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EDITORIAL

Dear esteemed readers of TurkJEM Family,

As put forward by World Health Organization in 1948 “Health is a state of complete physical, mental, and social well-being not merely the absence of disease, or infirmity.” Having no consensus around a single definition of well-being, the most consensuses are around the presence of positive emotions and moods, satisfaction with life, fulfillment, and positive functioning.

Researchers include factors like physical well-being, economic well-being, social well-being, development and activity, emotional well-being, psychological well-being, life satisfaction, domain-specific satisfaction, engaging activities, and work. COVID-19 pandemic has negatively affected almost all of the factors cited above independent of age, gender, and nationality. As of January 2021 there are globally confirmed over 102 million COVID-19 cases and 2.2 million deaths and the pandemic is in no way expected to cease within a few months. Traditionally, health-related quality of life has been linked to patient outcomes and has generally focused on deficits in functioning. Thus COVID-19 not only deteriorated deficits in the functioning of humankind but have a holistic negative impact on human wellbeing. The negative emotions that are threatened by natural disasters call for making available traditional methods of intervention for clinical syndromes. In a time where most beds and doctors are devoted to the pandemic, there seems to be no solution that could be rendered by clinical efforts. During such times positively focused, strengths-based approaches, one that relies on the individual’s existing capacities for effective functioning seems to be an effective way of contributing to upheaval wellbeing. Instead of stigmatizing individuals who are already disadvantaged, we believe a strengths approach can be empowering and provide a valuable new avenue for taking on the task of managing the demands of a world in upheaval. Strengths cover Wisdom and knowledge, courage, interpersonal strengths, civic strengths, strengths that protect against excess and strengths that forge connections to the larger universe and provide meaning. But beyond all, it seems that we are in an era of equity, social justice, and environmental considerations.

The spring edition of TJEM has these distinguished publications: “Prevalence and awareness of hypertension in seven distinct geographic regions of Turkey: SEMT HT study”, “Publication outcomes for oral presentations at Congresses of Endocrinology and Metabolıı Diseases of Turkey: analysis of twenty years “, “Clinical spectrum and outcome of patients with Graves’ disease: a single center experience from a tertiary care institution of the Kashmir valley, India”, “Evaluation of oxidative stress with a new method in differentiated thyroid cancer patients on thyrotrophin suppression treatment”, “Patients with ectopic posterior pituitary: report of six cases“, “Investigation of survivin promoter -31 G/C polymorphism and survivin levels in acromegaly “, “Pheochromocytoma: 16 years of experience in a single center “, “Evaluation of influenza, pneumococcus, zoster, measles, diphtheria and pertussis vaccinatıon rates in patients with type 1 and type 2 diabetes mellitus; a single center experience from Turkey”, “Metabolic age: a new predictor for metabolic syndrome”,”Does blood glucose regulation in adults with type 2 diabetes affect exocrine pancreatic functions?”, “Serum irisin levels in cigarette smokers “, “MTHFR C677T polymorphism in Turkish women with polycystic ovary syndrome”, “Pharmacotherapy and neoteric dietary approaches for polycystic ovary syndrome: a systematic review “, “SRY-positive 46XX testicular disorder of sex development as a rare cause of male hypergonadotropic hypogonadism: a case report “,”A case report of dapagliflozin-induced nodular vasculitis”.

Wish you all a very healthy and pleasant spring time.

With my best regards,

Nilgün Başkal MD
Editor-in-Chief

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Prevalence and Awareness of Hypertension in Seven Distinct Geographic Regions of Turkey: The SEMT HT Study

Türkiye'nin Yedi Farklı Coğrafi Bölgesinde Hipertansiyon Sıklığı ve Farkındalığı: TEMD HT Çalışması

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Abstract

Objective: This study aimed to assess the prevalence, the level of awareness, and the factors that increase hypertension in Turkey. **Material and Methods:** A cross-sectional survey with a multi-stage probability sampling was conducted. A total of 9604 people were screened; of them, 9316 had a complete clinical examination. The ages, genders, personal and family histories of hypertension, diabetes, coronary artery diseases were recorded. Their systolic and diastolic blood pressures (BP), heights, weights, waist, and hip circumferences were measured. Hypertension was defined as taking medication for hypertension or BP ≥140/90 mmHg. **Results:** According to the JNC-7 criteria, 22.1% of the population had prehypertension, 36.5% had hypertension (15.3% stage 1 and 21.2% stage 2 hypertension), and 41.4% were normal population. Of the population, 30% had an awareness of hypertension. The prevalence of hypertension was higher in Mediterranean, Central Anatolia, and Black Sea Regions and lower in South-East Anatolia, Aegean Regions, while it was similar in Marmara and East Anatolia Regions when compared to the general population of Turkey. Though rural life, advancing age, increasing body mass index, and waist to height ratio >0.5 were the factors responsible for an increased prevalence of hypertension, smoking was found to decrease the prevalence. **Conclusion:** The data of the SEMT hypertension study indicated that more than one-third of the adult population was hypertensive in Turkey. Furthermore, only one-third of the hypertensive adult population showed awareness of their hypertension.

Keywords: Hypertension; prevalence; Turkey; risk factors; SEMT study; JNC-7

Özet

Amaç: Çalışmanın amacı, Türkiye'de hipertansiyon prevalansını, farkındalık düzeyini ve hipertansiyon prevalansını arttıran faktörleri değerlendirmekti. **Gereç ve Yöntemler:** Çok aşamalı olasılık örneklemesi ile kesitsel bir anket çalışması yapıldı. Toplam 9604 kişi tarandı, 9316'sının tam klinik muayenesi yapıldı. Yaş, cinsiyet, kişisel ve ailesel hipertansiyon, diyabet ve koroner arter hastalığı öyküleri kaydedildi. Sistolik ve diyastolik kan basınçları (KB), boy, vücut ağırlığı, bel ve kalça çevresi ölçümleri yapıldı. Hipertansiyon tanısı, hipertansiyon için bir ilaç almak veya KB ≥140/90 mmHg olarak tanımlandı. **Bulgular:** JNC-7'ye göre popülasyonun %22,1'inde prehipertansiyon, %36,5'inde hipertansiyon (%15,3'ünde evre 1 ve %21,2'sinde evre 2) ve %41,4'ünde normal tansiyon vardı. Hipertansiyon farkındalığı %30 idi. Hipertansiyon prevalansı Akdeniz, İç Anadolu ve Karadeniz Bölgelerinde daha yüksek, Güneydoğu Anadolu, Ege Bölgeleri'nde daha düşük, Marmara ve Doğu Anadolu Bölgeleri'nde ise Türkiye genel nüfusu ile benzer oranda bulundu. Kırsal yaşam, artan yaş, artan beden kitle indeksi ve bel/boy oranı >0,5 olması hipertansiyon prevalansını arttıran faktörler iken, sigara kullanımının düşük prevalans ile ilişkili olduğu bulundu. **Sonuç:** TEMD hipertansiyon çalışması verileri, Türkiye'de yetişkin nüfusun 1/3'ünden fazlasının hipertansif olduğunu göstermiştir. Dahası hipertansif yetişkin popülasyonun sadece 1/3'ünün hipertansiyonlarının farkında olmasıdır.

Anahtar kelimeler: Hipertansiyon; prevalans; Türkiye; risk faktörleri; TEMD çalışması; JNC-7

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Introduction

Hypertension (HT) is a well-known modifiable risk factor for congestive heart failure, renal insufficiency, and coronary, cerebral, and peripheral vascular diseases (1). Epidemiological studies have shown that every 20 mmHg increase in systolic blood pressure (SBP) after the 4th decade in life is associated with a doubling of death rates for cerebral and coronary heart disease (2). At least 50% of cardiovascular diseases and 75% of cerebrovascular diseases are caused by HT. Moreover, randomized clinical studies have shown that these diseases can be easily prevented by controlling elevated blood pressure (3).

A high prevalence (25-55%) of hypertension has been reported in the adult population in most of the developed countries. Variation in rates of hypertension prevalence in different countries may result from different study protocols, environmental factors, and genetic susceptibilities (4). Adequate knowledge and epidemiological data regarding hypertension in developing populations are lacking. Modern dietary and lifestyle modifications, increased life duration, and rapid changes in social structure can lead to an increase in hypertension prevalence (5).

