumor sites play key roles in cancer progression. The production of these cytokines by epithelial cancer cells depends mainly on the NFκB transcription factor. Among the cytokines induced by NFκB, IL-6 has been identified as one of the most relevant myeloid-derived factors that promote tumor formation (25). Transcription factor NFκB, which is activated by many extra and intracellular stimuli including bacterial and viral signals via PRR (pattern recognition receptors; TLR and NLR), stress factors, signals triggered by tissue injury, as well as many cytokines (TNF, IL1), is one of the main regulators controlling inflammation in general and inflammationinduced cancer in particular (28)

Proinflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), are produced by stimulated inflammatory cells and epithelial cells during mucosal inflammation (29). IL-1β is known to activate the nuclear factor κB (NF-κB) signaling pathway and elicit IL-6 production (30). Proinflammatory cytokine IL-1β has a significant role in gastric carcinogenesis. Huang et all showed that IL-1R1-/- mice, which are unresponsive to IL-1β, were used to fully elucidate the specific role of IL-1β in linking gastric inflammation to DNA methylation induction. The results indicated that peripheral IL-1β challenge may induce proinflammatory cytokine release (29). Mc Load et all showed that inhibition of NF-kB in myeloid cells enhances lung tumorigenesis and, paradoxically, increases infiltration of neutrophils into the lungs. NF-kB-deficient neutrophils produced elevated levels of IL-1b, which was regulated by the serine protease cathepsinG (31). In present study, IL-1 levels was statistical ($p<0.05$) increased in the group 1 and 2 than control, however there was no statistically significant

difference between control groups and group3. Groups 2 and group 3 compared to group1, IL-1 level was significant decreased ($p<0.05$).

Interleukin-6 is a pleiotropic cytokine that regulates cell growth and differentiation of various tissues. It is known particularly for its role in the immune response and acute phase reactions. As a major mediator of the inflammatory response, IL-6 plays a key role in the inflammatory process by acting as both pro-inflammatory cytokine and anti-inflammatory myokine. The anti-inflammatory effect of IL-6 is mediated through its inhibition on tumor necrosis factor (TNF-α) and interleukin 1 (IL-1), and activations of interleukin-1 receptor antagonist (IL-1RA) and interleukin-10 (IL-10) (32). IL6 is produced by various cell types; however, myeloid cells (monocytes and macrophages) in acute inflammation and T-cells during chronic inflammation are considered the major sources of this cytokine (33). IL6 can also be produced by fibroblasts, keratinocytes, B cells, endothelial cells, and some types of tumor cells (34). In all these cases, expression of IL6 is regulated through activation of several transcription factors such as AP-1, C/EBPα, and particularly NF-κB. Because a variety of different transcription factors is able to activate expression of IL6, this cytokine is synthesized in virtually any type of inflammation with in various body tissues. The systemic level of IL6 often correlates with disease severity, e.g. during sepsis, obesity, diabetes, insulin resistance, inflammatory bowel disease, arthritis, and various types of cancer (35). Yan et al reported that the levels of IL-6 are elevated after stroke (36). Spittler et al. reported that macrophages exhibited an elevated capacity to phagocytose Escherichia coli in septic patients with serum IL-6 levels P1000 pg/mL (37). Yeh et all. showed that phagocytic and migratory ability of human peripheral monocytes following treatment with each CRC supernatant and IL-6–related blocking conditions. Similarly, rIL-6 or CRC-secreted IL-6 augmented the phagocytic and migratory ability of human monocytes and this activation was mediated by the gp130/ STAT3 pathway (38). In this present IL-1, IL-6, IL-8 levels were statistical ($p<0.05$) increased in the group 1 and 2 than control. There was no statistically significant difference between control groups and group3. When we compared group 1 to group 2, IL-6, level was no statistically significant difference, however IL-6 level was signifacntly decreased ($p<0.05$) compare to group 3 groups 2 compared to group3, IL-6, level was significant decreased ($p<0.05$)

Interleukin-8 (IL-8;also referred to as CXCL8) is a chemokine that plays apivotal role in acute inflammations and hence is an important biomarker for arange of diseases. During an injury or infection IL-8 is involved in the recruitment ofneutrophils from blood vessels to the affected tissue promoting angiogenesis. However,stimulants such as pro-inflammatory cytokines (e.g. TNF-α and interleukin-1),cellular stress, or bacterial and viral products, also trigger cells to express IL-8 proteins, activating neutrophils that release their toxic intracellular contents causing damage to host tissue and resulting in acute inflammation states. Often, elevated IL-8 levels are also associated with the progression of numerous chronic diseases including rheumatoid arthritis, inflammatory bowel disease, psoriasis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome(ARDS), atherosclerosis, central nervous system trauma, development of malignant cancer, and chronic liver disease (39)

Interleukin-8 (IL-8), alternatively known as CXCL8, is a proinflammatory CXC chemokine. Expression of IL-8 is primarily regulatedby activator protein and/or nuclear factor-nB–mediated transcriptional activity, although additional hormone response elements andNF-IL-6 consensus sites have been characterizedon the IL-8 gene promoter. Accordingly, expression of IL-8 has been shown to be regulatedby a number of different stimuli including inflammatory signals (e.g., tumor necrosis factor a, IL-1h), chemical andenvironmental stresses (e.g., exposure to chemotherapy agents andhypoxia), and steroid hormones (40). In a study by Kushi et al., both cerebrospinal fluid and serum levels of IL-8 were significantly higher after 72 h (3 days) in the fatality group compared to those in the survivor group (41). In our study showed that IL-8 level was statistical ($p<0.05$) increased in the group 1 and 2 than control but there was no statistically significant difference between control groups and group3. When we compared group 1 to group 2, IL-8 level was no statistically significant difference, however there was statistically significant decreased ($p<0.05$) between groups 1 and group3. Groups 2 compared to group3, IL-8 level was significant decreased ($p<0.05$)

Neopterin is secreted by peripheral blood monocytes/ macrophages stimulated by gamma-interferon which in itself

is produced as a result of T lymphocyte activation. The detection of neopterin in different body fluids in vivo, have led to the investigation of its value as a tumor marker and a prognostic factor for malignancies. Earlier studies have demonstrated increases in neopterin levels in relation to tumor type and stage (42). Neopterin measurements not only allow evaluating the extent of cellular immuneactivation but also the extent of oxidative stress and increased production of reactive oxygen species (ROS) (43). Hausen et all showed that diagnostic value of neopterin for hematological malignancies, high levels have been reported especially in patients with active leukemia and lymphoma, with substantially reduced levels in patients in remission(44). Prommeggar et al.(45) investigated the prognostic value of neopterin in 110 patients scheduled for lung cancer surgery. They found that preoperative neopterin levels were a valuable prognostic marker with regard to survival, and recommended that patients who were clinically operable should be reassessed before undertaking surgery if found to have high neopterin levels. In our study Neoptrinin level was signifacntly increased in the group 1 and 2 than control but there was no statistically significant difference between control groups and group3. When we compared group 1 to group 2, Neoptrinin level was no statistically significant difference. There was statistically significant decreased ($p<0.05$) between groups 1 and group3. Groups 2 compared to group3, Neoptrinin level was significant decreased ($p<0.05$).

Both IL1 and IL6 can recruit immune cells into a site of developing tumor or tumor microenvironment, there by enhancing inflammation. These data as well as stimulation of malignant proper ties of epithelial and cancer cells by cytokines IL1 and IL6 are directly related to the above mentioned ability of IL1 and IL6 to trigger activation of proto oncogenic transcription factors such as NF κB and STAT3 responsible for angiogenic, immunosuppressive, and antiapoptotic effects in the tumor microenvironment (28). IL-8 signaling also regulates the activity of the mitogen activated protein kinase (MAPK) signaling cascade that constitutes a number of serine/threonine kinases that are colocalized via their interaction with scaffolding proteins in close proximity to cell-surface receptors. Activation of MAPK signaling is consistent with the cell proliferation and cell survival promoting effects of IL-8 that have been reported in neutrophils, and endothelial and cancer cell lines (46).

To summarize, it can be stated that IL1 and IL6 are dramatically important components of the tumor microenvironment, displaying multiple functions. These cytokines take part at all stages of oncogenesis: from initiation to tumor, invasion, and metastasis of already established malignant and mutant epithelial cells. When IL1 and IL6 enhance local inflammatory response, which results in accelerating growth and progression of tumors, Neopterin levels have been shown to increase in many different malignant tumors, and its overexpression in the setting of tumor development and progression of disease makes it a potential indicator of neoplastic growth. There is significant support for targeting IL-8 signaling (andthat of its associatedproangiogenic CXCchemokines) in numerous solid tumors (e.g., gastric, pancreatic,melanoma, ovarian, bladder, and prostate).

These cytokines levels were decreased after treatment, There was no statistically significant difference after three traetment. These findings show how effective the treatment is. In the light of this information, we think that these markers can be used during the diagnosis and treatment of bladder cancer. All these characteristics make cytokines interesting novel targets for cancer therapy. We aim to work in a larger and different population of patients with progronosis.

References

1.Krajewski W, Kołodziej A, Dembowski J, Zdrojowy R, Genetic and immunologic determinants of intravesical BCG therapy in non-muscle-invasive urothelial bladder cancer, Postepy Hig Med Dosw (Online). 2014 Mar 20;68:291-300.)

2.Greenberg AS, Nordan RP, McIntosh J, Caluo JC, Scow PO, Jablons D. Il-6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes : A possible role for Il-6 in cancer cachexia. Cancer Res 1992; 52:4113-4116.

3. Oka M, Yamamoto K, Takahashi M, Hakozaki M, Abe T, et al. Relationship between serum levels of IL-6, vari-

ous disease parameters and malnutrition in patients with esophageal squamous cell carcinoma. Cancer Res 1996; 56:2776-2780.

4. Martin F, Santolaria F, Batista N, Milena A, Gonzalez-Reimers E, Brito MJ, et al. Cytokine levels (IL-6 and IFN-gamma), acute phase response and nutritional status as prognostic factors in lung cancer. Cytokine 1999; 11:80-86. Kemik ve ark. Kolon Kanseri ve Il6 Van Tıp Dergisi, Cilt:17, Sayı:2, Nisan/2010 45

5. Hatada K, Miki C. Nutritional status and postoperative cytokine response in colorectal cancer patients. Cytokine 2000; 12:1331-1336.

6. Yanagawa H, Sone S, Takahashi Y, Yano H, Shinohara T, Oguna T. Serum levels of IL-6 in patients with lung cancer. Br J Cancer 1995; 71:1095-1098.

7. Dosquet C, Schaetz A, Faucher C, Lepage E, Wautier JL, Richard F, et al. Tumor necrosis factor-alpha, Il-1β and IL-6 in patients with renal cell carcinoma. Eur J Cancer 1994; 30A: 162-167.

8. Watanabe S, Mu W, Kahn A, Jing N, Li JH, Lan HY, et al. Role of JSK/STAT payhway in IL-6-induced activation of vascular smooth muscle cells. Am J Nephrol 2004; 24(4):387- 392.

9. Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. J Cell Sci 2004; 117(8):1281-1283.

10. Heinrich PC, Behrmann I, Haan S, et al. Principles of IL-6-type signaling and its regulation. Biochem J 2003; 374(1):1-20. 11. Ashizawa T, O

11. Strassmann G, Fong M, Kenney JS, Jacob CO. Evidence for the involvement of IL-6 in experimental cancer cachexia. J Clin Invest 1992; 89:1681-1684.

12.Pike MC Synderman R: Structure and function of monocytes and macrophages.In : Arthritis and allied conditions. McCarty DJ, Koopman WJ (Eds). Lea and Febiger pp : 347-375, 1993.)

13.Ankara Tıp Mecmuası (The Journal Of The Faculty Of Medıcıne) Vol. 47 : 495-504, 1994).

14.International Journal of Hematology and Oncology Number: 2 Volume: 20 Year: 2010).

15. Trepat X, Chen Z, Jacobson K. Cell migration. Compr Physiol. 2012;2:2369-2392.[PubMed] [DOI]).

16. Campbell LM, Maxwell PJ, Waugh DJ. Rationale and Means to Target Pro-Inflammatory Interleukin-8 (CXCL8) Signaling in Cancer. Pharmaceuticals (Basel). 2013;6:929-959.[PubMed] [DOI]

17. Jun Shi and Pin-Kang Wei, Xiaotan Sanjie decoction inhibits interleukin-8-induced metastatic potency in gastric cancer World J Gastroenterol. 2015 February 7; 21(5): 1479-1487

18. Yanfeng Yang, Xuepeı Zhang, Dongkuı Song And Jınxıng We, Association between vascular endothelial growth factor gene polymorphisms and bladder cancer risk, . Molecular And Clınıcal Oncology 2: 501-505, 2014

19.Akshay Bhat, Andreas Heinzel, Bernd Mayer, Paul Perco, Irmgard Mühlberger, Holger Husi, Axel S. Merseburger, Jerome Zoidakis, Antonia Vlahou, Joost P. Schanstra, Harald Mischak, Vera Jankowski, Research Artıcle Protein Interactome Of Muscle Invasive Bladder Cancer, Plos One | Doı:10.1371/Journal.Pone.0116404, January 8, 2015

20. Cohen SM, Shirai T and Steineck G: Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol Suppl: 105-115, 2000.

21. Baena AV, Allam MF, Del Castillo AS, et al: Urinary bladder cancer risk factors in men: a Spanish case-control study. Eur J Cancer Prev 15: 498-503, 2006.

22. Benjamin A. Teply and Jenny J. Kim, Systemic therapy for bladder cancer – a medical oncologist's perspective

J Solid Tumors. 2014 ; 4(2): 25–35. doi:10.5430/jst.v4n2p25.

23.Fathia El-Sharkawi, Mahmoud El Sabah, Zeinab Hassan and Hussein Khaled, The biochemical value of urinary metalloproteinases 3 and 9 in diagnosis and prognosis of bladder cancer in Egypt, El-Sharkawi et al. Journal of Biomedical Science 2014, 21:72.

24. Guarino V, Castellone MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. Mol Cell Endocrinol. 2010;321(1): 94-102

25. Kemal Beksac, Cigdem Sonmez, Bahadir Cetin, Gorkem Kismali, Tevhide Sel, Yildirim Tuncer, Funda Kosova, Evaluation of proinflammatory cytokine and neopterin levels in women with papillary thyroid carcinoma , Int J Biol Markers 2016; 00(00): e000-e000

26.Oberholzer, Andreas MD; Oberholzer, Caroline MD; Moldawer, Lyle L., Cytokine signaling- regulation of the immune response in normal and critically ill states, Critical Care Medicine Issue: Volume 28(4) Supplement, April 2000, pp N3-N12, Copyright: © 2000 Lippincott Williams Wilkins, Inc. Publication Type: [Signal Transduction In Critical Care Medicine: Scientific Reviews

27. Anne M Lewis1,2, Sheelu Varghese1,3, Hui Xu1 and H Richard Alexander*1,3 interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment, Journal of Translational Medicine 2006, 4:48

28. O. S. Dmitrieva1,2, I. P. Shilovskiy2, M. R. Khaitov2, and S. I. Grivennikov1,Interleukins 1 and 6 as Main Mediators of Inflammation and Cancer, Biochemistry (Moscow), 2016, Vol. 81, No. 2, pp. 80 90

29. Fung-Yu Huang, Annıe On-On Chan, Asıf Rashıd, Danny Ka-Ho Wong, Waı-Kay Seto, Chı-Hın Cho, Chıng-Lung Laı and Man-Fung Yuen, Interleukin-1β İncreases The Risk Of Gastric Cancer Through İnduction of Aberrant Dna Methylation İn A Mouse Model, Oncology Letters 11: 2919-2924, 2016

30. Kohase M, May LT, Tamm I, Vilcek J and Sehgal PB: A cytokine network in human diploid fibroblasts: Interactions of β-interferons, tumor necrosis factor, platelet-derived growth factor, and interleukin-1. Mol Cell Biol 7: 273-280, 1987.

31. Allyson G. McLoed, Taylor P. Sherrill, Dong-Sheng Cheng, Vassilis Georgoulias, Rinat Zaynagetdinov, Timothy S. Blackwell, McLoed et al, Neutrophil-Derived IL-1b Impairs the Efficacy of NFkB Inhibitors against Lung Cancer,., 2016, Cell Reports 16, 1–13

32.Hu Chen, Teck Kian Choo, Jingfeng Huanga, YiWang, Yuanjun Liu, Mark Platt, et all; Label-free electronic detection of interleukin-6 using horizontally aligned carbon nanotubes, Materials and Design 90 (2016) 852–857

33. Kishimoto, T. (2005) Interleukin 6: from basic science to medicine – 40 years in immunology, Annu. Rev. Immunol., 23, 1-21.

34. Kishimoto, T. (1989) The biology of interleukin-6, Blood, 74, 1-10.

35. Neurath, M. F. (2014) Cytokines in inflammatory bowel disease, Nat. Rev. Immunol., 14, 329-342.

36.Yan et al., 2012)(Yan, J., Greer, J.M., McCombe, P.A., 2012. Prolonged elevation of cytokine levels after human acute ischaemic stroke with evidence of individual variability. J. Neuroimmunol. 246 (1–2), 78–84 (Mar 16).).

37. A. Spittler, M. Razenberger, H. Kupper, M. Kaul, W. Hackl, G. Boltz-Nitulescu, et al., Relationship between interleukin-6 plasma concentration in patients with sepsis, monocyte phenotype, monocyte phagocytic properties, and cytokine production, Clin. Infect. Dis. 31 (2000) 1338–1342.)

38. Kun-Yun Yeh, Tsung-Han Wu, Tai-Ling Wu, Colorectal cancer cell-derived interleukin-6 enhances the phagocytic capacity and migration of THP-1 cells Cytokine 79 (2016) 82–89.

39. R. Sharma, S.E.Deacon, D.Nowaka, S.E.George, M.P. Szymonik, A.A.S. Tang, et all; Label-free electro chemical impedance biosensor to detect human interleukin-8 in serum with sub-pg/mlsensitivity, Biosensors and Bioelectronics, 80 (2016) 607–613

40.DavidJ.J. Waugh and CatherineWilson, The Interleukin-8 Pathway in Cancer, Clin Cancer Res 2008;6735 14(21) November 1, 2008

41. H. Kushi, T. Saito, K. Makino, N. Hayashi, IL-8 is a key mediator of neuro-inflammation in severe traumatic brain injuries, Acta Neurochir. Suppl. 86 (2003) 347–350.

42.Sibel Alpar, Guzin G. Ersoy, Ozlem K. Kurt, Nazire Ucar, Ahmet Aydın, Bahar Kurt, The Diagnostic Role of Neopterin in Lung Cancer, International Journal of Hematology and Oncology, Number: 2 Volume: 20 Year: 2010)

43. Sonia Centi, SaraTombelli, Mariarita Puntoni, ClaudioDomenici et all; Detection of biomarkers for inflammatory diseases by an electrochemical immunoassay: The case of neopterin, Talanta 134 (2015) 48–53.

44. Hausen A, Fuchs D, Grünewald K, et al. Urinary neopterine in the assessment of lymphoid and myeloid neoplasia, and neopterine levels in haemolytic anaemia and benign monoclonal gammopathy. Clin Biochem 15: 34-7, 1982.

45.Prommegger R, Widner B, Murr C, et al. Neopterin: A prognostic variable in operations for lung cancer. Ann Thorac Surg 70: 1861-4, 2000.

46. David J.J. Waugh and Catherine Wilson; The Interleukin-8 Pathway in Cancer, Clin Cancer Res 2008;6735, 14(21) November 1, 2008.

S082

METABOLOMIC PROFILING IN DISTINCT TYPES OF LEUKEMIA

Ayşe Zehra Gül[1], Şahabettin Selek[1], Somer Bekiroğlu[2], Metin Demirel[1], Fatma Betül Çakır[3], Bülent Uyanık[4]

[1] Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Türkiye

[2] Tübitak Marmara Research Center, National Biological and Chemical Test Center, Gebze Kocaeli, Türkiye

[3] Bezmialem Vakıf University, Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye

[4] Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkiye

Introduction: The most common types of the hematological malignancies (HM) that cover the 9.8 percent of new cancer cases are leukemia, Non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), multiple myeloma, myelodysplastic syndrome and myeloproliferative neoplasms, respectively (1). In the simplest term, leukemia and lymphoma are defined as maturation disorders of white blood cells in the bone marrow. The underlying causes have been shown to be mostly genetical predisposition and epigenetic changes. Leukemia represents early-stage maturation problems in white blood cells entering the bloodstream in high amounts while lymphoma is considered as a local tumor of lymphoid tissues. With early diagnosis and advanced molecular analyzes significantly increase survival, novel targeted therapies can provide cure in several types of leukemia. Although a significant increase in survival is observed in all HM subtypes when grouped according to the year of diagnosis between 2010 and 2016 (5), the mortality due to leukemia and lymphoma still covers 6 (female) - 8 (male) percent of all cancers, emphasizes the importance of early diagnosis and effective treatment (3).

There are multiple metabolic profiling studies in leukemia and lymphoma literature yielding disrupted levels of metabolites as biomarker candidates in both the early detection and identifying therapeutical targets. State-of-the-art analytical chemistry techniques as nuclear magnetic resonance (NMR) and mass spectrometry (MS) make that possible covering the majority of metabolite spectrum and allowing quantitation of hundreds of metabolites without specific standard use and calibration requirement.

The aim of the study was to perform phenotypic profiling of HM patient serums and to reveal altered metabolites with their implemented pathways for better understanding of disease-driving causes and for considering tailored treatment options targeting these metabolic aberrations. Besides, easy implementation of the untargeted MS methodology contributes to the future construction of a workflow for HM detection to be used in routine hospital laboratories in the metabolomics era. Yet, emerging results need to be validated with wider cohorts and omics information for reaching generalized concepts and each participant should be evaluated within the frame of personalized medicine approach.

Methods: Inclusion criteria were initial diagnosis of HM patients on their active disease phase with no previous chemotherapy treatment. Exclusion criteria were as follows: drug-smoking use, presence of secondary tumors, malnutrition, presence of any chronic inflammatory disease.

Serum sample preparation steps included the filtration with hydrophilic membrane (0.45 µ) and transferring 200 µL from each to MS vials for the untargeted HRMS experiments. Serum samples of acute myeloid leukemia, chronic lymphocytic leukemia, and non-Hodgkin's lymphoma patients (30 participant for each) were investigated on the Q-Exactive mass spectrometer (Thermo Fisher Scientific) high-resolution MS platform coupled with UltiMate 3000 UHPLC (Dionex, Germany) system. Both positive (ESI+) and negative electrospray ionization (ESI−) modes were applied, and the chromatographic separation was performed at 4 °C UHPLC autosampler temperature with Fortis SpeedCore C18 1.7 µm (100x2.1 mm) column. Operation parameters were set as following: a spray voltage of 3.8 kV, a m/z scan range of 150-1500 Da, a capillary and heater temperature of 320 °C, an S-lens RF level of 50 V, a sheath gas flow rate of 45 and an auxiliary gas flow rate of 10 arbitrary units. Data were acquired with the full scan

(MS1) and MS/MS modes.

Statistical analysis: Univariate statistics of clinical parameters and metabolite concentration values were conducted through the SPSS Version 27.0. An initial principal component analysis (PCA) was performed to determine distribution trends of samples between groups and to exclude extreme values. Improving discrimination, analytes with variable significance score above 1 were selected and enhanced models covering these were created with the partial least squares regression (PLS) analysis. Group-specific differential serum m/z values were detected. The functional analysis using the Mummichog algorithm (MetaboAnalyst 5.0) was performed on HRMS intensity data.

Results: Patient socio-demographic and the clinical parameters have been shown in Table 1.

Table 1. Socio-demographic and clinical parameters of the groups. Values are presented as mean ± standard deviation for normally distributed variables, and as median (interquartile range) for non-normally distributed ones (ns: nonsignificant, *0.01<p<0.05; **0.001<p<0.01; ** *p<0.001 significance level). η2, ANOVA effect size.

	NHL (n=27)	**CLL (n=30)**	**AML (n=29)**	**Control (n=30)**	***p* value**
Sex (male/female)	17/13	26/6	15/15	15/15	0.05*
Age (year)	70.5 (59.75-76)	71 (60.25-76)	56.5 (38.5-78.25)	43 (38.75-47.25)	0.001***
BMI (kg/m^2)	26.2 ± 2.3	26.8 ± 2.9	27.3 ± 2.6	25.8 ± 1.9	ns
Fasting glucose (mg/dL)	116.69 ± 34.7	99 ± 13.36	120.64 ± 29.39	94.54 ± 9.79	0.001*** (η^2=0.21)
Creatinine (mg/dL)	0.98 ± 0.27	0.98 ± 0.19	0.85± 0.4	0.68 ± 0.06	0.01** (η^2=0.13)

All PLS-DA models of the HM and control comparisons concluded with a clear separation (Figure 1). Significantly altered m/z values between HM groups and controls with the VIP score>1 was shown in Figure 2. The enrichment test of functional analysis using the Mummichog algorithm concluded the most significant pathways as purine metabolism in AML; amino sugar metabolism with squalene and cholesterol biosynthesis in CLL; and glycine, serine, alanine, and threonine metabolism in NHL serums (p<0.05, MetaboAnalyst, Figure 3).

Figures

Figure 1. a-The scores plot display of the PLS-DA model from the AML/ control comparison (MetaboAnalyst). **b-** Significantly altered m/z values between AML-control groups with the VIP score >1. **c-** The enrichment test of functional analysis (Mummichog algorithm).

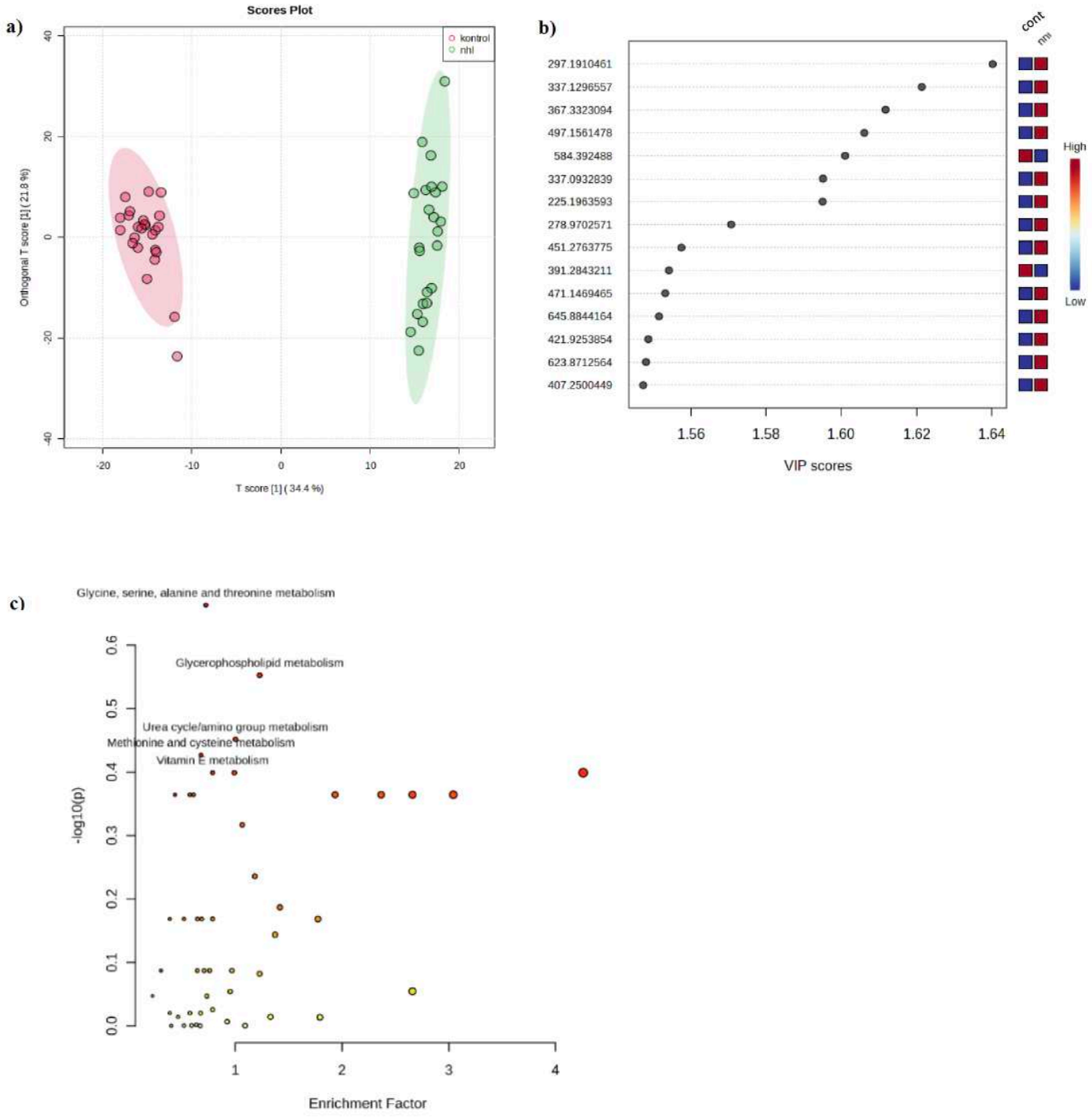


Figure 2. a-The scores plot display of the PLS-DA model from the CLL/ control comparison (MetaboAnalyst). **b-** Significantly altered m/z values between CLL-control groups with the VIP score >1. **c-** The enrichment test of functional analysis (Mummichog algorithm).

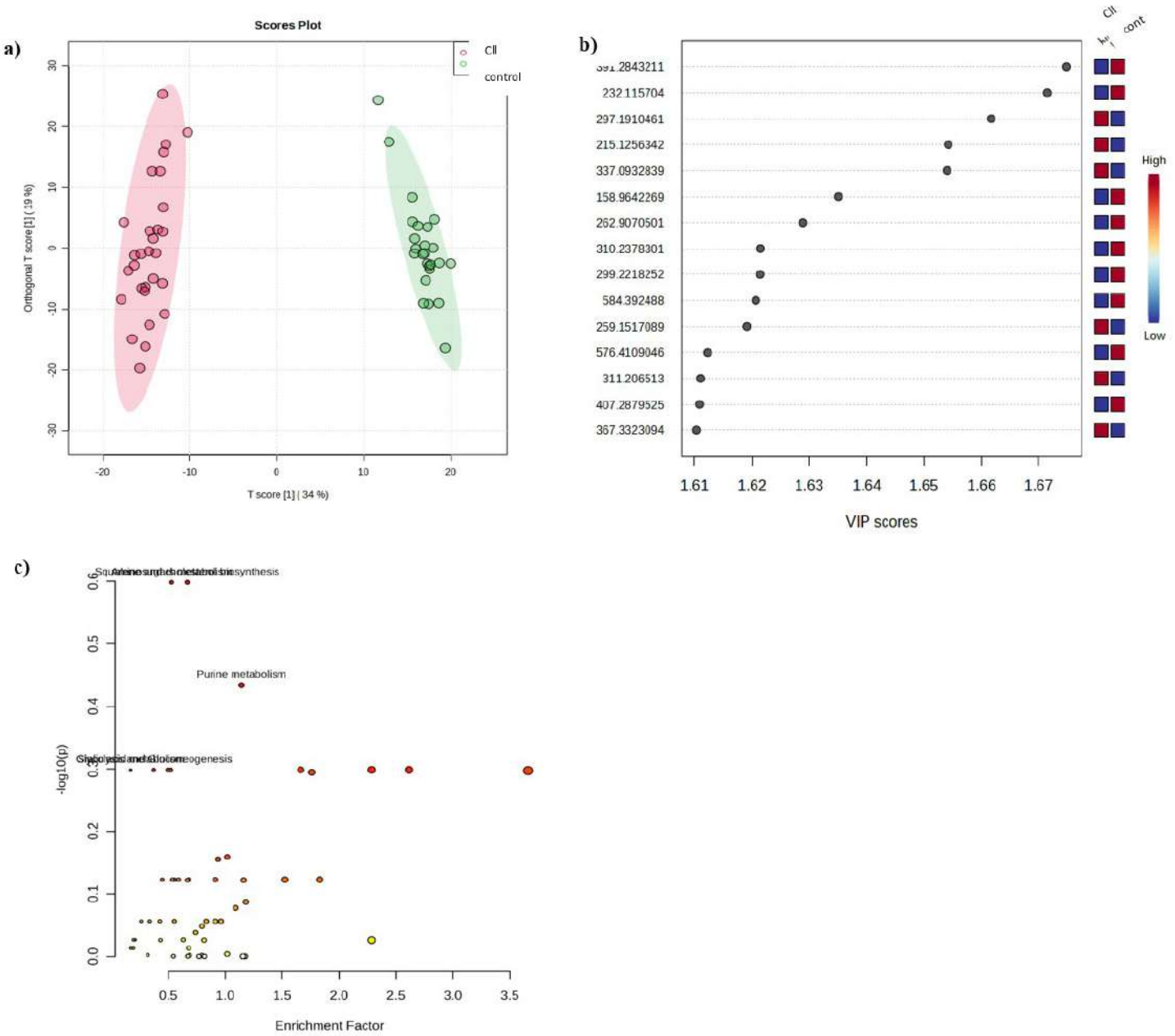
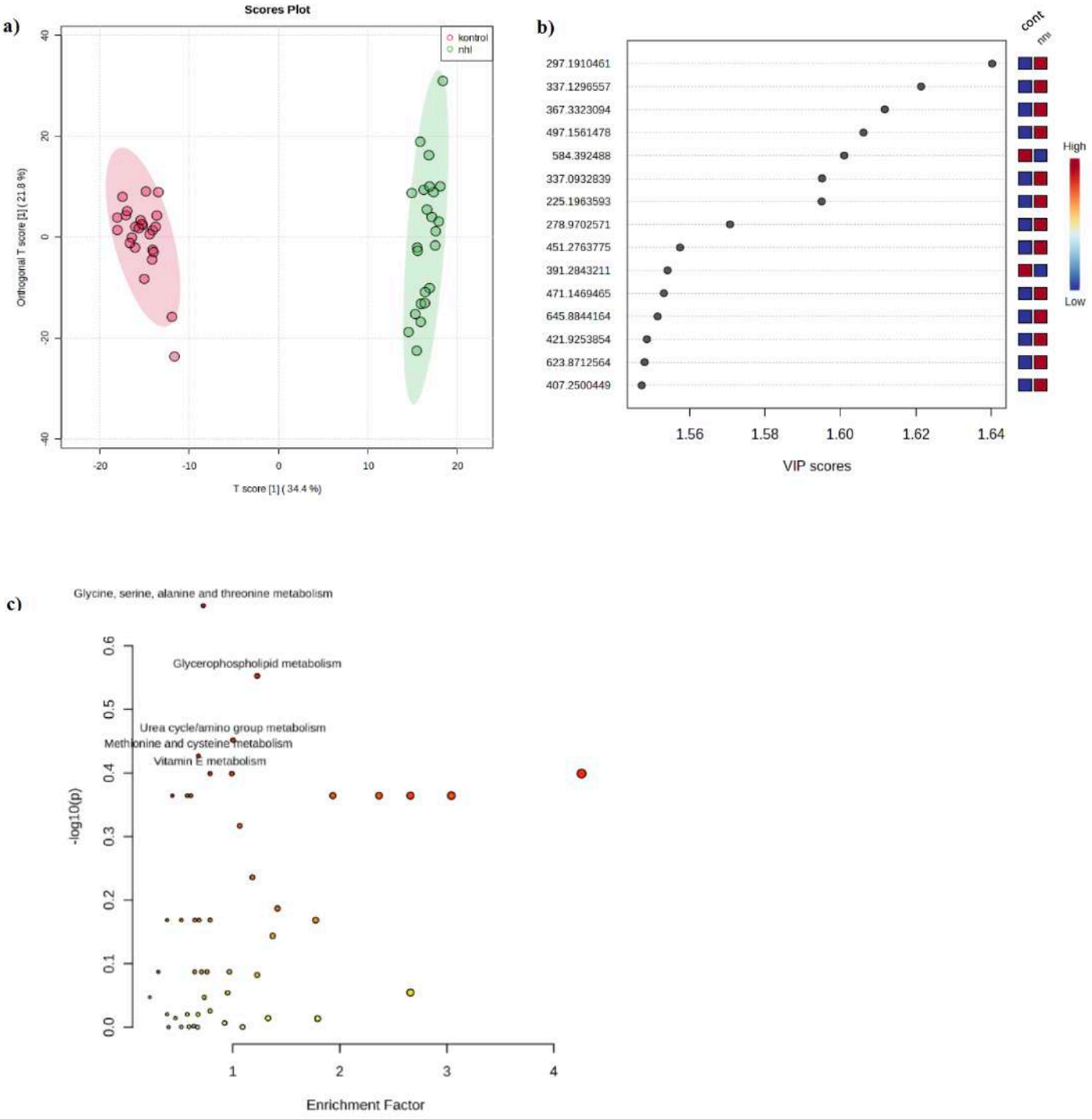


Figure 3. a-The scores plot display of the PLS-DA model from the NHL/ control comparison (MetaboAnalyst). **b-** Significantly altered m/z values between NHL-control groups with the VIP score >1. **c-** The enrichment test of functional analysis (Mummichog algorithm).



Discussion

The common hematological malignancies included in the study as AML, CLL and NHL contain complex subgroups and could have a very heterogeneous clinic across patients. Yet, individual serum profiles with similar metabolic deviations and adaptations in body fluids may reflect the general dynamics of pathogenetic processes and the population of oncogenic blasts/ white blood cells (17, 18).

Essential metabolic functions and analyte dependence for cell division, survival, increased energy demand in cancer cells are considered universal changes in a wide variety of tumors (19, 20). Therefore, typical pathway activations and metabolite changes observed in cancer cells could also be regarded for the HM subtypes included in the study (18, 21). The detection of pathways and their relevant biological function were carried out specific to each HM group and significant changes compared to control individuals were determined using the highly sensitive LCMS methodology with untargeted workflow.

Looking into group-specific pathways, the most differential change in AML patient serums was found as purine metabolism. Considering the frequent myeloblastic storm intervals, this finding may stem from the increased catabolism of structural proteins and nucleotides in aberrantly proliferated cells (22). In support of this, several studies targeted purine pathway blocking the cell proliferation, migration and survival in AML (23-25). Even some combinational chemotherapeutic interventions were shown to act on the purine biosynthesis (26, 27).

Altered purine pathway was also recorded in the CLL patient serums refer to the common mechanisms involved in these leukemia types. However, the CLL pathway analysis ended with amino sugar metabolism and squalene/ cholesterol biosynthesis being at the top affected bioprocesses. Amino sugar metabolism upregulation in CLL was demonstrated in a similar work (28). In this study, the amino sugar metabolism dysregulation was linked to the lymphocyte developmental steps as CD40 stimulation in CLL. Relating to this, another study concluded that CLL cells have high levels of O-GlcNAcylated proteins (29). Since the process of O-GlcNAcylation is reported as a potent tumor promoter, its advanced levels in CLL is associated with poor prognosis (30).

NHL patient serums on the other hand displayed the glycine, serine, alanine, and threonine metabolism as the most affected pathway. This metabolic axis mainly indicates the energy utilization efficiency (31) and the maintenance of the activated one-carbon and Krebs cycle in cancer cells (32). Interestingly, a former cell culture study supported this aberrant axis with showing altered levels of multiple pathway amino acids as well as inter-molecular correlations in various NHL subtypes (33). The reason behind these metabolite changes were linked to the de novo synthesis of the amino acids in the compensation of activated glucose utilization pathways for energy need.

Overall, determining the myeloid or lymphocytic characteristics in terms of metabolomic fingerprinting is of great importance to reveal the causes behind aforementioned findings (34). The findings of the current study revealing altered metabolic pathways in the distinction of HM groups contribute to the existing literature of lymphoma/leukemia pathogenesis and provide valuable insights for disease-specific interventions. The untargeted profiling study in HM groups constitutes a preliminary workflow that offers shortcut targets for metabolism research rather than drawing exact assumptions on the relevant findings. Another practical outcome is the demonstration that patient-based diagnosis and treatment practices can be carried out using LCMS technology, which seems likely to enter all routine laboratories in the future, and a methodology that includes easy sample preparation. At this point, personal screening and an individual-oriented approach provide great time savings and treatment success in complex diseases that contain highly heterogeneous subgroups and genomic infrastructures as in the case of HM.

Research funding: The study was supported by Bezmialem Vakif University Scientific Research Projects Unit (Project No: 20211203) and TÜBİTAK 1002 - A Module (Project No: 122S7442, 01/09).

Author Contributions: Ayse Zehra Gul: Methodology, Statistical analysis, Writing & editing. **Sahabettin Selek**: Conceptualization, Methodology, Statistical analysis, Review & editing. **Somer Bekiroglu**: Methodology. **Metin**

Demirel: Statistical analysis. **Fatma Betul Cakir**: sample acquisition. **Bulent Uyanik**: patient data acquisition and data interpretation. All authors have read and approved the manuscript.

Competing interests: The authors have no competing interests to declare.

Ethical approval: The study was performed at Bezmialem Vakıf University Pharmaceutical Application and Research Center and TÜBİTAK Marmara Research Center between 27.02.2023 and 05.06.2023 with the permission of BVU Ethics Committee (No:2021/391). All participants were given informed consent to the clinical study following the Declaration of Helsinki protocol.

References

1. Ries L, Eisner M, Kosary C, Hankey B, Miller B, Clegg L, et al. SEER (surveillance, epidemiology and end results) Cancer Statistics Review, 1973–1999, National Cancer Institute. Bethesda, Maryland. 2002.

2. Guillerman RP, Voss SD, Parker BR. Leukemia and lymphoma. Radiologic Clinics. 2011;49(4):767-97.

3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA: a cancer journal for clinicians. 2021;71(1):7-33.

4. Kantarjian HM, Keating MJ, Freireich EJ. Toward the potential cure of leukemias in the next decade. Cancer. 2018;124(22):4301-13.

5. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA: A Cancer Journal for Clinicians. 2022;72(1):7-33.

6. Montalban-Bravo G, Garcia-Manero G. Novel drugs for older patients with acute myeloid leukemia. Leukemia. 2015;29(4):760-9.

7. Alseekh S, Aharoni A, Brotman Y, Contrepois K, D'Auria J, Ewald J, et al. Mass spectrometry-based metabolomics: A guide for annotation, quantification and best reporting practices. 2021;18(7):747-56.

8. Wishart DSJB. Advances in metabolite identification. 2011;3(15):1769-82.

9. Bakhtiar R, Lohne J, Ramos L, Khemani L, Hayes M, Tse FJJoCB. High-throughput quantification of the anti-leukemia drug STI571 (Gleevec™) and its main metabolite (CGP 74588) in human plasma using liquid chromatography–tandem mass spectrometry. 2002;768(2):325-40.

10. Boddy AV, Sludden J, Griffin MJ, Garner C, Kendrick J, Mistry P, et al. Pharmacokinetic investigation of imatinib using accelerator mass spectrometry in patients with chronic myeloid leukemia. 2007;13(14):4164-9.

11. Awidi A, Salem II, Najib N, Mefleh R, Tarawneh BJLr. Determination of imatinib plasma levels in patients with chronic myeloid leukemia by high performance liquid chromatography–ultraviolet detection and liquid chromatography–tandem mass spectrometry: methods' comparison. 2010;34(6):714-7.

12. Picard S, Titier K, Etienne G, Teilhet E, Ducint D, Bernard M-A, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. 2007;109(8):3496-9.

13. Ramandi NF, Faranoush M, Ghassempour A, Aboul-Enein HYJCRiAC. Mass Spectrometry: A Powerful Method for Monitoring Various Type of Leukemia, Especially MALDI-TOF in Leukemia's Proteomics Studies Review. 2021:1-28.

14. Roy S, Josephson SA, Fridlyand J, Karch J, Kadoch C, Karrim J, et al. Protein biomarker identification in the

CSF of patients with CNS lymphoma. 2008;26(1):96.

15. Schmid R, Heuckeroth S, Korf A, Smirnov A, Myers O, Dyrlund TS, et al. Integrative analysis of multimodal mass spectrometry data in MZmine 3. 2023:1-3.