In Turkey, an Eastern Mediterranean developing country with a total population of about 83 million, 92.8% of people live in the cities and district centers. The Turkish population is relatively young, with a median age is 32.4 years; 23.1% of the population is under 15-years of age; 67.8% is between 15- 64 years, and 9.1% of the population is 65 years and older. In this relatively young population, the leading cause of death is cardiovascular disease (6).

Turkey has seven distinct geographic regions with different altitudes, weather conditions, lifestyles, dietary habits, and socioeconomic statuses that may play a key role in high hypertension prevalence. There is limited data about the nationwide or the regional distribution of hypertension in Turkey. We aimed to determine the prevalence of hypertension, the level of awareness, treatment, and control rates for hypertension, and the factors that increase the hypertension prevalence in the seven distinct areas of Turkey.

Material and Methods

Society of Endocrinology and Metabolism of Turkey (SEMT) hypertension (HT) Study, coordinated by the SEMT, was carried out in the seven geographic regions of Turkey. A multi-stage probability sampling was done. The study was approved by the Turkish Ministry of Health (Approval number 19.02.03/2088) and was conducted in accordance with the Declaration of Helsinki (DOH), 1964 on ethics and protection of human participants in medical research. We collected Household Verification Form (HVF) data from primary health care centers affiliated to Provincial Health Directorates. Men and non-pregnant women, who were between 20-83 years of age, were included in the study. At least three provinces of each geographic region of Turkey were randomly selected (Figure 1).

Initially, inhabitants of city centers, districts, and villages were planned by the stratified sampling method. Then cases were chosen with the random sampling method by using the HVF data. Age groups were classified as decades from the age of 20, up to the age of 80 and above.

Individual differences, such as gender, demographic, economic, social, and geographical situations were considered. The study protocol was given/explained about two weeks before in the regions where the study was to be conducted, and the participants were instructed to fast for 8-12 h before the collection of samples. About 100 participants were evaluated daily from 7 -10 a.m. in the health care centers. The waiting room of the participants was kept calm. Prior written informed consent from each of the participants was obtained. A total of 9604 people were screened; 9316 of them underwent clinical examination; 3383 of them underwent laboratory examination because of poor financial conditions. The numbers of participants from different regions were as follows: 1926 from the Mediterranean, 1964 from Central Anatolia, 1064 from South-East Anatolia, 661 from Aegean, 1270 from Marmara, 1199 from the Black Sea, and 1232 from East Anatolia Region.

Medical histories, including hypertension and other measurements, such as blood pressures, height, weight, waist circumference (WC), and hip circumference (HC) of

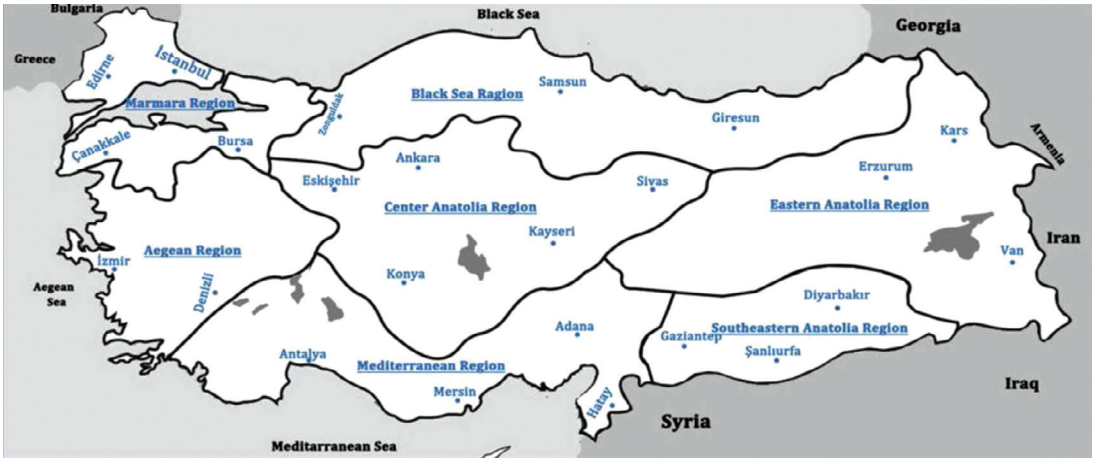

Figure 1. The provinces selected from each geographic region in Turkey.

the participants were taken by the specifically trained physicians. While measuring their height and weight, the participants were asked to remove items such as shoes and outer clothing that would constitute significant weight. Body mass index (BMI) was determined as weight (in kg/m^2; kg being weight, m being height). A participant was considered as underweight if their BMI was below 18.5 kg/m^2; normal if BMI was between 18.5-24.9 kg/m^2; overweight if between 25-29.9 kg/m^2; obese if between 30-39.9 kg/m^2; morbidly obese if ≥40 kg/m^2 (7). WC was measured between iliac processes and umbilicus, and HC was measured at the level of the largest protrusion of the hips. The standard abdominal obesity criterion was WC ≥100 cm for men and ≥90 cm for women. Other criteria for abdominal obesity for both genders include waist to hip ratio (WHR) >0.9 for men and >0.85 for women; and waist to height ratio (WHtR) >0.5 (8).

Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were measured with standard sphygmomanometers, twice with at least 15-min interval after a rest of 30-min in sitting position, and the mean of the two measurements was taken. None of the participants were permitted to take alcohol, tea, coffee, and smoke 30-min before the measurement. HT was defined as ≥140/90 mmHg for SBP and DBP, respectively. The Korotkoff phases 1 and 5 were utilized for SBP and DBP, respectively. The classification of hypertension was done under the JNC-7 criteria (9).

Statistical Analysis: SPSS version 13.0 (IBM, Illinois, Chicago, USA) was used for statistical analysis. The Chi-square test was employed to determine the differences in categorical variables between the groups. Student's t-test for parametric data and Mann-Whitney U test for nonparametric data were used to evaluate the differences of continuous variables among different independent groups. In order to determine the probability of socio-demographic and anthropometric variables associated with hypertension, the multiple logistic regression tests with the center and backward stepwise procedures were applied. The odds ratios with 95% confidence intervals from binary logistic regression were used for each model. Two-tailed p-values of <0.05 were considered as statistically significant.

Results

The mean systolic blood pressure (SBP) was 134.3±27.62 (males, 133.33±25.91; females, 134.85±28.53) mmHg, and the mean diastolic blood pressure (DBP) was 81.78±15.46 (males, 81.48±15.04; females, 81.95±15.7) mmHg. Normal blood pressure was recorded in 41.4% (42.4% of males, 40.8% of females) of the participants; 22.1% (23.1% of male, 21.5% of female) participants reported prehypertension, while 15.3% (14.5% of males, 15.7% of females) of the participants had stage 1 and 21.2% of the participants (19.9% of male, 22% of female) had stage 2 hypertension according to JNC-7 (Figure 2). Though the percentage of patients taking

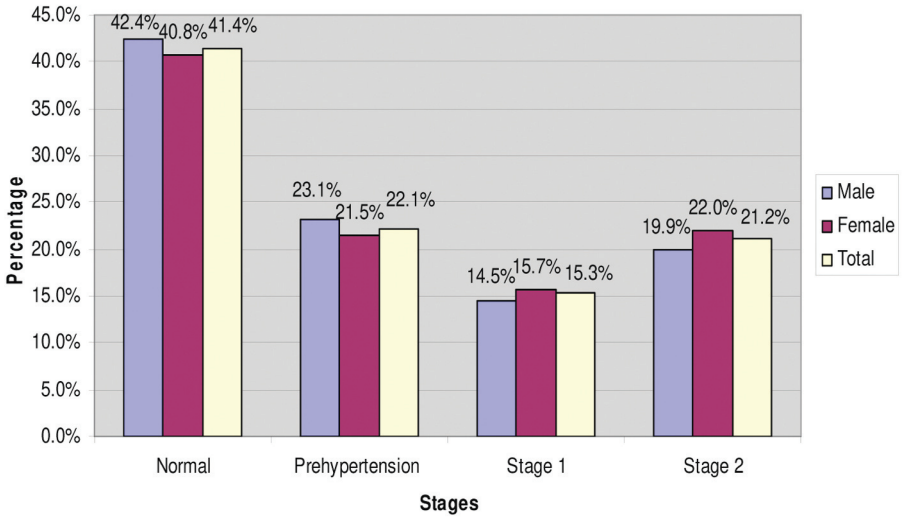

Figure 2. The distribution of HT types with respect to gender.

antihypertensive treatment was 57.3%, the rate of hypertension control was only 31.5%.