16. Shen X, Yan H, Wang C, Gao P, Johnson CH, Snyder MP. TidyMass an object-oriented reproducible analysis framework for LC–MS data. Nature Communications. 2022;13(1):4365.

17. Hanahan D, Weinberg RAJc. Hallmarks of cancer: the next generation. 2011;144(5):646-74.

18. Zuo F, Yu J, He XJFiO. Single-cell metabolomics in hematopoiesis and hematological malignancies. 2022;12:931393.

19. Komura D, Kawabe A, Fukuta K, Sano K, Umezaki T, Koda H, et al. Universal encoding of pan-cancer histology by deep texture representations. 2022;38(9).

20. Yoo HC, Yu YC, Sung Y, Han JMJE, medicine m. Glutamine reliance in cell metabolism. 2020;52(9):1496-516.

21. Stuani L, Sabatier M, Sarry J-EJBb. Exploiting metabolic vulnerabilities for personalized therapy in acute myeloid leukemia. 2019;17:1-17.

22. Di Marcantonio D, Martinez E, Kanefsky JS, Huhn JM, Gabbasov R, Gupta A, et al. ATF3 coordinates serine and nucleotide metabolism to drive cell cycle progression in acute myeloid leukemia. Molecular cell. 2021;81(13):2752-64. e6.

23. Yamauchi T, Miyawaki K, Semba Y, Nakao F, Sugio T, Sasaki K, et al. Paics, a De Novo Purine Synthetic Enzyme, Is a Novel Target for AML Therapy. Blood. 2019;134:1390.

24. Zhang P, Brinton LT, Gharghabi M, Sher S, Williams K, Cannon M, et al. Targeting OXPHOS de novo purine synthesis as the nexus of FLT3 inhibitor–mediated synergistic antileukemic actions. Science Advances. 2022;8(37):eabp9005.

25. Yamauchi T, Miyawaki K, Semba Y, Takahashi M, Izumi Y, Nogami J, et al. Targeting leukemia-specific dependence on the de novo purine synthesis pathway. Leukemia. 2022;36(2):383-93.

26. Hurrish KH, Su Y, Wiley S, Hou Z, Carter J, Kalpage H, et al. A Novel Isoflavone, ME-344, Enhances Venetoclax Antileukemic Activity Against AML Via Suppression of Oxidative Phosphorylation and Purine Biosynthesis. Blood. 2021;138:2238.

27. Hurrish KH, Su Y, Patel S, Ramage CL, Carter JL, Edwards H, et al. Enhancing anti-AML activity of venetoclax by isoflavone ME-344 through suppression of OXPHOS and/or purine biosynthesis. Research Square. 2023.

28. Chen Z, Simon-Molas H, Cretenet G, Valle-Argos B, Smith LD, Forconi F, et al. Characterization of metabolic alterations of chronic lymphocytic leukemia in the lymph node microenvironment. Blood, The Journal of the American Society of Hematology. 2022;140(6):630-43.

29. Shi Y, Tomic J, Wen F, Shaha S, Bahlo A, Harrison R, et al. Aberrant O-GlcNAcylation characterizes chronic lymphocytic leukemia. Leukemia. 2010;24(9):1588-98.

30. Spaner DE. O-GlcNAcylation in chronic lymphocytic leukemia and other blood cancers. Frontiers in immunology. 2021;12:772304.

31. Wu X, Liu C, Yang S, Shen N, Wang Y, Zhu Y, et al. Glycine-Serine-Threonine Metabolic Axis Delays Interver-

tebral Disc Degeneration through Antioxidant Effects: An Imaging and Metabonomics Study. Oxidative Medicine and Cellular Longevity. 2021;2021:5579736.

32. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer. 2013;13(8):572-83.

33. Eraslan Z. Metabolism in haematological cancer cells: University of Birmingham; 2021.

34. Song BH, Son SY, Kim HK, Ha TW, Im JS, Ryu A, et al. Profiling of metabolic differences between hematopoietic stem cells and acute/chronic myeloid leukemia. Metabolites. 2020;10(11):427.

S083

INVESTIGATION OF SERUM TRYPTOPHAN AND KYNURENINE METABOLITES IN BREAST CANCER PATIENTS

Mohammad Ahmad Bik[1], Karam Mazın Kamıl Gharab[2], Duygu Eryavuz Onmaz[1], Sedat Abuşoğlu[1], Ali Ünlü[1]

[1] Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

[2] Mustansiriyah University, National Diabetes Center For Treatment and Reaserch, Baghdad, Iraq

Introduction: Breast cancer affects women all over the world and presents differently for each individual. It is one of the most common and important causes of cancer incidence and mortality [1, 2]. According to GLOBOCAN 2020 data, breast cancer ranks first worldwide in both incidence and mortality [3]. With 2.26 million cases in 2020, breast cancer represents 24.5% of total cancer cases in women. In addition, 15.5% of cancer-related deaths in women are due to breast cancer, i.e. 685,000 deaths in 2020 [2, 3].

Tryptophan (TRP) and its metabolites play an important role in various physiological processes and also in cancer by promoting tumor progression through suppression of the antitumor immune response and increasing the malignancy of cancer cells [4, 5]. The kynurenine pathway is quantitatively the most important pathway of tryptophan metabolism and accounts for about 95% of the degradation of dietary TRP [6, 7]. The degradation of TRP in cancer patients is mainly mediated by increased activities of tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase 1 (IDO1) Stimulation of IDO1 and TRP degradation leads to the accumulation of TRP metabolites especially kynurenines (KYN), which can directly influence the immune response to the tumor [8, 9]. KYN is also converted to the neuroprotective metabolite kynurenic acid (KYNA), 3-hydroxyanthranilic acid (3-HAA), and picolinic acid or to the neurotoxic metabolites 3-hydroxy-kynurenine (3-HK) and quinolinic acid (QA)[10, 11]. In cancer survivors, an imbalance between neuroprotective and neurodegenerative metabolites in the kynurenine pathway was found to be associated with fatigue and depression. IDO and TDO are the first and rate-limiting enzymes of the kynurenine pathway. TDO is present mainly in the liver, whereas IDO is mainly present in other organs [6, 7, 12].

IDO expression proportionally increases to the progression of breast cancer. Elevated IDO expression is correlated with elevated micro-vessel density and poor prognosis in breast cancer [13, 14]. It also increases the concentration of KYN, an agonist of the aryl hydrocarbon receptor (AhR). Triple negative breast cancer cells may be more resistant to anoikis in vitro when the nuclear factor kappa B (NF-kB)/TDO/KYN/AhR axis is activated. Furthermore, blocking this axis can prevent the proliferation, invasion, and migration of these cells [15, 16]. Triple-negative breast cancer metastasis has been demonstrated to be decreased by in vivo suppression of TDO. In addition, advanced cancer stages and lower overall survival rates have been shown to be associated with increased TDO expression in breast cancer. In breast cancer therapy, blocking the IDO/TDO/KYN/AhR axis could be a useful strategy to halt disease progression. [15, 17, 18].

The aim of this study was to investigate the differences in serum levels of TRP and KYN metabolites between breast cancer patients and control subjects.

Methods:

This study included 50 breast cancer patients and 50 healthy controls. The age of patients were 55.2±38 while the age of control were 45.8±41. Prior to the analytical procedures, serum samples were taken from -80°C storage and thawed at room temperature. Subsequently, 300 µL of the serum sample was mixed with 100 µL of an internal standard (100 ng/mL donepezil, dissolved in methanol). To this mixture, 1000 µL of acetonitrile containing 1% formic acid was added and the sample was vortexed thoroughly for 30 seconds. The resulting sample was then centrifuged at 12000 rpm for 10 minutes. The resulting supernatant was collected in a glass tube and evaporated at room temperature under a stream of nitrogen gas. After evaporation, the sample was resuspended by diluting it in 250 µl of 0.1 % formic acid (25 % acetonitrile and 75 % water) and then vialized for analysis.

The chromatographic separation was carried out with a Shimadzu HPLC system by using a Phenomenex Luna C18 column. The mobile phase applied by gradient elution consisted of two components: Mobile phase A consisting of HPLC grade water containing 0.1% formic acid, and Mobile phase B consisting of acetonitrile containing 0.1% formic acid. Triple quadrupole mass spectrometer API 3200 detector with electrospray ionization interface (Applied Biosystems/MDS Sciex), was used for the analysis. Optimization parameters for the mass spectrometer included an ion spray voltage of 3000 V, a heating temperature set at 450 degrees, a curtain pressure at 15 psi, a GS1 ion source gas at 50 psi and GS2 ion source gas at 40 psi. The LC-MS/MS method used to optimize the tryptophan metabolites is shown in Table 1, and the operating parameters for LC-MS/MS are shown in Table 2. Statistical analysis of the data was performed using IBM SPSS Statistics version 21.0.

Table 1. LC-MS/MS method optimization criteria

LC MS/MS Parameter	*Q1 Mass (Da)*	*Q3 Mass (Da)*	*Time (msec)*	*DP (volts)*	*CE (volts)*	*CXP (volts)*
TRP	205.200	146.200	400	39.00	25.00	3.00
KYN	209.100	94.100	400	41.00	26.00	3.00
KYNA	190.200	144.000	200	70.00	20.00	3.00
3-HK	225.100	110.000	400	41.40	22.90	3.00
3-HAA	154.100	136.000	400	30.00	10.00	3.00
QA	168.000	124.000	500	60.00	30.00	7.00
donepezil	380.200	91.000	400	80.00	35.00	6.00

Table 2. LC-MS/MS operating parameters.

LC-MS/MS Instrumentation Parameters	*Value*
Autosampler	
Rinsing Volume	200 Ul
Needle Stroke	52 mm
Rinsing Speed	35 uL/sec
Sampling Speed	15 uL/sec
Purge Time	25 min
Pumping Mode	Binary flow
Total Flow	0.8 mL/min
Pumping Pressure Limits (min-max)	0-400 bar
Source/Gas	
Ion Source	Turbo spray
Curtain Gas	15.0
Collision Gas	6.0
İon Spray Voltage	3000.0
Temperature	450.0
Ion Source Gas1	50.0
Ion Source Gas2	40.0
Interface Heater	On
Analyzing Time	5min
Colon Type	PhenomeneLuna µl C18, 50x4,6 mm

Results: Our research findings showed that individuals who had breast cancer had significantly higher serum concentrations of KYN compared to the control cohort. They also had a significantly lower serum KYN/TRP ratio compared to the control group. Notably, there was no statistically significant discrepancy in serum concentrations of TRP, KYNA, 3-HK and 3-HAA between the breast cancer cohort and the putative healthy control group.

Table 3. Concentration and p-value of the analyte in the breast cancer patient and the control group

Parameter	*Breast cancer patient (n=50)*	*Control group (n=50)*	*p value*
TRP	27500 (8580-55400)	26250 (7750-75200)	0.751
KYN	286.5 (139.5-695.0)	232.5 (109.5-418.0)	**0.019***
KYN/TRP ratio	87.5 (13.2-207.1)	114.7 (33.1-246.0)	**0.011***
KNYA	8.69 (5.23-15.3)	8.86 (4.95-17.6)	0.677
3-HK	2.915 (0.84-5.97)	3.145 (1.10-13.60)	0.094
3-HAA	72.2 (18.9-247.0)	77.8 (34.6-185.0)	0.254

*Statistically significant value

Discussion: Breast cancer represents the predominant malignancy among females and continues to rank as the foremost contributor to mortality on a global scale [19]. Under normal physiological circumstances, tryptophan metabolism primarily involves TDO. However, during immune activation, particularly under the influence of proinflammatory cytokines, especially IFN-γ, there is a notable upregulation of IDO-1 expression [17, 20]. The degradation of tryptophan is recognized for its implications for tumor growth, promote immune evasion, and facilitate tumor-induced immunosuppression [21]. Increased IDO-1 activity has been observed in a wide range of inflammatory diseases, including cancer, infections, pregnancy, and allergies. This increased activity is associated with immunosuppression caused by TRP deficiency [20, 22, 23]. Several studies have demonstrated that high expression of IDO enzyme is predictive of shortened survival in various human malignancies, including various solid tumors (lung, endometrial, colorectal, ovarian, hepatocellular and breast cancers, malignant melanoma, gynecologic, cervical, esophageal and pancreatic ductal cancers, osteosarcoma and brain cancer)[24-29]. In addition, increased IDO expression leads to increased release of kynurenine, which impairs T cell functionality and thus promotes immune evasion and tumor progression in various human cancers [30].

The ratio of KYN/TRP serves as a common indicator or representation of IDO-1 enzyme activity [10, 31]. Our findings show that there is a significant difference in the concentrations of kynurenine and the KYN/TRP ratio between patients suffering from breast cancer and the healthy control group. However, there was no significant difference in other KYN metabolites.

In summary, investigating the role of altered kynurenine and KYN/TRP ratios in patients with breast cancer is a complex and multidimensional aspect of cancer biology. KYN has been noted for its ability to hinder the proliferation of type 1 helper T cells, leading to apoptosis. Furthermore, it interferes with the function of natural killer cells, reducing their efficacy in executing their killing function [28, 32]. Elevated serum concentrations of KYN have been associated with immunosuppression, which can promote both tumor progression and immune evasion [33]. In this study, we found that serum KYN levels significantly increased, while the KYN/TRP ratio significantly decreased in patients with breast cancer compared to the control group. Elevated kynurenine levels could be a biomarker for disease progression and indicate advanced stages of breast cancer. Due to its immunomodulatory properties, kynurenine may promote the growth and spread of cancer cells by suppressing the immune system in the tumor microenvironment. Our results show that the KYN/TRP ratio between patients suffering from breast cancer and the healthy control group decreases significantly. In contrast to the predicted increased kynurenine concentrations in cancer, the decreased KYN/TRP ratio in breast cancer suggests that the enzymes IDO and TDO, which are responsible for the conversion of tryptophan to kynurenine, may be suppressed or downregulated. It is critical to recognize that the role of kynurenine may be a consequence of the presence of the tumor and its interactions with the immune system rather than a direct trigger of breast cancer. In addition, therapeutic strategies targeting kynurenine signaling pathways, such as IDO inhibitors, are showing positive efficacy in preclinical and early clinical trials, suggesting that modulation of kynurenine levels may be an important approach to improving outcomes in breast cancer patients. It is also important to recognize that the main limitation of the study is the comparatively small population sample size. Therefore, further research and clinical trials are needed to determine safety and efficacy in a broader cohort of patients.

References

1. Zárate LV, Miret NV, Nicola Candia AJ, Zappia CD, Pontillo CA, Chiappini FA, et al. Breast cancer progression and kynurenine pathway enzymes are induced by hexachlorobenzene exposure in a Her2-positive model. Food Chem Toxicol. 2023;177:113822.

2. Girithar H-N, Staats Pires A, Ahn SB, Guillemin GJ, Gluch L, Heng B. Involvement of the kynurenine pathway in breast cancer: updates on clinical research and trials. British Journal of Cancer. 2023;129(2):185-203.

3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for

clinicians. 2021;71(3):209-49.

4. Perez-Castro L, Garcia R, Venkateswaran N, Barnes S, Conacci□Sorrell M. Tryptophan and its metabolites in normal physiology and cancer etiology. The FEBS journal. 2023;290(1):7-27.

5. Comai S, Bertazzo A, Brughera M, Crotti S. Tryptophan in health and disease. Advances in clinical chemistry. 2020;95:165-218.

6. Badawy AA-B. Kynurenine pathway and human systems. Experimental Gerontology. 2020;129:110770.

7. Platten M, Nollen EA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nature reviews Drug discovery. 2019;18(5):379-401.

8. Opitz CA, Somarribas Patterson LF, Mohapatra SR, Dewi DL, Sadik A, Platten M, et al. The therapeutic potential of targeting tryptophan catabolism in cancer. British journal of cancer. 2020;122(1):30-44.

9. Lyon DE, Walter JM, Starkweather AR, Schubert CM, McCain NL. Tryptophan degradation in women with breast cancer: a pilot study. BMC research notes. 2011;4(1):1-7.

10. Lovelace MD, Varney B, Sundaram G, Lennon MJ, Lim CK, Jacobs K, et al. Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. Neuropharmacology. 2017;112:373-88.

11. Krause D, Suh H-S, Tarassishin L, Cui QL, Durafourt BA, Choi N, et al. The tryptophan metabolite 3-hydroxyanthranilic acid plays anti-inflammatory and neuroprotective roles during inflammation: role of hemeoxygenase-1. The American journal of pathology. 2011;179(3):1360-72.

12. Li H, Lockwood MB, Schlaeger JM, Liu T, Danciu OC, Doorenbos AZ. Tryptophan and Kynurenine Pathway Metabolites and Psychoneurological Symptoms Among Breast Cancer Survivors. Pain Management Nursing. 2023;24(1):52-9.

13. Wei L, Zhu S, Li M, Li F, Wei F, Liu J, et al. High indoleamine 2, 3-dioxygenase is correlated with microves el density and worse prognosis in breast cancer. Frontiers in immunology. 2018;9:724.

14. Dill EA, Dillon PM, Bullock TN, Mills AM. IDO expression in breast cancer: an assessment of 281 primary and metastatic cases with comparison to PD-L1. Modern Pathology. 2018;31(10):1513-22.

15. Ala M. The footprint of kynurenine pathway in every cancer: A new target for chemotherapy. European Journal of Pharmacology. 2021;896:173921.

16. Kwiatkowska I, Hermanowicz JM, Przybyszewska-Podstawka A, Pawlak D. Not Only Immune Escape—The Confusing Role of the TRP Metabolic Pathway in Carcinogenesis. Cancers. 2021;13(11):2667.

17. Badawy AA. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. International ournal of Tryptophan Research. 2017;10:1178646917691938.

18. D'Amato NC, Rogers TJ, Gordon MA, Greene LI, Cochrane DR, Spoelstra NS, et al. A TDO2-AhR signaling axis facilitates anoikis resistance and metastasis in triple-negative breast cancer. Cancer research. 2015;75(21):4651-64.

19. Liu Y, Feng X, Lai J, Yi W, Yang J, Du T, et al. A novel role of kynureninase in the growth control of breast cancer cells and its relationships with breast cancer. Journal of cellular and molecular medicine. 2019;23(10):6700-7.

20. Onmaz DE, Tezcan D, Abusoglu S, Sak F, Yerlikaya FH, Yilmaz S, et al. Impaired kynurenine metabolism in

patients with primary Sjögren's syndrome. Clinical Biochemistry. 2023;114:1-10.

21. Puccetti P, Fallarino F, Italiano A, Soubeyran I, MacGrogan G, Debled M, et al. Accumulation of an endogenous tryptophan-derived metabolite in colorectal and breast cancers. PloS one. 2015;10(4):e0122046.

22. Ye Z, Yue L, Shi J, Shao M, Wu T. Role of IDO and TDO in cancers and related diseases and the therapeutic implications. Journal of Cancer. 2019;10(12):2771.

23. Meireson A, Devos M, Brochez L. IDO expression in cancer: different compartment, different functionality? Frontiers in immunology. 2020;11:531491.

24. Suzuki Y, Suda T, Furuhashi K, Suzuki M, Fujie M, Hahimoto D, et al. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. Lung cancer. 2010;67(3):361-5.

25. Huang A, Fuchs D, Widner B, Glover C, Henderson D, Allen-Mersh T. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. British journal of cancer. 2002;86(11):1691-6.

26. Ino K, Yamamoto E, Shibata K, Kajiyama H, Yoshida N, Terauchi M, et al. Inverse correlation between tumoral indoleamine 2, 3-dioxygenase expression and tumor-infiltrating lymphocytes in endometrial cancer: its association with disease progression and survival. Clinical Cancer Research. 2008;14(8):2310-7.

27. Inaba T, Ino K, Kajiyama H, Yamamoto E, Shibata K, Nawa A, et al. Role of the immunosuppressive enzyme indoleamine 2, 3-dioxygenase in the progression of ovarian carcinoma. Gynecologic oncology. 2009;115(2):185-92.

28. Yu J, Sun J, Wang SE, Li H, Cao S, Cong Y, et al. Upregulated expression of indoleamine 2, 3-dioxygenase in primary breast cancer correlates with increase of infiltrated regulatory T cells in situ and lymph node metastasis. Clinical and Developmental Immunology. 2011;2011.

29. Murr C, Bergant A, Widschwendter M, Heim K, Schrocksnadel H, Fuchs D. Neopterin is an independent prognostic variable in females with breast cancer. Clinical chemistry. 1999;45(11):1998-2004.

30. Chen J-Y, Li C-F, Kuo C-C, Tsai KK, Hou M-F, Hung W-C. Cancer/stroma interplay via cyclooxygenase-2 and indoleamine 2, 3-dioxygenase promotes breast cancer progression. Breast Cancer Research. 2014;16:1-14.

31. Mandarano M, Orecchini E, Bellezza G, Vannucci J, Ludovini V, Baglivo S, et al. Kynurenine/tryptophan ratio as a potential blood-based biomarker in non-small cell lung cancer. International journal of molecular sciences. 2021;22(9):4403.

32. Wang G, Cao K, Liu K, Xue Y, Roberts AI, Li F, et al. Kynurenic acid, an IDO metabolite, controls TSG-6-mediated immunosuppression of human mesenchymal stem cells. Cell Death & Differentiation. 2018;25(7):1209-23.

33. Zhai L, Bell A, Ladomersky E, Lauing KL, Bollu L, Sosman JA, et al. Immunosuppressive IDO in cancer: mechanisms of action, animal models, and targeting strategies. Frontiers in immunology. 2020:1185.

S094

RELIABILITY OF PROTEIN ANALYSIS IN COMPLETE URINE ANALYSIS

Fikret Akyurek[1], Fatma Sengul[2]

[1] Selçuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Türkiye

[2] Adiyaman University, Faculty of Pharmacy, Department of Biochemistry, Adiyaman, Türkiye

Introduction: Complete urine analysis is an essential diagnostic tool in clinical practice, encompassing various parameters such as proteinuria, hematuria, and urinary tract abnormalities. Proteinuria, the presence of excess protein in the urine, is a significant indicator of various conditions, including chronic kidney disease (CKD), preeclampsia, and acute kidney injury (AKI) (1-3). The detection and measurement of proteinuria are essential for the early diagnosis and management of these conditions. For instance, the use of urinary albumin measurement as the front-line test for proteinuria detection has been advocated to improve sensitivity and consistency in the early detection and management of CKD [1]. Additionally, proteinuria has been associated with adverse outcomes in hospitalized patients with COVID-19, where it was significantly linked to in-hospital AKI and mortality (3).

Various methods have been developed to assess and quantify proteinuria, including immunoassays and spot urine protein to creatinine ratio measurements, which have shown clinical equivalence to traditional laboratory methods (4). However, the accuracy of dipstick urinalysis in predicting significant proteinuria has been questioned, particularly in the context of pregnancy complications (5). Additionally, the prognostic significance of proteinuria has been emphasized in various conditions, such as diabetic nephropathy and lupus nephritis, where it has been associated with disease severity and outcomes (6, 7).

Under physiological conditions, daily urinary protein excretion is typically below 150 mg. The detection of protein excretion above this level, known as proteinuria, upon repeated measurements should not be overlooked, and further evaluation should be conducted. Elevated protein excretion levels are generally indicative of underlying kidney damage (8).

The commonly used method to assess whether there is protein excretion within normal limits is the measurement of protein in a 24-hour urine sample. However, as an alternative to this method, the calculation of the ratio of spot urine creatinine to total protein is also employed. While this ratio can serve as an approximate indicator of daily protein excretion in an individual with a body surface area of 1.73 m^2, there are instances where this method does not yield accurate results (9, 10).

In routine clinical practice, the presence of proteinuria is often identified using a method called complete urinary analysis before these methods. Urinary strips are sensitive to albumin; hence, they might not adequately diagnose various diseases where the excretion of molecules other than albumin is increased (such as in some benign conditions, multiple myeloma, and diabetic patients with microalbuminuria levels) (8).

In diabetic nephropathy patients, the presence of protein in urine strips is a late sign of the disease. This is because these strips only turn positive with an excretion of albumin above 300-500 mg daily. In cases of proteinuria at lower levels, protein excretion may not be detected (11).

The quantity of urinary protein in patients with proteinuria is used to predict the severity and prognosis of kidney disease. Studies have shown that the level of proteinuria at the time of diagnosis can independently predict the prognosis of kidney disease, regardless of the underlying condition. Albumin is semi-quantitatively measured in urine strips as an early indication of kidney function. However, if there is suspicion of any loss in kidney function, confirmation through quantitative measurement methods becomes necessary (12).

In this study, our aim was to investigate whether the screening method for kidney function aligns with the common standard method.

Methods: This study is a retrospective study. The results of 3739 patients, including complete urine analysis and 24-hour urinary protein analysis, were included in the study. Complete urine analyses were performed using original strips on the DRIUI FUS 200 (China) device. The negativity, trace, +1 (<30 mg/dl), +2 (30-100 mg/dl), +3 (100-300 mg/dl), +4 (>2000 mg/dl) readings in protein measurement with strips correspond to the concentrations provided alongside them. Among these values, individuals with negative and trace in dipstick protein results were classified as “0”, those with +1 positivity and +2 positivity were classified as “1’” and those with +3 to +4 positivity were

classified as "3". The cut-off value used for the TIT albumin test is 30 mg/dl. 24-hour urinary proteins were analyzed using original kits on the Roche Cobas 702 (USA) device through turbidimetric method. Quantitative protein measurement was based on 24-hour urine values. In the quantitative method, the 24-hour urine protein amount was categorized as "0" for those with 150 mg/dl, "1" for 150-300 mg/dl, and "2" for those with >300 mg/dl. Analyses were conducted according to these specified groups. Quantitative method evaluated in this study exhibited acceptable imprecision (CV<1.9%).

Statistical analyses were conducted using R version 4.1.2 (The R Foundation for Statistical Computing, www.r-project.org) in Austria, Vienna. The second-degree weighted Cohen's kappa (κ) value was calculated to assess the agreement in diagnosis between the screening test and the common standard method. Kappa scores were interpreted as follows: <0.40 indicating poor agreement between methods, 0.41-0.75 indicating moderate to good agreement, and >0.75 indicating excellent agreement.

Results: In this retrospectively designed study, the urine protein results of the patients were retrieved from the laboratory information system. According to the patients' strip protein results, the number of individuals in each group was as follows: n=2367 for negative, n=366 for trace, n=475 for +1 positive, n=408 for +2 positive, n=123 for +3 positive, and n=3 for +4 positive. According to the protein results from the turbidimetric method, the number of individuals in each group was: n=1437 for <150 mg/dl, n=948 for 150-300 mg/dl, and n=1354 for >300 mg/dl.

The weighted κ value for categorizing proteinuria patients into three categories based on the severity of proteinuria, as presented in the table, was an average of 0.33 (95% CI, 0.31 – 0.35) (Table 1). This value indicates a weak correlation among the methods of classifying patients.

Table 1: Comparison of the common standard method with the screening method.

	Common standard method			Total
Screening method	**0**	**1**	**2**	
0	1370	867	496	2733
1	59	80	744	883
2	8	1	114	123
Total	1437	948	1354	3739

According to the common standard method, the positive predictive value of the screening test was 93.3% and the negative predictive value was 50.1%. The specificity and sensitivity of the screening test were 95.3% and 40.8%, respectively.

Discussion: Proteinuria, the presence of an abnormal amount of protein in the urine, is a significant indicator of various health conditions, particularly kidney diseases. The diagnostic evaluation of proteinuria often involves the use of urine analysis methods, including dipstick testing, automated strip analysis, and other laboratory techniques (12). The fundamental dipstick technique stands as the quickest initial screening process, aiding in the early identification of kidney or urinary tract ailments in seemingly healthy or symptom-free individuals, aiming to prevent and slow the advancement toward CKD (13).

The common method utilized to evaluate proteinuria involves quantitatively measuring the protein concentration in a 24-hour urine sample. This method accommodates variations in protein levels throughout the day and day-to-day. Nonetheless, it is laborious and burdensome for patients and occasionally prone to inaccuracies that could notably impact its precision (14). Studies by Park et al. (15) and Lim et al. (16) have evaluated the diagnostic accuracy of urine reagent strips and dipstick testing for proteinuria, particularly in the context of chronic kidney disease. These

studies have highlighted the utility of dipstick testing and reagent strips in detecting proteinuria and related urinary abnormalities, particularly in resource-limited settings. In addition, studies by Lee et al. (17) and Kim et al. (18) have investigated the associations between dipstick proteinuria and the risk of cerebral infarction, coronary heart disease, and hypertensive crisis, highlighting the clinical relevance of dipstick testing for proteinuria in predicting long-term health outcomes. In summary, as indicated by various studies, the urine dipstick testing proves to be a valuable tool for the initial screening and assessment of proteinuria in both clinical and research settings. However, particularly in cases of proteinuria, confirmation through a turbidimetric method is also crucial.

The comparison of reactive strip devices with turbidimetric methods for detecting proteinuria is an interesting topic in the literature. According to studies, the role of reactive strip devices in detecting and assessing proteinuria is uncertain (1, 19). However, it is emphasized that it should be expressed as the total protein:creatinine ratio or albumin:creatinine ratio (20).

According to the study data, the results of the screening test can be considered successful in detecting healthy individuals. However, it has been observed that it does not adequately identify individuals with proteinuria or those at risk of kidney damage. This leads to an increase in false negative rates. The high false negativity might be attributed to the urinary strips' sensitivity to albumin, which may not adequately detect proteinuria related to other proteins. Additionally, there might be a possibility that the method used has low sensitivity.

We believe that the ability of urine dipstick testing in identifying patients with proteinuria is insufficient for early diagnosis of kidney failure. For routine monitoring of patients at risk for kidney diseases, we think that monitoring with spot urine protein analysis would be more effective than analyses conducted using urine dipsticks. Spot urine analysis, like complete urine analysis, is inexpensive, easy, and non-invasive. Additionally, we believe that periodic 24-hour urine protein analyses (every 6 months to 1 year) would also be beneficial. There is a strong correlation between the spot urine protein-to-creatinine ratio and the protein concentration in a 24-hour urine collection. The spot urine protein-to-creatinine ratio is a rapid and reliable test that eliminates the need for a 24-hour urine collection. In cases of suspicion, assessing proteinuria based on the protein-to-creatinine ratio, supported by evaluating the protein concentration in a 24-hour urine collection, would be appropriate to make decisions (14).

Limitations: The lack of demographic data, additional comorbidities, and information on medication usage among the patients is a limitation of this study. This retrospectively designed study requires support from prospective studies.

Conclusions: In conclusion, complete urine analysis, particularly the assessment of proteinuria, plays a critical role in the diagnosis, management, and prognostication of various medical conditions. The evolving understanding of proteinuria's clinical significance and the development of accurate measurement methods underscore its importance in clinical practice.

References

1. Lamb EJ, MacKenzie F, Stevens PE. How should proteinuria be detected and measured? Ann Clin Biochem. 2009;46(Pt 3):205-17.

2. Fishel Bartal M, Lindheimer MD, Sibai BM. Proteinuria during pregnancy: definition, pathophysiology, methodology, and clinical significance. Am J Obstet Gynecol. 2022;226(2s):S819-s34.

3. Chaudhri I, Moffitt R, Taub E, Annadi RR, Hoai M, Bolotova O, et al. Association of Proteinuria and Hematuria with Acute Kidney Injury and Mortality in Hospitalized Patients with COVID-19. Kidney Blood Press Res. 2020;45(6):1018-32.

4. Haigh WB, Guralski DM, Arrigo LM, Letsinger JT, Clarke M, MacIsaac RJ, et al. Development of a novel polyclonal antibody-based immunoassay for the quantitation of non-albumin urinary proteins. The Open Clinical

Chemistry Journal. 2013;6(1).

5. Waugh JJ, Clark TJ, Divakaran TG, Khan KS, Kilby MD. Accuracy of urinalysis dipstick techniques in predicting significant proteinuria in pregnancy. Obstetrics & Gynecology. 2004;103(4):769-77.

6. Engli KA, Handono K, Eko MH, Susianti H, Gunawan A, Kalim H. Proteinuria Severity in Lupus Nephritis is Associated with Anti-dsDNA Level and Immune Complex Deposit Location in Kidney. Journal of tropical life science. 2018;8(3).

7. Okada T, Nagao T, Matsumoto H, Nagaoka Y, Wada T, Nakao T. Clinical significance of microscopic haematuria in diabetic nephropathy in type 2 diabetes patients with overt proteinuria. Nephrology. 2013;18(8):563-8.

8. Barratt J, Topham P. Urine proteomics: the present and future of measuring urinary protein components in disease. Cmaj. 2007;177(4):361-8.

9. Cengiz ÖK, Atmış V. The Correlation Between Total Protein Level in 24-hour Urine Sample and Spot Urine Protein-to-creatinine Ratio in the Old Aged. European Journal of Geriatrics & Gerontology. 2021;3(3).

10. Teo BW, Loh PT, Wong WK, Ho PJ, Choi KP, Toh QC, et al. Spot urine estimations are equivalent to 24-hour urine assessments of urine protein excretion for predicting clinical outcomes. International journal of nephrology. 2015;2015.

11. Bakris GL. Microalbuminuria: what is it? Why is it important? What should be done about it? The Journal of Clinical Hypertension. 2001;3(2):99-102.

12. Aitekenov S, Gaipov A, Bukasov R. Detection and quantification of proteins in human urine. Talanta. 2021;223:121718.

13. Hajar F, Taleb M, Aoun B, Shatila A. Dipstick urine analysis screening among asymptomatic school children. N Am J Med Sci. 2011;3(4):179-84.

14. Kamińska J, Dymicka-Piekarska V, Tomaszewska J, Matowicka-Karna J, Koper-Lenkiewicz OM. Diagnostic utility of protein to creatinine ratio (P/C ratio) in spot urine sample within routine clinical practice. Critical Reviews in Clinical Laboratory Sciences. 2020;57(5):345-64.

15. Park JI, Baek H, Kim BR, Jung HH. Comparison of urine dipstick and albumin:creatinine ratio for chronic kidney disease screening: A population-based study. PLoS One. 2017;12(2):e0171106.

16. Lim D, Lee DY, Cho SH, Kim OZ, Cho SW, An SK, et al. Diagnostic accuracy of urine dipstick for proteinuria in older outpatients. Kidney Res Clin Pract. 2014;33(4):199-203.

17. Lee SM, Ha E, Ryoo JH. Urine Protein Levels Predict Future Development of Cerebral Infarction in Koreans. Yonsei Med J. 2022;63(1):66-71.

18. Kim BS, Yu MY, Park JK, Shin J, Shin JH. Association of Dipstick Proteinuria with Long-Term Mortality among Patients with Hypertensive Crisis in the Emergency Department. J Pers Med. 2022;12(6).

19. Collier G, Greenan MC, Brady JJ, Murray B, Cunningham SK. A study of the relationship between albuminuria, proteinuria and urinary reagent strips. Ann Clin Biochem. 2009;46(Pt 3):247-9.

20. Methven S, MacGregor MS, Traynor JP, O'Reilly DSJ, Deighan CJ. Assessing proteinuria in chronic kidney disease: protein–creatinine ratio versus albumin–creatinine ratio. Nephrology Dialysis Transplantation. 2010;25(9):2991-6.

S096

DETERMINATION OF POTENTIAL DRUG TYPES FOR NERVOUS SYSTEM DISEASES USING MULTI-TASK LEARNING TECHNIQUE

Aytun Onay

Türk Hava Kurumu Üniversitesi, Yazılım Mühendisliği Bölümü, Türkiye

Introduction: Drugs can be defined as small chemical molecules that bind to a target protein and alter the behavior of the protein [1]. The early-phase virtual screening of candidate drug molecules is the method that is utilized the most frequently in the process of culling possibly undesirable molecules from a drug library [2]. A ligand should have a number of conditions in order to be a successful drug candidate [3]. The molecular attributes encompassing absorption, distribution, metabolism, and excretion (ADME) hold significant relevance within the field of drug design [4, 5]. Therapeutic molecules should be orally bioavailable and target-selective, and their toxicity level should also be reduced [6, 7].

The process of withdrawing from drugs is frequently connected with substantial issues, including severe adverse effects and even fatalities. The liver, the cardiovascular system, or a great number of other organs might be the primary sites of these effects. Between the years 1969 and 2002, there were 2.3 million cases that were attributed to the adverse effects of drugs; yet, only 75 of the 6,000 pharmaceuticals had been withdrawn from the market [8]. According to the findings, 95 different drugs were taken off the market as a result of the deaths they caused between 1950 and 2013 [9]. The occurrence of adverse drug reactions (ADRs) continues to provide a significant obstacle in contemporary healthcare [10].

The two main divisions of the nervous system are the central nervous system (CNS) [11] and the peripheral nervous system (PNS). Damage to the NS results in disorders of the nervous system such as epilepsy, Alzheimer's, Parkinson's, Huntington's, MS, stroke, and ALS [12-15]. The application of computational approaches in the pharmaceutical sector has been widely adopted. However, there exists a pressing demand for novel strategies that might enhance and streamline the process of drug discovery and development [16-18]. New techniques for drug development include AI, deep learning, machine learning, and computational chemistry [19-24]. Training an effective learner requires a significant amount of labeled data. In order to learn a more accurate learner for each task, multi-task learning (MTL) [25-28] aims to increase generalization performance by collecting important information across numerous learning tasks. The CLUS software tool was used to apply the MTL algorithm to categorize NS drugs [29-31]. CORINA Symphony program [32] was used to compute 760 molecular properties and ToxPrint chemotype descriptors for all drug molecules. ToxPrint chemotypes are comprised of a collection of chemical characteristics as well as guidelines. The calculations were performed for each individual pharmacological molecule in order to facilitate a multi-target categorization process. The dataset includes approved drugs targeting the nervous system (NSADs) and withdrawn drugs that have been removed from the market (NSWDs).

Methods:

The NS drug dataset was used for the research presented here. Using the Clus Multi Task Learning (MTL) algorithm, a total of 182 drugs (the training set) belonging to a variety of disease groups (N02, N03, N04, N05, and N06) were used to construct the multi target classification (MTC) model. The Clus used MTC and regression techniques to organize drugs into disease categories. The drugs used in this research were obtained from the databases KEGG, PubChem, and DRUGBANK.

Models of MTC were generated using the CORINA Symphony tool to calculate a set of molecular identifiers for all drug molecules. To evaluate the model created in the research, two distinct test sets, which were not part of the training set, were employed. Table 1 provides comprehensive information regarding the training and test sets.

Table 1: The number of NS drug datasets for training and test groups.

NS Drug dataset	N02	N03	N04	N05	N06	ADs	WDs
Trainig group: 182 Drug Molecules (DM)	40	32	20	45	45	152	30
Test group_1: Original Model 18 DM	5	2	1	5	5	14	4
Test group_2: Rules-Pruned Model: 19 DM	2	2	4	6	5	16	3

The databases' presentations of drug compounds were in SDF format. SDF files contain the drug molecule's name, features, atom coordinates, bond types, atoms from which they are located and saved, and other pertinent information. The collection contained a total of 219 different pharmacological compounds. 182 of these are classified as NSADs. The remaining 37 drugs are from the NSWDs group. There is a relatively small amount of NSWDs contained inside the databases. Following the completion of the computation of the descriptors, the dataset was partitioned into the training set and two independent test sets (randomly selected from the data set). Test group_1 includes 18 DM and test group_2 contains 19 DM.

Table 2 displays the Anatomical Therapeutic Chemical (ATC) classification of the dataset containing our NS drugs.

Table 2: The ATC classification of our NS drug dataset.

KEGG ATC Classification
N02 Analgesics
N03 Anti-epileptics
N04 Anti-parkinson
N05 Psycholeptics
N06 Psychoanaleptics

In this study, the heuristic strategy employed in the MTL model was variance reduction [33]. It is a technique employed to enhance the accuracy of estimations acquired through the computational model. The pruning method employed was C.4.5. Additionally, the induction order chosen for the study was depth first, and random forest was applied to the model as an ensemble method. The diagram shown in Figure 1 illustrates the structural design of our framework.

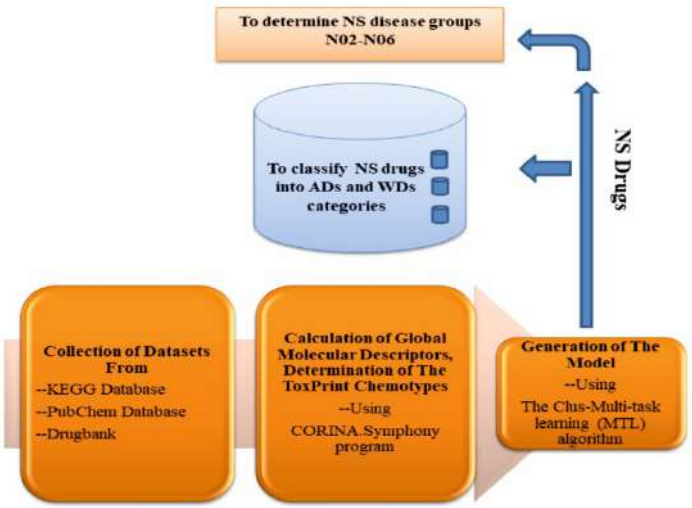


Figure 1: The architecture of the framework.

Determine the molecular characteristics

CORINA Symphony computed a total of 760 descriptors. Additionally, the system possesses a collection of 729 pre-established chemotypes that are used for the purpose of fingerprinting and profiling chemical information within the realm of toxicity. The properties calculated by the CORINA were illustrated in Figure 2.

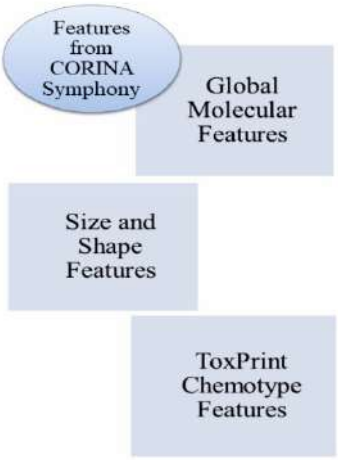


Figure 2: The features computed by the program

Data Preprocessing

The NS drug dataset needed to be cleaned, examined, and converted in order to generate computationally relevant data for each drug molecule. This was necessary because the source databases contained some information that was unrelated to the rest of the data. For this reason, preparation of the data was performed on the dataset. 760 descriptors are represented by the columns. The disease groups treated by NS drugs were categorized into multi-target classes, which were signified by the class label that corresponded to the approval or withdrawal status of the drug. For the purpose of data preprocessing, the MATLAB (R2015a) was performed [34].

The multi-task learning method

Data mining is the process of obtaining useful information from enormous datasets or databases by employing sophisticated methods and algorithms for searching. In this study, a classification model for NS drugs was developed by applying Clus to a dataset and then employing MTL methods. The system is based on a decision tree and a rule learning algorithm, and it operates within a predictive clustering framework. The categorization of the NS drugs challenge was transformed into a MTL problem. The mechanism known as MTL is an inductive transfer mechanism that primarily aims to enhance the performance of generalization. In the context of our learning problem, the first set of 760 attributes consisted of descriptive qualities intended for utilization in the formation of cluster descriptions. The latter two traits, referred known as target attributes, were intended to be predicted based on the descriptive attributes. The target attributes included disease categories and NS drugs withdrawal/approval status for forecasting purposes. Clustering = 761-762 highlighted the need for the tree-building clustering heuristics to be determined only on the basis of the target properties. The section in the settings file labeled "Tree" encompasses parameters that are specifically tailored to the process of tree learning. The selection of the heuristic for this run is guided by the principle of Variance Reduction, which suggests that among the various clustering heuristics available, the one chosen should prioritize the reduction of variance. The F-test was identified as a significant parameter utilized in the mtl_ds.s file. The F-test was employed to measure a test that effectively minimized variation. When the variance is reduced as much as possible, it leads to an increase in the cluster's homogeneity as well as an improvement in the model's ability to predict. The performance of the model created in the F-test for the classification of drug compounds was improved by 0.125. Both C4.5 and Random Forest were performed on the dataset in order to get the pruning and ensemble methods, respectively. By using the molecular descriptors, MTL was applied in order to categorize various drugs. On the dataset was partitioned into the training set and two different test set. The MTL model is created using the training set, and the test set is used to evaluate how well the model works. The training set contributes to the creation of the MTL model by altering its parameters. The measurement of classification accuracies in the context of multi-target classification was conducted. To facilitate the execution of MTL, two input files, namely mtl_ds.arff and mtl_ds.s, were prepared for MTL_DS, which represents the NS drug dataset. The file mtl_ds.s held the parameter settings, while mtl_ds.arff provided the training set. The output file were named mtl_ds.out. The training set, mtl_ds.arff, consisting of 182 drug molecules, was subjected to MTL procedure. The evaluation of the classification model involved the utilization of the weighted mean squared error (MSE) for nominal characteristics, Cramer's coefficient, and accuracy of multi-target classes. These metrics were employed to assess the quality of the model.