The prevalence of hypertension was 40.6% in Mediterranean Region, 41.4% in Central Anatolia, 23.2% in South-East Anatolia, 27.2% in Aegean Region, 33.8% in Marmara Region, 44.9% in the Black Sea Region, and 33.3% in East Anatolia, giving a total prevalence of 36.5% in Turkey (Table 1). The hypertension prevalence was higher in the populations of Mediterranean, Central Anatolia, and the Black Sea Regions, lower in South-East Anatolia and Aegean Regions (all $p<0.001$), and similar in Marmara and East Anatolia Regions when compared to the general population of Turkey.

The awareness of hypertension was 21.4% in the Mediterranean, 25.9% in Central Anatolia, 35.5% in South-East Anatolia, 44.8% in Aegean, 49.3% in Marmara, 26.8% in the Black Sea, and 27.6% in East Anatolia, giving a total of 30% in Turkey (Figure 3).

Hypertension was more common in the altitudes <200 m and >1000 m when compared with altitudes between 200-1000 m (50.4% and 50.3% vs. 34.2%, respectively, both $p<0.001$). Hypertension prevalence was higher (53.9%) in rural areas than in urban areas (45.1%) with $p<0.001$).

When different age groups were compared with the 20-29 age group, the hypertension prevalence up to the age of 79 registered an increase from 19% to 78% with the advancing age, but the rate decreased marginally for the group with age >79 yrs (all $p<0.001$) (Figure 4). The mean SBP value after the age of 30 and DBP after the age of

Table 1. Distribution of normotensive, prehypertensive, and stage-wise hypertensive participants with respect to the seven geographic regions of Turkey.

	Normal	Prehypertension	Stage 1	Stage 2	Total
Mediterranean	686 (35.6%)	458 (23.8%)	314 (16.3%)	468 (24.3%)	1926
Central	693 (35.3%)	457 (23.3%)	326 (16.6%)	488 (24.8%)	1964
South-East	630 (59.2%)	187 (17.6%)	117 (11%)	130 (12.2%)	1064
Aegean	361 (54.6%)	120 (18.2%)	77 (11.6%)	103 (15.6%)	661
Marmara	544 (42.8%)	296 (23.3%)	197 (15.5%)	233 (18.3%)	1270
Black Sea	406 (33.9%)	254 (21.2%)	204 (17%)	335 (27.9%)	1199
East	537 (43.6%)	285 (23.1%)	190 (15.4%)	220 (17.9%)	1232
Total	3857 (41.4%)	2057 (22.1%)	1425 (15.3%)	1977 (21.2%)	9316

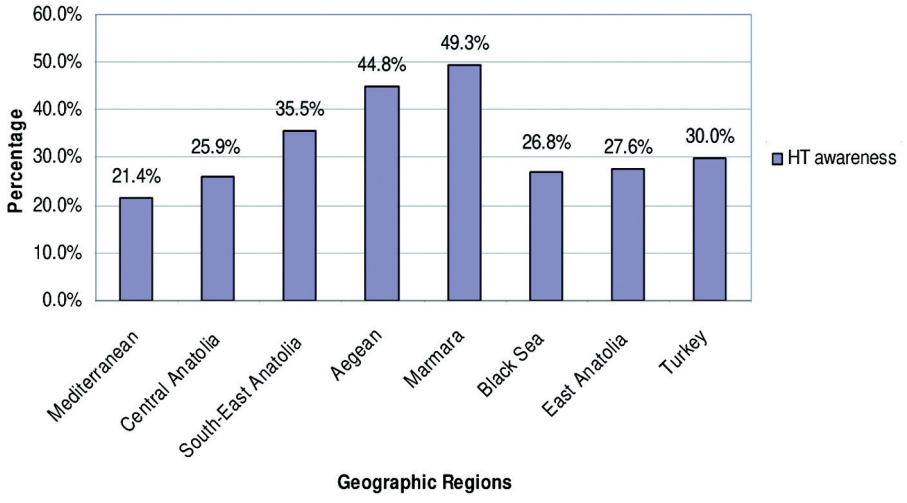

Figure 3. Hypertension awareness with respect to the seven geographic regions.

40 was recorded higher in women than in men across all age groups. After 80 years of age, SBP and DBP values became higher among men than women (Figure 5). The hypertension prevalence increased with the rise in BMI from 28% to 73% (Figure 6).

The logistic regression analysis revealed that participants living in the rural area had 1.2 times higher risk of hypertension as compared with the participants living in an urban area. When compared with the age group of 20-29 years, participants in age group 30-39 years had 1.3 times higher risk, 40-49 years of age had two times risk, 50-59 years of age group had four times, 60-69 years of age group had 6.8 times, 70-79 years of age group had 9.7 times, and finally the groups with age ≥80 years of age were 6.4 times more likely to develop hypertension. Smokers were 0.7 times less likely to suffer from HT ($p<0.001$) than the nonsmokers. Compared with standard reference, overweight participants were twice, obese participants were 3.2 times higher, and morbid obese participants were six times more likely to develop HT. Participants with waist/height ratio >0.5 were 1.3 times more prone to develop HT than those with a ratio of <0.5 (Table 2).

Discussion

Several national and regional studies about the epidemiology of hypertension in Turkey have been reported. The hypertension prevalence was estimated to be 33.7% in

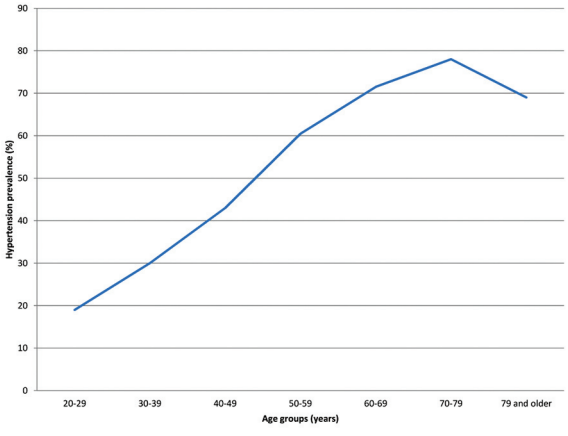

Figure 4. Prevalence of hypertension in Turkey by different age groups.

the TEKHARF study conducted by the Turkish Cardiology Association in the 1990 (10). The PatenT study documented the prevalence of general hypertension in Turkey as 31.8% in 2003 (11). The hypertension incidence in Turkey (HinT) study conducted in the population of the PatenT study in 2007 recorded the prevalence of hypertension to be 44.1% (12). The 2013 TURDEP II study showed that the hypertension prevalence was 31.4%, and the frequency of hypertension decreased by 11% in Turkey from that observed in the 2002 TURDEP I study (13).