Results

In this particular investigation, a method of classification known as MTC was performed. The constructed model was put to use in the process of determining NS disease groups as well as categorizing drugs AW categories. The findings of the accuracy rate (AR) and Cramer's coefficient for Test group_1**:** original model were determined and presented in Table 3, and the mean squared error (MSE) of the model was 1.0207. The model's performance is evaluated using the mean squared error (MSE), which is calculated as the average over all tasks. A lower value indicates superior performance, with a value of 0 indicating perfect model performance. According to AR results, multi-target classification performed better for the dataset. These results show that the classification approach can separate ADs and WDs and identify NS disease groupings. The obtained model can be used as rudimentary drug design filters. Based on the findings presented in Table 3, the accuracy rate (AR) was seen to be 0.67 for the dataset used to identify disease groups associated with NS, and 0.83 for the dataset employed to classify NS drugs as either approved or withdrawn. The findings of the testing error were presented in Table 3, encompassing a total of 18 drugs. The statistic known as Cramer's coefficient can take on values ranging from 0 to 1 and is utilized to determine the degree of correlation that exists between two nominal variables. Values that are near to 0 suggest that the association between the variables is not very strong, whereas values that are close to 1 indicate that the association is quite strong. The values of Cramer's coefficients for the determination of NS disease groups and the separation

of ADs and WDs, respectively, were 0.65 and 0.45. In terms of nominal features, the MSE was 1.02. From Table 3, of 5 N02, 3 were correctly predicted. The method missed 2 drugs for N02 group. It classified both drugs in the N03 group correctly. It also correctly classified 4 out of 5 drugs in the N05 group. The connection of misclassified drugs with other disease groups can also be investigated. Furthermore, the MTL technique accurately classified a total of 15 drugs into their respective groups, namely ADs and WDs, but it failed to correctly classify 3 compounds from the dataset, which included WDs.

Table 3: Accuracy rate (AR), Cramer's coefficient results of the original model.

Original Model						
Accuracy: 0.67 Cramer's coefficient: 0.65						
NS Drugs						
Real\Pred	**N02**	**N03**	**N04**	**N05**	**N06**	**Total**
N02	3	0	0	1	1	5
N03	0	2	0	0	0	2
N04	0	1	0	0	0	1
N05	0	0	1	1	3	5
N06	0	1	0	4	0	5
Total	3	4	1	6	4	18
Groups:						
Accuracy: 0.83 Cramer's coefficient: 0.45						
Real\Pred	**Approved**	**With-drawn**	**Total**			
Approved	14	0	14			
With-drawn	3	1	4			
Total	17	1	18			

According to the statistics shown in Table 4, the accuracy rate (AR) was found to be 0.58 for the dataset utilized in identifying disease categories linked with NS and 0.79 for the dataset employed in classifying NS drugs as either approved or withdrawn. The results related to the testing error were displayed in Table 3, which included a comprehensive analysis of 19 different pharmaceutical compounds. Regarding the nominal features, the mean squared error (MSE) was determined to be 1.24. According to Table 4, two drugs in the N03 group were classified correctly. In the N04 group, 2 of the 4 drugs were placed in the wrong groups. 4 out of 5 drugs in the N06 group were classified correctly. In the N05 group, 3 out of 6 drugs were classified correctly.

Table 4: Accuracy rate (AR), Cramer's coefficient results of the rules-pruned model.

Rules-Pruned Model						
Accuracy: 0.58 Cramer's coefficient: 0.59						
NS Drugs						
Real\Pred	**N02**	**N03**	**N04**	**N05**	**N06**	**Total**
N02	0	1	0	0	1	2
N03	0	2	0	0	0	2
N04	1	0	2	0	1	4
N05	1	2	0	3	0	6
N06	0	0	1	0	4	5
Total	2	5	3	3	6	19
Groups:						
Accuracy: 0.79 Cramer's coefficient: 0.21						
Real\Pred	**Approved**	**With-drawn**	**Total**			
Approved	14	2	16			
With-drawn	2	1	3			
Total	16	3	19			

Furthermore, while the MTL technique accurately classified a total of 15 drugs into the correct groups, namely ADs and WDs, it misclassified 4 compounds from the dataset, which contained ADs and WDs. In the drug design phase, the models developed in the study can be employed as a simple filter. In addition, some of the 24 rules obtained from the rules pruned model are given below as examples (Figure 3). These rule sets contain important information for AW drugs using 760 molecular identifiers. The rule sets can be used in NS drug design process.

```
Rule 2:
=======
IF bondC=Oacylhydrazide <= 0 AND
   XlogP > 4.39 AND
   chainalkaneCyclicethylC2(connectnoZ)
THEN [N04,Approved] [3.0,7.0]: 7

Rule 3:
=======
IF bondC=Oacylhydrazide <= 0 AND
   XlogP > 4.39 AND
   chainalkaneCyclicethylC2(connectnoZ)
   bondCXhalidearomatic-Xgeneric > 0.0 A
   Atoms > 39.0
THEN [N05,Approved] [5.0,6.0]: 6

Rule 4:
=======
IF bondC=Oacylhydrazide <= 0 AND
   XlogP > 4.39 AND
   chainalkaneCyclicethylC2(connectnoZ)
   bondCXhalidearomatic-Xgeneric > 0.0 A
   Atoms <= 39
THEN [N05,Withdrawn] [3.0,3.0]: 3
```

Figure 3: Rule sets obtained from the rules pruned model.

Discussion

The present study employed a MTL algorithm to analyze the NS drug dataset and proposed a highly efficient MTC approach for the computer-aided drug design based on their associated disease group. The classifier's performances were assessed on the dataset, revealing high classification accuracies. NS drugs that have been incorrectly classified as belonging to a different class by the created model have the potential to cure not just the target disease but also another condition. These drugs and their impact on diseases are also able to be investigated. In the literature, Yu et al. created simple central nervous system drug classification rules using a hybrid ensemble model. The model was developed using support vector machine and graph convolutional network methods on 940 market drugs, 315 CNS-active, and 625 CNS-inactive. Model accuracy was 0.96, and the F1 score was 0.95 [35]. Compared to their model, the MTC model is also capable of classifying potential NS drugs compounds into specific disease groups. Yosipof et al. developed the t-Distributed Stochastic Neighbor Embedding technique and applied other machine learning approaches to create a novel ensemble learning method named AL Boost. This method aims to effectively distinguish between drugs and nondrugs. Effective models were developed for drug categories such as antineoplastic compounds, cardiovascular system and nervous system drugs. The outcome was an improved classification rate above 0.81 [36]. In our study, NS candidate drug molecules can be classified according to Kegg ATC. Additionally, Zhao et al outperformed more established and well-known machine learning algorithms with their MTL-based QSAR models, which they used to predict active compounds [37].

The results of this study indicate that applying a MTL approach with molecular descriptors is more effective in classifying NS drugs into approved and withdrawn categories. During the process of developing new drugs, the model that was built can serve as a simple filter.

Acknowledgments: We are deeply thankful to Molecular Networks GmbH Computerchemie for CORINA Symphony program.

Research Funding: None declared.

Author contribution: Author has accepted responsibility for the entire content of this manuscript and approved its submission.

Competing Interest: The author has no conflicts of interest.

Informed Consent: Not applicable.

Ethical Approval: Not applicable.

References

1. Wang Y, Xing J, Xu Y, Zhou N, Peng J, Xiong Z. In silico ADME/T modelling for rational drug design. Q Rev Biophys 2015;2:1-28.

2.Reddy S, Pati S P, Potukuchi P K, Sastry G N. Virtual screening in drug discovery: a computatinal perspective. Curr Protein Pept Sci 2007;8:329-351.

3.Clark D E and Pickett S D. Computational methods for the prediction of drug-likeness. Drug Discovery Today 2000;5:49–58.

4.Ekins S, Shimada J, Chang C. Application of data mining approaches to drug delivery. Adv Drug Deliver Rev 2006;58:1409-1430.

5.Du BX, Xu Y, Yiu SM, Yu H, Shi JY. ADMET property prediction via multi-task graph learning under adaptive auxiliary task selection. iScience 2023;26.

6.Yusof I, Shah F, Hashimoto T, Segall M D, Greene N. Finding the rules for successful drug optimization. Drug

Discovery Today 2014;19:680-687.

7.Zheng M, Hu X, Yan A, Bajorath J. Computational Methods for Drug Design and Discovery : focus on China. Trends Pharmacol Sci 2013;34:549-559.

8.McNaughton R, Huet G, Shakir S. An investigation into drug products withdrawn from the EU market between 2002 and 2011 for safety reasons and the evidence used to support the decision making. BMJ Open 2014;4:4221-4226.

9.Onakpoya I J, Heneghan C J, Aronson J K. Delays in the post-marketing withdrawal of drugs to which deaths have been attributed: a systematic investigation and analysis. BMC Med 2015;26:1-11.

10.Huang SM, Lesko LJ, Temple R. Adverse Drug Reactions and Interactions. Pharmacol Ther Princ to Pract 2009;265–73.

11.Ghorbanzad'e M., Fatemi M H. Classification of central nervous system agents by least squares support vector machine based on their structural descriptors: A comparative study. Chemometr Intell Lab Syst 2012;110:102-107.

12.Chen Y, Pang J, Ye L, Zhang Z, Lin S, Lin N, et al. Disorders of the central nervous system: Insights from Notch and Nrf2 signaling. Biomed Pharmacother 2023;166: 115383

13.Nguyen TT, Dung Nguyen TT, Vo TK, Tran NMA, Nguyen MK, Van Vo T, et al. Nanotechnology-based drug delivery for central nervous system disorders. Biomed Pharmacother 2021;143:112117.

14.Chen Y, Wang H, Wang W zhi, Wang D, Skaggs K, Zhang HT. Phosphodiesterase 7(PDE7): A unique drug target for central nervous system diseases. Neuropharmacology 2021;196:108694.

15.Mozafari N, Ashrafi H, Azadi A. Targeted drug delivery systems to control neuroinflammation in central nervous system disorders. J Drug Deliv Sci Technol 2021;66:102802.

16.Cavasotto CN, Di Filippo JI. Artificial intelligence in the early stages of drug discovery. Arch Biochem Biophys 2021;698:108730.

17.Elbadawi M, Gaisford S, Basit AW. Advanced machine-learning techniques in drug discovery. Drug Discov Today 2021;26:769–77.

18.Reker D. Practical considerations for active machine learning in drug discovery. Drug Discov Today Technol 2019;32–33:73–9.

19.Parvatikar PP, Patil S, Khaparkhuntikar K, Patil S, Singh PK, Sahana R, et al. Artificial intelligence: Machine learning approach for screening large database and drug discovery. Antiviral Res 2023;220:105740.

20.Chen H, Engkvist O, Wang Y, Olivecrona M, Blaschke T. The rise of deep learning in drug discovery. Drug Discov Today 2018;23:1241–50.

21.Moingeon P, Kuenemann M, Guedj M. Artificial intelligence-enhanced drug design and development: Toward a computational precision medicine. Drug Discov Today 2022;27:215–22.

22.Vijayan RSK, Kihlberg J, Cross JB, Poongavanam V. Enhancing preclinical drug discovery with artificial intelligence. Drug Discov Today 2022;27:967–84.

23.Patel V, Shah M. Artificial intelligence and machine learning in drug discovery and development. Intell Med 2022;2:134–40.

24.Yang S, Kar S. Application of artificial intelligence and machine learning in early detection of adverse drug reactions (ADRs) and drug-induced toxicity. Artif Intell Chem 2023;1:100011.

25.Zhang Y and Yang Q. An overview of multi-task learning. National Science Review 2018;5:30-43.

26.Caruana R. Multitask Learning. Machine Learning, 1997;28:41-75.

27.Zhao Z, Qin J, Gou Z, Zhang Y, Yang Y. Multi-task learning models for predicting active compounds. J Biomed Inform 2020;108:103484.

28.Ye S, Zhao W, Shen X, Jiang X, He T. An effective multi-task learning framework for drug repurposing based on graph representation learning. Methods 2023;218:48–56.

29.Struyf J, Zenko B, Blockeel H, Vens C, Dzeroski S. Clus: User's Manual 2011:1-31.

30.Kocev D, Vens C, Struyf J, and Dzeroski S. Ensembles of multi-objective decision trees. In Proceedings of the 18th European Conference on Machine Learning, Springer 2007:624-631.

31.Ross Quinlan J. C4.5: Programs for Machine Learning. Morgan Kaufmann, San Francisco, CA, USA;1993.

32. CORINA Symphony program; 2023. Available from: https://mn-am.com/products/corinasymphony/

33. Marquet T, Oswald E. A Comparison of Multi-task Learning and Single-Task Learning Approaches. Lect Notes Comput Sci (including Subser Lect Notes Artif Intell Lect Notes Bioinformatics). 2023;13907:121–38.

34.MathWorks: Math. Graphics. Programming; 2023. Available from: https://www.mathworks.com/products/matlab.html

35. Yu TH, Su BH, Battalora LC, Liu S, Tseng YJ. Ensemble modeling with machine learning and deep learning to provide interpretable generalized rules for classifying CNS drugs with high prediction power. Brief Bioinform. 2022;23:1–13.

36 .Yosipof A, Guedes RC, García-Sosa AT. Data mining and machine learning models for predicting drug likeness and their disease or organ category. Front Chem. 2018;6:1–11.

37. Zhao Z, Qin J, Gou Z, Zhang Y, Yang Y. Multi-task learning models for predicting active compounds. J Biomed Inform. 2020;108:103484.

S117

COMPARISON OF ADVANCED GLYCATION END PRODUCT (AGE) AND ZINC LEVELS IN PATIENTS WITH DIABETES MELLITUS AND DIABETIC NEPHROPATHY

Alev Kural[1], Nazlı Helvacı[2], Özgür Can[3], Kürşad Nuri Baydili[4]

[1] Health Sciences University Bakırköy Dr. Sadi Konuk Teaching and Research Hospital Medical Biochemistry Department

[2] Health Sciences University Hamidiye School of Medicine Medical Biochemistry Department

[3] Health Sciences University Haydarpaşa Numune Teaching and Research Hospital Nephrology Departmen

[4] Health Sciences University Hamidiye School of Medicine Biostatistics and Medical Informatics Department

Introduction: A category of metabolic disorders, identified as diabetes mellitus (DM), is characterised by hyperglycaemia resulting from abnormal insulin production, activity or both. DM is linked to persistent hyperglycemia, which can lead to organ dysfunction and long-term damage, particularly to the kidneys, nerves, eyes, heart, and blood vessels [1].

Diabetic nephropathy (DN) is the primary cause of end-stage renal disease globally [1, 3], one of the most prevalent significant consequences of DM, and is related with mortality and morbidity among DM patients [4-6]. DN is one of the main long-term diabetic microangiopathies [1, 3].

The development of DN is mediated by lipids, lipoproteins, antioxidant enzymes, and advanced glycation end products (AGEs) [7].

AGEs are long-lived chemical intermediates formed when chemically reactive sugars or reactive oxoaldehyde react with lipids, proteins and nucleic acids through a non-enzymatic process [8, 9]. Given the essential role of the kidney in the clearance and reabsorption of AGEs from the circulation, increased accumulation of AGEs may increase renal dysfunction and DM-related kidney disease [9].

DN has been linked to oxidative stress. Antioxidant deficit can be the result of a lack of vitamins such as E and C, or by a decrease in the production of enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) owing to zinc (Zn) insufficiency [10]. Inadequate dietary Zn intake and low serum Zn levels have been related to an increased risk of cardiovascular disease, DM and DN. Although many factors play a role in the development of DN, inadequate Zn intake also appears to contribute to DM-related kidney damage [11]. Given the potential modulatory effects of Zn on antioxidant activities, increased Zn status in people with diabetes may help counterbalance the negative effects of oxidative stress and assist avoid DM-related problems [10].

Serum Zn levels in individuals with DM and DN may be regarded medically important and can be utilized as a nephropathy detection marker when combined with other nephropathy detection indicators. The relationship between AGE and Zn levels in DM and DN patient groups has not been clarified. In this study, we aimed to investigate the relationship between AGE and Zn levels in DM and DN patient groups and to compare these levels with healthy individuals.

Methods:

Study Population: After agreement from the institutional ethics committee (University of Health Sciences Hamidiye Scientific Research Ethics Committee, Date of Approval: 11.08.2023, Decision No:15/31, Registration No: 23/477), the samples from volunteers and patients for this prospective interventional study was collected over a 2-month period in the University of Health Sciences Haydarpasa Numune Training and Research Hospital and performed at University of Health Sciences Hamidiye School of Medicine, Medical Biochemistry Department. During

the research time frame, data were collected from 27 healthy volunteers, 37 patients with DM, and 46 patients with DN whose overall age range is from 18 to 75 years. Demographic data were collected on inclusion.

AGE Measurements: Serum were diluted 1:50 with phosphate-buffered saline (PBS; pH 7.4). Fluorescence intensity emission maximum was recorded as 485 nm and excitation maximum was recorded as 360 nm (spectrofluorimeter Biorad). Fluorescence intensity was expressed in polyethylene-bound units (AU) protein species [12].

Zn Measurements: In alkaline solution, Nitro-PAPS interacts with Zinc to generate a purple complex with an absorbance of 575 nm. İron and copper interference are substantially reduced by pH and chelating additions [13].

Serum samples underwent treatment with reagent 1 (R1) and reagent 2 (R2) respectively, followed by measurements conducted using atomic absorption spectrophotometry (AAS).

R1: Borate buffer 370 mM pH 8.20, salicylaldoxime 12.5mM, dimethylglyoxime 1.25 mM, surfactants and preservatives.

R2: Nitro-PAPS 0.40 mM.

Statistical analysis: Statistical analysis of the study employed IBM SPSS (Statistical Package for Social Sciences) Statistics 26.0 software. Descriptive statistical methods, including mean, standard deviation, and minimum-maximum, were utilized for data evaluation. Normality of data distribution was assessed using the Shapiro-Wilk test. In cases of non-normal distribution, the Mann Whitney-U test was performed to compare two variables. The distinctiveness between the patient and control groups was determined via the implementation of Pearson's Chi-Square test. The results were then analysed based on a significance level of $p<0.05$, alongside a 95% confidence interval.

Results: HbA1c levels exhibited significant increase in the DM and DN groups as opposed to the healthy group ($p<0.001$) (Figure 1). Creatinine values were notably higher in DN group when compared to the other 2 groups ($p<0.001$) (Figure 1, Table 1).

Additionally, serum AGE values revealed notable elevation in the DN group when compared to the healthy group ($p=0.012$) (Figure 2), while Zn values showed significant increase in the healthy group as against the DM and DN groups ($p=0.039$) (Figure 2, Table 1).

Based on these results, there is a negative correlation between AGE and Zn values. Additionally, Zn values were significantly higher in the healthy group, whereas AGE values were higher in the DM and DN groups compared to the healthy group.

Table 1: HbA1c, creatinine, AGE and Zn values for DM, DN and control groups

	Healthy Group	DM	DN	p
Age	31,5 (20-63)	55 (20-77)	64 (30-78)	<0,001*
HbA1c (%)	5,3 (4,8-5,8)	7,1 (5,4-10,9)	6,8 (5,3-11,3)	<0,001*
Creatinine (mg/dL)	0,64 (0,57-0,85)	0,69 (0,44-1,05)	1,34 (0,68-5,86)	<0,001*
AGE (AU)	17 (3-27)	18 (8-28)	20,5 (10-32)	0,012*
ZN (µg/dL)	60 (34-118)	53,5 (23-90)	52 (6-99)	0,039*

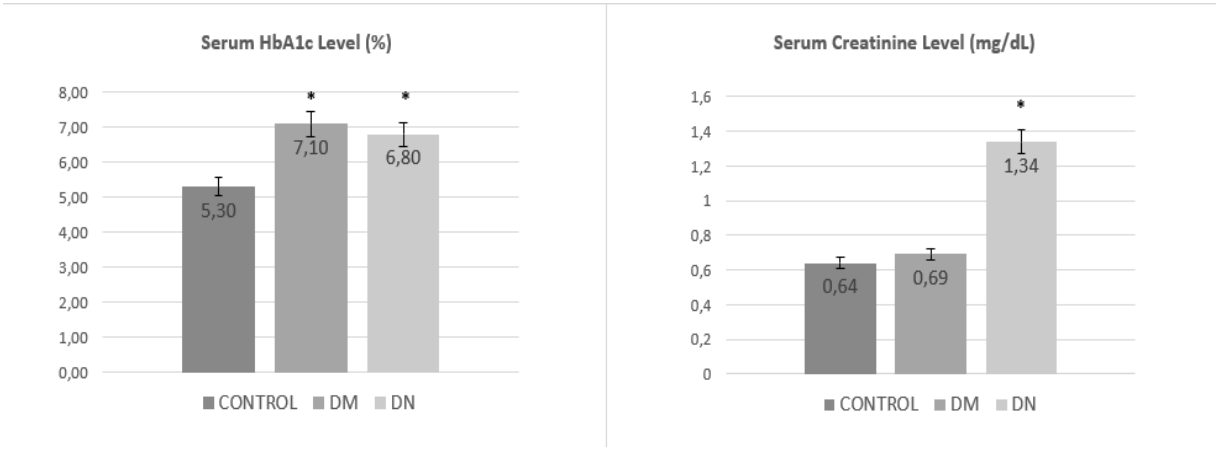


Figure 1: Serum HbA1c and creatinine levels for DM, DN and control groups. *Statistically significant compared with the control group, $p<0.05$.

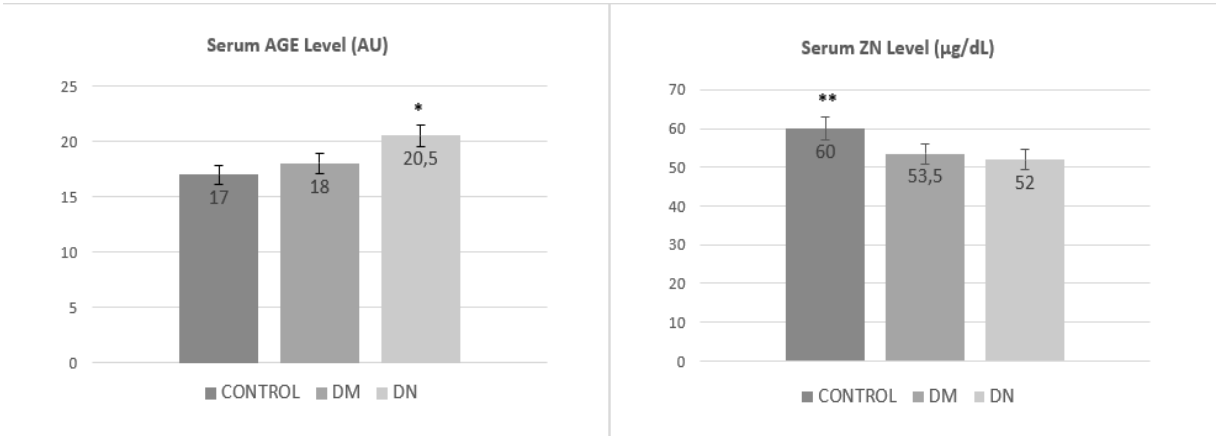


Figure 2: Serum AGE and Zn levels for DM, DN and control groups. *Statistically significant compared with the control group, $p<0.05$. **Statistically significant compared with the DM and DN patient group, $p<0.05$.

Discussion: DM is a condition resulting from irregular glucose levels or insufficient tolerance in conjunction with an inadequate insulin reaction [14]. Microangiopathy, which can proceed to DN, is one of the most critical clinical characteristics of DM. DN affects over a third of individuals with type 1 and type 2 DM and is associated with considerable mortality and morbidity [15].

AGEs have been linked to the pathogenesis of several chronic illnesses, such as diabetes, kidney disease, and neurodegenerative disorders. It is essential to note that AGEs are not exclusively derived from dietary sources but undergo endogenous production as well. These findings suggest a potential role for AGEs as a therapeutic target in the treatment of various pathologies [16]. Many research have been conducted to investigate the impact of AGEs on cell dysfunction and their role in the complications and development of DM [17, 18]. Studies have indicated a correlation between the rise in AGE concentration and the occurrence of DM.

The data from this study in diabetic patients also support the literature. In our study, AGE levels were found to be significantly higher in DN subjects compared to DM and healthy subjects, and higher in DM subjects compared to healthy subjects. The course of the disease was positively correlated with AGE levels, as well as creatinine levels. An increase in creatinine levels was observed to coincide with an increase in AGE levels. Furthermore, no significant correlation was found between AGE levels and HbA1c levels.

A study demonstrated a correlation between the level of serum AGE and the type of DM. Serum AGE levels were elevated in comparison to type 1 DM patients among type 2 DM patients as found by this study [8]. Another study by Koyama et al. showed a positive correlation between AGE fluorescence results and uric acid levels in serum samples of patients [19]. These findings suggest that AGEs contribute to the onset of microangiopathic complications in DM and thus DN.

DN is a global public health issue with a rising incidence. Zn concentration has been shown to be reduced in DN patients. Why Zn concentrations are reduced in DN patients has not yet been clearly explained, but several factors may be implicated. Examples include inadequate dietary intake, poor gastrointestinal absorption or phosphate-binding drugs [20, 21].

The study's data revealed significantly lower Zn values in the DM and DN patient group compared to the healthy group. Additionally, a negative correlation was observed between Zn values and creatinine and AGE values. These findings suggest that the disease progresses, AGE and creatinine values increase while renal function decreases and Zn levels decrease.

A substantial relationship was discovered between decreased renal function and lower Zn concentrations in DN patients, suggesting that another reason for decreased Zn concentrations is increased urinary Zn excretion [22].

The mechanism involving Zn concentrations in decreased renal function and thus reduced glomerular filtration rate (GFR) is still unclear. Some study results have found that Zn has a key role in lowering oxidative stress, which is thought to be responsible for decreased renal function [23].

Another pathogenic mechanism that causes the onset of DN is the stress exerted by reactive oxygen species (ROS) in the kidney (Figure 3) [24-26]. According to a research conducted by Ozcelik et al., in diabetic rats treated with zinc sulfate, it was observed that metallothionein decreased ROS through Zn-mediated activation, thus reducing the amount of kidney damage [24].

In addition to metallothionein upregulation, nuclear factor-erythroid 2-related factor 2 (Nrf2) also plays a role in the antioxidant mechanism of Zn. Nrf2 is an intracellular antioxidant that plays an important role in defense against glutathione S-transferase, SOD and other agents of neutralization that activate ROS [26]. In one research, Nrf2 discovered that Zn protects diabetic rats. The results were linked to the down-regulation of superoxide dismutase 1 (S0D1), superoxide dismutase 2 (SOD2) and additional proteins associated with oxidative stress [27].

Another mechanism by which Zn protects the kidney in DM is apoptosis [28, 29]. In the study conducted by Zhang et al., it was shown that Zn reduced apoptosis by inhibiting the passage of cytochrome-C, caspase 3 and caspase 9 from the mitochondria to the cytosol [28].

In another study by Zhou et al, diabetic rats treated with Zn supplementation showed a decrease in microalbuminuria and glomerular damage levels along with a decrease in blood glucose levels [30]. In another study in which Zn supplementation was administered, it was observed that renal metallothioneins were up-regulated with a decrease in 24-hour albuminuria levels [31, 32].

Considering the antioxidant effects of Zn, corrected Zn concentrations in DM and DN patients may eliminate the damaging effects of oxidative stress and may also eliminate advanced complications of diabetes. Therefore, Zn supplementation may be beneficial in the near future as a preventive agent in the development of advanced disease complications in DM patients, while in DN patients it may be beneficial in restoring renal function and reducing symptoms related to disease complications.

References

1. Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int 2021;8:1497449.

2.Valencia WM, Florez H. How to prevent the microvascular complications of type 2 diabetes beyond glucose control. BMJ 2017;1:17;356:i6505.

3. Nagib AM, Elsayed Matter Y, Gheith OA, Refaie AF, Othman NF, Al-Otaibi T. Diabetic Nephropathy Following Posttransplant Diabetes Mellitus. Exp Clin Transplant 2019;4:17(2):138-146.

4. Long AN, Dagogo-Jack S. Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. J Clin Hypertens 2011;4:13(4):244–251.

5. Maggiore U, Budde K, Heemann U, Hilbrands L, Oberbauer R, Oniscu GC, et al. Long-term risks of kidney living donation: review and position paper by the ERA-EDTA DESCARTES working group. Nephrol Dial Transplant 2017;2:1;32(2):216-223.

6. Thipsawat S. Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature. Diab Vasc Dis Res 2021;11-12:18(6):14791641211058856.

7. Butt N, Bano F, Ghani M, Ahmed AM, Majeed N. Association of serum advanced glycation (AGEs) end products, apolipoprotein-B and zinc in severity of T2DM retinopathy. Pak J Pharm Sci 2021;3:34(2 Suppl):803-808.

8. Indyk D, Bronowicka-Szydełko A, Gamian A, Kuzan A. Advanced glycation end products and their receptors in serum of patients with type 2 diabetes. Sci Rep 2021;6: 24;11(1):13264.

9. Koska J, Gerstein HC, Beisswenger PJ, Reaven PD. Advanced Glycation End Products Predict Loss of Renal Function and High-Risk Chronic Kidney Disease in Type 2 Diabetes. Diabetes Care 2022;3:1;45(3):684-691.

10. Al-Timimi DJ, Sulieman DM, Hussen KR. Zinc status in type 2 diabetic patients: relation to the progression of diabetic nephropathy. J Clin Diagn Res 2014;11:8(11):CC04-8.

11. Parham M, Amini M, Aminorroaya A, Heidarian E. Effect of zinc supplementation on microalbuminuria in patients with type 2 diabetes: a double blind, randomized, placebo-controlled, cross-over trial. Rev Diabet Stud. 2008 Summer;5(2):102-9.

12. Basınoğlu F, Kural A, Aydan Sağlam Z, Koldaş M, Döventaş Y, Seval H, et al. The Relation Between Fib ronectin Levels and Oxidation Glycosylatıon End Products in Type 1 Diabetic Patients. Erciyes Medical Journal 2010; 32(2), 81-86.

13. Makino T. A sensitive, direct colorimetric assay of serum zinc using nitro-PAPS and microwell plates. Clin Chim Acta 1991;3:29;197(3):209-20.

14. Zhou Y, Chi J, Lv W, Wang Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19) Diabetes Metab Res Rev 2021;2:37(2):e3377.

15. Bakris GL. Recognition, pathogenesis and treatment of different stages of nephropathy in patients with type 2 diabetes mellitus. Mayo Clin Proc 2011;5:86(5):444–56.

16. Sergi D, Boulestin H, Campbell FM, Williams LM. The role of dietary advanced glycation end products in metabolic dysfunction. Mol Nutr Food Res. 2021;1:65(1):e1900934.

17. Saulnier PJ, Wheelock KM, Howell S, Weil EJ, Tanamas SK, Knowler WC, et al. Advanced glycation end products predict loss of renal function and correlate with lesions of diabetic kidney disease in American indians with type 2 diabetes. Diabetes 2016;12:65:3744–3753.

18. Cohen MP, Ziyadeh FN, Chen S. Amadori-modified glycated serum proteins and accelerated atherosclerosis in diabetes: Pathogenic and therapeutic implications. J Lab Clin Med 2006;5:147:211–219.

19. Koyama Y, Takeishi Y, Arimoto T, Niizeki T, Shishido T, Takahashi H, et al. High serum level of pentosidine, an advanced glycation end product (AGE), is a risk factor of patients with heart failure. J Card Fail 2007;4:13:199–206.

20. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The Physiological, Biochemical, and Molecular Roles of Zinc Transporters in Zinc Homeostasis and Metabolism. Physiol Rev 2015;6:95:749–784.

21. Takagi K, Masuda K, Yamazaki M, Kiyohara C, Itoh S, Wasaki M, et al. Metal ion and vitamin adsorption profiles of phosphate binder ion-exchange resins. Clin. Nephrol. 2010;1:73:30–35.

22. Damianaki K, Lourenco JM, Braconnier P, Ghobril JP, Devuyst O, Burnier M, et al. Renal handling of zinc in chronic kidney disease patients and the role of circulating zinc levels in renal function decline. Nephrol Dial Transplant 2020;7:35:1163–1170.

23. Li MS, Adesina SE, Ellis CL, Gooch JL, Hoover RS, Williams CR. NADPH oxidase-2 mediates zinc deficiency-induced oxidative stress and kidney damage. Am J Physiol Cell Physiol 2017;1:312:C47–C55.

24. Özcelik D, Nazıroglu M, Tunçdemir M, Çelik Ö, Öztürk M, Flores-Arce MF. Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats. Biol Trace Elem Res 2012;12:150:342–349.

25. Pan HZ, Zhang L, Guo MY, Sui H, Li H, Wu WH, et al. The oxidative stress status in diabetes mellitus and diabetic nephropathy. Acta Diabetol 2010;12:47: (1 Suppl):71–76.

26. Apostolova MD, Choo KH, Michalska AE, Tohyama C. Analysis of the possible protective role of metallothionein in streptozotocin-induced diabetes using metallothionein-null mice. J Trace Elem Med Biol 1997;4:11:1–7.

27. Yang F, Li B, Dong X, Cui W, Luo P. The beneficial effects of zinc on diabetes-induced kidney damage in murine rodent model of type 1 diabetes mellitus. J Trace Elem Med Biol 2017;6:42:1–10.

28. Zhang X, Zhao Y, Chu Q, Wang ZY, Li H, Chi ZH. Zinc modulates high glucose-induced apoptosis by suppressing oxidative stress in renal tubular epithelial cells. Biol Trace Elem Res 2014;5:158:259–267.

29. Wang S, Nie P, Lu X, Li C, Dong X, Yang F, et al. Nrf2 participates in the anti-apoptotic role of zinc in Type 2 diabetic nephropathy through Wnt/β-catenin signaling pathway. J Nutr Biochem 2020;10:84:108451.

30. Zhou Z, Sun X, Kang YJ. Metallothionein protection against alcoholic liver injury through inhibition of oxidative stress. Exp Biol Med 2002;3:227:214–222.

31. Tang Y, Yang Q, Lu J, Zhang X, Suen D, Tan Y, et al. Zinc supplementation partially prevents renal pathological changes in diabetic rats. J Nutr Biochem 2010;3:21:237–246.

32. Gembillo G, Visconti L, Giuffrida AE, Labbozzetta V, Peritore L, Lipari A, et al. Role of Zinc in Diabetic Kidney Disease. Nutrients 2022;3:24;14(7):1353.

S120

GOJIBERRY POTENTIATES THE EFFECT OF L-CARNITINE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA IN VITRO

Mine Ergüven[1], Haniyeh Behrouzizad[2], Esin Çetin Aktaş[3], Ayhan Bilir[4]

[1] İstanbul Aydın University, Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Türkiye

[2] İstanbul Aydın University, Institute of Graduate Studies, Food Safety and Nutrition Program, İstanbul, Türkiye

[3] İstanbul University, As Detae, Department of Immunology, İstanbul, Türkiye

[4] Atlas University, Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye

Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm associated with the presence of the BCR activator of RhoGEF and GTPase-ABL proto-oncogene 1/non-receptor tyrosine kinase (BCR-ABL1) fusion gene [1,2]. CML is detected in approximately one in four of the leukemias and occurs in 1 or 2 out of every 100.000 people. The BCR-ABL1 fusion gene results in an abnormal protein called Bcr-Abl1 which is detected as the cause of CML formation. As a result of this gene-protein signal cascade, uncontrolled proliferation of abnormal white blood cells that can not function is observed [1,2]. BCR-ABL gene and protein signaling system resists intrinsic and extrinsic apoptotic pathways by triggering the pathways as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), nuclear factor kappa B (NF-κB) and increasing the expressions of antiapoptotic proteins (Bcl.. etc.) which all have a role in the cell proliferation and growth by the inhibition of an extrinsic apoptosis inducer tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [2,3].

Although CML is sensitive to chemotherapy based on tyrosine kinase inhibitors such as imatinib mesylate and the treatments are generally successful, the resistance mechanisms developed by the cell have required innovative anti-cancer combinations and new anti-neoplastic drugs [3-5]. High concentrations of drugs or drug combinations to achieve plasma and cell therapeutic drug concentrations lead to high toxicity and many patients lose their lives from the side effects of chemotherapy, not from cancer itself [6]. Today, many clinicians and researchers pay attention to naturally obtained antineoplastic drugs with high activity and low toxicity. Traditional Chinese herbs with anti-cancer properties which are involved in the goji berry (GB) have attracted considerable interest in recent years [5].

Goji (also known as wolfberry), the fruit of *Lycium barbarum* L., is used as a traditional medicinal food in Asian countries [5-7]. Goji fruits contain polysaccharides, vitamins E, B, and C as well as various essential amino acids including eight essential exogenous amino acids and organic acids such as polyphenols (Catechins or hyperosides), and ferulic or chlorogenic acids and their derivatives, trace elements such as zinc, iron, copper, and germanium [5-7]. GB has many biological effects such as anti-aging effects, neuroprotection, increased immune system endurance, increased metabolism, improved control of glucose and other diabetes symptoms, anti-glaucoma effects, immunomodulation, anti-tumor activity, and cytoprotection *in vivo* and *in vitro* studies [5-7].

Fatty acid oxidation (FAO) is a biochemical process in which fatty acid (FA) molecules are metabolized to acetyl-CoA in mitochondria by multiple beta oxidation cycles [8, 9]. The metabolism of fatty acids, FAO, needs co-factor carnitine. L-carnitine (LK, 4-trimethylamino-3-hydroxybutyric acid) is synthesized endogenously in the kidney, liver, and brain using lysine and methionine amino acids as well as getting exogenously from food [8, 9]. Carnitine transports fatty acids from cytosol to mitochondrial matrix [8, 9]. Studies have shown that FAO is an important energy pathway supporting cancer cell proliferation, growth, and survival (Resistance) in addition to carbohydrates (Aerobic glycolysis and Warburg effect) [9-12]. Accordingly, the treatment modalities targeted at LK have begun to be preferred [9-22].

In addition, studies are showing that LK leads to apoptosis-mediated antiproliferative effects alone and in combination with some of the cancer drugs (Synergistic effects) in some types of cancer, while the opposite publications

explain that LK is more likely to cause cancers and reduce the effectiveness of different treatment modalities such as cancer drugs or radiotherapy can also be found [13-22]. However, studies have also shown that LK deficiency plays a role in the pathogenesis of some types of cancer by a mechanism not associated with obesity and the amount of fat in the human body [22]. There are also some studies showing that there is a connection between cancer-related fatigue and cachexia pathogenesis which are very complex processes involving many cytokines and LK deficiency, but some of the others show just the opposite result [23, 24].

Leukemia inhibitory effects of GB have been studied in myeloid leukemia (AML) [25, 26]. Studies with LK in leukemic cancer type are mostly about AML and other leukemia types, and the effect of CML-related inhibitory effect of LK has been detected in very few studies [9, 27, 28]. There are no studies in which the combination of GB with the high FA content and LK, a fatty acid transporter, in leukemia cells including CML. Our goal in doing this study was to investigate the effects of GB with LK in CML along with the underlying signaling paths. We targeted to obtain low cytotoxic cancer treatment with this combination.

Methods:

Goji berry

The fruits of GB investigated in the present study were cultivated in China and were obtained from a website. Fruits are usually sold in open boxes and sun-dried small packs, as well as juices, concentrates, and infusions as well as capsules [6]. Before the extracts of GB fruit were prepared, the genus and species of GB had been confirmed by two botanical specialists from Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany.

L-carnitine

One tablet of the food supplement L-carnitine (CarnipureTM, Now, IL, USA) was used in the experiment. It contains 1.0 g L-carnitine tartrate with cellulose, vegetable-derived stearic acid and magnesium stearate, silica, and vegetable coating.

The preparation of goji berry extract

GB fruits were dried at room temperature in the dark for 30 days. They were cut into small pieces by cutting with a scalpel. 30 g of small pieces of fruits were extracted by refluxation in a Soxhlet Apparatus for almost 24 hours using 100 ml of methanol, 30 ml of petroleum ether, and 50 ml of chloroform [29,30]. Methanol, petroleum ether, and chloroform were removed by rota evaporator. The extractable compound amount (EC) was determined. The extract of GB was stored at -20^{o}C for post-applications such as cell culture [29,30].

The evaluation of anti-oxidant capacity

The determination of the total phenolic amount

The total amount of phenolic compounds in the extracts was determined by the application of colorimetric Slinkard and Singleton methods, i.e. the usage of the Folin-Ciocalteu solution, with some minor modifications [29]. After the application of the Folin-Ciocalteu solution to extracts, the absorbance was read against a blank at 760 nm [29]. Standards were prepared by using gallic acid (Sigma G7384, USA). The results obtained were evaluated by using the gallic acid standard curve equation as “mg gallic acid equivalents/g fruit” [29].

The determination of the total flavonoid amount

The total flavonoid content of the extracts was determined using the standard calorimetric method with some minor modifications [29]. Extracts and catechin standards (Fluka 22110, Germany) were placed in microplate wells. Then aliquots were mixed with distilled water, 5% sodium nitrite solution, aluminum trichloride, and 1 M sodium hydroxide. They were allowed to stand in the dark for 2 hours. The absorbance was read against blank at 510 nm [29]. The obtained results were evaluated with the catechin standard curve equation as “mg catechin equivalents/g

fruit" [29].

Suspension cell culture of CML

Human CML cell line as K562 (ATCC® CCL-243™) was provided from American Type Culture Collection (ATCC) and they were cultured in RPMI-1640 supplemented with 1 % antibioticantimycotic solution, 1.0 mM sodium pyruvate, 1.5 g / L sodium bicarbonate, 10% fetal bovine serum (All from Gibco, Thermo Fisher Scientific, Inc., USA). Cells were grown in flasks in an incubator (37 °C, 5% CO_2) [2].

Experiment design

300.000 CML cells were plated in 3 wells containing 3 ml RPMI-1640 medium and kept in the incubator for 1 hour. GB and LK were applied singly and in combination. Test groups were determined as follows: 1. The control group (Sterile distilled water), 2. The GB group, 3. The LK group, 4. The GB+LK group as the combination group.

Total cell number

500.000 K562 cells were plated in wells containing 5 ml RPMI-1640 medium for the determination of the total cell number. Single GB, LK, and a combination of them were applied and incubated for 72 hours in the incubator. The total cell number was determined by automated cell counting by using a PI kit-based commercial kit (A starter kit, ChemoMetec A/S, Allerod, Denmark). K562 cells were harvested at 24 and 72 hours and then lysed [31, 32]. The lysed cells were transferred to special cassettes covered with PI. The DNA was ligated to PI and stained. The number of cells was determined by measuring PI fluorescence in the cell counter [31, 32].

Cell viability and apoptosis

The application principles of flow cytometric annexin-V-fluorescent isothiocyanate/ (Annexin-V-FITC) and propidium iodide (PI) dual dye-based test kit (FITC Annexin V Apoptosis Detection Kit I, BD Pharmingen-556570, Germany) were followed with some minor modifications to determine cell viability and apoptosis rates [31,32]. Fluorescence measurements were done by using a flow cytometry instrument (BD FACSCalibur™, Becton Dickinson, USA) and the measurements were analyzed with the CellQuest and WinMDI programs [31,32]. The number of viable cells in the lower left (Anneksin V-, PI-), early apoptotic in the lower right cells (Anneksin V+, PI-), apoptotic cells in the upper right (Anneksin V +, PI+), dead cell populations in the upper left at the flow cytometry panels were observed. However, total apoptotic cell percentages were obtained by summing up early and late apoptotic rates of cells [31,32].