The current study presents the latest update about the prevalence of hypertension and represents Turkey's seven geographical regions in detail. It has reported hypertension in approximately 21 million adult indi-

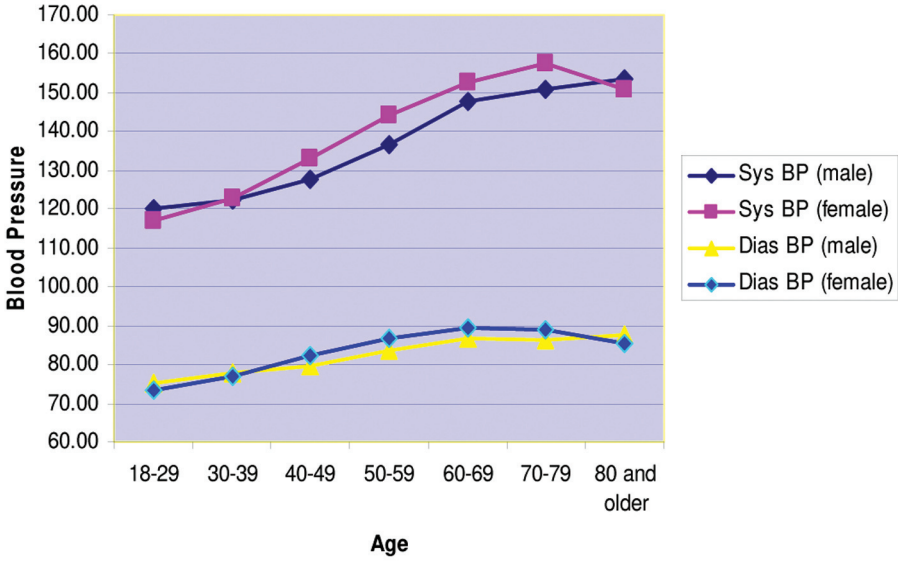

Figure 5. The mean systolic and diastolic BP with respect to age and gender.

viduals over 20 years of age in Turkey and showed that only 30% of them became aware of the situation. The awareness rate was highest (49.3%) in the Marmara region and lowest (21.4%) in the Mediterranean Region. This rate is worse than that of the PatenT study, which documented approximately 15 million hypertensive adults, with 40% of the adults with HT awareness. These data indicate a rising trend of HT prevalence in Turkey and a declining trend in awareness level about HT. Several studies have reported awareness levels ranging from 26% to 60.2% (11).

In this study, the hypertension prevalence was found higher in the Mediterranean, Central Anatolia, and Black Sea regions and lower in South-East Anatolia and Aegean Regions. Different altitudes, weather conditions, lifestyles, dietary habits, and socioeconomic statuses of the regions may explain this difference. Importantly, it was noticed that the higher the unawareness in the region, the higher was the prevalence of hypertension.

Hypertension was more common in the altitudes <200 m and >1000 m when compared with altitudes between 200-1000 m. Chronic hypoxia at high altitudes leads to elevated blood pressure by the activation of the sympathetic nervous system in the healthy subjects (14). Studies also showed that factors such as decreased basal nitric

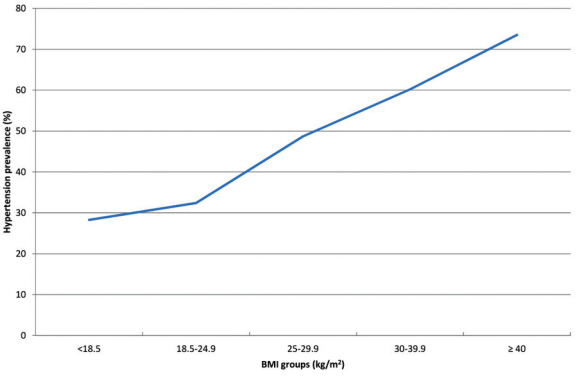

Figure 6. Prevalence of hypertension in Turkey by Body Mass Index (BMI).

oxide (NO) production, and increased NO consumption caused by increased blood viscosity, and increased blood hemoglobin might cause increased blood pressure with chronic hypoxia (15,16). A meta-analysis indicated that every 100 m increase in altitude led to a 2% increase in the prevalence of hypertension (17). The current study also found high hypertension prevalence in the regions with altitudes <200 m. Despite abundant information on prevalence at high altitude, there is little information about the effect of low altitude on blood pressure. Fiori et al. reported that hypertension was more frequent in low altitudes than in middle altitude in the subjects from Central Asia (18). High blood pressure levels in low altitude re-

Table 2. Multiple logistic regression of hypertension prevalence.

	Odds ratio	%95 CI
Rural	1.244*	1.113-1.390
Age		
20-29	1	
30-39	1.346*	1.146-1.581
40-49	2.048*	1.742-2.407
50-59	3.964*	3.329-4.721
60-69	6.844*	5.603-8.361
70-79	9.719*	7.558-12.497
≥80	6.415*	3.788-10.864
Female	0.962	0.866-1.069
DM history	1.199	0.997-1.441
Smoke	0.683*	0.605-0.772
BMI		
<18.5 (underweight)	0.710	0,492-1.123
18.5-24.9 (normal)	1	
25-29.9 (overweight)	1.4360	0.974-2.095
30-39.9 (obese)	2.264*	1.541-3.327
≥40 (morbid obese)	4.241*	2.610-6.890
Waist/Height (>0.5)	1.326*	1.147-1.532
Altitude		
<200 m	1.093	0.875-1.366
200-1000 m	1	
>1000 m	1.091	0.873-1.363

*p<0.001

gions may be due to high sea salt exposure and excessive consumption of dried fish, although the exact cause is unknown (19).

Findings in our study showed similarities to those in developing countries such as China and India, where the hypertension prevalence was found higher in rural areas than in urban areas (20,21). There is a lack of effective public health measures in rural areas in developing countries, and most of the people live without social insurance. Hypertension is also associated with several individual indicators, such as education, occupation, and socioeconomic status, and regional and national economic conditions, but these relationships remain complex and unexplored. Possible reasons for these associations are lower birth weight and heavier job stress in lower socioeconomic status groups. On the other hand, higher awareness, better medical care, better prevention, and monitoring could reduce the prevalence of hypertension in higher socioeconomic status groups (20,21). In developed countries such as Europe and the USA, hypertension prevalence was found higher in urban areas due to their lifestyles, eating habits, higher job strains (22-24) than in developing countries. The PatenT study showed that hypertension prevalence was not significantly different between the rural and urban populations (32.9% versus 31.1%) in Turkey (11).

In this study, the hypertension prevalence was higher in females than in males. The findings from previous nationwide surveys in Turkey (TEKHARF, HinT, and PatenT) have suggested that women show a higher prevalence of hypertension (10-12) than men. The monitoring of trends and determinants in Cardiovascular diseases (MONICA) study by WHO indicated that the problem of high blood pressure was almost universally more common in women than in men, especially in older age groups (25). In all 22 country populations, women in the 35-44 age group had lower SBP than men. In contrast, after this age group, SBP in women started to increase with age, and 34 of 41 study groups reported higher SBP values in women aged 55-64 years than men (25).

In the current study, although univariate analysis showed a relationship between increased hypertension prevalence with increasing BMI, WC, WHR, and WHtR, the multiple logistic regression analysis indicated that BMI and WHtR were the main determinants of hypertension prevalence. Although BMI, WC, and WHR are widely used as obesity indices for hypertension, diabetes, and other cardiovascular diseases, some authors have reported that BMI was a strong predictor of CVD mortality for whites, and also WC, WHR, or WHtR might be a better predictor for MetS or other CVD risks in different populations (26-29). WHtR was investigated in the NDNS and Health Survey for England, and it was found that both men and women with normal BMI but WHtR ≥0.5 had increased cardiometabolic risk factors, not only when they were compared to those with normal BMI and WHtR<0.5, but also with those with overweight BMI and WHtR<0.5 (30,31). Sayeed et al. highlighted the role of WHtR as an important predictor for hypertension (32). The hypertension rate was sig-

nificantly higher in the upper quintiles of BMI, WHR, and WHtR, and BMI, WHR, and WHtR significantly correlated with SBP and DBP. Logistic regression analysis, which considers hypertension as a dependent variable, showed a higher odds ratio with WHtR than BMI and WHR (32,33).

We found that the prevalence of smoking was lower in the hypertensive group than in the normotensive group, and smoking was found to affect hypertension prevalence inversely by multiple logistic regression analysis. Although smoking can cause an acute rise in blood pressure levels in normotensive and hypertensive individuals, epidemiological studies using standard office measurements reported that blood pressure levels were lower or equal in regular daily smokers than nonsmokers (34-37). When the researchers used ambulatory blood pressure monitoring (ABPM), Mikkelsen et al. reported that smokers reported lower mean daytime systolic BP measurements; Green et al. reported lower daytime diastolic BP measurements (36,38). Mikkelsen et al. suggested that this situation might lead to an adaptive change in the sympathetic nervous system against nicotine use for many years (38). They also emphasized that smoking might help reduce stress, which is associated with rising blood pressure. However, this result can lead to a misleading evaluation of the effects of smoking on health. It is well known that chronic smoking has a negative effect on endothelial function and arterial stiffness, resulting in cardiovascular diseases (39). Our study evaluated the current smoking status without indicating life-course-adjusted cigarette smoking. Morillo et al. showed that smokers with or without antihypertensive medication had significantly higher daytime SBP and DBP values (34). This finding was also corroborated by other studies conducted on normotensive and hypertensive patients (40-44). A lower smoking rate in hypertensive patients than in normotensive individuals in our study may be associated with the patients' reluctance to take extra risks due to smoking.