Apoptotic and anti-apoptotic protein levels

The Bicinkochromic Acid (BCA) method which was suitable for microplate was used to assess the protein concentrations of the samples [31,32]. The study range for this method was determined as 20-2000 µg/ml. Protein standards were prepared by using bovine serum albumin [31, 32].

Caspase-3 levels

The application principles of the test kit (Sigma-Aldrich CASP-3C, USA) were followed by some minor modifications to determine of caspase-3 levels in our study [31,32]. Absorbances were measured by using an ELISA reader (Thermo Scientific Multiskan GO UV/Vis) at 405 nm within 1-4 minutes and then they were reported. Absorbances were converted to concentration (nmol) with the calibration equations obtained from pNA standard curves [31,32].

Caspase-8, Caspase-9, Bax and Bcl-2 levels

The application guides of the test kit to investigate changes in the levels of caspase-8 (USCN SEA853Hu, Germany), caspase-9 (USCN SEA627Hu, Germany), Bcl-2-related X protein as bax (USCN SEB343Hu, Germany), and bcl-2 (USCN SEA778Hu, Germany) were followed by some minor modifications in this current study [31,32].

Protein standards and samples were put in caspase-8, caspase-9, bax, and bcl-2 antibody-coated microplate wells. The standard range is 0-40 ng/ml for caspase-8, 0-20 ng/ml for caspase-9, 0-100 ng/ml for bcl-2, and 0- 25 ng /ml for bax [31,32].

After the application of the primary antibody, the secondary antibody, and the chromogenic substrate [3,3',5,5-tetramethylbenzidine (TMB)], the absorbance was measured at 450 nm by an ELISA reader (Thermo Scientific Multiskan GO UV/Vis) and results were reported [31,32]. Protein concentrations of the samples were determined by using protein standard curve plots. Absorbances were converted to concentration units of ng/ml, pg/ml, and µmol [31,32].

Transmission Electron Microscopy (TEM)

Cells were fixed with 2.5% glutaraldehyde and post-fixed with 1% osmium tetroxide for 30 min at 4°C, respectively [31,32]. Cells were incubated in 1% uranyl acetate for 30 min at 4°C, dehydrated in a graded acetone series, and embedded in Epon 812. Samples were cut using a rotating-blade microtome (Leica, Heerbrugg, Switzerland), and 70-nm-thick sections were mounted on copper grids. Sections were subsequently stained with 5% uranyl acetate and counterstained with Reynold's lead citrate. Sections were examined using a JEOL JEM 1011 transmission electron microscope [31,32].

Statistical analysis

The effects of GB, LK, and the combination group on CML cells in comparison to the control group and each other were processed by using the SPSS program (SPSS, Turkey). The Shapiro-Wilks test and ANOVA test were used. The results were shown as means±standart deviation (SD). The difference was considered statistically significant when $p<0.05$.

Results:

Phenolic-flavonoid levels in goji berry

The antioxidant capacity was determined by the phenolic and flavonoid content. The amount of phenolic compound [Gallic acid equivalent (GAE)] for each gram of the weight of GB is 3.45 ± 0.44 mg GAE/g extract and the amount of flavonoid compound [Catechin equivalent (CAE) for each gram of GB weight] 2.5 ± 0.27 mg CAE/g.

Total cell number

The results of the effects of GB, LK, and combination groups on the total number of cells for 72 hours are shown in Figure 1. The mean number of cells in the experimental groups was found to be low in comparison to the control group at the statistically significant level for 72 hours (24^{th} h P_{GB} <0.001, P_{LK} <0.01, P_{GB+LK} <0.0001; 72^{nd} h P_{GB} <0.001, P_{LK} <0.01, P_{GB+LK} <0.00001). The cell number of GB was found to be lower than the LK group for 72 hours at a statistically significant level (24^{th} h: P_{LK} <0.05; 72^{nd} h: P_{LK}<0.05). The cell number average of GB + LK was lower than the GB group and LK at the statistically significant level (24^{th} h: P_{GB} <0.01; 72^{nd} h: P_{GB}<0.0001; 24^{th} h: P_{LK} <0.001; 72^{nd} h: P_{LK}<0.000001).

Cell viability and apoptosis

The alterations in cell viability and apoptosis are shown in **Figure 2.** The viable cell ratios in the experimental groups were found to be low in comparison to the control group at the statistically significant level for 72 hours (Fig. 2A) (24^{th} h: P_{GB} <0.000001, P_{LK} <0.00001, P_{GB+LK}<0.0000.; 72^{nd} h: P_{GB} <0.0000001, P_{LK}<0.0001, P_{GB+LK} <0.0000.). The GB group was found to be lower than the LK group at a statistically significant level for 72 hours (24^{th} h: P_{LK}<0.01; 72^{nd} h: P_{LK}<0.01). The viable cell ratio of GB + LK was determined to be lower than the GB group and the LK group at the statistically significant level (24^{th} h: P_{GB} <0.000001; 72^{nd} h: P_{GB}<0.000000001; 24^{th} h: P_{LK} <0.0000001; 72^{nd} h: P_{LK}<0.0000.).

Apoptotic cell ratios for 72 hours (Fig 2B) in the experimental groups were found to be high in comparison to the

control at the statistically significant level (24th h: P_{GB} <0.0000001, P_{LK}<0.00001, $P_{GB + LK}$<0.0000001; 72nd h: P_{GB} <0.0000001, P_{LK}<0.00001, $P_{GB + LK}$ <0.0000001). The apoptotic cell ratio of the GB group was found to be higher than the mean of the LK group at a statistically significant level (24th h: P_{LK}<0.01; 72nd h: P_{LK}<0.0001). The apoptotic cell ratio of GB + LK was found to be higher in the statistically insignificant level than the mean of GB (P_{GB}> 0.05), but it's higher than the mean of LK (P_{LK} <0.001) at the 24th h at the statistically significant level. The apoptotic cell ratio of GB+LK was found to be higher than the GB group (P_{GB}<0.0000001) and the LK group (P_{LK} <0.0000001) at the 72nd h at the statistically significant level.

Dead cell ratios for 72 hours (Fig. 2C) in the experimental groups were found to be high in comparison to the control at the statistically significant level (24th h: P_{GB} <0.001, P_{LK}<0.001, $P_{GB + LK}$ <0.0000.; 72nd h: P_{GB} <0.0001, P_{LK} <0.01, $P_{GB + LK}$ <0.0000.). The dead cell ratio of the GB group was found to be higher than the LK group at the 24th h at the statistically significant level (P_{LK}<0.01), but this ratio was detected to be lower at the 72nd h at the statistically significant level (P_{LK}<0.01). The dead cell ratio of the GB + LK group was determined to be higher than the GB group (24th h: P_{GB} <0.0000000000001; 72nd h: P_{GB}<0.0000.) and the LK group (24th h: P_{LK} <0.0000.; 72nd h: P_{LK}<0.000000001) at the statistically significant level for 72 hours.

Apoptotic protein levels

Caspase-3 levels (Fig. 3A) in the experimental groups were found to be high in comparison to the control at the statistically significant level (24th h: P_{GB}<0.000001, P_{LK}<0.0001, P_{GB+LK}<0.000001; 72nd h: P_{GB}<0.000001, P_{LK}<0.0001, P_{GB+LK}<0.0000.) for 72 hours. The caspase-3 levels of GB were found to be higher than the LK group at the statistically significant level (24th h: P_{LK} <0.001; 72nd h: P_{LK} <0.001). The caspase-3 levels of the GB + LK group were found to be higher than the GB group and LK at the statistically significant level (24th h: P_{GB} <0.001; 72nd h: P_{GB}<0.00001; 24th h: P_{LK} <0.05; 72nd h: P_{LK}<0.000001).

Caspase-8 levels for 72 hours (Fig. 3B) in the experimental groups were detected higher than the control group at the statistically significant level (24th h: P_{GB}< 0.05, P_{LK}<0.0000001, P_{GB+LK}<0.00000001; 72nd h: P_{GB}<0.01, P_{LK}<0.000000001, P_{GB+LK}<0.0000.). The caspase-8 levels of the GB group were lower than the LK group for 72 hours at the statistically significant level (24th h: P_{LK} <0.00001; 72nd h: P_{LK}<0.00001). The caspase-3 levels of GB + LK were found to be higher than the GB and LK group at the statistically significant level (24th h: P_{GB} < 0.000001;72nd h: P_{GB}<0.0000001; 24th h: P_{LK} <0.01; 72nd h: P_{LK}<0.01).

Caspase-9 levels for 72 hours (Fig. 3C) in the experimental groups were found to be high in comparison to the control group at the statistically significant level (24th h: P_{GB}< 0.00000001, P_{LK}<0.05, P_{GB+LK}<0.05; 72nd h: P_{GB}<0.0000., P_{LK}<0.05, P_{GB+LK}<0.05). The caspase-9 levels of the GB group were higher than the LK group for 72 hours at the statistically significant level (24th h: P_{LK} <0.0000001; 72nd h: P_{LK}<0.0000.). The caspase-9 levels of the GB + LK group were found to be low in comparison to the mean caspase-9 levels of GB at the statistically significant level, but it's higher than the mean of the LK group at a statistically insignificant level for 72 hours. (24th h: P_{GB} <0.0000001; 72nd h: P_{GB}<0.0000.; 24th h: P_{LK}> 0.05; 72nd h: P_{LK}> 0.05)

Bax levels for 72 hours (Fig. 3D) in the experimental groups were detected to be higher than the control group at the statistically significant level (24th h: P_{GB}< 0.00000001, P_{LK}<0.05, P_{GB+LK}<0.001; 72nd h: P_{GB}<0.0000., P_{LK}<0.05, P_{GB+LK}<0.0001). The bax levels of the GB group were higher than the LK group for 72 hours at the statistically significant level (24th h: P_{LK} <0.00000001; 72nd h: P_{LK}<0.00000001). The caspase-9 levels of the GB + LK group were found to be low in comparison to the GB group at the statistically significant level, but it's higher than the LK group at a statistically significant level for 72 hours (24th h: P_{GB} <0.00000001; 72nd h: P_{GB}<0.00000001; 24th h: P_{LK}< 0.05; 72nd h: P_{LK}< 0.05).

Anti-apoptotic protein levels

Bcl-2 levels of LK and GB + LK groups were found to be lower than the control group at the statistically significant

level at the 24th and 72nd hours (24th h: P_{LK} <0.05, P_{GB+LK} <0.001; 72nd h: P_{LK} <0.001, P_{GB+LK} <0.000001) (Fig. 3E). Bcl-2 levels of the GB group were found to be higher than the control group at the statistically significant level for all hours (24th h: P_{GB} <0.00001; P_{GB} <0.000000001). The bcl-2 levels of GB were higher than the LK group at the statistically significant level for 72 hours (24th h: P_{LK} <0.00001; 72nd h: P_{LK}<0.0000.). The bcl-2 levels of the GB + LK group were found to be lower in the statistically significant level than the GB and LK groups (24th h: P_{GB} < 0.0000001;72nd h: P_{GB}<0.0000.; 24th h: P_{LK} <0.01; 72nd h: P_{LK}<0.01).

Transmission electron microscopy

Cells in the control group exhibited normal morphology characterized by fine-textured nuclear chromatin, intact nuclear membrane, tubular structured mitochondria, intact cytoplasmic membrane, and many microvilli under TEM evaluation. Mitotic cells were also observed in this group (Fig. 4A). Cells belonging to the GB group had fine-textured nuclear chromatin, intact nuclear membrane, a high number of damaged mitochondria, some vacuoles in cytoplasm, intact cytoplasmic membrane, and a low number of microvilli under TEM evaluation. A high number of cells with an apoptotic appearance was usually observed (Fig. 4B). The LK group showed fine-textured nuclear chromatin, intact but invaginated nuclear membrane, less damaged mitochondria, some vacuoles in cytoplasm, a low number of autophagic vacuoles, an intact cytoplasmic membrane, high number of lipid droplets and a low number of microvilli under TEM evaluation. Cells with apoptotic appearance were also observed rarely (Fig. 4C). The cells of the combination group had fine-textured nuclear chromatin, intact nuclear membrane, less number of damaged mitochondria, a high number of autophagic vacuoles in cytoplasm, intact cytoplasmic membrane, a very high number of lipid droplets and a low number of microvilli under TEM evaluation. A high number of cells with an apoptotic appearance was also observed (Fig. 4D).

Discussion: In our study, we observed that GB inhibited the proliferation of CML cells. Even though we detected the same effect in the LK group, the inhibitory effect of LK was weaker than GB. The combined use of LK which reduces fatty acid content by high oxidation with GB consisting of high fatty acid levels exhibited a synergistic effect via the inhibition of CML cell proliferation at the highest level.

GB has been shown in many studies that demonstrate therapeutic effects as well as enhancing therapeutic effects in different types of cancer alone and in combination with various therapeutic protocols (Chemotherapy, immunotherapy, and radiotherapy) (4, 26, 29, 33-45). For example, Hsu *et al.* (2017) found that the carotenoid extracts of *Lycium barbarum* L. and nanoemulsion forms of their own inhibit the growth of colon cancer cells [42]. Another study done by Zhu *et al.* (2017) showed that 4 GBs phenolic amides with newly identified function inhibit the proliferation of glioma stem cells at various grades [29]. Chen *et al.* (2015) determined that GB (Polysaccharides) with immunotherapy agent cytokine interferon-alpha (IFN-α) shows a synergistic effect in renal carcinoma cells via the induction of intrinsic apoptosis [43]. Lu and Cheng (1991) found that GB alone (Polysaccharides) shows no effect on lung cancer inhibition however when GB application was combined with radiation therapy in lung cancer, this combination shows a synergistic effect [44]. GB leads to intrinsic apoptosis in human cervical cancer cells HeLa cells [39] and prostate cell lines [45]. Gan et al. (2001) worked with GB at AML cells and found that GB induces apoptosis via the activation of caspase-3 activity [25].

In our study, we found that GB was the 2nd most effective group after the combination group for the inhibition of CML proliferation. We found that GB inhibited CML cell proliferation consistent with previous studies in different cancer types through the induction of intrinsic apoptosis [45,46]. We found that caspase-9 activation and subsequent caspase-3 activation proceeded through the increase of bax levels and the decrease of bcl-2 levels. A high number of damaged mitochondria was observed at the TEM evaluation. A high number of damaged mitochondria also confirmed that GB exerts its' effect through intrinsic apoptosis.

In addition, it's shown in many studies that LK shows apoptosis-mediated anti-proliferative effects in some types of cancer alone, and increases the effects of some antineoplastic drugs while the opposite publications explained that GB is more likely to cause cancers and reduce the effectiveness of different treatment modalities such as chemothe-

rapy or radiotherapy [14-22]. The study conducted by Park *et al*. (2012) can be given as an example. It was shown that carnitine sensitizes TRAIL-resistant cancer cells to TRAIL-induced cell death (Extrinsic apoptosis pathway) by increasing bax expressions [14].

Fan and colleagues (2009) found that LK induces Fas ligand-mediated apoptosis in liver cancer and it also reduces bcl-2 expression [15]. They concluded that this inhibition mechanism is the result of LKs' effects as the trigger of extrinsic and intrinsic apoptotic pathways in these cells [15]. Roscilli and colleagues (2013) applied LK and curcumin alone and in combination on colon cancer cells and found that the development of advanced adenocarcinoma lesions has completely been stopped by the synergistic effect [19]. In another study conducted by Huang et al. (2012), histone deacetylase (HDAC) inhibitor as LK and protease inhibitör as bortezomib were used in combination on hepatoma cells *in vitro* and *in vivo* to determine their synergistic inhibitory effect [21].

In contrast to these studies, Ibrahim and colleagues (2014) found that co-administration of LK with tamoxifen increases the lifetime of mice bearing Ehrlich Acid carcinoma, but LK decreases tamoxifens' antitumor activity by blocking apoptosis [16]. In another study by Tas *et al*. (2017), LK was found to protect colonic mucosa cells from the effects of abdominopelvic radiotherapy [17]. Altun and colleagues showed that LK has a protective effect on neuroblastoma by the inhibition of cisplatin-induced toxicity and apoptosis induced by oxidative stress [20]. Zhu *et al.* (2015) investigated the toxic effect of cyclophosphamide used commonly in cancer on male germ cells and found that the use of LK reduces cyclophosphamides' effect via apoptosis inhibition and autophagy modulation. Although this work is not related to cancer, it supports the anti-apoptotic effect of LK [18].

Studies with LK in leukemic cancer type are mostly in AML and other leukemia types, and the inhibitory effect of LK on CML has been detected in very few studies [9, 27, 28]. In a study by Thomadaki and colleagues (2008), LK alone and in combination with dinuclear copper (II) complex were applied to AML and CML cells. The concentration-dependent ascending inhibition of combination in comparison to singly applied LK was observed in both cell lines as the decreased proliferative activity of AML cells and the increased necrotic appearance of CML cells [28]. In a study by Rogalidou *et al*. (2010), acylcarnitine levels were investigated in children with acute leukemia before and after therapy at various times, it was found that there is a decrease in carnitine levels and the application of carnitine supplementation to patients during chemotherapy is considered to be appropriate approach [27]. A study by Wu *et al*. (1998) showed that a carnitine transporter (CT) 2 is overexpressed in AML cells and the silencing of its' gene reduced the growth and viability of AML cells [26].

In our study, LK was found to be the least effective group among all applications, because it led to low CML cell proliferation inhibition. We found that LK induces apoptosis with caspase-3 activation, as in other groups, but this apoptosis initiation by LK is done differently through caspase-8 which leads to extrinsic apoptotic pathways. The lowest significant increase at bax levels and the lowest significant decrease at bcl-2 levels with low caspase-9 levels were detected in this group, it seems that LK leads to both extrinsic and intrinsic apoptosis as previously shown by Fan and colleagues [15]. Because caspase-9 levels were higher than caspase-8 levels, it can be thought that LK exerts its effect dominantly through an extrinsic pathway. Low numbers of damaged mitochondria were also observed during the evaluation of the electron microscope. This result obtained from TEM could also explain low bax and bcl-2 levels which were found at the LK group. We also thought that this effect of LK can be achieved by the accumulation of FAs in the form of lipid droplets in the cytoplasm of cancer cells through increasing the transport of cytotoxic FAs [8].

In our study, we found that the combination of LK and GB shows a synergistic effect. The combination was the most effective group in comparison to monotherapy groups in terms of high CML cell proliferation inhibition and high cell viability reduction. We found that the combination group induces apoptosis with caspase-3 activation similar to single test groups. The lowest increase at bax levels and the lowest decrease at bcl-2 levels with low caspase-9 levels were also detected in this group as well as LK, therefore, the combination group can use both extrinsic and intrinsic apoptosis to exert its effect. The highest number of cells that had an apoptotic cell-like appearance was

found in this group. In addition, a smaller number of damaged mitochondria, a high number of autophagic vacuoles, and a very high number of lipid droplets were also determined in this group. It can be thought that FAs in GB can provide cytotoxic FAs such as ceramides for the induction of apoptosis and LK transfers these toxic fatty acids of GB into the cancer cell. This may explain the reason for the synergistic effect. However, the reason for the mechanism of action of the combination as an extrinsic pathway remains unclear. It's not known why the combination group chose to use an extrinsic pathway dominantly instead of an intrinsic pathway.

Author contributions: The author(s) have (has) accepted responsibility for the entire content of this manuscript and approved its submission. **Mine Ergüven:** Conceived and designed analysis, collected data, contributed analysis tools, performed analysis, wrote the paper; **Haniyeh Behrouzizad:** collected data, contributed analysis tools, performed analysis; **Esin Çetin Aktaş:** contributed analysis tools, performed analysis; **Ayhan Bilir:** contributed analysis tools, performed analysis**.**

Competing interests: The authors state no conflict of interest.

Research funding: None declared.

Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Lee JY, Lee SM, Yoon HK, Kim KH, Choi MY, Lee WS. A case of synchronous multiple myeloma and chronic myeloid leukemia. Blood Res 2017;52:219-221.

2. Pereira WO, De Carvalho DD, Zenteno ME, Ribeiro BF, Jacysyn JF, Sardinha LR, et al. BCR-ABL1-induced downregulation of WASP in chronic myeloid leukemia involves epigenetic modification and contributes to malignancy. Cell Death Dis 2017;8:e3114.

3. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343-3356.

4. Zhang Q, Lv X, Wu T, Ma Q, Teng A, Zhang Y, et al. Composition of Lycium barbarum polysaccharides and their apoptosis-inducing effect on human hepatoma SMMC-7721 cells. Food Nutr Re 2015;59:28696.

5. Yao R, Heinrich M, Weckerle CS. The genus Lycium as food and medicine: A botanical, ethnobotanical and historical review. J Ethnopharmacol 2018;212:50-66.

6. O'Brien ME, Borthwick A, Rigg A, et al. Mortality within 30 days of chemotherapy: a clinical governance benchmarking issue for oncology patients. Br J Cancer 2006;95:1632-1636.

7. Jeszka-Skowron M, Zgoła-Grześkowiak A, Stanisz E, Waśkiewicz A. Potential health benefits and quality of dried fruits: Goji fruits, cranberries and raisins. Food Chem 2017;221:228-236.

8. Qu Q, Zeng F, Liu X, Wang QJ, Deng F. Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. Cell Death Dis 2016;7:e2226.

9. Wu Y, Hurren R, MacLean N, Gronda M, Jitkova Y, Sukhai MA, et al. Carnitine transporter CT2 (SLC22A16) is over-expressed in acute myeloid leukemia (AML) and target knockdown reduces growth and viability of AML cells. Apoptosis 2015;20:1099-1108.

10. Shao H, Mohamed EM, Xu GG, Waters M, Jing K, Ma Y, et al. Carnitine palmitoyltransferase 1A functions to repress FoxO transcription factors to allow cell cycle progression in ovarian cancer. Oncotarget 2016;7:3832-3846.

11. Pucci S, Zonetti MJ, Fisco T, Polidoro C, Bocchinfuso G, Palleschi A, et al. Carnitine palmitoyl transferase-1A (CPT1A): a new tumor specific target in human breast cancer. Oncotarget 2016;7:19982-19996.

12. Deep G, Schlaepfer IR. Aberrant Lipid Metabolism Promotes Prostate Cancer: Role in Cell Survival under Hypoxia and Extracellular Vesicles Biogenesis. Int J Mol Sci 2016;17:1061.

13. Chen Y, Wang Y, Huang Y, Zeng H, Hu B, Guan L, et al. PPARα regulates tumor cell proliferation and senescence via a novel target gene carnitine palmitoyltransferase 1C. Carcinogenesis 2017;38:474-483.

14. Park SJ, Park SH, Kim JO, Kim JH, Park SJ, Hwang JJ, et al. Carnitine sensitizes TRAIL-resistant cancer cells to TRAIL-induced apoptotic cell death through the up-regulation of Bax. Biochem Biophys Res Commun 2012;428:185-190.

15. Fan JP, Kim HS, Han GD. Induction of apoptosis by L-carnitine through regulation of two main pathways in Hepa1c1c 7 cells. Amino Acids 2009;36:365-372.

16. Ibrahim AB, Mansour HH, Shouman SA, Eissa AA, Abu El Nour SM. Modulatory effects of L-carnitine on tamoxifen toxicity and oncolytic activity: in vivo study. Hum Exp Toxicol 2014;33:968-979.

17. Tas S, Ozkan OF, Cikman O, Kiraz A, Akgun Y, Karaayvaz M. L-carnitine has a protective effect on the colonic mucosa during abdominopelvic radiotherapy in rats. Acta Cir Bras 2016;31:615-620.

18. Zhu B, Zheng YF, Zhang YY, Cao YS, Zhang L, Li XG, et al. Protective effect of L-carnitine in cyclophosphamide-induced germ cell apoptosis. J Zhejiang Univ Sci B 2015;16:780-787.

19. Roscilli G, Marra E, Mori F, Di Napoli A, Mancini R, Serlupi-Crescenzi O, et al. Carnitines slow down tumor development of colon cancer in the DMH-chemical carcinogenesis mouse model. J Cell Biochem 2013;114:1665-1673.

20. Altun ZS, Güneş D, Aktaş S, Erbayraktar Z, Olgun N. Protective effects of acetyl-L-carnitine on cisplatin cytotoxicity and oxidative stress in neuroblastoma [published correction appears in Neurochem Res 2010;35:679. Erbayrktar, Zübeyde [corrected to Erbayraktar, Zübeyde]]. Neurochem Res 2010;35:437-443.

21. Huang H, Liu N, Yang C, Liao S, Guo H, Zhao K, et al. HDAC inhibitor L-carnitine and proteasome inhibitor bortezomib synergistically exert anti-tumor activity in vitro and in vivo. PLoS One 2012;7:e52576.

22. Arioz DT, Kanat-Pektas M, Tuncer N, Koken T, Unlu BS, Koken G, et al. L-Carnitine: a new insight into the pathogenesis of endometrial cancer. Arch Gynecol Obstet 2015;291:1147-1152.

23. Sakamoto A, Tsukahara Y, Gomi D, Fukushima T, Kobayashi T, Matsushita H, et al. Plasma L-carnitine levels in terminally ill cancer patients receiving only palliative care. Ann Palliat Med 2017;6:S52-S57.

24. von Haehling S, Anker SD. Treatment of cachexia: An overview of recent developments. Int J Cardiol 2015;184:736-742.

25. Gan L, Wang J, Zhang S. Inhibition the growth of human leukemia cells by Lycium barbarum polysaccharide. Wei Sheng Yan Jiu 2001;30:333-335.

26. Huang Y, Tan A, Shen Y, Lu J. Scavenging effect of total flavonoids of lyceum barbarum L on active oxygen radicals and inhibitory effects on heat output from L1210 cells. Wei Sheng Yan Jiu 1998;27:109-115.

27. Rogalidou M, Evangeliou A, Stiakaki E, Giahnakis E, Kalmanti M. Serum carnitine levels in childhood leukemia. J Pediatr Hematol Oncol 2010;32:e61-e69.

28. Thomadaki H, Karaliota A, Litos C, Scorilas A. Enhanced concentration-dependent cytotoxic effect of the dinuclear copper(II) complex of L-carnitine [Cu2(L-carnitine)2Cl2(H2O)2]Cl2, compared to L-carnitine or copper chloride dihydrate, in human leukemic cell lines. J Med Chem 2008;51:3713-3719.

29. Zhu PF, Dai Z, Wang B, Wei X, Yu HF, Yan ZR, et al. The Anticancer Activities Phenolic Amides from the Stem of Lycium barbarum. Nat Prod Bioprospect 2017;7:421-431.

30. Bitis L, Kultur S, Melikoglu G, Ozsoy N, Can A. Flavonoids and antioxidant activity of Rosa agrestis leaves. Nat Prod Res 2010;24:580-589.

31. Erguven M, Oktem G, Kara AN, Bilir A. Lithium chloride has a biphasic effect on prostate cancer stem cells and a proportional effect on midkine levels. Oncol Lett 2016;12:2948-2955.

32. Erguven M, Yazihan N, Aktas E, abanci A, Li CJ, Oktem G, et al. Carvedilol in glioma treatment alone and with imatinib in vitro. Int J Oncol 2010;36:857-866.

33. Zhang XJ, Yu HY, Cai YJ, Ke M. Lycium barbarum polysaccharides inhibit proliferation and migration of bladder cancer cell lines BIU87 by suppressing Pi3K/AKT pathway. Oncotarget 2017;8:5936-5942.

34. Georgiev KD, Slavov IJ, Iliev IA. Antioxidant Activity and Antiproliferative Effects of Lycium barbarum's (Goji berry) Fractions on Breast Cancer Cell Lines. Folia Med (Plovdiv) 2019;61:104-112.

35. Wawruszak A, Czerwonka A, Okła K, Rzeski W. Anticancer effect of ethanol Lycium barbarum (Goji berry) extract on human breast cancer T47D cell line. Nat Prod Res 2016;30:1993-1996.

36. Jiao R, Liu Y, Gao H, Xiao J, So KF. The Anti-Oxidant and Antitumor Properties of Plant Polysaccharides. Am J Chin Med 2016;44:463-488.

37. Ceccarini MR, Vannini S, Cataldi S, et al. In Vitro Protective Effects of Lycium barbarum Berries Cultivated in Umbria (Italy) on Human Hepatocellular Carcinoma Cells. Biomed Res Int 2016;2016:7529521.

38. Shen L, Du G. Lycium barbarum polysaccharide stimulates proliferation of MCF-7 cells by the ERK pathway. Life Sci 2012;91:353-357.

39. Zhu CP, Zhang SH. Lycium barbarum polysaccharide inhibits the proliferation of HeLa cells by inducing apoptosis. J Sci Food Agric 2013;93:149-156.

40. Mao F, Xiao B, Jiang Z, Zhao J, Huang X, Guo J. Anticancer effect of Lycium barbarum polysaccharides on colon cancer cells involves G0/G1 phase arrest. Med Oncol 2011;28:121-126.

41. Li G, Sepkovic DW, Bradlow HL, Telang NT, Wong GY. Lycium barbarum inhibits growth of estrogen receptor positive human breast cancer cells by favorably altering estradiol metabolism. Nutr Cancer 2009;61:408-414.

42. Hsu HJ, Huang RF, Kao TH, Inbaraj BS, Chen BH. Preparation of carotenoid extracts and nanoemulsions from Lycium barbarum L. and their effects on growth of HT-29 colon cancer cells [published online ahead of print, 2017 Mar 7]. Nanotechnology 2017;28:135103.

43. Chen S, Liang L, Wang Y, Diao J, Zhao C, Chen G, et al. Synergistic immunotherapeutic effects of Lycium barbarum polysaccharide and interferon-α2b on the murine Renca renal cell carcinoma cell line in vitro and in vivo. Mol Med Rep 2015;12:6727-6737.

44. Lu CX, Cheng BQ. Radiosensitizing effects of Lycium barbarum polysaccharide for Lewis lung cancer. Zhong Xi Yi Jie He Za Zhi 1991;11:611-582.

45. Luo Q, Li Z, Yan J, Zhu F, Xu RJ, Cai YZ. Lycium barbarum polysaccharides induce apoptosis in human prostate cancer cells and inhibit prostate cancer growth in a xenograft mouse model of human prostate cancer. J Med Food 2009;12:695-703.

46. Xiong L, Deng N, Zheng B, Li T, Liu RH. Goji berry (Lycium spp.) extracts exhibit antiproliferative activity via

modulating cell cycle arrest, cell apoptosis, and the p53 signaling pathway. Food Funct 2021;12:6513-6525.

S128

EFFECT OF STORAGE PERIOD ON BLOOD ETHANOL LEVELS

Tuba Çakmak[1], Giray Bozkaya[2]

[1] University of Health Sciences, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

[2] University of Health Sciences, Izmir Faculty of Medicine, Izmir Bozyaka Training and Research Hospital, Department of Medical Biochemistry, İzmir, Türkiye

INTRODUCTION: The accuracy of ethanol measurement results is very important for laboratory and forensic institutions. Ethanol is the most common cause of traffic accidents caused by foreign substances[1]. The ethanol measurement result reported by the laboratories can influence decisions about who is at fault for traffic accidents. For this reason, all analysis stages of ethanol measurement have been tried to be standardized. The Institute for Clinical and Laboratory Standards (CLSI) has set standards on this topic [2]. In our country, these procedures are carried out according to the directive of the Ministry of Health titled 'Procedures and Principles of Ethanol Analysis Procedures in Blood Samples' [3]. According to this directive, in ethanol analysis, a back up sample is taken simultaneously with the test sample and stored at -20 °C for 6 months. During this period, the back up sample is sent to the Institute of Forensic Medicine if requested by an official request letter from the judicial authorities.

In terms of the reliability of ethanol results, it is of great importance that the preanalytical stage is carried out in accordance with the guidelines [4]. Errors that occur during the collection, transportation, analysis and storage of the sample affect the measurement result [5].

In our study, we aimed to investigate whether there is a difference between the ethanol levels of 114 samples that were stored in our laboratory for 6 months and will be destroyed at the end of this period and the first measured ethanol levels. Thus whether the results obtained by storing and reanalyzing the test sample are reliable.

Methods: This study was conducted at University of Health Sciences Izmir Bozyaka Training and Research Hospital, Medical Biochemistry Laboratory, Turkey from July 2022 to September 2022. Our study was approved by the ethics committee of our hospital and was done in accordance with the helsinki declaration.

Ethanol samples that came to our laboratory for 3 months were included in our study (n=114). These samples; they were accepted to our laboratory after their characteristics such as transport conditions, tube level and hemolysis were evaluated.Only 51 of the stored samples had an initial measured ethanol level of >10 mg/dl. This value (10 mg/dl) is the lower limit of detection for the reagents used in our study.

Whole blood samples were collected in gray stoppered 4 mL tubes containing sodium fluoride plus K3EDTA (Vacusera, Disera A.S., Izmir, Turkey) as additive according to CLSI standards [2]. Immediately after collection, whole blood samples were turned upside down 9-10 times to mix with the contents of the tube. The sample was centrifuged at 3000×*g* for 15 minutes in our laboratory. The plasma obtained using the test sample was quickly analyzed and the same plasma was immediately aliquoted into an ependorf. This plasma was stored airtight and at -20 °C until reanalyzed. The plasma of the test sample was used because it was not possible to dissolve and separate the plasma of the back up sample stored as whole blood.

After 6 months of storage, frozen samples that had to be destroyed were brought to room temperature and analyzed immediately. Ethanol analysis was performed using the enzymatic method with alcohol dehydrogenase on Roche Cobas-6000 (Roche Diagnostics, Mannheim, Germany) [6]. Analysis was performed using the original ethanol kit (Roche Diagnostics, Ethanol Gen.2).

The ethanol levels of 51 samples that were due for disposal at the end of 6 months of storage at -20 °C in our hospital were compared with the ethanol levels measured in the test sample 6 months ago. Statistical Package for the Social Sciences version 21 was used for statistical analysis. The distribution of variables was evaluated with the Kolmogorov-Smirnov test.The variables were found to conform to normal distribution and the data were compared with the paired sample-t test. The significance level was determined as $p<0.05$.

RESULTS: The group of 51 samples was statistically evaluated and a decrease in plasma ethanol concentrations was observed. The difference between ethanol concentrations measured before storage and concentrations measured after 6 months is shown in the table (Table 1). A statistically significant difference was observed between the two measurements according to the paired sample t test ($p<0,001$).

	Ethanol concentration (mg/dL) (median ± SD)		
Storage period (-20 °C)	Initial	Post storaged	P
6 months (n=51)	201,03 (±127,71)	169,47 (±110,87)	< 0,001
Table 1: Evaluation of initial and poststorage plasma ethanol concentrations			

DISCUSSION: In blood ethanol analysis, the change in ethanol concentration during storage is very important as one of the samples is stored for later measurement [7,8]. During the 6-month storage period, a verification test may be requested at any time by judicial authorities. The ethanol concentration in this verification test must not be statistically significantly different from the concentration in the first measured test sample. Therefore, determining the change in the ethanol test after 6 months of storage gives us an indication of if this practice can be used. We compared ethanol concentrations at the end of the 6 month storage period with concentrations measured before storage. It was important that we obtained statistically significantly lower results

we found that the ethanol concentration of the sample stored at -20 °C as plasma in our laboratory was no longer stable after 6 months. we determined that the sample stored as plasma could not be used for ethanol confirmation testing.

Ethanol losses have been shown to be largely due to evaporation. In our study, this loss was minimized by keeping the samples sealed.

In the study by Meyer et al., 68 whole blood samples were stored at -20 °C for 6 months and no significant decline was seen at the end of this period [9]. They performed their studies by modified ADH method (Bonnichsen & Theorell 1951; Biicher & Redetzki 1951) and gas chromatography method (Meyer 1978). We think that this difference occurred in our study because unlike that study, the plasma of the test sample was stored and analyzed in the auto-analyzer.

Mandic-Radic et al. grouped 120 urine and whole blood samples and analyzed them for ethanol at 4 and -20 degrees Celsius. Thıs study was done sımılarly to our study with alcohol dehydrogenase using the roche commercial assay. Similar to our study, statistically significant reductions were observed in samples stored at 20 °C for more than 6 months (15% reduction) (n=15). In the same study, no statistically significant reduction was found in samples stored at -20 °C for 1-3 months (only 4% reduction) (n=15) [10].

There are mostly older publications on studies related to the change of storage time in ethanol concentrations, and these are generally from a forensic point of view [11-15]. there are many studies on postmortem blood ethanol concentrations [16-19]. Our study did not deal with postmortem ethanol analysis. we contributed to ethanol reporting, which is a social and forensic situation in people living with alcohol use.

In the study by Kocak et al. 80 samples were stored and analyzed for 2,3,4 or 5 months. In all 4 sample groups, a decrease in plasma ethanol concentrations was observed, similar to our study [20]. In the same study, % decreases in ethanol concentrations were found to be directly proportional to storage time. Although this study supports our study, we did not calculate decreases in shorter storage times.

According to the guidelines, it is recommended to keep the whole blood taken with the study sample as a back up sample. Although the guidelines only recommend this, over time, practices have emerged in the field that the plasma of the study sample can also be stored. In this study, we have shown that this practice is not reliable enough to reflect the actual ethanol result of the test sample. In addition, the guidelines do not specify whether and how to store rejected samples after centrifugation and plasma extraction. According to our study, we concluded that storing the plasma of these samples in laboratories for 6 months would be an unnecessary practice due to the decrease in ethanol concentrations. In addition, by showing that this practice is unnecessary, we have prevented both excess material consumed and unnecessary storage space. We have also prevented laboratories from giving false ethanol results using these samples.

As a result, each laboratory may want to determine its own reliable storage conditions and operation according to its own possibilities, but we have demonstrated that a different practice for ethanol analysis is unnecessary. We confirmed that the ethanol confirmation test cannot be performed by storing the plasma of the initial sample and must be performed using a back up sample stored as whole blood in forensic laboratories.

References

1. Beck KH, Kasperski SJ, Caldeira KM, Vincent KB, O'Grady KE, Arria AM. Trends in alcohol□related traffic risk behaviors among college students. Alcoholism: Clinical and Experimental Research. 2010 Aug;34(8):1472-8.

2. Blood Alcohol Testing in the Clinical Laboratory, CLSI C60-A. Approved Guideline, C60AE, Clinical and Laboratory Standards Institute 2014.

3. Procedures and Principles of Ethanol Analysis Procedures in Blood Samples February 2017 [Online]. Available from: http://e-belge.saglik.gov.tr adresinden 8d3b7682-df03-4f2e-b8fb-20727dac068

4. Froede RC. The laboratory management of a medicolegal specimen. Annals of Clinical & Laboratory Science. 1976 May 1;6(3):251-5.

5. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. London: Elsevier Health Sciences; 2012.

6. Rodionov YV, Keppen OI, Sukhacheva MV. A photometric assay for ethanol. Applied Biochemistry and Microbiology. 2002 Jul;38:395-6.

7. Olds ML, Kosecki PA, Jones AW. Preanalytical Factors Influencing the Stability of Ethanol in Antemortem Blood and Urine Samples. Journal of Analytical Toxicology. 2023 Jun 1;47(5):403-16.

8. Shan X, Tiscione NB, Alford I, Yeatman DT. A study of blood alcohol stability in forensic antemortem blood samples. Forensic science international. 2011 Sep 10;211(1-3):47-50.

9. Meyer T, Monge PK, Sakshaug J. Storage of blood samples containing alcohol. Acta Pharmacologica et Toxicologica. 1979 Oct;45(4):282-6.

10. Mandic-Radic S, Dzingalasevic G, Lukovic N. Stability of ethanol in blood and urine samples. Journal of Medical Biochemistry. 2007;26(3):241.

11. Laurens JB, Sewell FJ, Kock MM. Pre-analytical factors related to the stability of ethanol concentration during storage of ante-mortem blood alcohol specimens. Journal of Forensic and Legal Medicine. 2018 Aug 1;58:155-63.

12. Kosecki PA, Abbott LA, Raines ME. Large-scale reanalysis of refrigerated antemortem blood samples for ethanol content at random intervals. Journal of Forensic Sciences. 2021 Sep;66(5):1966-72.

13. Kristoffersen L, Stormyhr LE, Smith-Kielland A. Headspace gas chromatographic determination of ethanol: the use of factorial design to study effects of blood storage and headspace conditions on ethanol stability and acetaldehyde formation in whole blood and plasma. Forensic science international. 2006 Sep 12;161(2-3):151-7.

14. Kosecki PA, Canonico E, Abbott L. Ethanol stability in unpreserved refrigerated antemortem blood. Journal of Forensic Sciences. 2023 Mar;68(2):682-7.

15. Tiscione NB, Vacha RE, Alford I, Yeatman DT, Shan X. Long-term blood alcohol stability in forensic antemortem whole blood samples. Journal of analytical toxicology. 2015 Jul 1;39(6):419-25.

16. Kosecki PA, Canonico E, Abbott L. Ethanol stability in unpreserved refrigerated antemortem blood. Journal of Forensic Sciences. 2023 Mar;68(2):682-7.

17. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic science international. 2007 Jan 5;165(1):10-29.

S130

METABOLIC EFFECTS OF ORAL TITANIUM DIOXIDE IN JUVENILE RATS: INSIGHTS FROM NMR-BASED METABOLOMICS ANALYSIS

Fatmanur Köktaşoğlu[1], Metin Demirel[1], Kübra İzler[2], Fatih Gültekin[3], Eray Metin Güler[4], Somer Bekiroğlu[5], Şahabettin Selek[1]

[1] Bezmialem Vakif University, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey

[2] University of Health Sciences, Hamidiye Institute of Health Sciences, Istanbul, Turkey

[3] Lokman Hekim University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

[4] University of Health Sciences, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey

[5] Tubıtak Marmara Research Center, Institute of Chemical Technology, Kocaeli, Turkey

INTRODUCTION: Titanium dioxide (TiO_2), designated as the food additive E171, is utilized in various food products following its safety approval by the Food and Drug Administration (FDA) in 1966 and subsequent endorsement by the Codex Alimentarius organization in 1969. Its application in the food industry is primarily attributed to its capacity to confer a distinct white coloration and an attractive sheen to food items. [1, 2]. This decision to approve titanium dioxide (E171) as a food additive was made without establishing a reliable daily intake amount. Subsequent research revealed that this substance is not only absorbed from the gastrointestinal tract but can also penetrate and accumulate in bodily tissues. This accumulation has been associated with various histopathological and physiological alterations, sparking significant controversy within the scientific and regulatory communities [3-7]. In response to the scientific recommendations and concerns raised by the French Agency for Food, Environment and Occupational Health and Safety (ANSES), the Risk Assessment and Research Office of the Dutch Food and Consumer Product Safety Authority (NVWA), and numerous food-related non-governmental organizations, the European Food Safety Authority (EFSA) announced in 2021 the removal of titanium dioxide (E171) from its list of food additives considered safe. This decision reflects a significant shift in regulatory stance based on evolving scientific understanding and public health considerations.[8-10]. The Turkish Food Codex Food Additives Regulation, formulated by the Turkish Ministry of Agriculture and Forestry, underwent a revision on October 13, 2023. This updated regulation stipulates that, effective from April 1, 2024, the production of food containing TiO_2 will be prohibited. This regulatory change aligns with the evolving global perspective on the safety and use of TiO_2 in food products. [11]. It has been observed that children, who predominantly consume products such as chewing gum and confectionery, experience a higher level of oral exposure to TiO_2 compared to adults [12]. While the daily TiO_2 exposure rate in adults is estimated to be between 0.2 and 1 mg/kg, in children, this rate has been demonstrated to be significantly higher, ranging from 1 to 3 mg/kg [13, 14]. This distinction underscores the heightened exposure risk of TiO_2 in the children's age group, particularly through their consumption patterns of specific food products.

Metabolomics encompasses the systematic study and analysis of small molecules, typically those with a molecular weight under 1500 kDa, found within various biological fluids including serum, plasma, urine, cerebrospinal fluid (CSF), tissue homogenates, saliva, and semen. This analytical approach is instrumental in the qualitative and quantitative assessment of metabolites within these fluids [15]. The application of metabolomics facilitates the diagnosis and monitoring of disease progression, enables the tracking of drug effects and pharmacokinetic interactions, and, critically, aids in the identification of individual metabolic variances [16].

Globally, in the field of metabolomics studies, the most prevalent analytical techniques employed are Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). Although MS is acknowledged for its superior sensitivity, as evidenced by lower limit of detection (LoD) values, NMR is distinguished by its high specificity. NMR's high specificity renders it invaluable in the domain of biomarker discovery and metabolomics research. Its ability to quantify

hundreds of distinct molecules in a single sample empowers researchers to elucidate intricate metabolic pathways and interactions, thereby significantly contributing to the advancement of metabolomic studies.[17]

In the present investigation, our objective was to scrutinize the impacts of orally administered TiO_2,used food additive, utilizing the advanced scientific technique of metabolomics. To simulate child metabolism and specifically to observe the effects on younger organisms, three-week-old Sprague-Dawley rats were selected as the experimental model. The study was designed to conduct an in-depth analysis of changes at the organ level, with a particular focus on liver and kidney tissues. Through this metabolomic approach, we aimed to elucidate the underlying mechanisms of TiO_2's action within these organs.

Methods: Chemicals and instrumentation: All chemicals used in experimental process were commercially obtained Sigma-Aldrich (Darmstadt, Germany). The part of animal study of the Project was performed in Hamidiye Experimental Animal Production and Research Laboratory, Istanbul, Turkey with convenient ethical approved with document number 2020-01/15. 1D-H-NMR spectroscopy analysis were conducted commercially from Bezmialem Drug Application and Research Center with 500 MHz NMR spectrometer Bruker Avance. 2D HNMR spectroscopy analysis were applied with 700 MHz NMR spectrometer Bruker Avance III HD in TUBITAK Marmara Research Center, Gebze, Kocaeli.

Process of Experimental Animal:

Female Sprague-Dawley rats, aged 3 weeks and weighing between 27-38 grams, were procured for the study. These subjects were housed under controlled environmental conditions with a temperature range of 20-22°C and a relative humidity of 55-60%. The animals were provided with standard pellet feed and had access to water ad libitum. The oral dosage of E171-TiO_2 administered was determined based on methodologies described by Chen et al [18]. While the control group was administered ultrapure water, the treatment group received a dosage of 100 mg/kg titanium dioxide suspended in ultrapure water, delivered via oral gavage. There were 1 lost in control group during experimental process. At the end of 6 week animals were sacrified and their organs including liver, kidney, brain and heart were obtained.

Preparation of tissue samples and NMR Spectral Process:

100 mg were weightened per organs and 1 mL of 20 mM phosphate buffer at pH 7.4 were added. Homogenisation was conducted with bead-homogeniser and centrifuged at 10 000 g for 10 minutes. After the filtering with 0.22 µm cellulose acetate filter, 600 µL of the supernatan and 100 µL 1,75 mM 3-(Trimethylsilyl)-1-propanesulfonic acid-d6 sodium salt in deuterium oxide (D_2O) were mixed and transfered to 5 mm NMR tube. Quality control (Qc) samples were prepared with taking 100 µL each sample and 600 µL was taken from the mixture to prepare NMR sample of Qc. The concetrations of metabolites calculated as microgram for per 100 mg tissue.

^1H one-dimensional NMR experiments were conducted utilizing the nuclear Overhauser effect spectroscopy (NOESY, with the noesypr1d sequence) pulse sequences. These analyses were conducted on a Bruker Neo NMR system operating at 500 MHz. The experimental conditions were set to perform 128 scans at a controlled temperature of 22 ºC, targeting a spectral window of approximately 12 ppm centered on the water signal at 4.69 ppm. Each scan captured 64K data points with an inter-scan relaxation delay of 3 seconds. The NOESY experiments were tailored with specific parameters: a mixing time of 0.2 seconds for NOESY. Post-acquisition, the raw data were processed using version 4.1.3 of Bruker's TopSpin software. For accurate chemical shift referencing in the 1H NMR spectra, the DSS signal was calibrated to 0.000 ppm.

Metabolites were identified and quantified using Chenomx NMR Suite 10 professional (Chenomx Inc., Canada), leveraging data from 1D NOESY experiments. This software facilitated the manual integration of peak areas corresponding to specific proton signals of compounds in comparison with the internal standard, DSS. The quantification of metabolite concentrations was then calculated, utilizing a methodology that relies on the signal intensity of the

1H NMR spectrum, with DSS serving as the reference standard.

To validate the study's findings, two-dimensional (2D) heteronuclear 1H-13C Heteronuclear Single Quantum Coherence (HSQC) and homonuclear proton Total Correlation Spectroscopy (TOCSY) NMR spectroscopy experiments were conducted using the "hsqcetgpsisp2.2" and 1D NMR "dipsi2gpphzs" pulse sequences, respectively, from the standard pulse catalog of Bruker. These experiments were performed on quality control (Qc) samples, which were prepared by extracting 10 µL from each of the sample. Qc samples samples were generated in three variants: QcC, which was obtained from control samples; QcT, derived from the E171-TiO2-treated group; and QcG, which was the general QC encompassing all of the samples. The reason for running only QC samples with 2D-NMR was to save time, as studying a single sample with 2D-NMR takes about 24 hours. The experiments were carried out on a 700 MHz Bruker Neo NMR system, equipped with a QCI CryoProbe, at a temperature of 22 ºC. The spectral widths for the 1H (F2) and 13C (F1) dimensions in HSQC experiments were set at 11.7 and 185 ppm, respectively, while for TOCSY, they were 11.7 and 12.0 ppm, with 256 increments recorded in each spectrum. HSQC experiments involved 64 scans with a relaxation delay of 1.5 s per scan. In contrast, TOCSY experiments included 48 scans with a 2 s relaxation delay and a set mixing time of 80 ms for spin systems. Spectral processing employed a QSINC window function for all Free Induction Decays (FIDs), and the LPfc linear prediction procedure with coefficients of 60 and 48 for HSQC and TOCSY, respectively. The processed spectra were then transferred to the COLMARm 13C-1H HSQC, HSQC-TOCSY, and TOCSY Query and Verification database in Topspin ASCII format, as cited in "ACS Chemical Biology" (10(2), pp.452-459). The Deep Picker model 2 and Voigt function were utilized for peak picking and fitting, respectively. In the COLMARm database service, default values and query parameters were used, except for the 13C chemical shift cutoff, which was set at 0.4 ppm instead of the default 0.3 ppm. Finally, the metabolites identified by COLMARm in each QC sample were iteratively compared and further analyzed alongside the results obtained from the 1D NOESY qualitative and quantitative analyses.

Analysis of NMR data:

In the NMR spectroscopy analysis, DSS (4,4-Dimethyl-4-silapentane-1-sulfonic acid) was employed as the standard material for concentration determination, in addition to its role as a chemical shift calibration agent. It was added to the homogenate solution at a concentration of 250 µM. The concentration of metabolites were calculated relative to concentration of DSS. Univariate analysis, including the calculation of mean and median values of metabolites, was performed using SPSS version 26. The statistical significance of these metabolites was determined using Student's t-test or the Mann-Whitney U test, based on the homogeneity of variances, with a p-value threshold of <0.05 established for statistical significance. The metabolite list identified through NMR analysis was imported into MetaboAnalyst 5.0 [19] for data reduction and visualization. Prior to chemometric analysis, the data underwent several preprocessing steps. These steps included sample normalization by median, square root transformation, and auto scaling, specifically for liver tissue samples. Cube-root transformation was applied instead of square root in kidney tissue samples. The statistically significant differential metabolites were visualized using a Box Plot. Additionally, pathway analysis was conducted using MetaboAnalyst 5.0. Scatter plot visualization was generated with the Global test enrichment method. Relative-betweeness Centrality based topology analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) library for Rattus norvegicus.

RESULTS: Effects of E171-TiO_2 on Liver Metabolomics

There were 32 metabolites detected in liver tissue samples including 4-Aminobutyrate, Acetamide, Acetate, Acetoin, Alloisoleucine, Betaine, Creatine, Creatine phosphate, Creatinine, Cytidine, Dimethylamine, Ethanolamine, Fumarate, Glucose, Glutamate, Glycine, Guanidoacetate, Isobutyrate, Isoleucine, Lactate, Leucine, Methylguanidine, N-Acetylglucosamine, N-Nitrosodimethylamine, O-Acetylcholine, Pyruvate, Serine, Succinate, Thymine, Trimethylamine N-oxide, Uracil, 3-Methylhistidine. There were statistically significant different betwween groups in 4-Aminobutyrate, serine, Fumarate an Uracil. Subsequent to the processing of the raw data acquired through Nuclear Magnetic Resonance (NMR) spectroscopy, the task of annotating metabolites was undertaken. Following

this, the results of the fold change analysis, which focused on metabolites exhibiting significant differences between the studied groups, were rendered into a visual format. 4-Aminobutyrate, fumarate, serine and uracil were the metabolites which showed statisticallly significant difference as shown in box plot in Figure 1A. NMR spectroscopy possesses the capability to detect molecular concentrations that exceed micromolar thresholds. In the context of the control group, the concentrations of serine and fumarate were below the detection limit. Conversely, within the treatment group, quantitative analysis revealed the average concentrations to be 79.82 (±23.18) µM for fumarate and 572.75 (±178.92) µM for serine. The mean values of 4-Aminobutyrate in control group and treatment group were 326.85 (±223) and 460.87 (±135) µM. The values for uracil were 378.57 (118) in control and 281.21 (57) µM in treatment group.

The pathway enrichment analysis revealed alterations in Pyrimidine metabolism and in Alanine, Aspartate, and Glutamate metabolism, as depicted in Figure 2. The corresponding heat metabolites, their p-values, and their impact are detailed in Table 1.

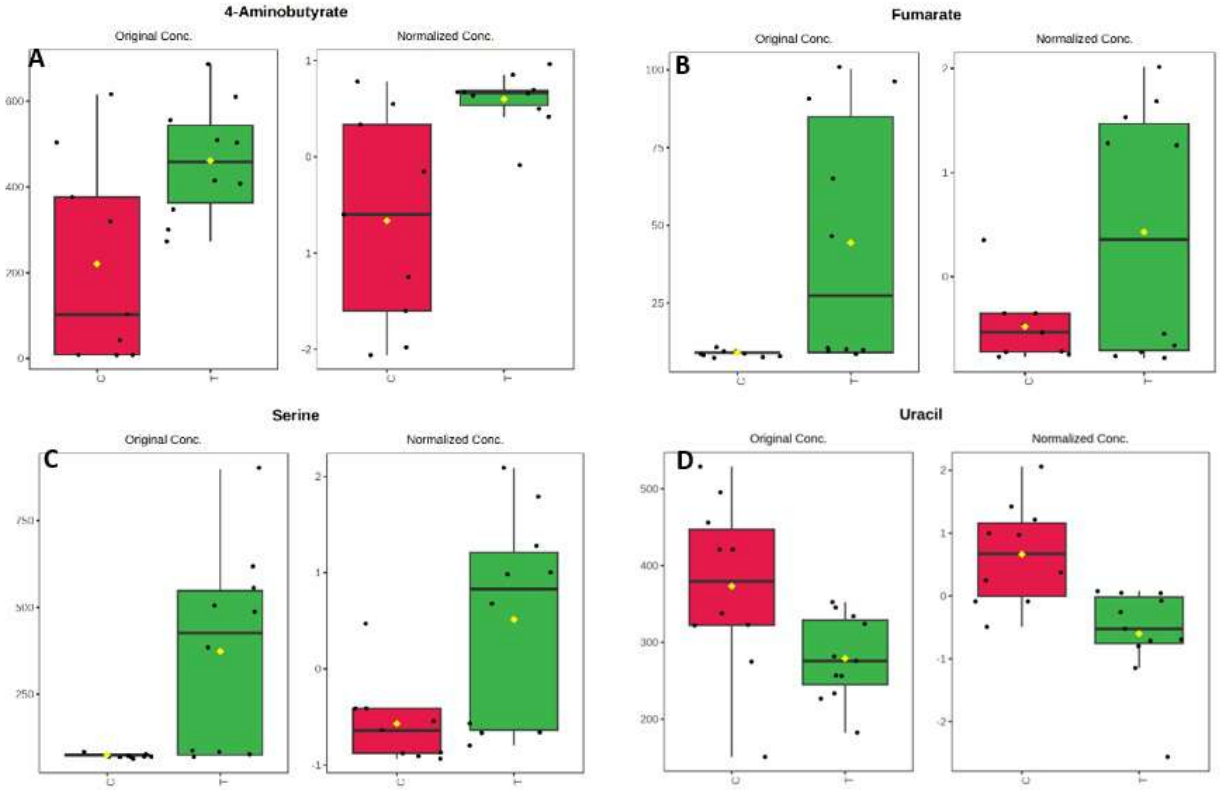


Figure 1. Boxplot illustrating the relative levels of certain significantly changed metabolites ($p < 0.05$) in the liver tissue as shown in A,B, C and D for 4-Aminobutyrate, fumarate, serine, and uracil, respectively. C=Control, depicted in red and T= E171-TiO_2 treated group shown in green. The Y-axis displays these levels in relative units. The data normalization was done median, square root transformation, and auto scaling, as analyzed using the Metaboanalyst program. The bar graphs represent the normalized mean values with a standard deviation. The boxplot spans from the 25th to the 75th percentile, with error bars showing the 5th and 95th percentiles.

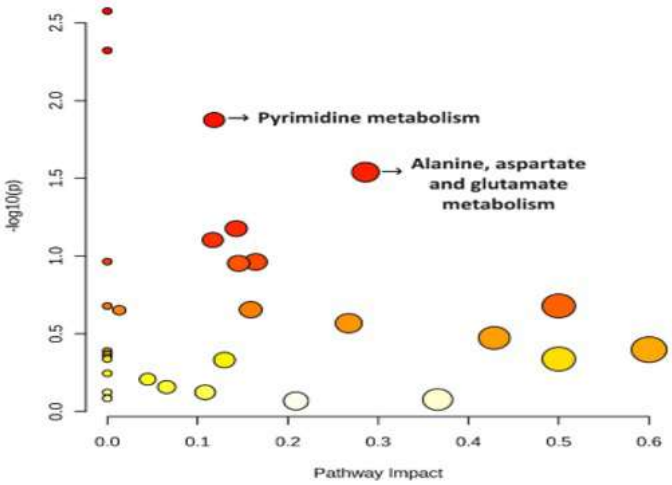


Figure 2. Pathway encrihment analysis generated with altered liver tissue metabolites depicts

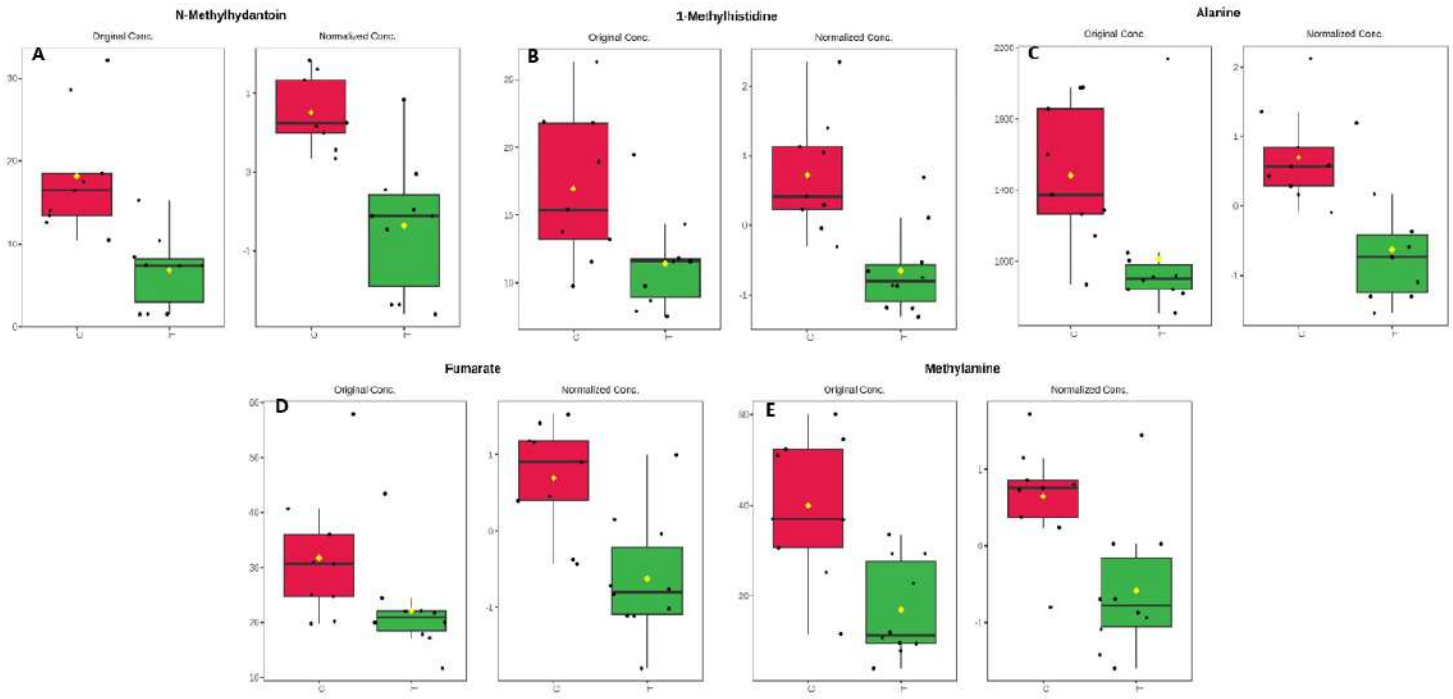


Figure 3. Boxplot graphs obtained with statistically significant metabolites A:N-Methylhydantoin, B:1-Methylhistidine, C:Alanine, D:Fumarate, E:Methylamine. C=Control, T= E171-TiO_2 treated group. The Y-axis displays these levels in relative units. The data normalization was done median, square root transformation, and auto scaling, as analyzed using the Metaboanalyst program. The bar graphs represent the normalized mean values with a standard deviation. The boxplot spans from the 25th to the 75th percentile, with error bars showing the 5th and 95th percentiles.

Table 4. . p values and impact of metabolites and pathways in liver tissues

	Total	Hits	P value	Hit metabolites	Impact
Pyrimidine metabolism	39	3	0.013333	Cytidine; Thymine, Uracil	
Alanine, aspartate and glutamate metabolism	28	6	0.028866	L-Alanine; L-Glutamate; 4-Aminobutanoate; Fumarate; Pyruvate; Succinate	

Effects of E171-TiO_2 on Kidney Metabolomics

52 metabolites were detected in kidney tissue samples. There was statistically significant reduction in N-methylhydantoin, 1-methylhistidine, alanine, fumarate, methylamine and cis-Aconitate levels comparing to controls as shown in Figure 3 in Box-plot graph. Although there was a statistically significance fold change in cis-Aconitate level, because of p value was upper the significance (0.05) that metabolite was not included in significant metabolite groups. Alterations in 5 metabolites in the kidney tissue samples were statistically significant. The mean levels of metabolites in control and E171-TiO_2 treated groups as N-methylhydantoin (18.6; 9.40), 1-methylhistidine (17.86; 11.36), alanine (1510.92; 1034.11), fumarate (17.86; 11.36), methylamine (40.32; 16.23) respectively. All level of significant metabolites level were lower in treatment group compared to control group.

In kidney tissue samples, there were alterations in Citrate cycle (TCA cycle), Tyrosine metabolism, Alanine, aspartate and glutamate metabolism, Pyruvate metabolism, Glyoxylate and dicarboxylate metabolism, Glycine, serine and threonine metabolism pathways. The impacted pathways and interested metabolites were shown in Figure 4 and Table 2, respectively.

Table 2. Influenced pathways and hit metabolites, their impacts and p values.

	Total	Hits	P value	Hit metabolites	Impact
Citrate cycle (TCA cycle)	20	4		Succinate; cis-Aconitate; Pyruvate; Fumarate	
Tyrosine metabolism	42	4	0.002304	L-Tyrosine; Figure 5. Pathway enrichment analysis of kidney tissue. Fumarate; Pyruvate;Acetoacetate	
Alanine, aspartate and glutamate metabolism	28	8		L-Aspartate; L-Alanine; L-Glutamate; 4-Aminobutanoate; L-Glutamine; Fumarate; Pyruvate; Succinate	0.6234
Pyruvate metabolism	22	4	0.005	Pyruvate;(S)-Lactate; Acetate; Fumarate	
Glyoxylate and dicarboxylate metabolism	32	7		cis-Aconitate; Glycolate; Glycine; L-Glutamate; Acetate; Pyruvate; L-Glutamine	0.209
Glycine, serine and threonine metabolism	34	6	0.015036	Betaine; Guanidinoacetate; Glycine; L-Threonine; Creatine; Pyruvate	

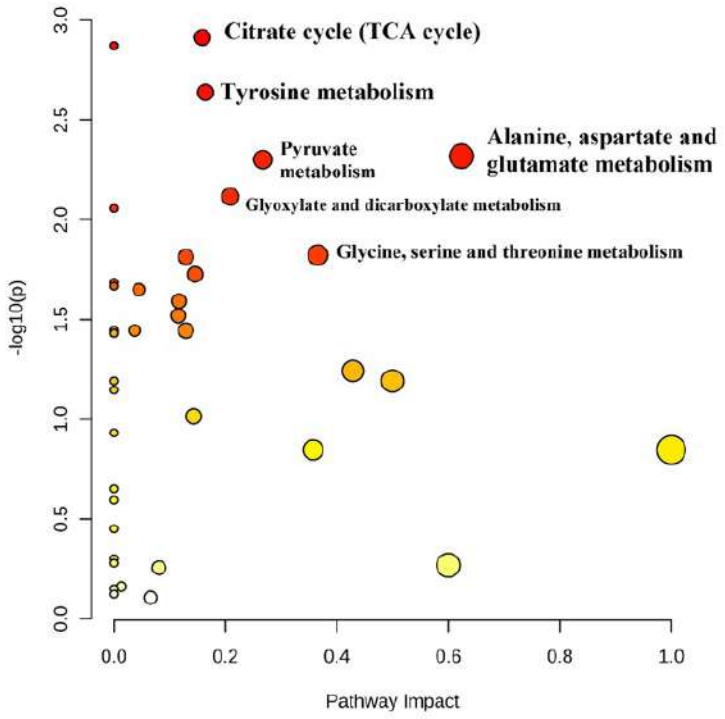


Figure 4. Pathway encrihment analysis generated with altered kidney tissue metabolites depicts influenced patyways. The diameter and colour of the circles have meaning about commenting analysis. The bigger circle is more impacted and the red is more powerful

DISCUSSION: Titanium dioxide, used as food additive, is particularly valued for its capacity to enhance the brightness and whiteness of food products. Despite the intricate legal regulations governing its use in the food industry, the specific mechanisms underlying the adverse effects associated with this compound remain largely unexplored and are not yet fully understood.With this study, we observed that altered pathways as Pyrimidine metabolism in liver tissue, and as TCA cycle, Pyruvate metabolism, and Glyoxylate and Dicarboxylate metabolism, Tyrosine metabolism in kidney tissue. Alanine, Aspartate, and Glutamate metabolism were the only affected pathway for both two organs.

In the context of liver tissue, the metabolomic impact of E171-TiO_2 was particularly pronounced in three molecules. Among these, 4-Aminoglutamate (commonly referred to as GABA), an inhibitory neurotransmitter, is noteworthy. Previous research has identified an elevation in GABA levels in association with obesity-induced hepatic lipid accumulation[20]. Intriguingly, the inhibition of GABA-transaminase, an enzyme critical in GABA synthesis, has been proposed as a therapeutic target for conditions like hyperinsulinemia and insulin resistance, both of which contribute to increased hepatic lipid levels [21]. The observed increase of this molecule in liver tissue following oral TiO2 administration may indicate a potential contribution to the exacerbation of fatty liver conditions.

The absence of fumarate and serine amino acids in the control group, in contrast to their presence in the E171-TiO_2-treated group, can be attributed to the limited sensitivity of NMR Spectroscopy. Employing quantitative NMR spectroscopy, which has a detection threshold at the micromolar level, a significant elevation in the levels of these molecules was noted in the E171-TiO_2-treated group compared to the control. Despite the lack of complete understanding of its underlying mechanism, studies have demonstrated an elevation in cellular fumarate levels in environments characterized by high glucose concentrations [22]. However, this hypothesis warrants further verification

through the analysis of other metabolites involved in energy metabolism.

A study investigating liver metabolite dynamics under hypoxic conditions revealed an increase in the serine metabolite, paralleling the findings of our study. This elevation in serine has been linked to the induction of enzymes within the pathway responsible for serine synthesis, which serves as a substrate in one-carbon metabolism [23] . Consequently, it can be inferred that E171-TiO_2 potentially induces liver damage via mechanisms associated with hypoxia-related pathways. [24].

Uridine, composed of uracil and ribose, has been documented to exert hepatoprotective effects, particularly in mitigating drug-induced hepatotoxicity and psychiatric disorders [25]. It is found that uracil could be decreased with age in a study conducted with rats [26]. This could be thought oral E171-TiO_2 exposure could be contribute to aging effects. The ingestion of E171-TiO_2 has been observed to induce an increase in the hepatic concentrations of these nucleobases, suggestive of potential oxidative stress-induced stimulation of purine catabolism or impaired glycogenesis. Furthermore, pathway analysis has revealed that E171-TiO_2 exposure elicits perturbations in the metabolic pathways governing purines and pyrimidines [27].

Pyrimidine metabolism, identified as one of the impacted pathways in liver tissue, has been linked to Non-Alcoholic Fatty Liver Disease (NAFLD). Inhibition of pyrimidine catabolism has been found to indirectly attenuate liver fibrosis [28]. Additionally, an upregulation of this pathway has been observed in hepatotoxicity induced by triptolide, a drug employed in autoimmune diseases known for its side effects [29]. Supported by these findings, it is plausible to propose that E171-TiO_2 may also promote an increase in pyrimidine metabolism. An another research has indicated a positive correlation between ubiquitin-conjugating enzyme E2T (UBE2T) and pyrimidine metabolism in Hepatocellular Carcinoma (HCC) [30]. This evidence may elucidate the potential cancer-like effects of TiO_2 through a similar mechanistic pathway.

Alterations in 5 metabolites in the kidney tissue samples were statistifically significant. N-methylhydantoin, a metabolite involved in Arginine and Proline metabolism, exhibited lower levels in the experimental group compared to the control (18.6 vs. 9.40, respectively). It has been documented that this metabolite is inversely correlated with the Urine Albumin-to-Creatinine Ratio (UACR) and positively correlated with Glomerular Filtration Rate (GFR). Furthermore, its reduction is noted in kidney-related metabolic disorders [31]. These findings, particularly the diminished levels of N-methylhydantoin in the presence of E171-TiO_2, provide key clues suggesting a potential impact on kidney functions. 1-Methylhistidine, often referred to as 1-Mhis, is categorized within the group of organic molecules identified as histidine derivatives. In a study, the patients with kidney cancer, had lower serum levels 1-Mhis, compared with healthy human serum [32]. These observations align with the findings of our study, where the control and E171-TiO_2-treated groups exhibited respective values of 17.86 and 11.36. However, it is noteworthy that while such results have been reported in tissues with Chronic Kidney Disease (CKD) in a previous study, they were not observed in healthy tissues [33]. This discrepancy indicates that further research is required to fully understand the behavior of this metabolite under different physiological conditions.

Research has demonstrated a reduction in the amino acid alanine metabolite in CKD kidney tissues, a finding that mirrors our study's results [34]. Given the catabolic nature of CKD, characterized by protein-energy wasting and the depletion of whole body and muscle proteins, it is conceivable that prolonged exposure to E171-TiO_2 (with average levels of 1510.92 in the control group and 1034.11 in the E171-TiO_2-treated group) may elicit similar effects [35].

Studies have shown an elevation in urinary fumarate levels, an intermediate component of the TCA cycle, in instances of renal failure attributed to CKD and diabetes [36, 37]. Conversely, research in cancerous tissues revealed lower fumarate levels compared to non-cancerous tissues, paralleling the findings of our study [38]. This variance between tissue types and associated biofluids may be attributable to organ-specific pathophysiological changes. In our pathway analysis, the pathways involving this metabolite were observed to be among the most significantly impacted.

In kidney tissue, several carbohydrate-related metabolic pathways, including the Citrate cycle (TCA cycle), Pyruva-

te metabolism, and Glyoxylate and Dicarboxylate metabolism, have been identified as being affected. Additionally, alterations have been observed in amino acid pathways, notably in Tyrosine metabolism; Alanine, Aspartate, and Glutamate metabolism; and in pathways related to Glycine, Serine, and Threonine metabolism. These findings suggest that oral consumption of titanium dioxide may adversely impact kidney function and contribute to abnormal effects on oxidative stress.

In the study, a notable variance was observed in the levels of the metabolite fumarate within both liver and kidney tissues. Specifically, it was discerned that the group treated with TiO_2 exhibited an increase in fumarate concentration in liver tissue, while concurrently displaying a decrease in its concentration in kidney tissue. This differential response highlights the distinct metabolic impacts of TiO_2 exposure in these two organ systems.

Our observations indicate that oral administration of TiO_2 leads to alterations at the tissue level. These changes are particularly pronounced and more readily quantifiable in kidney tissue. To corroborate these initial findings, there is a pressing requirement for expanded research, necessitating larger-scale studies to provide more comprehensive insights into the effects of TiO_2 on biological tissues.

1. JECFA. Thirtieth report of the joint FAO/WHO expert committee on food additives. FAO nutrition meetings report series. . 1969.

2. FDA. United States Food and Drug Administration - Summary of Color Additives for Use in the United States in Foods, Drugs, Cosmetics, and Medical Devices 2017 [Available from: https://www.fda.gov/industry/color-additive-inventories/summary-color-additives-use-united-states-foods-drugs-cosmetics-and-medical-devices.

3. Medina-Reyes EI, Delgado-Buenrostro NL, Díaz-Urbina D, Rodríguez-Ibarra C, Déciga-Alcaraz A, González MI, et al. Food-grade titanium dioxide (E171) induces anxiety, adenomas in colon and goblet cells hyperplasia in a regular diet model and microvesicular steatosis in a high fat diet model. Food and Chemical Toxicology. 2020;146:111786.

4. Dorier M, Béal D, Marie-Desvergne C, Dubosson M, Barreau F, Houdeau E, et al. Continuous in vitro exposure of intestinal epithelial cells to E171 food additive causes oxidative stress, inducing oxidation of DNA bases but no endoplasmic reticulum stress. Nanotoxicology. 2017;11(6):751-61.

5. Freyre-Fonseca V, Medina-Reyes EI, Téllez-Medina DI, Paniagua-Contreras GL, Monroy-Pérez E, Vaca-Paniagua F, et al. Influence of shape and dispersion media of titanium dioxide nanostructures on microvessel network and ossification. Colloids and Surfaces B: Biointerfaces. 2018;162:193-201.

6. Jensen DM, Løhr M, Sheykhzade M, Lykkesfeldt J, Wils RS, Loft S, et al. Telomere length and genotoxicity in the lung of rats following intragastric exposure to food-grade titanium dioxide and vegetable carbon particles. Mutagenesis. 2019;34(2):203-14.

7. Proquin H, Rodríguez-Ibarra C, Moonen CG, Urrutia Ortega IM, Briedé JJ, de Kok TM, et al. Titanium dioxide food additive (E171) induces ROS formation and genotoxicity: contribution of micro and nano-sized fractions. Mutagenesis. 2017;32(1):139-49.

8. ANSES. Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the risks associated with ingestion of the food additive E171. ANSES Maisons□Alfort, France; 2019.

9. NVWA. Netherlands Food and Consumer Product Safety Authority- Opinion on possible health effects of the foodadditive titanium dioxide (E171). 2019.

10. Additives EPoF, Flavourings, Younes M, Aquilina G, Castle L, Engel KH, et al. Safety assessment of titanium dioxide (E171) as a food additive. Efsa Journal. 2021;19(5):e06585.

11. T.C. Turkish Ministry of Agriculture and Forestry - TÜRK GIDA KODEKSİ GIDA KATKI MADDELERİ YÖNETMELİĞİ. 2023.

12. Winkler HC, Notter T, Meyer U, Naegeli H. Critical review of the safety assessment of titanium dioxide additives in food. Journal of nanobiotechnology. 2018;16:1-19.

13. Weir A, Westerhoff P, Fabricius L, Hristovski K, Von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environmental science & technology. 2012;46(4):2242-50.

14. Bachler G, von Goetz N, Hungerbuhler K. Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO2) nanoparticles. Nanotoxicology. 2015;9(3):373-80.

15. Muthubharathi BC, Gowripriya T, Balamurugan K. Metabolomics: Small molecules that matter more. Molecular omics. 2021;17(2):210-29.

16. Bingol K. Recent advances in targeted and untargeted metabolomics by NMR and MS/NMR methods. High-throughput. 2018;7(2):9.

17. Miggiels P, Wouters B, van Westen GJ, Dubbelman A-C, Hankemeier T. Novel technologies for metabolomics: More for less. TrAC Trends in Analytical Chemistry. 2019;120:115323.

18. Chen Z, Wang Y, Wang X, Zhuo L, Chen S, Tang S, et al. Effect of titanium dioxide nanoparticles on glucose homeostasis after oral administration. Journal of Applied Toxicology. 2018;38(6):810-23.

19. Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic acids research. 2009;37(suppl_2):W652-W60.

20. Geisler CE, Ghimire S, Hepler C, Miller KE, Bruggink SM, Kentch KP, et al. Hepatocyte membrane potential regulates serum insulin and insulin sensitivity by altering hepatic GABA release. Cell reports. 2021;35(13).

21. Geisler CE, Ghimire S, Bruggink SM, Miller KE, Weninger SN, Kronenfeld JM, et al. A critical role of hepatic GABA in the metabolic dysfunction and hyperphagia of obesity. Cell reports. 2021;35(13).

22. Frizzell N, Thomas SA, Carson JA, Baynes JW. Mitochondrial stress causes increased succination of proteins in adipocytes in response to glucotoxicity. Biochemical Journal. 2012;445(2):247-54.

23. Liang H, Song K. Comprehensive metabolomics and transcriptomics analysis reveals protein and amino acid metabolic characteristics in liver tissue under chronic hypoxia. Plos one. 2023;18(9):e0291798.

24. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. Cell Metab. 2017;25(1):27-42.

25. Setzer B, Lebrecht D, Walker UA. Pyrimidine nucleoside depletion sensitizes to the mitochondrial hepatotoxicity of the reverse transcriptase inhibitor stavudine. The American journal of pathology. 2008;172(3):681-90.

26. Son N, Hur HJ, Sung MJ, Kim M-S, Hwang J-T, Park JH, et al. Liquid chromatography–mass spectrometry-based metabolomic analysis of livers from aged rats. Journal of Proteome Research. 2012;11(4):2551-8.

27. An Y, Xu W, Li H, Lei H, Zhang L, Hao F, et al. High-fat diet induces dynamic metabolic alterations in multiple biological matrices of rats. Journal of proteome research. 2013;12(8):3755-68.

28. Luukkonen PK, Sakuma I, Gaspar RC, Mooring M, Nasiri A, Kahn M, et al. Inhibition of HSD17B13 protects against liver fibrosis by inhibition of pyrimidine catabolism in nonalcoholic steatohepatitis. Proceedings of the National Academy of Sciences. 2023;120(4):e2217543120.

29. Zhao J, Xie C, Wang K, Takahashi S, Krausz KW, Lu D, et al. Comprehensive analysis of transcriptomics and metabolomics to understand triptolide-induced liver injury in mice. Toxicology letters. 2020;333:290-302.

30. Zhu Z, Cao C, Zhang D, Zhang Z, Liu L, Wu D, et al. UBE2T-mediated Akt ubiquitination and Akt/β-catenin activation promotes hepatocellular carcinoma development by increasing pyrimidine metabolism. Cell Death & Disease. 2022;13(2):154.

31. Feng Q, Li Y, Yang Y, Feng J. Urine metabolomics analysis in patients with normoalbuminuric diabetic kidney disease. Frontiers in Physiology. 2020;11:578799.

32. Nizioł J, Ossoliński K, Tripet BP, Copié V, Arendowski A, Ruman T. Nuclear magnetic resonance and surface-assisted laser desorption/ionization mass spectrometry-based serum metabolomics of kidney cancer. Analytical and Bioanalytical Chemistry. 2020;412:5827-41.

33. Abbiss H, Maker GL, Gummer JP, Rawlinson C, Musk GC, Fleming PA, et al. Untargeted gas chromatography–mass spectrometry-based metabolomics analysis of kidney and liver tissue from the Lewis Polycystic Kidney rat. Journal of Chromatography B. 2019;1118:25-32.

34. Khattri RB, Thome T, Ryan TE. Tissue-specific 1H-NMR metabolomic profiling in mice with adenine-induced chronic kidney disease. Metabolites. 2021;11(1):45.

35. Stenvinkel P, Heimbürger O, Lindholm B. Wasting, but not malnutrition, predicts cardiovascular mortality in end-stage renal disease. Nephrology Dialysis Transplantation. 2004;19(9):2181-3.

36. You Y-H, Quach T, Saito R, Pham J, Sharma K. Metabolomics reveals a key role for fumarate in mediating the effects of NADPH oxidase 4 in diabetic kidney disease. Journal of the American Society of Nephrology: JASN. 2016;27(2):466.

37. Hanifa MA, Skott M, Maltesen RG, Rasmussen BS, Nielsen S, Frøkiær J, et al. Tissue, urine and blood metabolite signatures of chronic kidney disease in the 5/6 nephrectomy rat model. Metabolomics. 2019;15:1-16.

38. Nizioł J, Copié V, Tripet BP, Nogueira LB, Nogueira KO, Ossoliński K, et al. Metabolomic and elemental profiling of human tissue in kidney cancer. Metabolomics. 2021;17:1-15.

S138

EVALUATION OF THE EFFECT OF LITHIUM USE ON THYROID HORMONES AND INFLAMMATORY MARKERS

Rabia Zülal Bilge[1], Aslıhan Bilge Bektaş[1], Giray Bozkaya[2]

[1] University of Health Sciences,izmir Bozyaka Education and Research Hospital,department of Medical Biochemistry,izmir,türkiye

[2] University of Health Sciences,izmir Faculty of Medicine,izmir Bozyaka Education and Research Hospital,department of Medical Biochemistry,izmir,türkiye

INTRODUCTION: Bipolar disorder is a disease characterized by recurrent episodes of mania, depression, hypomania and mixed episodes, with remission periods in which subthreshold symptoms or no symptoms are observed outside the episodes, affecting functionality and leading to increased mortality and morbidity [1]. It is thought to be a mental disorder in which genetic and neurobiological mechanisms play a role in its etiology and triggered by biopsychosocial stressors [2]. Due to its recurrent characteristic, the time patients spend under maintenance treatment is much longer than acute treatment, and maintenance treatment is as important as acute treatment [3].

Lithium is the most effective mood stabilizer in the maintenance treatment of bipolar disorder in terms of protecting from both manic and depressive episodes and preventing suicide [4]. Lithium therapy is the main prophylactic treatment for bipolar disorders. Lithium accumulates in the thyroid gland in concentrations 3-4 times higher than in plasma [5,6]. Previous studies have shown that transient TSH elevations may occur, especially in the first 2-3 months of treatment [7,8]. Although thyroid function should be monitored continuously, no intervention is required as long as the elevations are moderate. Lithium affects normal thyroid function through multiple mechanisms. At the cellular level, it decreases thyroid hormone synthesis and release. It also decreases peripheral deiodination of tetraiodothyronine (T4) or thyroxine by decreasing the activity of the enzyme type I 5' deiodinase [9]. Lithium has been shown to decrease TSH secretion and cAMP activity. Hypothyroidism and clinically and/or ultrasonographically detected goiter are the most common thyroid abnormalities in patients receiving long-term lithium treatment. Lithium-induced hyperthyroidism is very rare. Lithium increases the tendency to thyroid autoimmunity in susceptible individuals due to its effect of increasing the activity of B lymphocytes and decreasing the ratio of circulating suppressor cells to cytotoxic T cells [10].

The results of previous studies show that lithium use also has some regulatory effects on the hematopoietic system. It increases granulocyte colony stimulating factor (G-CSF) in the bone marrow, increases the amount of neutrophils and increases the number of neutrophils in peripheral blood. This in turn affects the Systemic Immune Inflammatory Index (SII) [11,12].

In our study, we aimed to retrospectively search the effects of maintenance therapy on thyroid, creatinine and some hematologic parameters in lithium users.

Methods: The data of patients who received lithium treatment for bipolar disorder from the outpatient clinics of our hospital between May 2021 and May 2023 were collected retrospectively from the Hospital Information System. There were 41 patients who received lithium treatment for more than three months and had therapeutic drug monitoring more than twice.

Platelet, neutrophil and lymphocyte tests to calculate the Systemic Immune Inflammatory Index [SII= ((platelet count)*(neutrophil count))/lymphocyte count] were measured on a Sysmex XN-1000 Hematology Analyzer (Sysmex, Norderstedt, Germany). TSH, FT4 and creatinine tests were performed on Cobas 8000 Modular Analyzer System (Roche, Mannheim, Germany) using serum samples. The data obtained were evaluated by Kolmogorov-Smirnov, One-way ANOVA, Friedman and Paired Sample-T tests using Statistical Package for the Social Sciences

(SPSS) v22.0. The significance level was determined as $p<0.05$.

RESULTS: A total of 72 electronic patient files were accessed. Fifteen patients could not be included in the study because they had only 2 lithium level measurements. Similarly, 6 patients were excluded because they had less than 3 months of follow-up and 10 patients were excluded because they did not have simultaneous blood lithium level, hemogram and biochemistry data. Forty-one patients who had been receiving lithium treatment for more than three months, had more than two therapeutic drug monitoring and had simultaneous hemogram and biochemistry data were included in the study.

Participants' files were reviewed retrospectively, and blood lithium level, hemogram and biochemistry data at certain time intervals were included in the analyses.

The time periods to be used for repeated measurements of biochemical tests and hemogram values were determined as 4th-8th month (6 months) and 16th-20th month (18 months). For lithium levels, the 4th-8th month and 16th-20th month values were included in the analysis together with the baseline measurements.

Biochemical measurements (creatinine, TSH, free thyroxine (sT4), blood cell count levels) in electronic patient files and hospital laboratory system records were recorded together with concurrent lithium levels. The effects of lithium on other systems (e.g. gastrointestinal system, central nervous system, etc.) were not evaluated.

The mean age of the participants was 48.32 years and 36.58% of the sample was female.

The initial measurement values, the values between the 4th-8th months and 16th-20th months of the patients who were included in the study and who received lithium treatment were included in the One Way ANOVA analysis. No significant difference was found between lithium blood levels at all three time intervals (Table 1).

On the other hand, although there was a moderate increase in TSH, it was not statistically significant ($p>0.05$). Free T4 levels increased in the 4th-8th months of treatment compared to the 16th-20th months of treatment, but again not statistically significant ($p>0.05$) (Table 1).

Neutrophil, leukocyte values and systemic immune inflammatory index increased in the 4th-8th months of treatment compared to the 16th-20th months of treatment, but again not statistically significant ($p>0.05$). (Table 1).

In addition, creatinine values of the sample did not differ significantly between all three time intervals (Table 1).

DISCUSSION: Lithium is an effective mood stabilizer used in the treatment of bipolar disorders. Lithium is one of the drugs that affect thyroid functions most frequently among psychotropic drugs [12,13]. The most common thyroid gland side effects have been reported as goiter, subclinical or clinical hypothyroidism and rarely hyperthyroidism [12,14]. Lithium suppresses TSH activity. As a result of these changes in TSH activity, a decrease in T3 and T4 release occurs. It is thought that TSH release is stimulated in response to the decrease in thyroid hormone levels in order to maintain homeostasis in the HPT axis [15,16].