Conclusion

To sum up, the SEMT hypertension study indicated the prevalence of hypertension in more than one-third of the adult population in Turkey. Also, only one-third of the hypertensive adults showed awareness of their hypertension. Living in a rural area, older age increased BMI and waist to height ratio >0.5 were factors leading to a higher prevalence of hypertension, while the smoking rate was lower in hypertensive patients. This was the first study evaluating the characteristics of hypertension in 7 regions of Turkey. Significant differences were observed among the regions in terms of the prevalence and awareness about hypertension.

Overall, the data of the SEMT hypertension study has indicated that in the adult Turkish population, the prevalence of hypertension was high, but the awareness levels and treatment rates of hypertension were low, despite regional differences. It is suggested to conduct nationwide awareness programs on the risk factors of hypertension and the application of practical physical examination methods in primary health care centers for the prevention, early diagnosis, and control of hypertension.

Abbreviations

ABMP: Ambulatory blood pressure monitoring
BP: Blood Pressure
BMI: Body Mass Index
DM: Diabetes Mellitus
DBP: Diastolic blood pressure
HC: hip circumference
HT: Hypertension
JNC-7: Seventh report of the Joint National Committee (USA)
MONICA: Monitoring of trends and determinants in cardiovascular diseases
SEMT: Society of Endocrinology and Metabolism of Turkey
SBP: Systolic blood pressure
TURDEP-II: The Turkish Epidemiology Survey of Diabetes, Hypertension, Obesity and Endocrine Diseases
WC: waist circumference
WHtR: Waist to height ratio

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Fahri Bayram, Tevfik Sabuncu, Aydın Vedia Gedik, Demet Çorapçıoğlu, Ahmet Kaya; Design: Fahri Bayram, Tevfik Sabuncu, Özgür Demir; Control/Supervision: Aydın Vedia Gedik, Demet Çorapçıoğlu, Ahmet Kaya; Data Collection and/or Processing: Fahri Bayram, Tevfik Sabuncu, Özgür Demir; Analysis and/or Interpretation: Fahri Bayram, Tevfik Sabuncu, Özgür Demir, Mehmet Ali Eren; Literature Review: Fahri Bayram, Tevfik Sabuncu, Özgür Demir, Mehmet Ali Eren; Writing the Article: Fahri Bayram, Tevfik Sabuncu, Özgür Demir, Mehmet Ali Eren; Critical Review: Fahri Bayram, Tevfik Sabuncu; References and Fundings: Tevfik Sabuncu, Özgür Demir, Mehmet Ali Eren; Materials: Fahri Bayram, Tevfik Sabuncu.

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Publication Outcomes for Oral Presentations at Congresses of Endocrinology and Metabolic Diseases of Turkey: Analysis of Twenty Years

Türkiye Endokrinoloji ve Metabolizma Hastalıkları Kongresi Sözlü Sunumlarının Yayına Dönüşüm Oranları: Yirmi Yılın Analizi

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Abstract

Objective: This study aimed to analyze the publication rate for oral presentations at Congresses of Endocrinology and Metabolic Diseases of Turkey (CEMDT), their contribution to the literature, and variations over twenty years. **Material and Methods:** Presentations from 1997–2017 were accessed through congress booklets. PubMed and Google Scholar were used for the relevant literature search. **Results:** A total of 456 oral presentations were identified as having a publication conversion rate of 45.4% (207). These manuscripts were published in journals with a median impact factor (IF) of 2.79 (interquartile range (IQR): 1.77-3.58) and were found to receive a median of 9 (IQR: 3-21) citations. The publication rate in SCI/SCI-E indexed journals was 80.2% (166). The publication duration was a median of 20.6 months (IQR: 6-41 months). The multinational abstract rate was 6.8% (31), whereas 19.3% (88) of the abstracts comprised of retrospective data. The most frequent variation during the conversion to articles was a change in the order of the authors, which was observed in 65.71% (136) of cases. When the two ten-year periods from 1997-2006 and 2007-2017 are compared, the publication rate increased from 37.1% to 54.3% ($p<0.01$). **Conclusion:** This is the first study to evaluate the publication rate of the CEMDT presentations. The conversion rate for oral presentations to publication is high compared to studies from other branches of medical science in Turkey. However, it remains lower than the international congresses. There is a promising enhancement in the last ten years.

Keywords: Congress; endocrinology; Turkey; meeting bbstract; publications

Özet

Amaç: Türkiye Endokrinoloji ve Metabolizma Hastalıkları Kongresi (TEMHK) sözlü bildirilerin yayına dönüşüm oranını, literatüre katkısını ve 20 yıl sürecindeki değişimlerini analiz etmektir. **Gereç ve Yöntemler:** 1997-2017 yılları bildirilerine kongre kitapçıklarından ulaşıldı. İlgili literatür taraması PubMed ve Google Scholar veri tabanlarından yapıldı. **Bulgular:** Toplam 456 sözlü bildirinin, yayına dönüşüm oranı %45,4 (207) olarak tespit edildi. Bu yayınların medyan IF 2,79 (IQR: 1,77-3,58) dergilerde yayınlandığı ve medyan 9 (IQR: 3-21) atıf aldığı gözlendi. SCI/SCI-E indeksli dergilerde yayınlanma oranı %80,2 (166) idi. Yayınlanma süresi medyan 20,6 ay (IQR: 6-41 ay) idi. Çok ülkeli bildiri oranı %6,8 (31), retrospektif verilerden oluşturulan bildirilerin oranı %19,3 (88) idi. Makaleye dönüşümde en sık değişiklik, yazar sırasında değişim %65,71 (136) olarak gözlendi. İki on yıllık dönem olan 1997-2006 ile 2007-2017 karşılaştırıldığında, yayına dönüşüm oranlarında %37,1'den %54,3'e artış tespit edildi ($p<0,01$). **Sonuç:** TEMHK sözlü bildirilerinin yayınlanma oranı ilk kez değerlendirilmiştir. Sözlü sunumların makaleye dönüşüm oranı, ülkemizdeki diğer tıp bilim dallarının çalışmalarına göre yüksektir. Ancak uluslararası kongrelerle kıyaslandığında bir miktar geride kalmaktadır. Son 10 yıl içindeki artış umut vadetmektedir.

Anahtar kelimeler: Kongre; endokrinoloji; Türkiye; toplantı özeti; yayınlar

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Introduction

The Congress of Endocrinology and Metabolic Diseases of Turkey (CEMDT) is one of the most important national endocrinology congresses. The 41st Congress was held in 2019. Congresses are environments providing the platform for researchers to share their studies and further nurture their research in accordance with constructive recommendations and criticisms. After presentations, researchers receive feedback and are, therefore, benefitted from the experts in the field and other colleagues; however, this is generally a less meticulous assessment than that of indexed journals (1). The reviewer assessment process in journals does not just eliminate low-quality studies but also increases the quality of studies through revisions before publication (2).

The value of a scientific conference and its impact on the scientific society can be estimated by the rate of conversion of presentations to journal publications (3). The Cochrane review reported the conversion-to-publication rate as 24.6% for posters and 43.1% for oral presentations (4). As depicted in Table 1, in Turkey, the publication rate for oral presentations of studies in different branches of medical science varies from 20.4-42.3% (5-14).

There is no previous study about the publication rate for CEMDT presentations in scientific journals. The aim of this research is to fill the gap in the literature, to research the contribution of oral presentations given at the CEMDT to the literature, and to analyze the variations over twenty years.

Material and Methods

The pdf versions of the congress booklets from recent years were accessed through the official website of The Society of Endocrinology and Metabolism of Turkey (SEMT). The pdf versions of congress booklets from previous years were obtained from the SEMT. Abstracts of the Congresses held from 1997-2017 were included in the study. Due to the European Endocrine Congress being held in Turkey in 2009, there was no CEMDT held that year, so that year was excluded from our study. Congresses in 2000 and 2001 were organized in cooperation with other foreign foundations. We also excluded a total of 4 oral presentations in 2000 and 20 oral presentations in 2001, which included only foreign authors.