In our study, although TSH levels of the patients increased during this period, it was statistically insignificant. Free T4 levels increased in the 4-8 month period and decreased in the 16-20 month period.

In a retrospective study examining the effect of lithium on thyroid functions, it was shown that TSH elevation was related with the dose of lithium used and TSH elevation was found in high blood levels of lithium. In our study, the mean lithium blood level was found to be 0.71 mmol/L and the fact that lithium blood levels were similar in all three time intervals may explain the lack of change in thyroid function tests. [17]

In addition, the fact that 41 of 72 patients with bipolar disorder who were newly started on lithium for 2 years were included in the study with regular examinations is consistent with the fact that less than 15 percent of the sample had follow-up laboratory examinations in the study conducted in Sweden. Both studies have shown that the guideline recommendations for bipolar disorder are not followed [18].

Previous data support that lithium use increases granulocyte colony stimulating factor (G-CSF) in the bone marrow, increases the amount of neutrophils and increases the number of neutrophils in peripheral blood [17]. In previous years, these effects of lithium have led it to be considered as an alternative treatment option in cases of neutropenia developing with cytotoxic agents [18]. There are conflicting data regarding the effect of lithium on platelets [19]. In our study, although platelet blood levels increased in a two-year period, it was not statistically significant. In the blood parameters of the patients included in our study, neutrophils and leukocytes increased in the 4-8-month period. However, laboratory values showed a downward trend in the 16-20 month period. Systemic immune inflammatory index increased in the 4-8 month period and decreased in the 16-20 month period.

The effect of lithium on renal function is important. Previous studies have reported that polyuria and tubular damage occurred in approximately 20% of patients after lithium use. In a large sample study conducted to determine renal damage developing with lithium use, it was reported that renal damage was 0.5-1.2% in patients who used lithium for more than 15 years [20]. It has been shown that nephrogenic side effects are generally reversible in the short and medium term, but become permanent in treatments lasting longer than 15 years [21]. When we analyzed previous studies, it was reported that lithium may lead to a decrease in glomerular filtration rate, but the magnitude of the effect was not clear [20]. It is stated that the most important factor in nephropathy developing due to lithium use is the duration of lithium use [20]. The duration of lithium use was shorter in the patients included in our study and no significant change was found in repeated creatinine measurements. Therefore no difference could be achieved.

The limitations of our study were the small sample size and the fact that it was performed with only 2 years of data. In addition, since it was performed retrospectively, we did not have information about thyroid function tests and the addition of non-psychotropic agents that may affect creatinine. The strength of our study was the monitoring of the progression of the parameters we determined as treatment onset, acute and chronic stage.

In the future, there is a need for randomized controlled studies with longer duration, with more cases and including the evaluation of risk factors.

REFERENCES

1. Yatham LN, Kennedy SH, Parikh SV, Schaffer A, Bond DJ, Frey BN, et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2018 guidelines for the man”agement of patients with bipolar disorder. Bipolar disorders. 2018;20:97-170.

2. Altınbaş K, editor. Mood Disorders Diagnosis and Treatment. 2nd Edition. Istanbul:Bahçıvanlar Printing Industry I.C.;2021

3. Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon DA, et al. The long-term natural history of the weekly symptomatic status of bipolar I disorder. Archives of general psychiatry. 2002;59:530-537.

4. Geddes JR, Burgess S, Hawton K, Jamison K, Goodwin GM. Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. American Journal of Psychiatry. 2004;161:217-222.

5. Altınbaş K, Yeşilbaş D, Ince B, Cansiz A, Silan F, Özdemir Ö, et al. İki Uçlu Bozukluk Hastalarında Lityuma Yanıt ile GSK-3β Polimorfizmi İlişkisinin Değerlendirilmesi. Turk Psikiyatri Dergisi. 2018;29:73-78.

6. El-Mallakh RS, Wyatt RJ. The Na, K-ATPase hypothesis for bipolar illness. Biological psychiatry. 1995;37:235-244.

7. Oliveira JL, Silva Júnior GB, Abreu KL, Rocha ND, Franco LF, Araújo SM, Daher ED.et al. Nefrotoxicidade por lítio. Revista da Associação Médica Brasileira. 2010;56:600-606.

8. Rogers MP, Whybrow PC. Clinical hypothyroidism occurring during lithium treatment: two case histories and a

review of thyroid function in 19 patients. American Journal of Psychiatry. 1971;128:158-163.

9. Stein RS, Beaman C, Ali MY, Hansen R, Jenkins DD, Jumean HG. Lithium carbonate attenuation of chemotherapy-induced neutropenia. New England Journal of Medicine. 1977;297:430-431.

10. Shimizu M, Hirokawa M, Manabe T, Shimozuma K, Sonoo H, Harada T. Lithium associated autoimmune thyroiditis. Journal of clinical pathology. 1997;50:172-174.

11. Kleiner J, Altshuler L, Hendrick V, Hershman JM. Lithium-induced subclinical hypothyroidism: review of the literature and guidelines for treatment. Journal of clinical psychiatry. 1999;60:249-255.

12. Friedman E, Levinson D, Connell TA, Singh H. Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. Biological psychiatry. 1993;33:520-525.

13. Kusalic M, Engelsmann F. Effect of lithium maintenance therapy on thyroid and parathyroid function. Journal of Psychiatry and Neuroscience. 1999;24:227.

14. Eşel E, Sofuoğlu S, Gönül AS, Turan MT, Baştürk M, Tutuş A, et al. Bipolar Hastalarda Kısa ve Uzun Süreli Lityum Tedavisinin Tiroid Hormonları Üzerine Etkisi. Türk Psikiyatri Dergisi 2001;12:195-201

15. Livingstone C, Rampes H. Lithium: a review of its metabolic adverse effects. Journal of Psychopharmacology. 2006;20:347-355.

16. Phelps J, Coskey OP. Low and very low lithium levels: Thyroid effects are small but still require monitoring. Bipolar Disorders. 2023;00:1-7.

17. Bosi A, Ceriani L, Elinder CG, Bellocco R, Clase CM, Landen M, et al. Quality of laboratory biomarker monitoring during treatment with lithium in patients with bipolar disorder. Bipolar Disorders. 2023;25:499-506.

18. Chadha VD, Bhalla P, Dhawan D. Uptake and retention of 65Zn in lithium-treated rat liver: role of zinc. Digestive and Liver Disease. 2010;42:446-450.

19. Boton R, Gaviria M, Batlle DC. Prevalence, pathogenesis, and treatment of renal dysfunction associated with chronic lithium therapy. American Journal of Kidney Diseases. 1987;10:329-45.

20. Lepkifker E, Sverdlik A, Iancu I, Ziv R, Segev S, Kotler M. Renal insufficiency in long-term lithium treatment. Journal of Clinical Psychiatry. 2004;65:850-856.

21. Bendz H, Aurell M, Balldin J, Mathe AA, Sjödin I. Kidney damage in long-term lithium patients: a cross-sectional study of patients with 15 years or more on lithium. Nephrology Dialysis Transplantation. 1994;9:1250-1254.

POSTER FULL TEXTS

P007

NOISE EXPOSURE OF MEDICAL LABORATORY EMPLOYEES

Metin Özdemir[1], Tülay Özdemir[2]

[1] Samsun Gazi State Hospital, Department of Medical Microbiology, Samsun, Türkiye

[2] Samsun Gazi State Hospital, Department of Medical Biochemistry, Samsun, Türkiye

Introduction: Discomfort due to ambient noise has been noted among laboratory employees (1). The effects of noise on the human body are not limited to hearing alone; they also include systemic and mental effects (2). Among the adverse health effects of noise, there is evidence suggesting that it can lead to irritability, sleep disorders, increased risk of cardiovascular disease, and attention disturbances (3). In addition to physiological stress caused by noise, studies have shown a significant increase in errors due to background noise in operating rooms (4). While noise pollution in hospitals has been overlooked for a long time, it has recently become the subject of more research (5,6). Although noise in laboratories has been considered in risk assessments, the exposure of employees and the investigation of noise in terms of laboratory safety have not been explored (7,8). Existing studies in the literature have been conducted either outside or inadequately according to standards (5,6,8,9).

Various publications have discussed noise measurements in hospital environments and the adverse effects of noise on hospital personnel (6,9,10). However, there is a lack of studies that provide a standardized analysis of noise levels and employee exposure to noise in laboratory environments, specifically in microbiology or biochemistry laboratories. This study aims to investigate whether the data obtained from measurements in our hospital, evaluated according to regulations, can determine whether noise poses a significant risk to laboratory safety (11).

This study presents the exposure of laboratory employees at Samsun Gazi State Hospital to noise, along with comparisons of ambient noise levels and measurements from other selected units.

Methods:

Ethics: The study was conducted with the approval of the Samsun University Clinical Research Ethics Board (SÜ-KAEK-2023 13-15).

Study design: As part of the periodic risk analysis of Samsun Gazi State Hospital, accredited by TETRA-Test in Izmir, Türkiye, ambient noise and exposure measurements were conducted in various sections, including the laboratory, on December 29, 2022. The measurements were performed using the TS EN ISO 9612 and TS EN ISO 1996-2 methods and were reported on March 23, 2023.

A Delta Ohm HD-2110 portable noise measurement device was calibrated using the CESVA Model CB006-Acoustic Calibrator before conducting ambient noise measurements (according to TS-EN-ISO/9612 standards). In each laboratory room, a Svantec SV104 noise dosimeter sensor was placed on the collar of a volunteer employee for personal task-based noise exposure measurements (according to TS-ISO/1996-2 standards).

Ambient noise measurements were conducted at a height of 1.2 m from the ground, with sensors placed on tripods at least 1 m away from walls, while doors and windows were closed for 2 hours.

Exposure measurements were performed on the collar of a staff member in each environment, approximately 10 cm from the external auditory canal, for the entire shift duration (420 minutes).

Noise measurements were taken under indoor atmospheric conditions of approximately 22°C temperature, 38% humidity, 1010 hPa pressure, and 0 m/s air velocity (Va). Measurements were not taken in noise-free areas such as

storage areas.

Records were analyzed by the relevant company in a computerized environment, and equivalent continuous sound pressure level (Leq: dBA) and weighted noise level exposure normalized to an 8-hour working day (LEX,8h: dBA), C-weighted peak sound pressure level (LcPk: dBC, Lpeak: dBC) were reported. Laboratory measurement results were evaluated according to regulations, and the results were compared with measurements from other areas. The level, duration, and frequency of noise were determined to assess whether the noise was harmful and disturbing. Noise levels (Leq) and their time-weighted averages (LEX,8h) in the report were compared. Additionally, the number of noise-generating devices in the laboratory unit areas and the laboratory unit areas were compared.

Results

Laboratory areas and device numbers are shown in Table 1. The ceiling heights of all areas are 2.52 m, with suspended ceilings in place, consisting of vinyl-coated plaster panel coverings without noise-absorbing properties.

Table 1. Laboratory Areas and Number of Devices

Laboratory	Area	Analyzer	Centrifuge	Refrigerator	Air Conditioner	Deionized Water Device	Biological Safety Cabinet	Total
Biochemistry	114.74 m^2	7	5	5	3	2	-	22
ELISA/Serology	112.71 m^2	7	3	5	2	2	-	19
Bacteriology	36.30 m^2	3	-	2	1	-	2	6
Pathology Macroscopy	8.27 m^2	-	-	-	1	-	1	2
Nuclear Medicine	50,0 m^2	1	-	1	1	-	-	3

The highest ambient measurement (Leq/Lpeak) was 90 dBA/127 dBC in the boiler room, and the highest employee exposure (Lex, 8h/LcPk) was 102,7 dBA/154,2 dBC in the laundry room. Ambient (Leq/Lpeak) and exposure (Leq/Lpeak) measurement results in laboratories were as follows:

- Biochemistry laboratory: 70,3 dBA/122,4 dBC, 72,9 dBA/113,9 dBC
- Bacteriology laboratory: 67,6 dBA/118,5 dBC, 72,2 dBA/106,5 dBC
- Pathology Macroscopy room: 69,9 dBA/121,1 dBC, 75,7 dBA/125 dBC
- Nuclear Medicine unit: 72,9 dBA/117,8 dBC, 68,7 dBA/126,8 dBC

The measurement results are shown in Table 2.

Table 2. Noise Measurement Results

Measurement Location	Ambient Measurement Results		Measurement Strategy	Exposure Duration (min)	Exposure Measurement Results	
	Leq (dBA)	Lpeak (dBC)			Lex, 8h (dBA)	LcPk (dBC)
Bacteriology Lab	67,6	118,5	Task-Based Noise Exposure	420	72,2	106,5
ELISA/Serology Lab	69,1	120,1	Task-Based Noise Exposure	420	69,2	106,9
Biochemistry Lab	70,3	122,4	Task-Based Noise Exposure	420	72,9	113,9
Emergency Lab	69,8	124,1	Task-Based Noise Exposure	420	72,6	107,7
Sterilization Center	69,1	125,5	Task-Based Noise Exposure	420	90,6	128
Transfusion Center	64,6	116,1	Task-Based Noise Exposure	420	70,4	128,9
Pathology Macroscopy	69,9	121,1	Task-Based Noise Exposure	420	75,7	125
Nuclear Medicine	72,9	117,8	Task-Based Noise Exposure	420	68,7	126,8
Palliative Care	77,6	121,3	Task-Based Noise Exposure	420	70,1	120,9
General Intensive Care 1	69	119,3	Task-Based Noise Exposure	420	75,8	118,4
General Intensive Care 5th	68,9	125,5	Task-Based Noise Exposure	420	75,8	140,7
Infection Service	69,7	120,7	Task-Based Noise Exposure	420	69,3	116,9
Orthopedic Service 10th	72,1	125	Task-Based Noise Exposure	420	70,4	109,2
ENT Service 7th	68,2	122,7	Task-Based Noise Exposure	420	67,5	107,7
Urology Service 5th	68,1	123,8	Task-Based Noise Exposure	420	65,6	110,9
General Surgery 4th	68,7	113,6	Task-Based Noise Exposure	420	65,5	109,7
Neurosurgery Service 3rd	64,6	118,4	Task-Based Noise Exposure	420	63,1	102,2
Day Care Service	72,6	124,3	Task-Based Noise Exposure	420	61,1	111,4
Emergency Observation	66,6	118,8	Task-Based Noise Exposure	420	71,3	123,4
Emergency Yellow Area	70,7	121	Task-Based Noise Exposure	420	69,4	123,3
Emergency Trauma	72,4	113,5	Task-Based Noise Exposure	420	69,9	120,7
Boiler Room	90	127	Task-Based Noise Exposure	420	78,5	120
Laundry Room	86,5	126,1	Task-Based Noise Exposure	420	102,7	154,2

Discussion

Except for the boiler room and laundry room units, there were no measurement values above the values specified in the European Parliament directive and the regulation on the protection of workers from risks related to noise in Türkiye (11,12). Ambient and exposure measurements in laboratory areas were below the minimum action value (80 dBA) and the maximum peak sound level (Lpeak/LcPk) was measured below the minimum action value (130 dBC). However, exposures below these values, when prolonged, pose some health risks to employees (7).

Contrary to the environmental measurement results, where an equivalent continuous sound pressure level of 72,9 dBA was determined in nuclear medicine unit but exposure measurement was 68,7 dBA lower, in Biochemistry and Bacteriology laboratories, an equivalent continuous sound pressure level of 70,3 dBA and 67,6 dBA, respectively, was determined, but exposure measurements were 72,9 dBA and 72,2 dBA higher, respectively. There seems to be

exposure difference in these areas. The relatively higher exposure in Biochemistry and Bacteriology laboratories can be explained by continuous operation and a higher number of devices per unit area.

The ambient measurement value in the sterilization center is similar to that in the laboratories. However, the high exposure values can be explained by the narrowness of the working area and the high number of devices.

In a study conducted in Greece in hospital environments, ambient noise values of 73 dBA were found in the laundry unit, lower than our study, and ambient noise values of 66 dBA in the bio-pathology laboratory were similar to our measurements (13).

The exposure values of our laboratory environments were similar to a study conducted by Loupa et al. in 2013 in an automotive repair shop (69,3 ± 3.4) (14).

Intensive care units, patient wards, and outpatient clinics are generally investigated in hospital environments (15-17). It has been suggested that noise pollution in these units is a factor affecting the physical and mental health of patients and caregivers (16). Our study shows similar ambient noise levels to these units. The noise standards for these units are lower (45 dB). However, laboratory areas are considered industrial spaces, and higher standards are set (80 dB). Nevertheless, specific limit values and action values for the laboratory environment are not currently established.

Contrary to many studies, in our study, we tried to provide complete information about measurement equipment, device calibration, device location, and recording duration. We avoided taking averages and reported them as Leq values (18,19). Wallis et al. drew attention to this issue (8).

Improve occupational health is an important topic in the EFLM Guidelines For Green And Sustainable Medical Laboratories (20). There is a need to expand its scope to include improvement of laboratory environment noise and exposure.

Our study has some limitations. The hearing loss levels due to noise exposure of the employees were not included in this study. A long-term, multidisciplinary cohort study monitored by long-term measurements is needed for such a study.

Conclusions

In our laboratory design and device configuration, measurements were below the minimum exposure action values specified in the regulations, which is 80 dBA (Lex,8h), and did not require the use of personal protective equipment such as headphones. However, personal task-based noise exposure, except for nuclear medicine, was higher than ambient measurement values in other laboratory sections. Employees are likely to experience relatively high noise exposure due to continuous and intense work pace. It was concluded that our laboratories are not high-risk areas for hearing loss related to noise exposure. However, existing noise levels are at an ergonomic level that can affect employee performance, increase stress, and cause attention disturbances. Attention should be paid to this issue when preparing regulations. Laboratories are not entirely safe environments in terms of noise. Noise control of sources should be implemented to completely or partially eliminate the harmful effects of noise.

This study conducted noise measurements and cross-sectional analysis in the laboratory environment using methods compliant with standards. However, for determining the physical, social, and psychological effects of noise in the laboratory environment, further studies with long-term personal observations are needed. Laboratory architectural planning, device selection, and placement should consider the noise factor. Employee rotation should be ensured. Periodic measurements should be conducted to monitor ambient noise levels and personal exposures.

Research ethics: The study was conducted with the approval of the Samsun University Clinical Research Ethics Board (SÜKAEK-2023 13-15).

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Research funding: None declared.

References

1. Günay İc, Alayunt Fn, Çakmak B. Mikrobiyoloji Laboratuvarlarinda Ergonomi. Mühendislik Bilimleri ve Tasarım Dergisi. 2017;5:41-7.

2.Raja RV, Rajasekaran V, Sriraman G. Non-auditory effects of noise pollution on health: A perspective. Indian Journal of Otolaryngology and Head & Neck Surgery. 2019; 71:1500-1.

3.Basner M, Babisch W, Davis A, Brink M, Clark C, Janssen S, et al. Auditory and non-auditory effects of noise on health. The lancet. 2014; 383:1325-32.

4.Kurmann A, Peter M, Tschan F, Mühlemann K, Candinas D, Beldi G. Adverse effect of noise in the operating theatre on surgical-site infection. Journal of British Surgery. 2011; 98:1021-5.

5.Oliver D. David Oliver: Tackling noise pollution in hospital. BMJ. 2022; 377:1027.

6.Yildirim H, Mayda AS. Determination of noise levels in the outpatient clinics of medical faculty hospital at Duzce University. Konuralp Medical Journal. 2019; 11:235-41.

7.Derçin K, Küçük B, Kaya E, Doğan NF, Aral M. Tıbbi Mikrobiyoloji Laboratuvarı, Kan Transfüzyon Merkezi ve Merkezi Sterilizasyon Ünitesinde Risk Değerlendirmesi. Turk Mikrobiyol Cemiy Derg. 2023; 53:10-20

8.Wallis R, Harris E, Lee H, Davies W, Astin F. Environmental noise levels in hospital settings: A rapid review of measurement techniques and implementation in hospital settings. Noise & Health. 2019; 21:200-16.

9.Scquizzato T, Gazzato A, Landoni G, Zangrillo A. Assessment of noise levels in the intensive care unit using Apple Watch. Critical Care. 2020; 24:1-3.

10.Delaney L, Litton E, Van Haren F. The effectiveness of noise interventions in the ICU. Current Opinion in Anesthesiology. 2019; 32:144-9.

11.Çalışanların gürültü ile ilgili risklerden korunmalarına dair yönetmelik;2013. Avaible from: https://www.mevzuat.gov.tr/File/GeneratePdf?mevzuatNo=18647&mevzuatTur=KurumVeKurulusYonetmeligi&mevzuatTertip= 5

12.Consolidated text: Directive 2002/49/EC of the European Parliament and of the Council of 25 June 2002 relating to the assessment and management of environmental noise;2021. Avaible from: http://data.europa.eu/eli/dir/2002/49/2021-07-29

13. Loupa G, Katikaridis A, Karali D, Rapsomanikis S. Mapping the noise in a Greek general hospital. Science of The Total Environment. 2019; 646:923-9.

14.Loupa G. Case study: Health hazards of automotive repair mechanics: Thermal and lighting comfort, particulate matter and noise. Journal of occupational and environmental hygiene. 2013; 10:135-46.

15.Elliott RM, McKinley SM, Eager D. A pilot study of sound levels in an Australian adult general intensive care unit. Noise Health. 2010; 12:26-36 .

16.Pal J, Taywade M, Pal R, Sethi D. Noise pollution in intensive care unit: a hidden enemy affecting the physical

and mental health of patients and caregivers. Noise & health. 2022; 24:130.

17.Juang DF, Lee CH, Yang T, Chang MC. Noise pollution and its effects on medical care workers and patients in hospitals. International Journal of Environmental Science & Technology. 2010; 7:705-16.

18.Frontiers Production Office. Erratum: Methods for measuring and identifying sounds in the intensive care unit. Front Med (Lausanne). 2023; 10:1183690.

19.ISO 1996-2:2017 Acoustics Description, measurement and assessment of environmental noise Part 2 Determination of sound pressure levels;2017. Avaible from: https://www.iso.org/standard/59766.html

20.The EFLM Task Force "Green & Sustainable Laboratories". EFLM Guidelines For Green And Sustainable Medical Laboratories;2022. Avaible from: https://www.eflm.eu/upload/docs/EFLM-GREEN-LAB-BOOKLET.pdf

P026

INVESTIGATION OF PCSK9 AND CETP POLYMORPHISMS IN DIABETIC NEPHROPATHY

Nazlı Helvacı[1], Alev Kural[2], Sibel Kuraş[1], Özgür Can[3], Fatih Hacımustafaoğlu[1]

[1] Health Sciences University Hamidiye School of Medicine Medical Biochemistry Department

[2] Health Sciences University Bakırköy Dr. Sadi Konuk Teaching and Research Hospital Medical Biochemistry Department

[3] Health Sciences University Haydarpaşa Numune Teaching and Research Hospital Nephrology Department

Introduction: Diabetic nephropathy (DN), a complication of diabetes mellitus (DM), is a chronic metabolic disease characterized by gradual impairment of renal function and albuminuria. It is a microvascular complication. DN is commonly acknowledged as the primary cause of end-stage kidney disease [1-3]. It has been stated that DN can affect up to 40% (25-40%) of diabetic patients [4, 5]. A progressive decrease in glomerular filtration rate, glomerular hypertension, increased urinary albumin excretion and renal failure or nephrotic syndrome are the outcomes of DN, which is characterized by thickness of the basement membrane of the glomerulus, glomerular sclerosis, and mesangial hypertrophy [5, 6]. When blood glucose levels exceed renal capacity, glucose cannot be reabsorbed by renal ultrafiltration and glucose levels in the fluid increase in the early stages of type 2 diabetes (T2DM). Increased glucose levels also increase both osmotic pressure and urine volume [4]. The primary risk factors associated with the development of DN are chronic hyperglycemia and hypertension. Typically, annual screening for microalbuminuria should start 5 years after the patient has been diagnosed with type 1 diabetes, and then annually thereafter. For T2DM, screening should take place at the time of diagnosis and annually thereafter. It is important to adhere to these recommendations. The etiology of DN is characterized by a multifaceted and intricate process that remains inadequately comprehended [3].

Dyslipidaemia is a significant risk factor for the development of cardiovascular diseases and DN. Cardiovascular disease is recognized as the primary cause of mortality in individuals with DN [7]. Monitoring the lipid profile as well as glycaemic control may delay the development of DN or other advanced complications of DM [8]. Dyslipidaemia contributes to the course of nephropathy by impairing the activity of many proteins involved in cholesterol metabolism (such as cholesteryl ester transfer protein (CETP)), resulting in increased LDL cholesterol, increased triglyceride levels, and decreased HDL cholesterol. It also hastens inflammation by increasing extracellular matrix synthesis and the development of proteinuria [9].

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a zymogen, is mainly produced by hepatocytes within the endoplasmic reticulum, as well as in the kidney and gut. It functions as a serine kinase. Following autocatalytic cleavage, it is released into the plasma [10]. The PCSK9 protein, encoded by the *PCSK9* gene, controls the number of LDL receptors. PCSK9 is significantly involved in the metabolism of LDL cholesterol and cardiovascular well-being by stimulating the process of lysosomal degradation of the LDL receptor [11]. *PCSK9* gain-of-function polymophisms cause cardiovascular disease by accelerating LDL receptor degradation and therefore increasing plasma LDL levels [12, 13]. The rs505151 polymorphism located within the cysteine-rich C-terminal domain of exon 12 c.2009A>G (E670G) is implicated in regulating self-processing PCSK9. The removal of this specific genetic region has been observed to result in the buildup of processed protein. Therefore, single nucleotide polymorphisms (SNPs) within this region may potentially be linked to modified enzyme activity and may contribute to the variability in the expression of PCSK9 [13]. The role of circulating levels of PCSK9 in DN patients has been previously studied, but the effect of the rs505151 polymorphism has not been studied.

CETP protein, encoded by the *CETP* gene, is involved in the regulation of plasma HDL, LDL and lipoprotein (a) levels and is also a therapeutic target [14]. Variants in the CETP gene may affect these cholesterol levels. By transferring cholesteryl esters from HDL particles to apolipoprotein B-containing particles, and partially exchanging them

for triglycerides, CETP plays a crucial role in HDL cholesterol metabolism [15]. The *CETP* rs5882 (I405V, A>G) and rs708272 (TaqIB, G>A) polymorphisms located within the highly polymorphic CETP gene locus demonstrate an association with decreased CETP levels. Furthermore, this association is correlated with elevated HDL levels and decreased LDL levels [23, 26]. TaqIB and I405V polymorphisms are found in non-coding regions of the *CETP* gene but affect enzyme activity [16, 17].

Given all of this knowledge, our objective was to assess the potential impact of rs505151, rs708272, and rs5882 polymorphisms in DN patients by performing a case-control design.

Methods:

Study Population: After agreement from the institutional ethics committee (University of Health Sciences Hamidiye Scientific Research Ethics Committee, Date of Approval: 14.10.2022, Decision No:23/7, Registration No: 22/499), the samples from volunteers and patients for this prospective interventional study was collected over 5-month in the University of Health Sciences Haydarpasa Numune Training and Research Hospital and performed at University of Health Sciences Hamidiye School of Medicine, Medical Biochemistry Department. During the research time frame, data were collected from 30 healthy volunteers and 54 patients with DN whose overall age range is from 18 to 75 years. Demographic data were collected on inclusion.

Genotype Distributions: Genomic DNA was extracted from the collected blood specimens using the GeneAll Exgene Blood SV DNA isolation kit (Cat. No. 105-101) following the manufacturer's protocol. Then, the concentrations of the DNA samples were measured with a fluorometric device (Denovix DS-11, USA).

Detecting variants of *PCSK9* gene rs505151 (A>G), *CETP* gene rs708272 (G>A) and rs5882 (A>G) performed by the touchdown polymerase chain reaction (PCR) method. A total of 25 µl PCR mix containing, 20 µl of "Before PCR Mix" and 5 µl genomic DNA samples were prepared for DNA amplification. A thermal cycler (C1000™; BioRad, CA, USA) was utilized to conduct PCR amplifications in a total volume of 25 µl. The PCR mixture was subjected to incubation for 3 minutes at a temperature of 95°C, which was pursued by 16 cycles of 20 seconds at 95°C, 25 seconds at 60-52°C (-0.5°C/cycle), and 50 seconds at 72°C. Subsequently, there were 20 cycles of 15 seconds at 95°C, 20 seconds at 52°C, and 40 seconds at 72°C. The final step was executed at 72°C for five minutes. Then, 3 µl of the "post-PCR" solution was added to the mixture amplified in the thermal cycler. The mixture was analyzed using the BioRad CFX96 Touch, a real-time quantitative PCR device. The mixture was incubated for 1 minute at 95°C and 5 minutes at 60°C for denaturation. SNPs were determined based on the melting curves.

Statistical Analysis: Statistical analysis of the study employed IBM SPSS (Statistical Package for Social Sciences) Statistics 26.0 software. Data were evaluated using descriptive statistical approaches such as mean, standard deviation, and minimum-maximum. Data distribution normality was tested using the Shapiro-Wilk examination. In scenarios where the distribution was non-normal, a Mann-Whitney U test was carried out to compare the two variables. The uniqueness of patient and control clusters was determined by implementing Pearson's Chi-Square test. The results were then analyzed based on a significance level of $p<0.05$, alongside a 95% confidence interval.

Results: No significant differences in genotype distribution were found between the patient and control groups concerning the rs505151 ($p= 0.305$), rs708272 ($p=0.657$), and rs5882 ($p=0.391$) polymorphisms. In addition, the allele frequency distributions of *PCSK9* (rs505151), *CETP* (rs708272), and (rs5882) did not exhibit a statistically significant difference between patient and control groups (Table 1).

Table 1: Genotype distribution and allelic distributions between patient and control groups

Genotype distributions of the PCSK9 and CETP gene polymorphisms in between DN and control groups			
	Patients	**Controls**	**p**
***PCSK9* (rs505151)**	**n=53**	**n=28**	
AA (Wild type)	38 (71.70%)	21 (75%)	
AG (Heterozygote)	13 (24.53%)	4 (14.29%)	
GG (Mutant)	2 (3.77%)	3 (10.71%)	
***CETP* (rs708272)**	**n=53**	**n=30**	
GG (Wild type)	11 (20.75%)	4 (13.33%)	
GA (Heterozygote)	29 (54.72%)	19 (63.33%)	
AA (Mutant)	13 (24.53%)	7 (23.33%)	
***CETP* (rs5882)**	**n=49**	**n=30**	
AA (Wild type)	27 (55.10%)	12 (40%)	
AG (Heterozygote)	6 (12.24%)	6 (20%)	
GG (Mutant)	16 (32.65%)	12 (40%)	

Allelic distributions of the PCSK9 and CETP gene polymorphisms in between DN and control groups			
	Patients	**Controls**	**p**
***PCSK9* (rs505151)**	**n=53**	**n=28**	
A (AA + AG)	51 (96.2%)	25 (89.3%)	0.334
G (GG)	2 (3.8%)	3 (10.7%)	
***CETP* (rs708272)**	**n=53**	**n=30**	
G (GG + GA)	40 (75.5%)	23 (76.7%)	0.903
A (AA)	13 (24.5%)	7 (23.3%)	
***CETP* (rs5882)**	**n=49**	**n=30**	
A (AA + AG)	33 (67.3%)	18 (60%)	0.508
G (GG)	16 (32.7%)	12 (40%)	

- Abbreviations: *PCSK9*, *Proprotein convertase subtilisin/kexin type 9*; *CETP, Cholesteryl ester transfer protein.*
- The data are given as n (%) (total number of genotypes (percentage of genotype)).

p: Pearson's Chi-Square test. Significance is set at $p < 0.05$

Genotype and allelic distributions of the *PCSK9* and *CETP* polymorphisms are shown in Figure 1 and Figure 2.

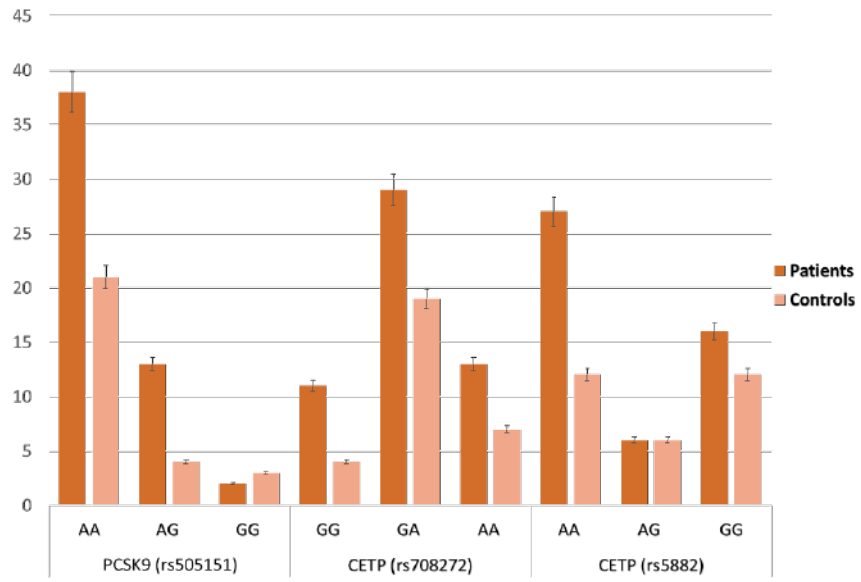


Figure 1: Genotype distributions of the *PCSK9* and *CETP* gene polymorphisms

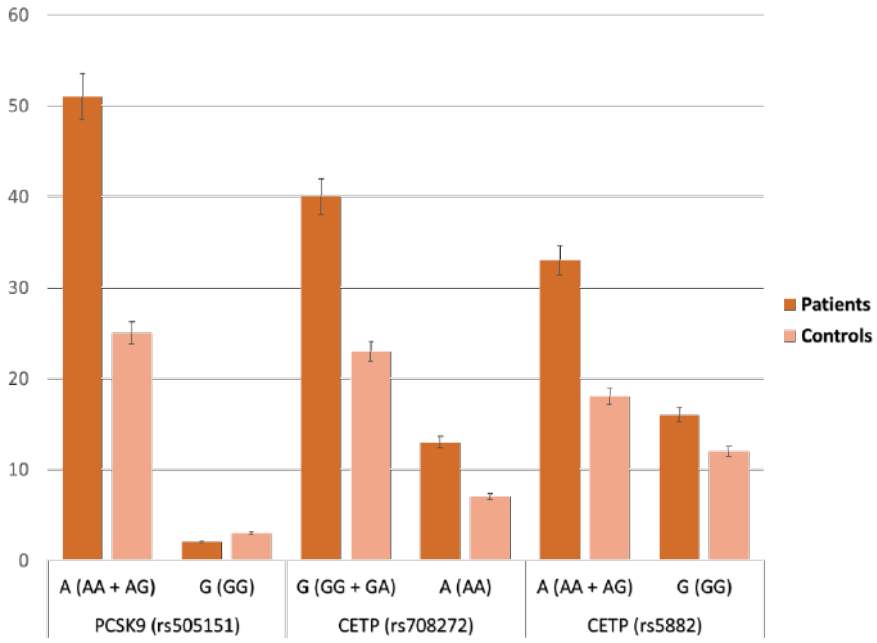


Figure 2: Allelic distributions of the *PCSK9* and *CETP* gene polymorphisms

Discussion: Occurring in approximately 40% of diabetics, DN is one of the advanced microvascular complications. The pathophysiology of DN is characterized by a complex interplay of several factors. Among these factors, dyslipidemia plays a significant role in the progression and development of DN. PCSK9 plays an important role in the maintenance of normal pancreatic islet function and diabetes progression. Islet autocrine lack of PCSK9 may result in increased LDL receptor number and LDL uptake therefore reducing β-cells' ability to secrete insulin [19]. Plasma levels of the PCSK9 protein were elevated in patients with T2DM in contrast to the control group [20].

In a study by Feng et al, they reported that increased PCSK9 accelerated inflammation in the kidneys of high-fat-fed diabetic mice. Additionally, it was shown by the researchers that the focused intervention of PCSK9 has the potential to significantly mitigate inflammation associated with DN and impede its advancement [21]. Another study has shown that serum PCSK9 levels are associated with renal dysfunction in patients with T2DM and that low PCSK9 levels in some patients may help to reduce chronic kidney disease [10].

PCSK9 levels are higher in patients with nephrotic syndrome, which is associated with hypercholesterolemia and hypertriglyceridemia, and PCSK9-lowering therapies are likely to help reduce proteinuria seen in nephrotic syndrome [22]. The study found that DN patients had higher levels of LDL cholesterol, total cholesterol, and triglycerides, as well as lower levels of HDL cholesterol [23]. High levels of both LDL cholesterol and remnant cholesterol were found to increase cardiovascular mortality in a study, and remnant cholesterol interacted with LDL cholesterol in people with T2DM and nephropathy [24]. Therefore, LDL cholesterol levels have an important effect in nephropathic patients. Increased circulating PCSK9 levels were positively correlated with inflammatory and oxidative stress markers in DN patients. Additionally, it was proposed that PCSK9 might be a non-conventional diabetes biomarker that might be used to identify Indian individuals who are at risk of having secondary problems from their diabetes [25].

According to a study, there is a higher prevalence of the minor allele G of the *PCSK9* rs505151 polymorphism among individuals diagnosed with cardiovascular disease [26]. In another study, it was found that AG polymorphism was more common in people with diabetic coronary artery disease and *PCSK9* expression levels were higher in G allele carriers than in A allele carriers [27]. In a study examining the effect of rs505151 polymorphism on lipid profile in healthy individuals, it was shown that there was no significant relationship, but it was shown to be affected

based on gender [28].

CETP is a protein that has both atherogenic properties in terms of decreasing HDL cholesterol level and anti-atherogenic role in terms of mediating reverse cholesterol transport [18]. The utilization of CETP inhibitors has demonstrated efficacy in mitigating the likelihood of developing new-onset diabetes, as well as enhancing glucose tolerance and insulin sensitivity [29].

In a study in which CETP mass was analyzed in patients with T2DM and DN, it was observed that CETP mass was higher and HDL cholesterol levels were lower in patients with T2DM; while HDL cholesterol levels were still low in patients with DN, CETP mass did not change significantly. Elevated CETP mass in type 2 diabetes is probably not responsible for the reduction of HDL cholesterol in nephropathy [30].

The *CETP* rs708272 AA allele was associated with lower CETP levels than the GG (wild-type) and GA (heterozygote) alleles. In a study conducted in patients with metabolic syndrome, rs708272 polymorphism was not found to be significant, while CETP levels were found to be higher [31]. Since the rs708272 polymorphism is situated in the *CETP* gene's first intron, it might be difficult to assess how CETP variation affects the likelihood of developing DN. Hadjadj et al discovered no significant differences in rs708272 polymorphisms in DN patients [32]. Although lower HDL cholesterol levels and HDL/LDL ratio were observed in women who developed diabetic kidney disease (compared those not developed), no difference was observed in terms of *CETP* rs708272 polymorphism [33].

In their study, Dizaji et al. observed that individuals with the rs5882 AA genotype had a protective effect against metabolic syndrome. However, this genotype did not demonstrate any significant impact on CETP protein levels [34]. A study showed that *CETP* polymorphisms (rs5882 and rs708272) did not affect on postprandial triglyceride levels in well-controlled T2DM patients [35].

In summary, our investigation did not yield statistically significant disparities between the rs505151, rs708272, and rs5882 polymorphisms in individuals with diabetic nephropathy (DN) when compared to the control group. The present investigation was subject to many limitations. There is a scarcity of studies that have examined the possible impact of *PCSK9* and *CETP* gene polymorphisms in patients with DN. This study is characterized by a single-center design and a limited sample size, rendering it cross-sectional. Additionally, in our study, we focused on examining only *PCSK9* and *CETP* gene polymorphisms without looking at circulating PCSK9 and CETP. The effect of circulating PCSK9 and CETP proteins on DN disease should also be investigated with a larger sample size. Our study has limited power to detect variants with small effect sizes; therefore, we cannot eliminate the possibility of a role for *PCSK9* and *CETP* genes, which are crucial to the dyslipidemia process, in the pathogenesis of DN. We suggest further analysis in larger independent cohorts to confirm these findings.

References

1. Hukportie DN, Li FR, Zhou R, Zheng JZ, Wu XX, Wu XB. Anthropometric Measures and Incident Diabetic Nephropathy in Participants With Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne) 2021;12:706845.

2. Qi C, Mao X, Zhang Z, Wu H. Classification and Differential Diagnosis of Diabetic Nephropathy. J Diabetes Res 2017;2017:8637138

3. Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int 2021;2021:1497449.

4. Jiang H, Zhang Y, Xu D, Wang Q. Probiotics ameliorates glycemic control of patients with diabetic nephropathy: A randomized clinical study. J Clin Lab Anal 2021;35:e23650.

5. Yashilha D, Shini-Rubina SK, Nanda KR, Anuba P. Association Between Monocyte-to-High-Density Lipoprotein (HDL) Cholesterol Ratio and Proteinuria in Patients With Type 2 Diabetes Mellitus: A Prospective Observational Study. Cureus 2023;15:e45783.

6. Rai B, Srivastava J, Saxena P. The Functional Role of microRNAs and mRNAs in Diabetic Kidney Disease: A Review. Curr Diabetes Rev 2023;20:aop.

7. McKay GJ, Savage DA, Patterson CC, Lewis G, McKnight AJ, Maxwell AP. Association Analysis of Dyslipidemia-Related Genes in Diabetic Nephropathy. PLoS One 2013;8:e58472.

8. Jha P, Das BK, Shrestha S, Majhi S, Chandra L, Sharma S, et al. Glycemic status, lipid profile and proteinuria in diabetic nephropathy. JNMA J Nepal Med Assoc 2010;49:143–146.

9. Singh J, Jain A, Bhamra R, Rathi V, Dhingra AK. The Mechanistic Role of Different Mediators in the Pathophysiology of Nephropathy: A Review. Curr Drug Targets 2023;24:104–117.

10. Feng Z, Liao X, Zhang H, Peng J, Huang Z, Yi B. Increased serum PCSK9 levels are associated with renal function impairment in patients with type 2 diabetes mellitus. Ren Fail 2023;45: 2215880.

11. Elewa U, Fernández□Fernández B, Mahillo□Fernández I, Martin□Cleary C, Sanz AB, Sanchez□Niño MD, et al. PCSK9 in diabetic kidney disease. Eur J Clin Invest 2016;46:779–786.

12. Galvão Lopes V, Fernandes de Oliveira V, Mendonça Munhoz Dati L, Naslavsky MS, Ferreira GM, Hirata MH. Dynamics of the personalities of PCSK9 on missense variants (rs505151 and rs562556) from elderly cohort studies in Brazil. J Biomol Struct Dyn 2023;3:1–9.

13. Xavier LB, Sóter MO, Sales MF, Oliveira DK, Reis HJ, Candido AL, et al. Evaluation of PCSK9 levels and its genetic polymorphisms in women with polycystic ovary syndrome. Gene 2018;644:129–136.

14. Yu C, Bakshi A, Watts GF, Renton AE, Fulton□Howard B, Goate AM, et al. Genome□Wide Association Study of Cardiovascular Resilience Identifies Protective Variation in the CETP Gene. J Am Heart Assoc 2023;12:e031459.

15. Boekholdt SM, Kuivenhoven JA, Hovingh GK, Jukema JW, Kastelein JJ, van Tol A. CETP gene variation: relation to lipid parameters and cardiovascular risk. Curr Opin Lipidol 2004;15:393–398.

16. Hou H, Ma R, Guo H, He J, Hu Y, Mu L, et al. Association between Six CETP Polymorphisms and Metabolic Syndrome in Uyghur Adults from Xinjiang, China. Int J Environ Res Public Health. 2017;14:653-666.

17. Kanca D, Gormus U, Tokat B, Eronat AP, Bugra Z, Ozturk O, et al. Additive Antiatherogenic Effects of CETP rs708272 on Serum LDL Subfraction Levels in Patients with CHD Under Statin Therapy. Biochem Genet

2017;55:168–182.