The authors of abstracts were classified as a single education and research hospital (ERH), single university hospital, multicenter studies, and other (single state hospitals, pharmaceutical industry). The province of the first author's organization was also recorded from the abstracts. The number of authors was enlisted. Studies performed in cooperation with branches of science outside of endocrinology were recorded. Presentations were categorized as clinical studies, experimental studies, and case reports.

The first author/final author and relevant keywords were entered in English; if no results were found, searches were repeated in Turkish. The relevant abstract was displayed on the same screen with a search for authors and keywords in Google Scholar and PubMed via a web-interface (PHP & HTML coding and MySQL database). Research results were entered into the database via the web interface.

Only if an article significantly overlapped with the authors, title, and content of the presentation abstract at a high rate was it accepted as a full publication. Specifically, a full publication was required to have an author list, including at least the first and last authors of the presentation, a title, including the related keywords as the abstract title, and apparently reproduce the material and methods of the presentation. Publications with different sample sizes (compared to the abstract) were accepted as a match if the aim of the study and methods were the same as the abstract and the author criteria were compatible (15,16).

If publications could not be reached, the most appropriate keywords for the presentation were selected, and all publications by the first/final author between the congress date and 2020 were searched again. Literature and relevant citation screening were completed in January-February 2020.

According to the Web of Science (Clarifying Analytics, Philadelphia, PA, USA) directory, articles were divided into two groups- SCI (Science Citation Index)/SCI-Expanded and other (Emerging Sources Citation Index, Index Copernicus, TÜBİTAK ULAKBİM TR directory, EBSCOhost, Embase, Scopus, etc.).

Table 1. Publication rate for oral presentations from various science branches in Congresses in Turkey.

Authors	Publication Date	Congress Years	Science Branches	Congress Name	Number of Publications from Oral Presentation/ Total Oral Presentations	Presentation-To-Publication Rate
Civan Kahve et al.(9)	2020	2012-2016	Psychiatry	National Psychiatry Congress	64/187	34.2%
Evman et al.(14)	2017	2011	Thoracic Surgery	National Thoracic Surgery Congress	5/23	21.7%
Özyurt et al.(6)	2012	2004,2006, 2008	Dermatology	National Dermatology Congress	29/134	21.6%
Ersoy(7)	2016	2009	Plastic, Reconstructive and Aesthetic Surgery	National Congress of Plastic, Reconstructive and Aesthetic Surgery	36/118	30.5%
Çekmecelioğlu et al.(12)	2019	2011-2014	Anesthesiology and Reanimation	Anesthesiology and Reanimation National Congresses	136/319	42.3%
Yüksel et al.(8)	2018	2012	Urology	Congress of the Society of Urological Surgery	34/103	33%
Aksüt et al.(11)	2019	2012, 2014, 2016	Cardiovascular Surgery	National Congresses of Turkish Society of Cardiovascular Surgery	279/675	41.3%
Kaya Mutlu et al.(5)	2013	2002,2004, 2006,2008	Physiotherapy	Physiotherapy Symposiums	37/181	20.44%
Bagatur et al.(10)	2019	2013,2014	Orthopedics and Traumatology	National Turkish Orthopedics and Traumatology Congresses	236/612	38.5%
Kalyoncu et al.(13)	2011	2005-2009	Rheumatology	National Rheumatology Congresses	28/87	32.1%

Five-year impact factors were noted from the InCites Journal Citation Reports (Clarivate Analytics). If the presentation was identified as published, the publication date, name of the journal, the duration from presentation to publication, the index site for the journal, the 5-year impact factor (IF), Q1/Q2/Q3/Q4 categories of the journal in SCI/SCI-E and changes during the conversion to an article were recorded. The total number of citations received by the article was divided by the duration between the year of publication and 2020 to calculate the citation/year value.

Our study did not require permission from an ethics committee as it used data open to public access.

Statistical Analysis

The descriptive data were represented as the median and interquartile range (IQR: difference between 25th and 75th percentiles) due to incompatibility with the normal distribution. Comparisons of numerical data used the Mann-Whitney U test. Categoric variables were assessed with the chi-square test. Multivariate analysis used binomial logistic regression to determine relationships between different variables. Inclusion criteria for the model were statistical significance in univariate analysis ($p<0.05$) and its potential relationship with publication. Statistical significance was accepted as $p<0.05$.

Results

Over 20 years, a total of 456 oral presentations were found to have a presentation-to-publication ratio of 45.4% (207). These publications were in journals with a median IF of 2.79 (IQR: 1.77-3.58) and received a total of 3697 (median: 9, IQR: 3-21) citations. The number of citations per year was computed as 1.26 citations/year per publication (IQR: 0.5-2.63). The publication rate in SCI/SCI-E indexed journals was 80.2% (166). The numbers of Q1/Q2/Q3/Q4 publications were 43/47/32/44, respectively. The median publication duration was 20.6 months (IQR: 6-41 months). The median number of presentation authors was 6 (IQR: 5-8).

There were 395 (86.6%), 41 (9%), and 20 (4.4%) presentations based on clinical studies, experimental studies, and case reports, respectively. Publication conversion rates were 170 (43%), 27 (65.9%), and 10 (50%) for clinical studies, experimental studies, and case reports, respectively (p=0.02) (Figure 1). Experimental studies had a significantly high rate of conversion to publication, in comparison to clinical studies (65.9% vs. 43%, p=0,005).

When classified according to presentation topics, the top three topics included thyroid at 25.2%, diabetes at 22.1%, and pituitary at 14.5%. However, no statistical difference was obtained in terms of the publication status according to the topic (Figure 2).

Universities gave 75.7% of the presentations (345), followed by education and research hospitals at 12% (55), multicenter reports at 10.9% (50), and other organizations at 1.3% (6). In terms of publication conversion rates for single universities and single ERB's has not shown any statistical significance. (42.9% vs. 40%; p=0.68). Multicenter studies manifested higher publication rates compared to single university-sourced studies (70% vs. 42.9%; p<0.01). Moreover, a higher rate of conversion to publication was identified for multicenter studies, compared to studies sourced in a single ERH (70% vs. 40%; p=0.02) (Figure 3).

As compared to presentations authored exclusively by endocrinology researchers, presentations made in cooperation with branches of science outside of endocrinology were observed to have a higher rate of conversion to publication (50.7% vs. 36.8%; p=0.001). Presentations conducted in cooperation with researchers from other countries comprised 6.8% (31). Multinational presentations also demonstrated a higher rate of conversion to publication than the presentations from single countries (74.2% vs. 43.3%; p=0.001). The presentations involving retrospective data were 19.3% (88). Studies encompass-

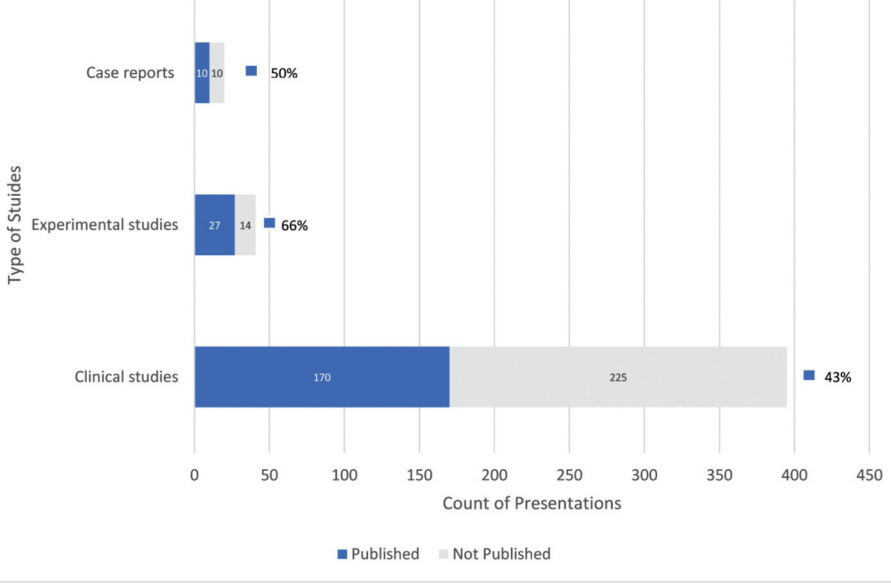

Figure 1. Presentation-to-publication rates vs. type of studies, Chi-square test (p=0.02).