18. Goodarzynejad H, Boroumand M, Behmanesh M, Ziaee S, Jalali A. Cholesteryl ester transfer protein gene polymorphism (I405V) and premature coronary artery disease in an Iranian population. Bosn J Basic Med Sci 2016;16:114–120.

19. Lu F, Li E, Yang X. The association between circulatory, local pancreatic PCSK9 and type 2 diabetes mellitus: The effects of antidiabetic drugs on PCSK9. Heliyon 2023;9:e19371.

20. Ibarretxe D, Girona J, Plana N, Cabré A, Ferré R, Amigó N, et al. Circulating PCSK9 in patients with type 2 diabetes and related metabolic disorders. Clínica e Investigación en Arteriosclerosis 2016;28:71–78.

21. Feng Z, Liao X, Peng J, Quan J, Zhang H, Huang Z, et al. PCSK9 causes inflammation and cGAS/STING pathway activation in diabetic nephropathy. The FASEB Journal 2023;37:e23127.

22. Doiron S, Paquette M, Baass A, Bollée G, Cardinal H, Bernard S. Association between circulating PCSK9 and proteinuria in nephrotic syndrome: A cross-sectional study. Clin Biochem 2022;109–110:51–56.

23. Azadi SM, Fadaei R, Omid-Shafaat R, Hosseini J, Moradi N. Elevated angiopoietin-like protein 3 serum levels in diabetic nephropathy patients and its association with renal function and lipid profile. BMC Nephrol 2023;24:172.

24. Yu D, Wang Z, Zhang X, Qu B, Cai Y, Ma S, et al. Remnant Cholesterol and Cardiovascular Mortality in Patients With Type 2 Diabetes and Incident Diabetic Nephropathy. J Clin Endocrinol Metab 2021;106:3546-3554.

25. Waiz M, Alvi SS, Khan MS. Association of circulatory PCSK-9 with biomarkers of redox imbalance and inflammatory cascades in the prognosis of diabetes and associated complications: a pilot study in the Indian population. Free Radic Res 2023;57:294–307.

26. Devi K, Bhargave A, Ahmad I, Yadav A, Gupta R. Association study of *PCSK9* SNPs (rs505151 & rs562556) and their haplotypes with CVDs in Indian population. Ann Hum Biol 2023;50:56–62.

27.Mohamed SH, Hassaan MMM, Ibrahim BA, Sabbah NA. *PCSK9* E670G (rs505151) Variant and Coronary Artery Disease Risk Among Diabetics. Genet Test Mol Biomarkers 2021;25:615–623.

28. Rojas C, Ramírez H, Salazar LA, Kalergis AM, Gálvez AS, Escobar□Vera J. Characterization of *LDLR* rs5925 and *PCSK9* rs505151 genetic variants frequencies in healthy subjects from northern Chile: Influence on plasma lipid levels. J Clin Lab Anal 2019;33:e23001.

29. Piko P, Jenei T, Kosa Z, Sandor J, Kovacs N, Seres I, et al. Association of CETP Gene Polymorphisms and Haplotypes with Cardiovascular Risk. Int J Mol Sci 2023;24:10281.

30. Kawahara R, Yoshino M, Miyamae M, Tanaka A, Iwamoto Y. Study of CETP mass in the type 2 diabetic patients with nephropathy. Diabetes Res Clin Pract 2000;50:260–261.

31. Maroufi NF, Farzaneh K, Alibabrdel M, Zarei L, Cheraghi O, Soltani S, et al. Taq1B Polymorphism of Cholesteryl Ester Transfer Protein (CETP) and Its Effects on the Serum Lipid Levels in Metabolic Syndrome Patients. Biochem Genet 2016;54:894–902.

32. Hadjadj S, Gallois Y, Simard G, Bouhanick B, Passa P, Grimaldi A, et al. Lack of relationship in long□term type 1 diabetic patients between diabetic nephropathy and polymorphisms in apolipoprotein ε, lipoprotein lipase and cholesteryl ester transfer protein. Nephrology Dialysis Transplantation 2000;15:1971–1976.

33. Russo GT, Giandalia A, Romeo EL, Muscianisi M, Ruffo MC, Alibrandi A, et al. HDL subclasses and the common CETP TaqIB variant predict the incidence of microangiopatic complications in type 2 diabetic women: A

9 years follow-up study. Diabetes Res Clin Pract 2017;132:108–117.

34. Dizaji BF, Rivandi M, Javandoost A, Saberi Karimian M, Raei A, Sahebkar A, et al. Association of genetic polymorphisms of PON1 and CETP with the presence of metabolic syndrome; the effects of genotypes on their serum activity and concentrations. Egyptian Journal of Medical Human Genetics 2018;19:43–48.

35. Gavra P, Kolovou V, Papazafiropoulou AK, Melidonis A, Iraklianou S, Mavrogeni S, et al. The influence of gene polymorphisms on postprandial triglyceride response after oral fat tolerance test meal in patients with diabetes mellitus. Int J Clin Pract 2019;73:e13432.

P040

PREVALENCE OF DIABETIC NEPHROPATHY IN TREATED DIABETICS: SINGLE-CENTER STUDY

Berina Hasanefendić[1, 2], Snežana Unčanin[2, 3], Emir Šeherčehajić[2], Suzana Tihić-kapidžić[1], Velda Smajlbegović[4], Lajla Halilović[1], Denis Hasković[2, 5]

[1] Clinical Biochemistry and Immunology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2] Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Clinic For Nephrology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[4] Clinic For Oncology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[5] Clinical Pathology, Cytology and Human Genetics, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Introduction: Diabetic nephropathy is a microvascular complication of diabetes mellitus characterized by the presence of persistent proteinuria (high microalbuminuria and macroalbuminuria). Diabetic nephropathy is a kidney disease with complications in the form of various forms of secondary glomerular disease with functional and structural changes, the most significant of which are three histological changes in the glomeruli: mesangial expansion, thickening of the glomerular (tubular) basement membrane, and glomerulosclerosis (nodular and diffuse) [1]. The pathophysiological pathway of diabetic nephropathy is based on hyperglycemia, which leads to the production of reactive oxygen species and the activation of pathways, including protein kinase C, polyol, hexosamine, and advanced glycation end products. A significant feature is pronounced inflammation manifested by an increase in cytokines and chemokines, including IL-6, MCP-1 (monocyte chemoattractant protein-1), TGF-beta (transforming growth factor-beta) and VEGF (vascular endothelial growth factor), causing inflammation fibrosis and increased vascular permeability. Podocytopathy occurs, resulting in albuminuria. The resulting systemic and intraglomerular hypertension results in proteinuria. Proteinuria causes epithelial-mesenchymal cell transformation leading to fibroblasts and chronic tubular injury [2].

Most guidelines recommend screening with urine albumin/creatinine ratio (ACR; normal >30mg/g creatinine), either from the first morning urine (preferably) or from random samples. The pathological result is repeated once or twice over several months for consistency. Screening begins at diagnosis of type 2 diabetes and usually 5 years after the onset of type 1 diabetes [3].

Diabetic nephropathy is defined by evidence of proteinuria ≥300mg/day in diabetics. Although urinary albumin is recognized as an early marker, significant glomerular damage has already occurred when albumin appears in the urine. Therefore, new urinary biomarkers are needed to identify patients who are at risk of developing kidney damage. A proteomic study of a condition collectively called non-albumin proteinuria (NAP) identified several putative early biomarkers such as α-1 microglobulin, β-1 microglobulin, nephrin and cystatin C. Morning urine protein precipitation and subsequent resolution by 2D electrophoresis also identified another putative urinary biomarker is kininogen-1 [4].

Treatment of diabetic nephropathy targets four areas: cardiovascular risk reduction, glycemic control, blood pressure control, and inhibition of the renin-angiotensin system (RAS). Modification of risk factors, including smoking cessation and optimal lipid control strategies, is essential to reduce cardiovascular risk. A target value of HbA1C of 7% in type 2 diabetes leads to a lower risk of microvascular complications, including nephropathy [2].

The aim of this study is to examine the differences between laboratory and physical parameters between diabetics on oral and insulin therapy, as well as between diabetics with and without diabetic nephropathy.

Methods: This cross-sectional, observational study was conducted between July and August 2022 at the Health Center of Sarajevo Canton in Sarajevo, Bosnia and Herzegovina.

Participants

The study included 100 patients of both sexes over the age of 18, who suffer from diabetes mellitus type 2 and who use oral antidiabetics and those who are on insulin therapy of varying duration. The criteria for inclusion in the study were patients over 18 years old with diabetes mellitus type 2, patients with complete medical documentation of proven diabetes mellitus type 2 and diabetic nephropathy. The criteria for exclusion from the study were patients with a present urinary infection, patients with acute inflammatory disease and malignant diseases, patients younger than 18 years and pregnant women. The duration of the disease was determined for all patients by reviewing their medical records.

Samples

For the analysis, blood samples from the cubital vein were used primarily to determine the level of glucose in the blood, HbA1c, lipid status and creatinine of the patient, as well as urine samples that were further analyzed in order to assess the kidney function of the patients. Both types of samples were taken in the early morning hours.

A biochemical analyzer Olympus AU400e, Beckmann Coulter was used to determine blood glucose, creatinine and lipids and a biochemical analyzer Adams A1c, HA-8180T, Arkray, Inc. was used to determine HbA1c. A fully automated urine analyzer test strip UC□3500 (Sysmex, Kobe, Japan) was used for quantitative measurement of protein concentration in urine. Test tapes (Meditape UC-9A and UC-11A, Sysmex, Kobe, Japan) were used in the analysis. All patients had their blood pressure measured during the doctor's examination using a mercury sphygmomanometer. Patients' glomerular filtration was calculated using the formula: GF [ml/min/1.73m2] = 186 x (serum creatinine [μmol/l] x 0.0113) - 1.154 x age [years] - 0.203 (x 0.742 for women).

Ethical statement

This study was approved by the Ethics Committee of Health Center of Canton Sarajevo (No. 01-06-3245-2/22) and all principles of the Declaration of Helsinki were followed.

Statistical analysis

The collected data were archived in Microsoft Office Excel 2016 and the statistical program IBM SPSS Statistics 25.00 was used for statistical data processing. T-test was used to assess significant statistical differences between two groups. The threshold of statistical significance was set at the conventional level of $p \leq 0.05$.

Results: Out of a total of 100 patients, 69 were using insulin therapy, while the rest of the patients used oral therapy that included oral antidiabetics. Table 1. shows the age, physical and biochemical characteristics between patients using oral or insulin therapy. Significance was only observed when comparing the presence of hypertension in these two groups of patients ($p = 0,02$).

Table 1. Characteristics of patients (n = 100)

	Total	Oral therapy	Insulin therapy	p value
Age	69,71 ± 10,17	71,27 ± 8,57	68,88 ± 10,43	0,65
Duration of DMT2 (age)	14,93 ± 10,19	10,48 ± 7,00	16,06 ± 9,74	0,22
Presence of hypertension	61 (61%)	23 (37,7%)	38 (62,3%)	**0,02**
Presence of hyperlipidemia	63 (63%)	22 (34,92%)	41 (65,08%)	0,25
HbA1c (%)	8,45 ± 1,58	8,43 ± 1,88	8,46 ± 1,44	0,14
Blood sugar (mmol/l)	9,45 ± 3,89	9,56 ± 3,49	9,34 ± 4,16	0,1
GF (min/ml)	58,74 ± 23,81	58,41 ± 18,54	58,95 ± 27,23	0,5

Abbreviations: DMT2 – diabetes mellitus type 2; GF – glomerular filtration.

In our study, 77% patients was with diabetic nephropathy and those patients were older and with longer duration of diabetes mellitus type 2. As shown in table 2, big difference was shown in the concentration of total proteins between patients with and without diabetic nephropathy with statistical significance of 0,0004, respectively. Significance was also shown in systolic blood pressure and glomerular filtration, p = 0,0005 and p = <0,05, respectively.

Table 2. Comparision of biochemical and physical parameters between patients with and without diabetic nephropathy

	Without diabetic nephropathy	With diabetic nephropathy	p value (Cl%)
Age	65,11 ± 10,40	71,15 ± 10,08	0,9 (81,95%)
Duration of DMT2 (age)	12,58 ± 5,45	15,93 ± 11,29	0,2 (74,76%)
SBP (mmHg)	145,19 ± 21,48	134,40 ± 18,69	**0,005 (99,43%)**
DBP (mmHg)	82,50 ± 7,96	78,85 ± 11,52	0,2 (75,81%)
Hyperlipidemia (mmol/l)	5,18 ± 1,08	4,48 ± 1,27	0,3 (61,64%)
HbA1c (%)	9,21 ± 1,70	8,17 ± 1,53	0,4 (58,85%)
Blood sugar (mmol/l)	10,13 ± 3,4	9,00 ± 4,03	0,4 (58,15%)
GF (min/ml)	99,84 ± 3,95	58,74 ± 23,81	**<0,05 (100%)**
Creatinine (µmol/l)	111,42 ± 10,60,	117,65 ± 57,02	0,2 (73,13%)
Total proteins (mg/dl)	15,47 ± 5,64	67,22 ± 16,61	**0,0004 (99,96%)**

Abbreviations: DMT2 – diabetes mellitus type 2; SBP – systolic blood pressure; DBP – diastolic blood pressure; GF – glomerular filtration.

Discussion: The aim of this study is to examine the differences between laboratory and physical parameters between diabetics on oral and insulin therapy, as well as between diabetics with and without diabetic nephropathy. This study included 100 patients who suffer from diabetes mellitus type 2 and who use oral antidiabetics and those using insulin therapy. All of them suffered from diabetes mellitus type 2 more than 10 years. Comparing the presence of hypertension in these two groups of patients significance was observed (p = 0,02). Study of Sun D et al. follows our results, which concluded that diabetes mellitus type 2 may causally affect hypertension, whereas the relationship from hypertension to diabetes mellitus type 2 is unlikely to be causal [5]. Study which included 321 type − 2 diabetic

patients showed high prevalence of hypertension in type - 2 diabetes, elevated blood pressure was detected in 70.5% of the patients [6). Several studies from our country follow our result too and already presented the occurrence of hypertension in diabetics [7,8,9].

In our study out of 100 diabetics, 77 patients developed diabetic nephropathy. Extensive meta-analysis conducted in China included more than 79 thousands participants showed that prevalence of diabetic nephropathy is high. Their results showed that prevalence of diabetic nephopathy was 21,8% [10]. As shown in table 2, most patients had already defined risk factors for the development of diabetic nephropathy [11], which in this case are duration of diabetes, hypertension, dyslipidemia and hyperglycemia. Comparing the biochemical and physical parameters between diabetics with and without diabetic nephropathy statistical significance was shown in total proteins, systolic blood pressure and glomerular filtration. Glomerular filtration in patients with diabetic nephropathy was 58,74 ± 23,81, which would place those patients in stage 4, according to the staging of Gheith O et al. [12]. Based on the latest data, it was established that the opposite time trend in the prevalence of albuminuria and the decrease in glomerular filtration values in diabetics, despite the regression of microalbuminuria (reduced prevalence of albuminuria), continues the decline in glomerular filtration. This increased divergence between albuminuria and reduced values of glomerular filtration differs from the classic view, that albuminuria always precedes and leads to a progressive decrease in glomerular filtration [13].

In patients with diabetes mellitus type 2, hypertension usually exists before the onset of kidney disease. Obesity is assumed to be a common risk factor responsible for hypertension. It has been proven that hypertension additionally affects impaired kidney function and directly contributes to the deterioration of cardiovascular conditions. Proteinuria always precedes the hypertensive phase in diabetics, and then deterioration of renal functions contributes to the degradation of cardiovascular functions. The severity of high blood pressure in patients with diabetic nephropathy increases with each stage of chronic kidney disease, which in turn worsens renal function and ultimately 90% of patients approach end-stage renal disease. An individual's susceptibility to developing high blood pressure and kidney disease is caused by a variety of metabolic and hemodynamic changes shared by most diabetics [14]. Based on the American Diabetes Association's (ADA) study, a 10 mmHg reduction in systolic blood pressure was associated with a reduction in diabetic microvascular complications, including nephropathy. ADA recommends a blood pressure reduction target of <140/90 mmHg [15]. High blood pressure and high HbA1c are selected as significant risk factors for increasing proteinuria, which is a significant predictor of glomerular filtration rate (GFR) decreasing in patients with diabetic nephropathy [16].

Our study has some limitations, it is a single-center study and it included patients from only one area of our country. In further research, it would be necessary to include a larger number of patients with diabetic nephropathy from more health institutions and in the future to use more laboratory parameters or some of the new markers to monitor the progression of the disease.

To conclude, diabetics who used insulin therapy had higher values of HbA1c, glomerular filtration, a higher incidence of hypertension and hyperlipidemia than patients who used oral antidiabetics. Diabetic patients who developed renal nephropathy had significantly higher concentrations of protein in urine and lower values of SBP, DBP, lipids, HbA1c, blood sugar and GF in contrast to patients without renal nephropathy. Our results emphasize the importance of monitoring the mentioned parameters in diabetics for the purpose of timely actions.

References:

1. National Kidney Foundation. KDOQI Clinical Practice Guideline for Diabetes and CKD: Am J Kidney Dis. 2012;60:850-886.

2.Varghese RT, Jialal I. Diabetic Nephropathy. StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534200/

3. Lim A. Diabetic nephropathy - complications and treatment. International Journal of nephrology and renovascular disease. 2014;7:361–381.

4. Sulaiman MK. Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. Diabetology & metabolic syndrome. 2019;11:7.

5. Sun D, Zhou T, Heianza Y, Li X, Fan M, Fonseca VA, et al. Type 2 Diabetes and Hypertension. Circ Res. 2019;124:930-937.

6. Naseri MW, Esmat HA, Bahee MD. Prevalence of hypertension in Type-2 diabetes mellitus. Ann Med Surg (Lond). 2022;78:103758.

7. Dizdarevic-Bostandzic A, Begovic E, Burekovic A, Velija-Asimi Z, Godinjak A, Karlovic V. Cardiovascular Risk Factors in Patients with Poorly Controlled Diabetes Mellitus. Med Arch. 2018;72:13-16.

8. Jusupović F, Avdić D, Mahmutović J, Rudić A, Pašalić A, Branković S, et al. The presence of risk factors for diabetes mellitus type 2 in patients of family practice medicine. JHSCI. 2011;1:23-7.

9. Mališ S, Savić-Radojević A, Kovačević M, Pavlović D. Oxidative stress and other risk factors associated with diabetic nephropathy in type 2 diabetes mellitus. Biomedicinska istražavanja. 2018;9:27–36.

10. Zhang XX, Kong J, Yun K. Prevalence of Diabetic Nephropathy among Patients with Type 2 Diabetes Mellitus in China: A Meta-Analysis of Observational Studies. J Diabetes Res. 2020;2020:2315607.

11. Tziomalos K, Athyros VG. Diabetic Nephropathy: New Risk Factors and Improvements in Diagnosis. Rev Diabet Stud. 2015;12:110-8.

12. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and risk factors. J Nephropharmacol. 2016;5:49–56.

13. Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int. 2021;2021:1497449.

14. Nazar CM. Mechanism of hypertension in diabetic nephropathy. J Nephropharmacol. 2014;3:49-55.

15. American Diabetes Association. 9. Microvascular complications and foot care. Diabetes Care. 2016;3:S72–S80.

16. Kovačević M, Kovačević M, Prodanović V, Čančar O, Mališ S, Čančar V, et al. Analysis of risk factors for progression of diabetic nephropathy in patients with type 2 diabetes. Srpski arhiv za celokupno lekarstvo. 2021;149:295-300.

P042

THE INVESTIGATION of KISSPEPTIN, SPEXIN and GALANIN in EUTHYROID WOMEN with HASHIMOTO'S THYROIDITIS

Ümmügülsüm Can[1], Sadinaz Akdu[2], Ahmet Hamdi Aktan[2]

[1] Konya City Hospital

[2] Fethiye State Hospital

Introduction: Anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies that are elevated and cause the destruction of thyroid cells are the hallmarks of Hashimoto thyroiditis (HT), an autoimmune thyroid disease. Insulin resistance (IR) and several other conditions, such as central obesity, dyslipidemia, endothelial dysfunction, and atherosclerosis, have been definitively linked to hypothyroidism. Additionally, changes occurring in appetite and body weight (BW) are linked to hypothyroidism. Patients with hypothyroidism frequently gain weight despite having less appetite [1].

The contribution of circulating levels of galanin, kisspeptin, and spexin to such alterations in patients with hypothyroidism and euthyroidism remains unclear.

The gene of kisspeptin-1 (KiSS-1) encodes a family of peptides known as kisspeptins, which were first identified as metastasis suppressor peptides. It has been proposed that the hormone leptin, derived from adipocytes, is capable of controlling the expression of KiSS-1 in the hypothalamus [2]. By controlling hypothalamic gonadotropin-releasing hormone (GnRH) neurons through the kisspeptin receptor, kisspeptin influences pituitary-gonadal function, which in turn mediates fertility. Furthermore, it has been observed that kisspeptins are crucial in controlling body composition, feeding behavior, glucose homeostasis, and cardiac function [3].

It has been demonstrated that kisspeptin and galanin can counteract IR by boosting insulin sensitivity and energy metabolism. Kisspeptin inhibits obesity by acting as an anorexigenic factor, whereas galanin increases appetite by promoting food intake and BW. The peripheral tissues like adipose tissue, macrophages, skeletal and cardiac muscles, and pancreatic islets, as well as the central and peripheral nervous systems, all exhibit high levels of galanin expression. By binding to its three subtype galanin receptors (GalR1, GalR2, and GalR3), galanin also controls the release of insulin and pituitary hormones, inflammation, learning, memory, pain, nutrition, and reproduction [4].

Spexin, a peptide hormone with 14 amino acids, is extensively expressed in both peripheral and central tissues. When it comes to metabolic stress, spexin is secreted into the bloodstream and involved in various metabolic processes, including blood pressure, blood glucose and lipid metabolism, food intake, energy balance, and BW [5].

HT may influence metabolic parameters and heighten the risk of obesity. As far as we are concerned, no research has looked into galanin, kisspeptin, or spexin in females with euthyroid hypothyroidism. Thus, we tried to find out if HT influences the levels of circulating kisspeptin, spexin, and galanin in the current study. Additionally, in euthyroid HT women, we sought to assess the correlation between serum concentrations of kisspeptin, spexin, and galanin and metabolic parameters like IR and lipid parameters, as well as thyroid autoimmunity markers like anti-TPO and anti-Tg antibodies.

Methods: The study comprised 45 euthyroid HT women (mean age 38.4±10.5 years) and 45 healthy control women (mean age 38.1±9.4 years). Individuals having previously experienced thyroid dysfunction, diabetes mellitus (DM), hypertension, liver or lung disorders, cancer, renal, coronary heart, or rheumatologic diseases, as well as those receiving thyroid medication or other medications, were not included in the study. Furthermore, the subjects exhibiting abnormal concentrations of thyroid-stimulating hormone (TSH) or free triiodothyronine-4 (fT4) were excluded. Thyroid ultrasonography (USG) and positive anti-thyroid antibodies were the basis for the diagnosis of hypothyroidism (HT). Individuals diagnosed with HT had moderate-to-severe parenchymal heterogeneity on thyroid USG and

concurrent positive anti-TPO and anti-Tg antibody tests. Even so, the participants in the control group were in good health and had no prior history of thyroid or other diseases.

The calculation of body mass index (BMI) was detected with the division of weight (kg) by height (m^2), and the calculation known as fasting serum glucose (mmol L) x fasting serum insulin (μU mL)/22.5 was used to determine the homeostatic model assessment for insulin resistance (HOMA-IR). IR ≥2.5 was accepted for HOMA-IR scores [6].

Blood samples: After a 12-hour fasting, all blood samples were drawn from antecubital veins, and the sera were separated and frozen at 70°C until the analyses were performed. The chemiluminescence method (Cobas E 601 Hormone Auto-analyser, Roche Diagnostic System, Rotkreuz, Switzerland) was used to measure the serum fasting insulin, TSH, fT3, fT4, anti-TPO, and anti-Tg values. Commercially available kits based on standard procedures were used to measure serum fasting glucose, triglycerides (Tg), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels on the Architect C 8000 System (Abbott Laboratories, Abbott Park, Illinois, USA). Following the manufacturer's instructions, the concentrations of serum kisspeptin, spexin, and galanin were determined using a sandwich enzyme-linked immunosorbent assay (ELISA, Bioassay-Technology Laboratories Co., Ltd., Zhejiang, China).

Statistical analysis: The Statistical Package for the Social Sciences software, version 22.0 was used to assess all statistical analyses of the study findings(SPSS Inc., Chicago, IL, USA). To determine whether the data were appropriate for the normal distribution, the Kolmogorov-Smirnov test was employed. For parametric variables, the student's *t*-test was used to compare the groups, and for non-parametric variables, the Mann-Whitney U test was employed. For normally distributed variables, descriptive analyses were presented using mean±standard deviation (SD), and for non-normally distributed variables, median and range (min-max). To record potential correlations between parametric and non-parametric variables, respectively, Pearson's and Spearman's correlation analyses were carried out. Every subject was also included in the correlation analysis, which was also conducted.

Results: The biochemical parameters and baseline characteristics of the groups are displayed in Table 1. Such factors as age, aspartate transaminase (AST), alanine transaminase (ALT), fasting insulin and glucose, Tg, HDL-C, LDL-C, TC, HOMA-IR, fT3, fT4, and TSH did not significantly differ between the patients and controls (all parameters, $p>0.05$). Anti-TPO and anti-Tg levels in the patient group were significantly higher ($p<0.001$) than those in the controls for each. Additionally, the BMI scores of the patients were higher than those of the controls ($p<0.05$).

Under the findings presented in Table 1 and Figures 1 and 2, the patients had higher levels of kisspeptin and galanin than those in the controls ($p<0.01$) for each. Table 1 and Figure 3 demonstrate that the serum spexin levels in the patient and control groups were similar ($p=0.333$). Thyroid autoantibodies, kisspeptin, spexin, galanin, and lipid parameters were not correlated in light of our findings.

Kisspeptin and galanin, however, were found to have a positive correlation ($p<0.05$, $r=0.786$) (Table 2).

Discussion: To our knowledge, the present study is the first to examine the molecules of galanin, kisspeptin, and spexin in euthyroid hypothyroid patients. Nevertheless, it is still unclear what clinical significance these markers have for euthyroid hypothyroidism patients. In euthyroid women with HT, we investigated the relationship between anti-TPO, anti-Tg, glucose, and lipid metabolism and the levels of serum kisspeptin, spexin, and galanin. The levels of spexin, fT3, fT4, TSH, Tg, TC, HDL-C, LDL-C, and HOMA-IR were similar in both groups; however, serum kisspeptin, galanin, anti-TPO, anti-Tg, and BMI were observed to be increased in the patient group. Additionally, we discovered a positive correlation between kisspeptin and galanin.

Proinflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are responsible for the autoimmune disease known as HT. In people with IR, a correlation has been found between these proinflammatory cytokines and anti-TPO antibody levels [1]. In a previous study, the authors of the study asserted that regardless of

thyroid function, hyperlipidemia, IR, and atherosclerosis were linked to thyroid autoimmune disease [7].

In our study, we discovered elevated levels of kisspeptin and galanin among the patients with euthyroid hypothyroidism and normal lipid, glucose, insulin, and HOMA-IR levels. Although no metabolic disorders were detected in our study group, elevated levels of galanin and kisspeptin during the initial phase suggested that these markers might be linked to the etiology of HT. We believe that more research is necessary to fully understand the relationship between the changes in kisspeptin and galanin levels and the inflammatory process observed in these diseases.

Three related bioactive peptides, kisspeptin, spexin, and galanin, have functions in mood, behavior, and regulation of energy homeostasis. In both human and animal models, kisspeptin influences glucose-stimulated insulin secretion (GSIS), food intake, and/or energy expenditure [8].

In a study, it was shown that obese patients had higher levels of kisspeptin than those in the controls, but lower levels of adiponectin and that higher levels of kisspeptin were linked to IR [9]. Leptin levels in the serum have been demonstrated to be significantly lower in mice recipients of streptozotocin injections. Hypothalamic KiSS-1 is suppressed in uncontrolled DM due to defective leptin [2]. In the study carried out by Song et al. [10], it was observed that obese people with T2DM fed a high-fat diet (HFD) and having an ob/ob leptin deficiency had higher levels of KiSS-1 in their serum and livers. Through the stimulation of cyclic adenosine monophosphate (cAMP) protein kinase A (PKA)-responsive element binding protein (CREB) [cAMP-PKA-CREB] signaling, the hepatic production of increased kisspeptin is secondary to glucagon levels, ultimately acting on β-cells to suppress GSIS. It was discovered that in HFD-induced obese mice and ob/ob leptin-deficient mice, kisspeptin gene ablation increases GSIS and improves insulin sensitivity [10]. In a similar vein, adipocytes from type 1 DM (T1DM) rats had increased KiSS-1 mRNA levels in comparison to non-diabetic rats. Such a circumstance could reveal that insulin regulates adipocyte KiSS-1 expression in a significant way [11]. Conversely, in a different investigation, Kolodziejski et al. [12] discovered that, in comparison to non-obese controls with a BMI of ≤25 kg/m2, circulating levels of kisspeptin and spexin were lower in obese patients with a BMI of ≥35 kg/m2. There is a negative correlation between plexin and kisspeptin and BMI, HOMA-IR, insulin, glucagon, leptin, and active ghrelin [12]. Moreover, adult female KiSS-1r KO mice showed significantly higher BW, leptin levels, and adiposity with impaired glucose tolerance. The KiSS-1r KO female rats exhibited no decreased thyroid hormone secretion, despite feeding less and exhibiting significantly lower respiratory rate, energy expenditure, and locomotor activity. Additionally, the researchers hypothesized that the changes in kisspeptin signaling may be a direct or indirect cause of metabolic dysfunction, DM, or obesity in humans [13]. Another study found that whereas KiSS-1 expression was downregulated in the female hypothalamus and male pituitary gland, it was elevated in the fat tissue of both male and female rats after an 18-hour fasting. The study also suggested that adipocytes might be a source of kisspeptin in circulation; in contrast, obese HFD-fed rats showed a decrease in KiSS-1 mRNA in their fat tissue and an increase in KiSS-1 mRNA in their hypothalamus. Interestingly, refeeding for six hours brought fat KiSS-1 mRNA levels back to normal in male rats, but not in females [14]. Kisspeptin-10 (Kp-10) administered peripherally to male rats resulted in a decrease in total oxidant status and malondialdehyde, as well as an increase in antioxidant superoxide dismutase (SOD) and catalase (CAT) levels, suggesting that Kp-10 has protective effects on liver metabolism [15]. A recent study showed that there were reductions in the number of kisspeptin-immunoreactive neurons in the arcuate nucleus (ARC) and KiSS-1 mRNA-expressing neurons in the hypothalamus of female rats treated with propylthiouracil, which resulted in hypothyroidism. Based on this finding, kisspeptin expression in ARC is inhibited when thyroid hormone levels are low [16]. Kisspeptin has immunomodulatory, antioxidant, and trophoblastic migration effects. Fetal weight, glutathione peroxidase, SOD1, CAT, and fT3 and fT4 levels were all elevated by daily Kp-10 treatment. It was reported that daily Kp-10 treatment enhances placental morphology and fetal development in hypothyroid rats, inhibits placental oxidative damage, and boosts the placenta's expression of growth factors and antioxidant enzymes [17].

Based on our study findings, kisspeptin may be a key player in the pathophysiology of euthyroid hypertrophy and offers a therapeutic target for the development of new diagnostic tools for the prevention and treatment of HT and related disorders. We believe that additional research on humans and animals is necessary to fully understand the

intricate effects of kisspeptin.

Numerous central and peripheral receptor-mediated processes, such as feeding, pain, energy homeostasis, anterior pituitary hormone regulation, and reproduction, are linked to galanin [18]. In research, it was demonstrated that galanin caused a significant reduction in IR rats [19]. Another study revealed that obese children had significantly higher serum levels of leptin and galanin. Galanin has also been shown to increase insulin sensitivity, and such a connection implies that the galanin peptide, raising appetite, plays a role in the development of obesity and its associated conditions, like dyslipidemia. In the study, the researchers emphasized that the orexigenic peptide galanin was linked to the development of obesity and related metabolic disorders [20]. Additionally, it was discovered that metabolic syndrome was associated with significantly higher serum galanin levels. Also, a significant positive correlation was observed between serum galanin and HbA1c, Tg, HOMA-IR, and fasting blood glucose, indicating a potential role for neuropeptides in the pathophysiology of the condition [21]. The expression of anterior pituitary galanin is induced by estrogen and basal conditions and requires thyroid hormone. In a study, the administration of levothyroxine sodium (T_4) was stated to reverse a six-fold decrease in steady-state anterior pituitary galanin mRNA levels observed in hypothyroid rats, suggesting that thyroid hormones act directly at the pituitary level to regulate the expression of the galanin gene [22].

Our findings on galanin are compatible with those reported by previous studies.

As a recently identified adipokine, the novel spexin peptide plays a critical role in the regulation of obesity and associated metabolic disorders [5]. The findings regarding the function of spexin in IR, obesity, and DM are still up for debate. Spexin gene expression is 14.9 times downregulated in the omental and subcutaneous fat of obese individuals. Spexin causes a 32% reduction in caloric intake in rats, leading to weight loss. A study also indicated that spexin may be a novel hormone regulating BW and may be used in the treatment of obesity. As a result, variations in both acute and long-term nutritional cues can affect spexin levels [23]. Significant reductions in serum spexin levels were observed in a study examining obese children. Additionally, compared to obese children without IR, obese children with IR had lower serum spexin levels. Based on the study, the researchers concluded that spexin is closely related to β-cell function in obese children and may protect the process of glucose homeostasis [24]. The results of a study looking at the relationship between exercise and circulating spexin levels in obesity and T2DM revealed that plasma spexin levels were lower in obese individuals with or without T2DM and had a negative correlation with blood pressure and adiposity markers. Exercise was found to significantly increase spexin levels [25]. In animals with T2DM and DIO, spexin reduces appetite, controls lipid and carbohydrate metabolism, and enhances insulin sensitivity. Moreover, spexin also regulates the decrease of serum concentrations of adiponectin and the decrease of leptin, lowers lipid content, and changes the levels of IL-6 and TNF-α protein in the liver to influence the hormonal and metabolic profiles in DIO mice with T2DM. Researchers hypothesized in a study that spexin is a potential target for the treatment of DIO and T2DM and may be linked to the development of obesity and T2DM [26].

We found that serum spexin was similar in the patient and control groups. Based on our study findings, the patients with a high titer of anti-TPO and anti-Tg were found to have increased serum kisspeptin and galanin levels, suggesting there was a relationship between kisspeptin and galanin, and thyroid antibodies. The immune system linked to the thyroid may be able to enhance the production of kisspeptin and galanin, which in turn is linked to the pathogenesis of HT by controlling appetite and BW. To fully understand the connection between kisspeptin, galanin, and HT, further studies are required. Thyroid hormone, IR, and lipid levels were unaffected in our group of Hashimoto's patients, and their values showed no difference substantially from those in the controls. In Hashimoto's patients, early and subclinical impairment of these markers primarily points to the presence of an autoimmune illness.

This study has several limitations. First, our sample size was small.

Conclusions: In conclusion, we consider that based on our study findings, kisspeptin, spexin, and galanin will shed light on a novel perspective on the etiology of HT and related metabolic diseases. Future research on those bioactive peptides may provide a deeper insight into the pathophysiology of HT and pinpoint more exact molecular mecha-

nisms.

Acknowledgments

Author Contribution statement

Ummugulsum Can: Conceptualization) (lead); writing – review and editing – original draft (lead); formal analysis (lead)

Sadinaz Akdu: Software (lead); Methodology (lead)

Ahmet Hamdi Aktan: Conceptualization (supporting); Writing – original draft (supporting)

Ethics

The study was approved by the ethical board of the Necmettin Erbakan University, IRB NUMBER:2021/3223, and informed consent was obtained from all participants.

Conflict of interest

Ummugulsum Can, Sadinaz Akdu and Ahmet Hamdi Aktan: had no conflict of interest to declare in relation to this article.

Research funding

Ummugulsum Can, Sadinaz Akdu and Ahmet Hamdi Aktan declared none research funding.

Data Statement

All data generated or analyzed during this study are included in this article.

References

1. Blaslov K, Gajski D, Vucelić V, Gaćina P, Mirošević G, Marinković J, et al. The Associatıon of Subclınıcal Insulın Resıstance wıth Thyroıd Autoımmunıty ın Euthyroıd Indıvıduals. Acta Clin Croat. 2020;59(4):696-702.

2. Castellano JM, Navarro VM, Roa J, Pineda R, Sánchez-Garrido MA, García-Galiano D, et al. Alterations in hypothalamic KiSS-1 system in experimental diabetes: early changes and functional consequences. M.Endocrinology. 2009;150(2):784-94.

3. Wolfe A, Hussain MA. The Emerging Role(s) for Kisspeptin in Metabolism in Mammals. Front Endocrinol (Lausanne). 2018;9:184.

4. Fang P, She Y, Zhao J, Yan J,Yu X, Jin Y, et al. Emerging roles of kisspeptin/galanin in age-related metabolic disease. Mech Ageing Dev. 2021;199:111571.

5. Türkel İ, Memi G, Yazgan B. Impact of spexin on metabolic diseases and inflammation: An updated minireview. Exp Biol Med (Maywood). 2022;247(7):567-73.

6. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.

7. Tamer G, Mert M, Tamer I, Mesci B, Kilic D, Arik S. Effects of thyroid autoimmunity on abdominal obesity and hyperlipidaemia. Endokrynol Pol. 2011;62(5):421-8.

8. Mills EG, Izzi-Engbeaya C, Abbara A, Comninos AN, Dhillo WS. Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour. Nat Rev Endocrinol. 2021;17(2):97-113.

9. Calcaterra V, Nappi RE, Pelizzo G, De Silvestri A, Albertini R, De Amici M, et al. Insulin resistance and potential modulators of ovarian reserve in young reproductive-aged women with obesity and type 1 diabetes. Gynecol Endocrinol. 2021;37(9):823-30.

10. Song WJ, Mondal P, Wolfe A, Alonso LC, Stamateris R, Ong BW, et al. Glucagon regulates hepatic kisspeptin to impair insulin secretion. Cell Metab. 2014;19(4):667-81.

11. Dudek M, Kołodziejski PA, Pruszyńska-Oszmałek E, Sassek M, Ziarniak K, Nowak KW, et al. Effects of high-fat diet-induced obesity and diabetes on Kiss1 and GPR54 expression in the hypothalamic-pituitary-gonadal (HPG) axis and peripheral organs (fat, pancreas and liver) in male rats. Neuropeptides. 2016;56:41-9.

12. Kołodziejski PA, Pruszyńska-Oszmałek E, Korek E, Sassek M, Szczepankiewicz D, Kaczmarek P, et al. Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women. Physiol Res. 2018;67(1):45-56.

13.T olson KP, Garcia C, Yen S, Simonds S, Stefanidis A, Lawrence A, et al. Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. J Clin Invest. 2014;124(7):3075-9.

14. Brown RE, Imran SA, Ur E, Wilkinson M. KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. Mol Cell Endocrinol. 2008;281(1-2):64-72.

15. Aydin M, Oktar S, Yonden Z, Ozturk OH, Yilmaz B. Direct and indirect effects of kisspeptin on liver oxidant and antioxidant systems in young male rats. Cell Biochem Funct. 2010;28(4):293-9.

16. Tomori Y, Takumi K, Iijima N, Takai S, Ozawa H. Kisspeptin expression is decreased in the arcuate nucleus of hypothyroid female rats with irregular estrus cycles. Neurosci Res. 2017;117:35-41.

17. Santos BR, Dos Anjos Cordeiro JM, Santos LC, Barbosa EM, Mendonça LD, Santos EO, et al. Kisspeptin treatment improves fetal-placental development and blocks placental oxidative damage caused by maternal hypothyroidism in an experimental rat model. Front Endocrinol (Lausanne). 2022;13:908240.

18. Šípková J, Kramáriková I, Hynie S, Klenerová V. The galanin and galanin receptor subtypes, its regulatory role in the biological and pathological functions. Physiol Res. 2017;66(5):729-40.

19. Azin F, Khazali H. Neuropeptide galanin and its effects on metabolic and reproductive disturbances in female rats with estradiol valerate (EV) - Induced polycystic ovary syndrome (PCOS). Neuropeptides. 2020;80:102026

20. Acar S, Paketçi A, Küme T, Demir K, Gürsoy Çalan Ö, et al. Positive correlation of galanin with insulin resistance and triglyceride levels in obese children. Turk J Med Sci. 2018;48(3):560-8.

21. Alotibi MN, Alnoury AM, Alhozali AM. Serum nesfatin 1 and galanin concentrations in the adult with metabolic syndrome. Relationships to insulin resistance and obesity. Saudi Med J. 2019;40(1):19-25.

22. Hooi SC, Koenig JI, Abraczinskas DR, Kaplan LM. Regulation of anterior pituitary galanin gene expression by thyroid hormone. Brain Res Mol Brain Res. 1997;51(1-2):15-22.

23.W alewski JL, Ge F, Lobdell H 4th, Levin N, Schwartz GJ, Vasselli JR, et al. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity (Silver Spring). 2014;22(7):1643-52.

24. Chen T, Wang F, Chu Z, Sun L, Lv H, Zhou W, et al. Circulating Spexin Decreased and Negatively Correlated with Systemic Insulin Sensitivity and Pancreatic β Cell Function in Obese Children. Ann Nutr Metab. 2019;74(2):125-31.

25. Khadir A, Kavalakatt S, Madhu D, Devarajan S, Abubaker J, Al-Mulla F, et al. Spexin as an indicator of bene-

ficial effects of exercise in human obesity and diabetes. Sci Rep. 2020;10(1):10635.

26. Skrzypski M, Kołodziejski PA, Pruszyńska-Oszmałek E, Wojciechowicz T, Janicka P, Krążek M, et al. Daily Treatment of Mice with Type 2 Diabetes with Adropin for Four Weeks Improves Glucolipid Profile, Reduces Hepatic Lipid Content and Restores Elevated Hepatic Enzymes in Serum. Int J Mol Sci. 2022;23(17):9807.

Table 1: Clinical and demographic characteristics of control and Hashimoto Thyroiditis subjects

	Hashimoto Thyroiditis Subjects (n=45)	Controls (n=45)	p-Value
Age (yrs)	38.4 ± 10.5	38.1 ± 9.4	0.874
AST, IU/L	15 (10-25)	15 (9-27)	0.546
ALT, IU/L	16 (7-31)	14 (8-36)	0.802
Fasting insulin,µIU/mL	10.3 (3.8-56.5)	9.8 (2.9-17.3)	0.205
Fasting glucose, mg/dL	92 (69-106)	92 (75-102)	0.831
Triglycerides, mg/dL	94 (36-254)	97 (43-289)	0.865
HDL-C, mg/dL	60.8 ± 11.2	56.3 ± 14.3	0.204
LDL-C, mg/dL	129.6 ± 36.3	114.5 ±28.7	0.90
Cholesterol, mg/dL	209.6 ± 39.7	193.7 ±34.3	0.110
BMI, kg/m2	25.3 (20.7-244.8)	24.0 (18.8-31.6)	<0.05
HOMA-IR	2.3 (0.8-14.8)	2.2 (0.5-3.9)	0.295
FT3, pg/mL	3.1 ± 0.5	3.1 ± 0.3	0.903
FT4, ng/dL	1.2 ± 0.1	1.2 ± 0.2	0.655
TSH, µIU/mL	2.1 (0.3-4.2)	1.8 (0.4-3.9)	0.157
AntiTg, IU/mL	232.8 (15.9-940.9)	13.5 (10.0-37.1)	<0.001
AntiTPO, IU/mL	97.8 (8.3-600.0)	9.6 (7.7-17.9)	<0.001
Galanin, ng/L	33.2 (6.0-109.4)	10.3 (2.4-240.2)	<0.01
Kisspeptin, ng/L	488.5 (67.10-1598.7)	209.2 (62.0-2112.7)	<0.01
Spexin, ng/L	2041.9 (99.0-4403.1)	2108.6 (7.2-4236.1)	0.333

BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance

Table 2: Spearman's correlation analyses were performed to investigate the association of biomarkers levels in the Hashimoto Thyroiditis subjects

		Galanin
Kisspeptin	r	0,786
		<0,05

FIG. 1. Comparison of serum kisspeptin levels (ng/L) in women with euthyroid Hashimoto thyroiditis and controls ($p < 0.01$)

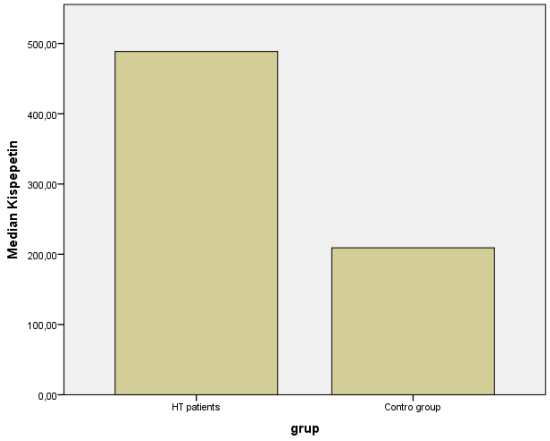


FIG. 2. Comparison of serum galanin levels (ng/L) in women with euthyroid Hashimoto thyroiditis and controls ($p < 0.01$)

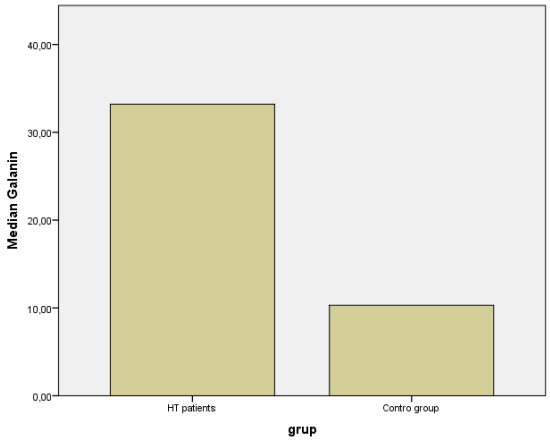
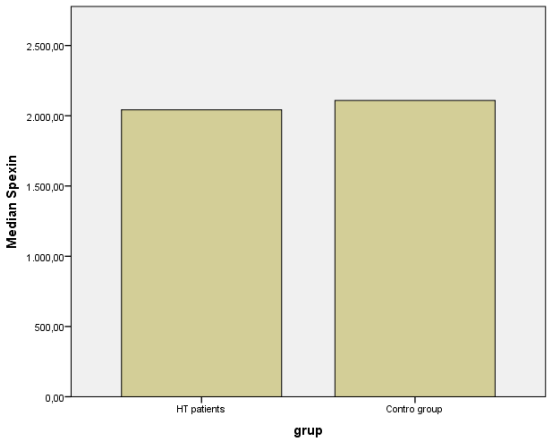


FIG. 3. Comparison of serum spexin levels (ng/L) in women with euthyroid Hashimoto thyroiditis and controls (p= 0.333)



P063

THE IMPORTANT ROLE OF SANGER SEQUENCING IN THE DETECTION OF THE MAGHREBIAN MUTATION C.525DELT IN THE SGCG GENE IN LIMB-GIRDLE MUSCULAR DYSTROPHY

Fatimazahra Smaili[1, 2], Khawla Zerrouki[1, 2], Fatima Ezzahra Aouni[1, 2], Ayad Ghanam[3, 4], Abddeladim Babakhouya[3, 4], Mariam Tajir[1, 2]

[1] Laboratory of Medical Genetics, Central Laboratory, Mohammed V1 University Hospital, Oujda, Morocco

[2] Epidemiology, Clinical Research and Public Health Laboratory, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

[3] Pediatrics Department, Mother and Child Hospital, Mohammed V1 University Hospital, Oujda, Morocco

[4] Laboratory of Immunohematology / Cellular Therapy For Adults and Children, Faculty of Medicine and Pharmacy of Oujda, Mohammed Premier University, Oujda, Morocco

Introduction: Limb-girdle muscular dystrophy R5 (LGMD-R5) or Gamma Sarcoglycanopathy is a genetic heterogeneous limb-girdle muscular dystrophy characterized by progressive degeneration of the muscles of the scapular and pelvic girdles. It is an autosomal recessive genetic disorder. LGMD-R5 is caused by mutations in the *SGCG* gene located on the long arm of chromosome 13 in 13q12 and coding for gamma-sarcoglycan [1,2]. Loss of gamma-sarcoglycan function in the dystrophin-associated sarcoglycan protein complex disrupts sarcolemma and causes degeneration of myofibers [3]. The founder mutation NM_000231.2(*SGCG*): c.525delT (p.Phe175Leufs) in exon 6 is the first to be tested in our context due to its frequency: it represents 65% of autosomal recessive LGMDs [4,5]. The objective of our work is to highlight the important role of Sanger Sequencing in the rapid and low-cost determination of this mutation.

Methods: We report a patient with limb-girdle muscular dystrophy recruited in the Medical Genetics Laboratory of the Mohammed VI University Hospital of Oujda. The blood sample was collected in an EDTA tube. Extraction of the patient's genomic DNA was performed using the QIAamp DNA Blood Mini Kit QIAGEN. Its quality and quantity were controlled by spectrophotometry. The amplification of exon 6 of the *SGCG* gene was performed by conventional PCR using the following primers: ex6_*SGCG*_forward TGGTGTCACTTATTTACTTCTGC and ex6_*SGCG*_reverse CATACATTATTCCAGCACATACC. The amplification reaction was performed in a 50 µL volume containing 50 ng of human genomic DNA, 1 µL of each primer, 25 µL DreamTaq Green PCR Master Mix, and 20µL pure water. PCR steps for this reaction are performed in a thermocycler as follows: denaturation (95°C for 10 min), hybridization (52°C for 45 s), and elongation (72°C for 1 min) for 35 cycles.

Then, PCR products were controlled by electrophoresis on 1% agarose gel and observed under UV.

The PCR-amplified fragments were sequenced by the Sanger method on Applied Biosystems ABI SeqStudio using the following steps: purification of PCR products using ExoSAP-IT solution, execution of the sequence reaction using BigDye Terminator 3.1 and finally the purification of the sequence reaction by SAM and BigDye XTerminator. The electropherogram obtained by the capillary electrophoresis was analyzed using the specific software "Sequence Scanner Software 2.0" and a comparison with the reference sequence was performed.

Next, this mutation was analyzed in the DNA of the patient's mother. The patient's father was not available for analysis.

Results: Amplification of the *SCGC* exon 6 by PCR and migration of PCR products on agarose gel showed the presence of specific bands (Figure 1).

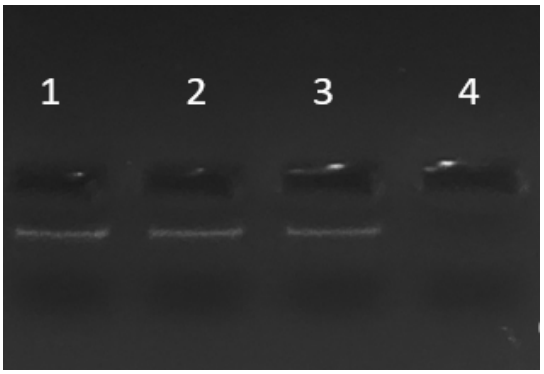


Figure 1: Gel electrophoresis with 1% agarose of the product amplified by conventional PCR of exon 6 of the *SGCG* gene (1: Patient, 2: Mother, 3: Control DNA, and 4: Negative control).

The c.525delT (p.Phe175Leufs) mutation was found in the patient in the homozygous state (Figure 2). Compared with the reference sequence, the electropherogram obtained in the patient showed the deletion of thymine at position 525 (Figures 2 and 3). Sequencing showed also the presence of this mutation in the heterozygous state in the patient's mother (Figure 4).

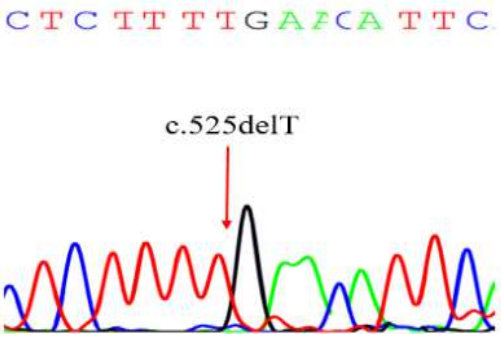


Figure 2: Electropherogram showing the homozygous mutation in exon 6 of the *SGCG* gene of the patient.

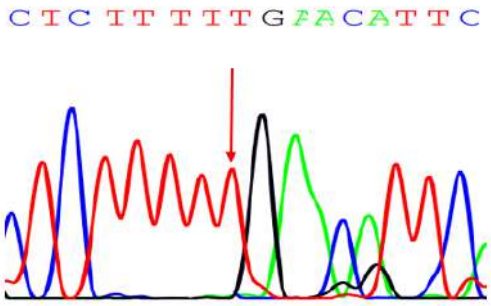


Figure 3: Electropherogram showing the normal sequence of the *SGCG* gene.

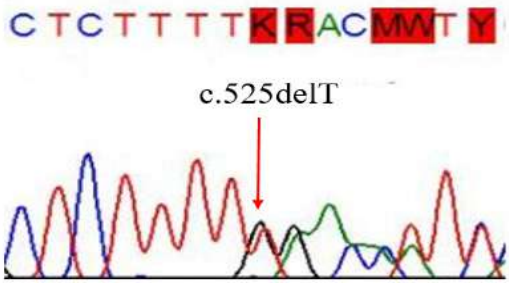


Figure 4: Electropherogram showing the mother's heterozygous mutation in exon 6 of the *SGCG* gene with the reading frame shift.

Discussion :

The sarcoglycans (gamma, alpha, beta, and delta) form a complex that plays an important role in the glycoprotein complex associated with dystrophin and protects the sarcolemma from damage induced by muscle contraction [6]. The absence of one of the sarcoglycans on the plasma membrane reduces the stability of the whole complex and disrupts the membrane integrity of muscle fibers [7].

The diagnosis of sarcoglycanopathy is based on the analysis of the four sarcoglycan genes, either by Sanger sequencing (gene-by-gene analysis), or by NGS (Next Generation Sequencing) using a panel of genes involved in neuromuscular disorders (LGMD panel for example) or exploring the whole exome.

Autosomal recessive LGMD is a frequent indication for mutation analysis in medical genetics. We proceed by searching for the mutation called Maghrebin c.525delT of the *SGCG* gene because of its high frequency [5]. At the complementary DNA level, the c.525delT mutation is a deletion of thymine at position 525 in exon 6 of the *SGCG* gene. At the protein level, the mutation leads to the substitution of phenylalanine for leucine at codon 175. Searching for this mutation first by Sanger sequencing is an interesting approach that provides rapid, targeted, and low-cost detection of this mutation, and therefore helps in the diagnosis of the disease.

Conclusion: Molecular analysis by Sanger sequencing plays an important role in the targeted, rapid, and low-cost detection of genetic mutations. It confirms the associated disease and provides adequate genetic counseling to the patient and his family.

References:

1. Bushby KM. The limb-girdle muscular dystrophies: multiple genes, multiple mechanisms. Hum Mol Genet 1999;8:1875–82.

2. Chabbi N, Angelini C, Rodriguez AA. An update of clinical, epidemiological, and psychosocial features in gamma-sarcoglycanopathy. Muscles 2023;2:164–76.

3. Seo YE, Stephen HB, Amber NK, Oliver CR, Sarah L, Adegboye K, et al. Systemic g-sarcoglycan AAV gene transfer results in dose-dependent correction of muscle deficits in the LGMD 2C/R5 mouse model. Mol Ther Methods Clin Dev 2023; 28:284–99.

4. White SJ, Uitte de Willige S, Verbove D, Politano L, Ginjaar IH, Breuning M, et al. Sarcoglycanopathies and the risk of undetected deletion alleles in diagnosis. Hum Mutat 2005;26:59.

5. El Kerch F, Ratbi I, Sbiti A, Laarabi FZ, Barkat A, Sefiani A. Carrier frequency of the c.525delT mutation in the SGCG gene and estimated prevalence of limb girdle muscular dystrophy type 2C among the Moroccan population. Genet Test Mol Biomarkers 2014;18:253–6.

6. Vainzof M, Souza LS, Gurgel-Giannetti J, Zatz M. Sarcoglycanopathies: an update. Neuromuscular disorders 2021;31:1021–7.

7. Israeli D, Cosette J, Corre G, Amor F, Poupiot J, Stockholm D, et al. An AAV-SGCG dose-response study in a γ-sarcoglycanopathy mouse model in the context of mechanical stress. Mol Ther Methods Clin Dev 2019;13:494–502.

P068

BONE TURNOVER MARKERS IN SYSTEMIC SCLEROSIS: IS THERE AN INCREASED RISK FOR OSTEOPOROSIS?

Nazlı Ecem Dal Bekar[1, 2], Gamze Tuna[3], A. Merih Birlik[3, 4], Gül Güner Akdoğan[1], G. Hüray İşlekel[3, 5]

[1] Izmir University of Economics, School of Medicine, Department of Medical Biochemistry, Izmir, Türkiye

[2] Technical University of Munich, Faculty of Life Sciences, Department of Molecular Life Sciences, Chair of Proteomics and Bioanalytics, Freising, Germany

[3] Dokuz Eylul University, Institute of Health Sciences, Department of Molecular Medicine, Izmir, Turkiye

[4] Dokuz Eylul University, Faculty of Medicine, Department of Immunology and Rheumatology, Izmir, Turkiye

[5] Dokuz Eylul University, Faculty of Medicine, Department of Medical Biochemistry, Izmir, Turkiye

Introduction: Systemic sclerosis (SSc) is a chronic inflammatory disease characterised by diffuse fibrosis of the skin and internal organs. Although the most clinically characteristic feature is fibrosis of the skin (scleroderma), the disease is called 'systemic sclerosis' because inflammatory, fibrotic and vascular changes can also be seen in internal organs such as the gastrointestinal tract, kidneys, lungs and heart. SSc is associated with significant psychosocial problems as it often affects young women and affects the physiognomy of patients. Sclerodermal changes affect morbidity, and visceral organ involvement affects mortality as well as morbidity (Erten & Turgay, 2012). Although the prevalence and incidence of the disease vary due to its chronic nature, the values reported in prevalence studies vary between 50-300 per million, while the annual incidence values vary between 0.9-19 per million. In the only study reported from Turkey, the prevalence of SSc was found to be 220 per million (Pamuk, 2015). Both genetic and environmental factors play a role in the pathogenesis of SSc.

Osteoporosis (OP) is a pathological condition characterized by low bone mass and reduced bone mineralisation. As an undesirable consequence, the loss of bone integrity leads to an increased risk of fracture (Loucks & Pope, 2005). Genetic background, changes in sex hormones, decreased levels of insulin-like growth factor and estrogen, nutritional deficiencies (vitamin D and calcium deficiency) and chronic inflammation are considered to be major risk factors for OP (Lawrence et al., 1998; Loucks & Pope, 2005). In addition, long-term glucocorticoid use during chronic inflammation is also associated with increased bone loss (Di Munno et al., 1995). According to a limited number of studies, scleroderma may be associated with an increased risk of osteoporosis due to its chronic inflammatory state, malabsorption due to gastrointestinal involvement, severe vitamin D deficiency, and medications widely used in the treatment of scleroderma such as prednisone (Loucks & Pope, 2005). However, there are still conflicting results regarding the quantification of burn turnover markers in scleroderma.

Bone turnover refers to the activity and number of bone cells, osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells). Regardless of age and health status, the bone cycle consists of two processes in bone tissue: bone resorption and bone formation. During resorption, osteoclasts remove small amounts of bone from different parts of the skeleton, creating resorption cavities. During resorption, bone tissue components are degraded and released into the bloodstream and excreted in the urine. The amount of bone constituents or their breakdown products in the blood and urine reflects bone resorption activity. Osteoblasts secrete molecules that fill the resorption cavity created by osteoclasts with osteoid, a collagen-rich connective tissue, and these molecules are released into the circulation. The molecules released by osteoblasts are markers of bone formation and their concentration in the blood reflects bone formation activity. Finally, bone formation is completed by mineralisation of the osteoid (Eastell & Szulc, 2017).

Type I collagen, which accounts for 90% of bone proteins, is synthesised as type I procollagen. During type 1 collagen synthesis by osteoblasts, the amino (-N) and carboxy (-C) propeptides are cleaved by extracellular endopepti-

dases. Serum levels of type 1 collagen carboxy-terminal propeptide (PICP) and amino-terminal propeptide (PINP) reflect changes in the synthesis of new collagen synthesised by osteoblasts in bone and fibroblasts in connective tissue (Kucukalic-Selimovic et al., 2013). Cross-links formed between adjacent collagen molecules stabilise and consolidate bone type 1 collagen. These cross-links connect the amino-terminal end of type 1 collagen to the pridinoline in the other molecule. During bone destruction, telopeptides cross-linked to collagen are released into the circulation as amino-terminal (NTX) and carboxy-terminal (CTX) fragments and excreted in the urine (Tekin et al., 2005). Both CTX-1 and PINP are considered as stable and sensitive biomarkers of bone turnover process for early diagnosis of OP (Kuo & Chen, 2017). Besides, PINP represents bone formation rate and has been shown to be more sensitive biomarker for OP (Garnero et al., 2008). To date, only two independent studies in scleroderma focused on serum CTX-1 and PINP levels separately [11-12]. In these studies, CTX-1 was found to be higher in SSc, whereas there was no significant result regarding PINP levels. Therefore, there is still a need for quantitative evaluation of bone turnover markers in the same group of patients. The aim of this study was to evaluate the bone turnover markers PINP and CTX simultaneously in the same group of patients and to investigate the possible correlation of the results with clinical findings such as disease subtype and modified Rodnan skin score.

Methods:

Sample Collection and Ethical Approval: Patients with SSc who were being monitored at the Immunology and Rheumatology Department of Dokuz Eylul University, Faculty of Medicine and healthy individuals who were administered at the same clinic for any reason but did not have autoimmune/autoinflammatory and/or chronic diseases were included in this study. Ethical approval was obtained from the Ethics Committee of Izmir University of Economics, Faculty of Medicine with the protocol number B.30.2.IEUSB.0.05.05-20-230. Informed consent was signed by each participant and blood samples were collected. All samples were centrifuged at 3000 rpm for 10 minutes and stored at -80 ^{o}C until further analysis.

Demographic and laboratory parameters such as age, CRP, ESR levels, disease subtype (limited and diffuse), autoantibody positivity, modified Rodnan skin score (mRSS) and disease duration (date of first Raynaud's phenomenon and date of first non-Raynaud's symptom) were recorded.

Quantification of bone turnover markers in serum samples: Serum samples were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits for the sensitive quantification of CTX-1 (Elabscience, USA) and PINP (AFG Bioscience, USA) molecules. The color at the end of the reaction was measured using a spectrophotometer at a specific wavelength. The concentrations of CTX-1 and PINP in the samples were calculated from a calibration curve plotted separately for each parameter using standards.

Statistical Analyses: Median and min-max values were used in the results for non-parametric data. Comparisons of numerical values between two groups were made using the Mann-Whitney U test. When there were more than two independent groups, the Kruskal-Wallis test was used to evaluate numerical data. Non-numerical data such as sex, presence of clinical findings was tested using the Chi-square test. The level of statistical significance was accepted as $p<0.05$. Spearman correlation test for non-parametric data was used to examine the relationship between variables. R was used for all statistical analyses and graphical designs.

Results:

Patient Characteristics: 26 patients and 20 healthy controls were included in this study. All patients and healthy subjects were female and the mean age was 50.88±4.15 and 49.6±5.88 for patients and healthy subjects, respectively ($p>0.05$). The clinical characteristics of the patients are shown in Table-1.

Serum CTX-1 and PINP levels and clinical correlations: Calibration curves were plotted between the concentration ranges of 0.14-2.39 ng/mL and 1.5-27 µg/L for CTX-1 and PINP, respectively (Fig. 1).

Serum CTX-1 levels were significantly higher in the patient group than in the HCs (0.49: 0.16-0.97 and 0.38: 0.17-

0.64, respectively, p=0.042), whereas serum PINP levels were dramatically lower in the patients than in the HCs (8.92: 1.18-23.17 and 18.30: 3.79-32.51, respectively, p=0.001). (Fig. 2A and 2B). Furthermore, diffuse SSc patients (n=7) had higher serum CTX-1 levels compared to patients with limited SSc (n=19) (0.59: 0.27-0.97 and 0.46: 0.16-0.96, respectively, p=0.043) (Fig. 3). According to the results of correlation analysis, serum CTX-1 levels had a significant positive correlation with mRSS scores and ESR levels (r=0.493, p=0.010 and r= 0.431, p=0.031, respectively) (Figure 4A and 4B). Except this, there was no significant correlation between clinical scores and CTX-1/PINP.

Discussion: Scleroderma is defined as a chronic inflammatory and rare autoimmune disease. Chronic inflammation and the use of glucocorticoids to treat the disease are known risk factors for osteoporosis. In particular, scleroderma patients with organ involvement may be exposed to long-term steroid use. On the other hand, a large proportion of patients have gastrointestinal system involvement and may have nutritional deficiencies due to malabsorption. Malabsorption and severe vitamin D deficiency in scleroderma are also one of the factors that may contribute to the increased risk of OP.

To date, there is a very limited number of studies focusing on the risk of OP in scleroderma. Allanore et al., investigated serum CTX-1 and type I C-terminal procollagen propeptide (PICP) levels in SSc and found significantly higher serum CTX-1 concentrations in patients compared to healthy controls (Allanore et al., 2003). They also showed a positive correlation between CTX-1 levels and CRP, ESR and mRSS scores. However, they found no difference in serum PICP levels between patients and healthy controls. In another study by Scheja et al, serum concentrations of PINP and PICP were determined by radioimmunoassay and no significant difference was found between patient and control groups (Scheja et al., 2000). In this study, we focused, for the first time, on serum CTX-1 and PINP levels simultaneously as biomarkers of bone resorption and formation, respectively. In SSc patients, we found significantly elevated CTX-1 levels and lower PINP levels. Moreover, diffuse SSc patients had higher serum CTX-1 levels compared to patients with limited SSc. There was also a positive strong correlation between serum CTX-1 levels and patients' mRSS scores and ESR levels. This is the first study to show a significant increase and a dramatic decrease in stable and sensitive bone resorption and formation markers, respectively in scleroderma. Furthermore, in accordance with our data we also suggest that bone resorption and/or bone formation rates can be strongly associated with clinical severity of SSc patients. This finding may be explained by the fact that the more severe clinical status of diffuse patients correlates with an increase in problems such as malabsorption and vitamin D deficiency. In addition, as would be expected, the more progressive patients are also exposed to a more intensive use of glucocorticoids.

Among many other factors, it has been suggested that, impaired type 1 collagen metabolism may be associated with an increased risk of osteoporosis in people with scleroderma. However, a limited number of studies have produced conflicting results and no consensus has been reached. Genetic background, changes in sex hormones, reduced levels of insulin-like growth factor and estrogen, nutritional deficiencies (vitamin D and calcium deficiency) and chronic inflammation are thought to be important risk factors for osteoporosis. In line with these risk factors, systemic sclerosis is characterized by a chronic inflammatory process and most patients have gastrointestinal involvement, leading to malabsorption and subsequent nutritional deficiencies. In addition, not only malabsorption but also progressive fibrosis of the skin contributes to severe vitamin D deficiency in scleroderma, which is also considered a significant risk factor for OP. Taken together, patients with scleroderma are at increased risk of developing osteoporosis. In this study, we demonstrated a strong tendency towards bone resorption in scleroderma with elevated CTX-1 and dramatically decreased PINP levels. These results are a preliminary indication that the risk of osteoporosis may be increased in SSc. This study also has the potential to contribute to treatment or clinical follow-up strategies for scleroderma patients. We believe that future studies focusing on the bone turnover process will address the lack of literature on this topic and may improve the quality of life of scleroderma patients.

Conclusion: Scleroderma may increase the risk of osteoporosis due to several factors, including chronic inflammation, glucocorticoid use, malabsorption and severe vitamin D deficiency. In this study, we showed a tendency for bone resorption rather than bone formation in scleroderma. Therefore, further research that comprehensively addresses the bone turnover process is needed to shed light on this issue and contribute to the patients' life quality.

Acknowledgement: The authors would like to thank Yağmur Yavaş and Ali Birdoğan for their technical support.

Table-1: Clinical characteristics of the SSc patients included in this study

Patients (n=26)	
Age, mean (SD)	50.88 (4.15)
Type of SSc, n (%)	
Diffuse	7 (27%)
Limited	19 (73%)
Disease duration* (years), mean (SD)	13.74 (10.90)
Autoantibodies positive, n (%)	
Antinuclear antibody	24 (92%)
Anti-Scl-70	15 (58%)
Anti-centromere antibody	10 (38%)
Modified Rodnan skin score, mean (SD)	4.15 (5.43)
UCLA SCTC GIT 2.0 score, mean (SD)	0.32 (0.32)
CRP, mean (SD)	4.83 (3.95)
ESR, mean (SD)	20.68 (12.90)
Medications, n (%)	
Corticosteroid (<7.5 mg/day), n (%)	6 (23%)
Proton pump inhibitor, n (%)	19 (73%)
Calcium channel blockers, n (%)	18 (69%)
Hydroxychloroquine, n (%)	6 (23%)
Immunosuppressants, n (%)	5 (19%)

Fig.1 Calibration curves for CTX-1 and PINP quantification

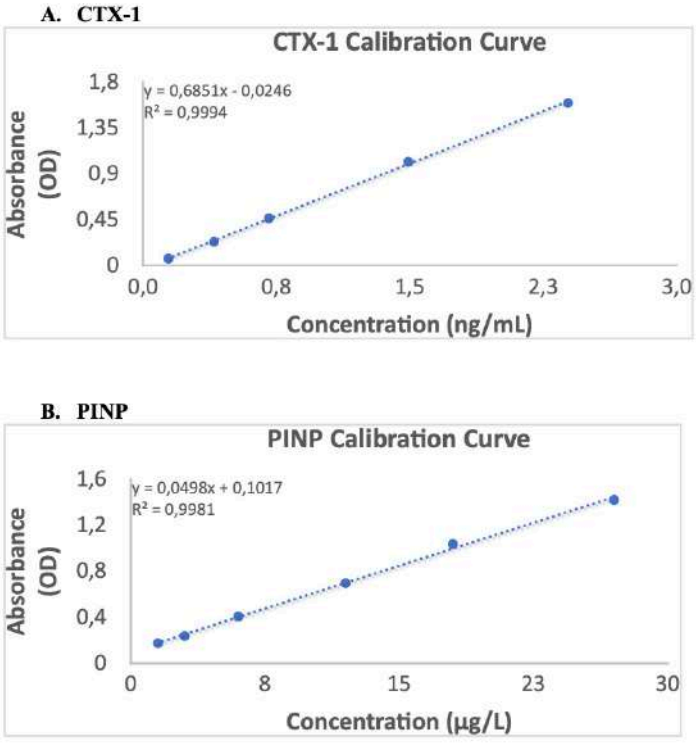
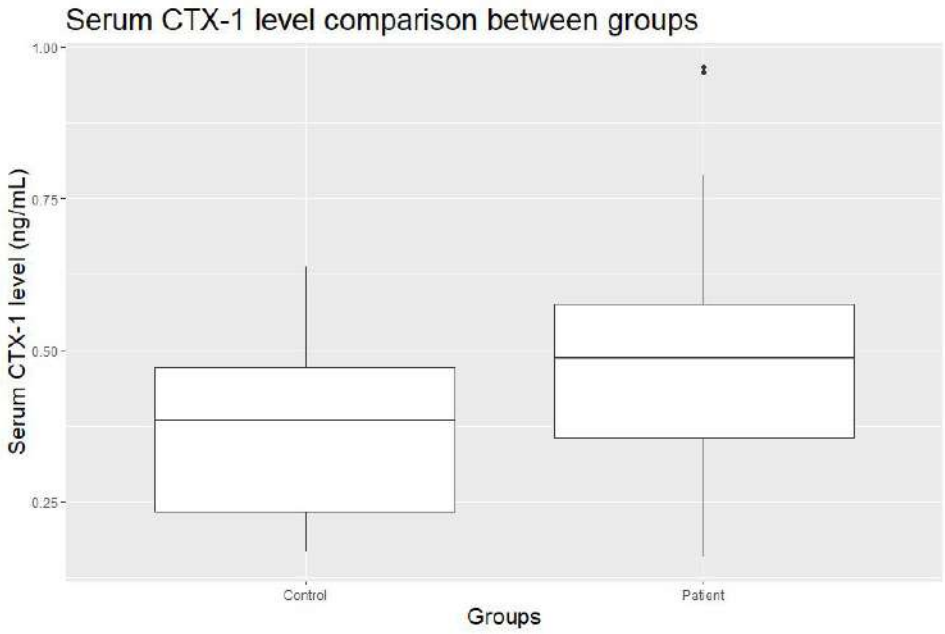


Fig.2 Comparison of serum CTX-1 and PINP levels between SSc patients and healthy individuals

A. Serum CTX-1 levels, (0.49: 0.16-0.97 and 0.38: 0.17-0.64, respectively, p=0.042)



B. Serum PINP levels, (8.92: 1.18-23.17 and 18.30: 3.79-32.51, respectively, p=0.001)

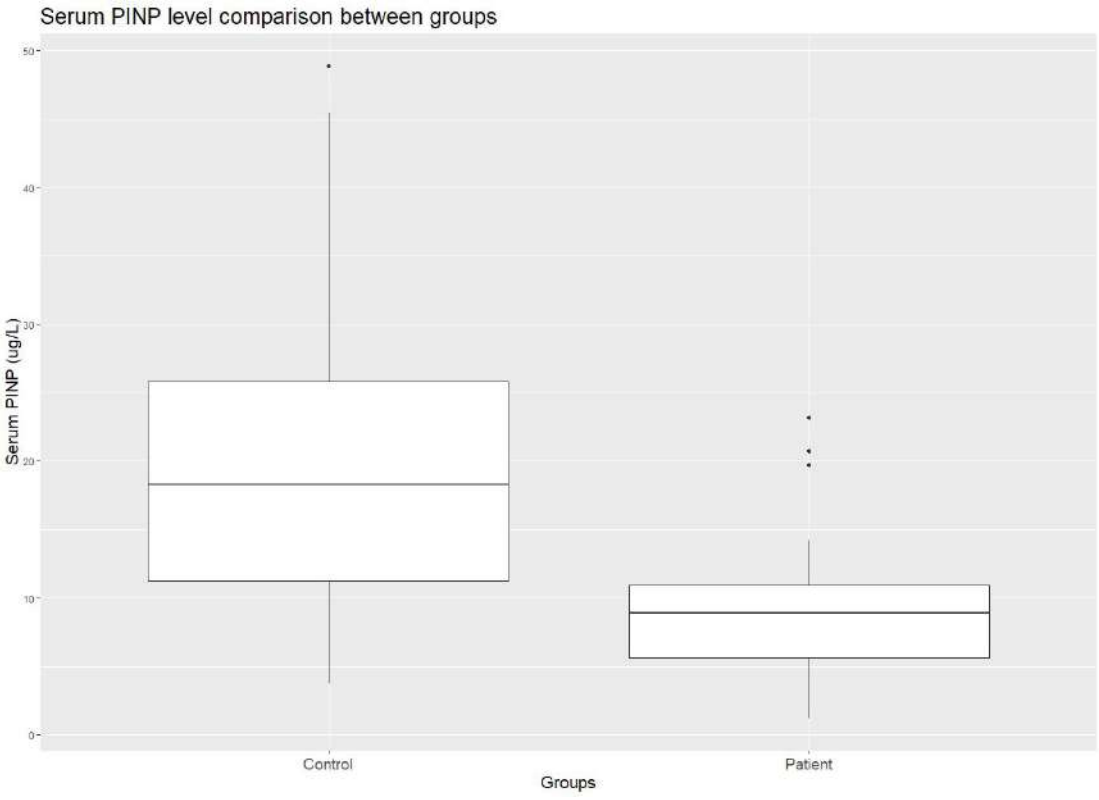


Fig.3 Serum CTX-1 levels in SSc patients with diffuse and limited subtypes, (0.59: 0.27-0.97 and 0.46: 0.16-0.96, respectively, p=0.043)

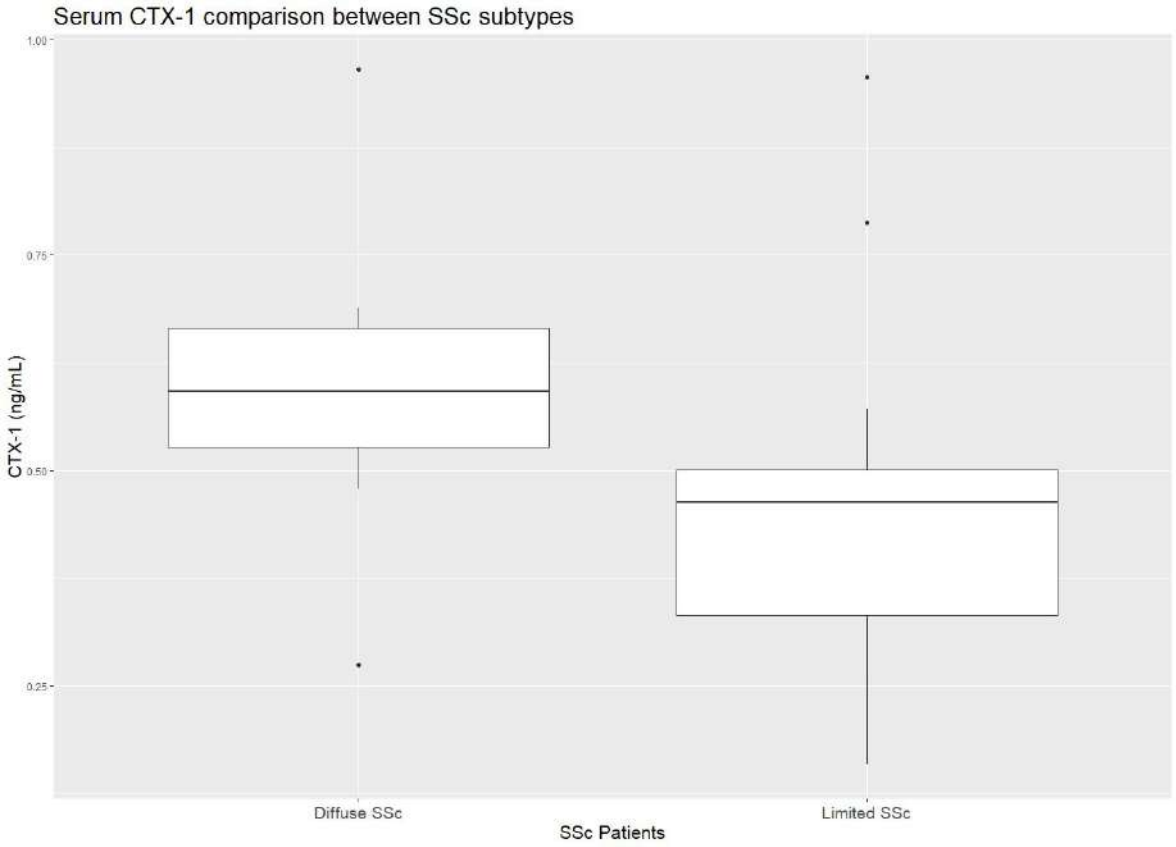
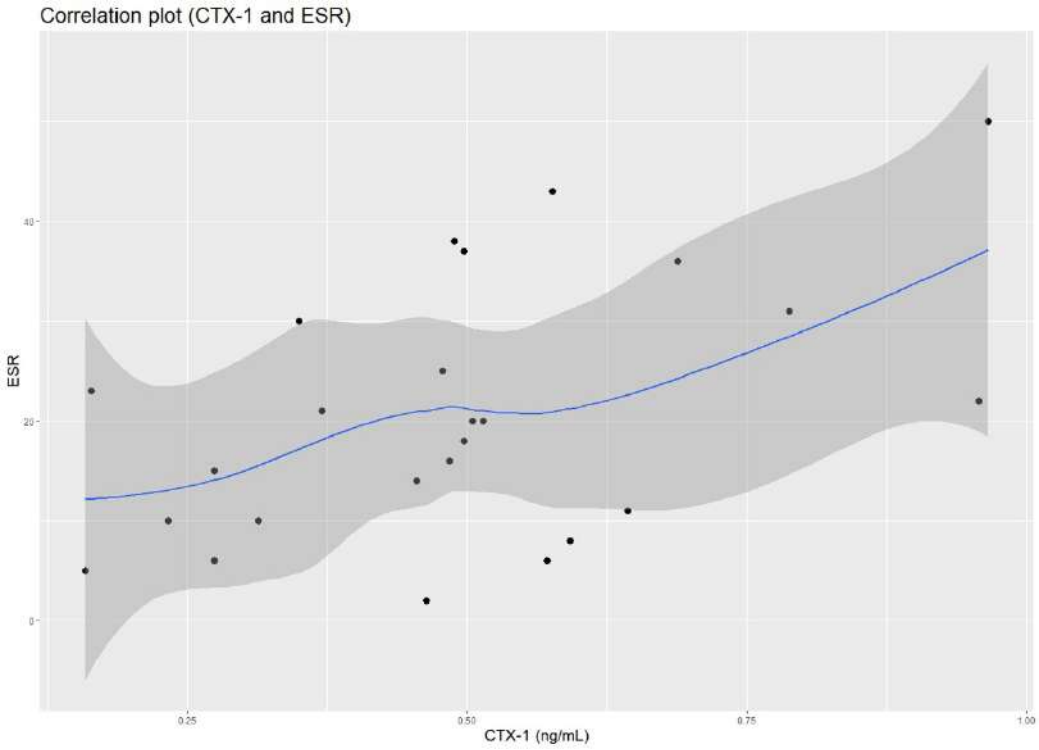
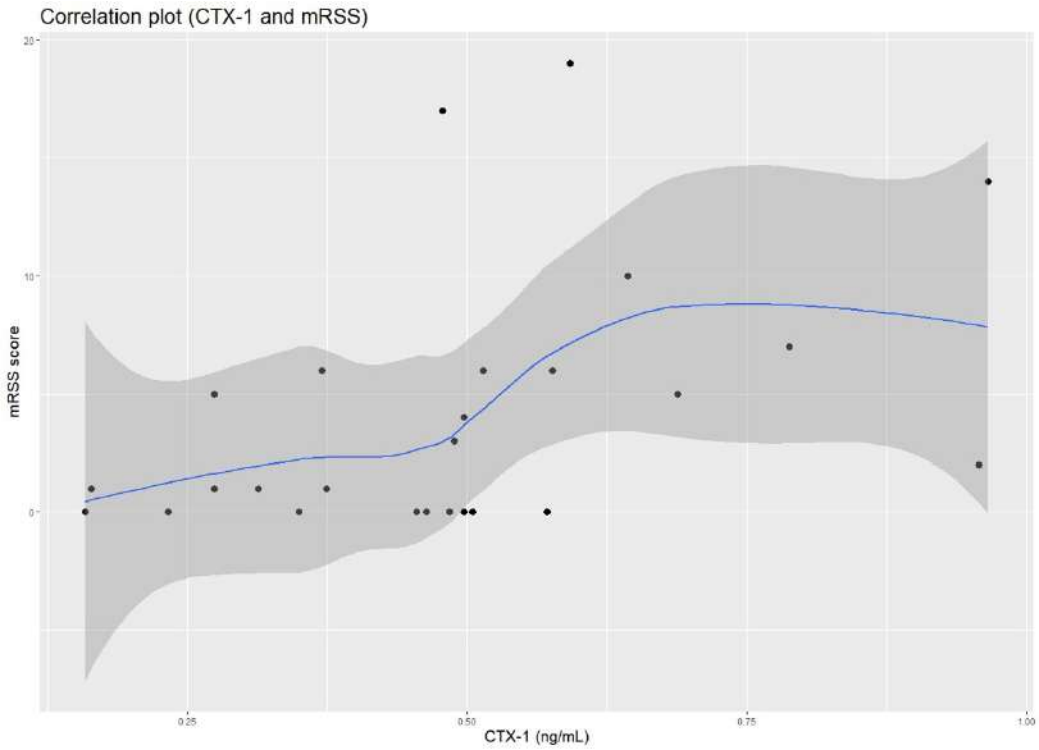


Fig.4 Correlation plots for CTX-1&ESR and CTX-1&mRSS score

A. Correlation plot for CTX-1 and ESR, (r= 0.431, p=0.031)



B. Correlation plot for CTX-1 and mRSS score (r=0.493, p=0.010)



Reference

Allanore, Y., Borderie, D., Lemarechal, H., Cherruau, B., Ekindjian, O. G., & Kahan, A. (2003). Correlation of Serum Collagen I CarboxyterminalTelopeptide Concentrations with Cutaneous andPulmonary Involvement in Systemic Sclerosis. *The Journal of Rheumatology*, *30*(1), 68–74.

Di Munno, O., Mazzantini, M., Massei, P., Ferdeghini, M., Pitaro, N., Latorraca, A., & Ferri, C. (1995). Reduced Bone Mass and Normal Calcium Metabolism in Systemic Sclerosis with and without Calcinosis. In *Clinical rheumatology* (Vol. 14, Issue 4).

Eastell, R., & Szulc, P. (2017). Use of bone turnover markers in postmenopausal osteoporosis. *The Lancet Diabetes and Endocrinology*, *5*(11), 908–923. https://doi.org/10.1016/S2213-8587(17)30184-5

Erten, Ş., & Turgay, M. (2012). Sistemik Sklerozda Oksidatif Stres. *ADÜ Tıp Fakültesi Dergisi*, *13*(3), 37–41.

Garnero, P., Vergnaud, P., & Hoyle, N. (2008). Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. *Clinical Chemistry*, *54*(1), 188–196. https://doi.org/10.1373/clinchem.2007.094953

Kucukalic-Selimovic, E., Valjevac, A., & Hadzovic-Dzuvo, A. (2013). The utility of procollagen type 1 N-terminal propeptide for the bone status assessment in postmenopausal women. *Bosn J Basic Med Sci*, *13*(4), 259–265.

Kuo, T. R., & Chen, C. H. (2017). Bone biomarker for the clinical assessment of osteoporosis: Recent developments and future perspectives. *Biomarker Research*, *5*(1). https://doi.org/10.1186/S40364-017-0097-4

Lawrence, R. C., Helmick, C. G., Arnett, F. C., Deyo, R. A., Felson, D. T., Giannini, E. H., Heyse, S. P., Hirsch, R., Hochberg, M. C., Hunder, G. G., Liang, M. H., Pillemer, S. R., Steen, V. D., & Wolfe, F. (1998). Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis and Rheumatism*, *41*(5), 778–799. https://doi.org/10.1002/1529-0131(199805)41:5<778::AID-ART4>3.0.CO;2-V

Loucks, J., & Pope, J. E. (2005). Osteoporosis in scleroderma. *Seminars in Arthritis and Rheumatism*, *34*(4), 678–682. https://doi.org/10.1016/j.semarthrit.2004.08.006

Pamuk, Ö. N. (2015). Sistemik Sklerozun Epidemiyolojisi. *Turkiye Klinikleri Journal of Rheumatology Special Topics*, *8*(2), 7–13.

Scheja, A., Wildt, M., Wollheim, F. A., & Saxne, T. (2000). Circulating collagen metabolites in systemic sclerosis. Differences between limited and diffuse form and relationship with pulmonary involvement*. *Rheumatology*, *39*, 1110–1113.

Tekin, Y., Erkin Bozdemir, A., & Barutçuoğlu, B. (2005). Biochemical Markers and Their Affecting Factors in Assessing Osteoporosis. *Türk Klinik Biyokimya Dergisi*, *3*(2), 73–83.