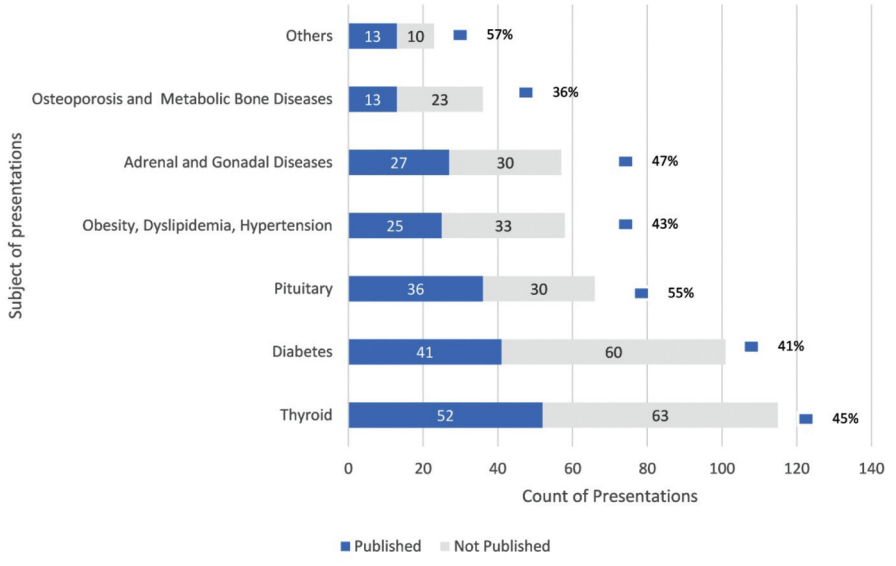

Figure 2. Classification of presentations based on topic and publication status, Chi-square test (p>0.05).

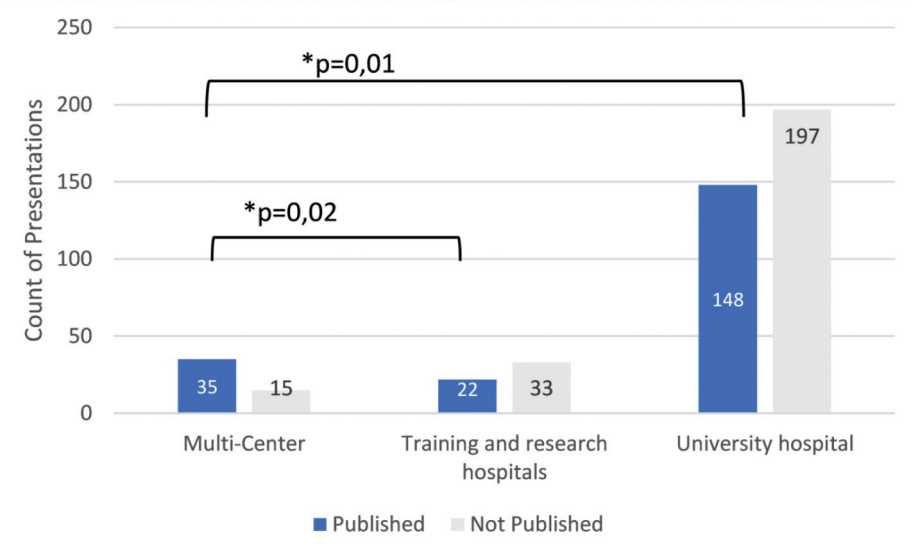

Figure 3. Presentation-to-publication rate according to the study center.

ing retrospective data were observed to be converted to publications at a lower rate when compared to studies produced from non-retrospective data (33% vs. 48.4%; p=0.009).

The multivariate logistic regression analysis for conversion to publication found that multicenter studies had twice the probability of being converted to publication as compared to single university research studies (Odds=2.161 [1.084-4.309]; p=0.029). The probability of publication for multi-unit studies was 1.6 times higher when compared to single unit research (Odds=1.6 [1.07-2.39]; p=0.02). The probability of publication for multinational studies was found to be 2.72 times compared to research from single countries (Odds=2.72 [1.07-6.86]; p=0.034). The publication probability of studies comprising retrospective data was reduced 1.7 times compared to research involving non-retrospective data (Odds=1/0.583 [0.352-0.295]; p=0.036).

The most frequent change during the con-

Table 2. Most frequently observed changes during conversion to articles (n=207).

Status of Change	% (n)
Change of the authors' order	65.7% (136)
Change in title	40.6% (84)
Increase in the number of authors	30.9% (64)
Increase in number of patients/data	29% (60)
Change in the last author	24.6% (51)
Decrease in the number of authors	23.7% (49)
Change in the first author	20.3% (42)
Decrease in the number of patients/data	5.8% (12)

version to articles were in the order of the authors at 65.71% (136). Other changes are detailed in Table 2. Fifteen articles (7.3%) were published a median of two months before being presented at the congress. The publication conversion numbers and rates based on provinces with the most presentations, according to the location of the organization of the first author, are illustrated in Figure 4.

When the two 10-year periods of 1997-2006 and 2007-2017 are compared, the publication conversion rates increased from 37.1% to 54.3% (p<0.01) (Table 3). While there was an increase in ERH presentations (6.4% vs. 18.5%; p<0.01), there was a decrease in university presentations (84.2% vs. 68.5%; p<0.01). There was a partial increase observed for multicenter studies (9.4% vs. 13%; p>0.05); however, this was statistically insignificant. There was no significant variation in terms of the province of the first author for the majority of provinces. However, a significant increase in presentations from the province of Kayseri (3.8% vs. 10%; p<0.05) was evident.

Discussion

The rate of conversion to publication for presentations at the CEMDT congresses was evaluated for the first time, and it was revealed that the conversion to publication rate was 45.4% for twenty years of oral presentations. Comparing the oral presentations for 1997-2006 and 2007-2017, an increase was observed in publication conversion rates from 37.1% to 54.3%. The 2018 Cochrane review explored 425 studies and reported the conversion to publication rates as 24.6% for posters and 43.1% for oral presentations, with an overall rate of 37.3% (4). The rates of conversion to publication for presentations manifest much variability according to the congress and the area of speciality, varying from 8.2-66% (17). When the 2007 Cochrane review (18) and the 2018 review were compared, a reduction in the overall rate from 44.5% to 37.3% was evident (4). In spite of the decrease in the general publication conversion

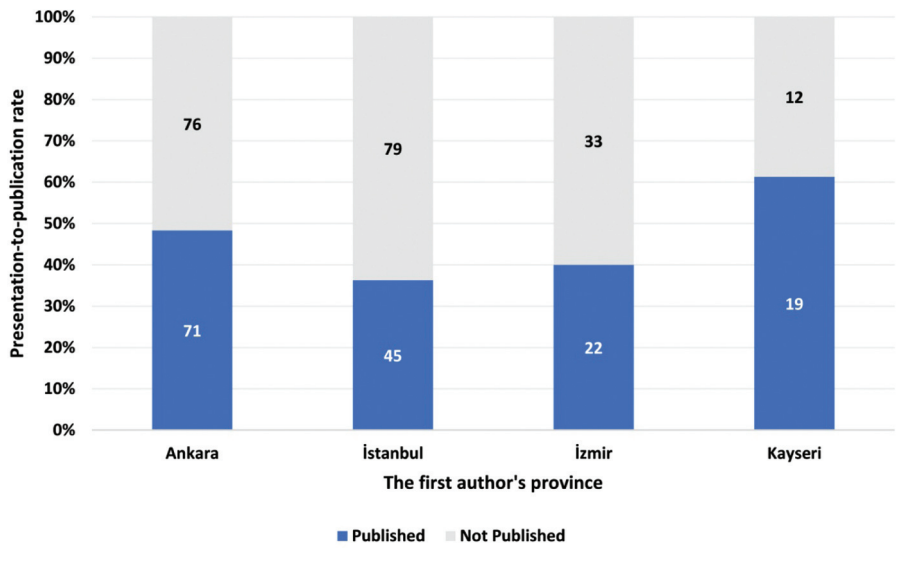

Figure 4. Presentation-to-publication rate based on the provinces with most presentations (Top four).

Table 3. Comparison of presentations for 1997–2006 and 2007–2017.

	1997-2006 n (%)	2007-2017 n (%)	p value
Total Number of Presentations	237 (52%)	219 (48%)	
Presentation-to-publication rate	37.1%	54.3%	P<0.001
Institution			p<0.01
University	197 (84.2%)	148 (68.5%)	*
Education and Research Hospitals	15 (6.4%)	40 (18.5%)	*
Multi-Center	22 (9.4%)	28 (13%)	
Institutional province of the first author			p=0.04
Ankara	80 (33.8%)	67 (30.6%)	
İstanbul	71 (30%)	53 (24.2%)	
İzmir	24 (10.1%)	31 (14.2%)	
Kayseri	9 (3.8%)	22 (10%)	*
Total of Other Provinces	53 (24.4%)	46 (21%)	
Publication duration (Median)	21 months (IQR: 6-47)	20 months (IQR: 6-37)	p>0.05
Index Category			P=0.38
SCI- (E)	68 (77.3%)	98 (82.4%)	
Non-SCI- (E)	20 (22.7%)	21 (17.6%)	
SCI-E Categories			P=0.14
Q1	24 (35.3%)	19 (19.4%)	
Q2	16 (23.5%)	31 (31.6%)	
Q3	16 (23.5%)	20 (20.4%)	
Q4	12 (17.6%)	28 (28.6%)	
Impact factors of journals published (Median)	2.79 (IQR: 1.90-5.54)	2.84 (IQR: 1.77-3.09)	p>0.05
Number of Citations per year of Articles Published (Median)**	1.28 (IQR: 0.59-3.30)	1.25 (IQR: 0.41-2.50)	p>0.05

*p<0.05.
** number of citations per year from the date of publication until February 2020.
SCI-E=Science Citation Index/Expanded; IQR= Interquartile range.

rates in the literature, an increase was observed for CEMDT. This increase between the two decades is an indirect indicator of the enhancement in the literature impact of the CEMDT.

The number of studies reviewing the publication conversion rates for international endocrinology congresses are limited compared to other branches of science and only include the analysis of international diabetes congresses (19,20). The first study to identify the publication rate for an international endocrine congress was completed with the participation of one of the current authors, and the publication conversion rate for oral presentations at the European Congress of Endocrinology (ECE 2014) was found to be 65% (21). It is promising that the conversion rate for oral presentations at the CEMDT has approached the level of international congresses in the last ten years. Furthermore, it may be regarded as an indicator that oral presentations are opted meticulously abiding by international selection standards.

When presentations are classified according to the type of organization, the multicenter studies reflected the highest publication rate, and studies in collaboration with departments outside of endocrinology also portrayed higher rates of publication conversion. This validated the importance of cooperation and interdisciplinary studies. The lack of observation of

a significant difference between publication conversion rates for single university and single ERH-related presentations may be a sign of objective assessment while choosing congress presentations.

Few studies are available regarding a bibliometric analysis of Turkish endocrinological research. In terms of metabolic bone disease, Turkey has a low publication output (22). Our study revealed that Osteoporosis and Metabolic Bone Diseases had the lowest rate in the presentation subjects and also the lowest publication conversion rate.

For national and international congresses, the rate of presentations published in a journal before presentation at the congress varies from 8.7-20.5% (9,21,23,24). This rate was low in our study at 7.3%, where most studies were found to be published after the congress. This leads to a consideration of the contribution of post-presentation CEMDT feedback to the process of conversion to articles.

The publication rate for presentations at national congresses in SCI/SCI-E journals varies from 64.3-66.1%, with this rate at 80.6% for the 2014 ECE (12,21,25). With reference to the CEMDT, the rate was in agreement with the international congresses.

The order of the authors reflected the most frequent change (65.7%) during the article conversion process. For an orthopedics congress, this rate was identified as 61.2% (26). Changes to the name of the first author had rates of 5.7-20.7% in the literature (14,26), while this rate was 20.3% in our study. This may be affected by the inclusion or exclusion of authors during the process of conversion to an article. There was an increase in patient/data numbers of 29% and a reduction of 5.8% during publication conversion. This situation may have affected the change in author numbers. Changes to the titles was in accordance with the literature (27).

The reason for non-publication of nearly half of the oral presentations is a topic requires clarification. A survey conducted with the authors whose presentations had not been converted to publications five years after the congress summarized the following top 3 responses regarding lack of publication- insufficient time to publish, still continuing the publication process, and problems experienced with co-authors (28). Strategies should be developed to monitor the process of publication conversion for oral presentations at the CEMDT.

Our study has some limitations. Studies were not classified according to evidence levels, whereas the rate of studies based on retrospective data was investigated. Google Scholar and PubMed were employed as the search engine, so publications in other indexes or not identified by the search engines may have been missed. Besides screening until at least three years after the presentation, there may have been articles that were still under journal review during the literature screening. During literature screening, despite using a convenient interface program to record the search and for the database, human errors may not be eliminated. Another limitation in terms of assessing the contribution of the congress to the literature was that only oral presentations were assessed, and posters were not included.

Conclusion

To the best of our knowledge, this is the first study to evaluate the publication rate of CEMDT oral presentations. The article conversion rate for oral presentations at the CEMDT is higher in comparison to studies in other branches of medical science in Turkey. However, it remains a little lower in comparison to international congresses. The increase in publication rate during the last ten years is promising.

Future perspectives and recommendations

Increasing the feedback received during the congress presentation, encouraging publication, and following the papers that are published after the congress will enhance academic production.

Using prospective data, collaboration with non-endocrine units, and multicenter, multinational design were determined as factors that increase the probability of publication as an article.

It will be useful to develop a website archiving all congress abstracts. This will help to track the abstracts that do not turn into a full-text article and also follow the differences between the abstract and the full text more clearly.

Publication outcome of posters needs to be evaluated in future research.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

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Clinical Spectrum and Outcome of Patients with Graves' Disease: A Single-Center Experience from a Tertiary Care Institution in the Kashmir Valley, India

Graves Hastalığı Olan Hastaların Klinik Spektrumu ve Sonuçları: Keşmir Vadisindeki (Hindistan) Üçüncü Basamak Bir Sağlık Kurumunda Tek-Merkezli Deneyim

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Abstract

Objective: Graves' disease (GD) is a common autoimmune disorder with variable outcomes. We aim to study the clinical manifestations and treatment outcome of GD in the post-iodization scenario. **Material and Methods:** The present study was designed as a cross-sectional study, in which a total of 180 patients with GD (127 males and 53 females) attending our center were reviewed retrospectively. The demographic data, modes of treatment, comorbidities, remission, and recurrence rates were determined for the patients. All patients were initially treated with anti-thyroid drugs (ATDs), with the subsequent management depending on the course of the disease. **Results:** The mean (±SD) age at diagnosis was 38.30 (10.73) years and the lag period between the onset of symptoms and the diagnosis was 5.12 (2.69) months, with the male patients having a significantly shorter duration of illness compared to females (4.36 vs. 5.44 months; P=0.015). Majority of the patients presented with the typical symptoms and signs associated with hyperthyroidism and/or goiter, although the atypical presentations were not uncommon. ATDs were the most preferred treatment modality employed to achieve clinical and biochemical remission. The mean duration of achieving euthyroidism and the normalization of TSH levels were 3.31±1.51 and 7.45±3.35 months, respectively. On follow-up at three months, 46.1% of the patients were euthyroid, with normalization of the TSH levels in 15.6% of them. Failure to achieve early remission/disease control was significantly higher in males (p=0.003) and smokers (p=0.036). Among the 72 patients who completed medical therapy, 49 patients achieved remission, of whom 20 patients relapsed with a first-year relapse rate of 20.4%. Disease relapse was significantly associated with higher initial 99 mTechnetium (99^{m}Tc) uptake (p=0.022) and higher grade of goiter (p=0.026) at presentation. The logistic regression analysis revealed male gender (p=0.048) and orbitopathy (p=0.036) as the independent risk factors predicting relapse of the GD. **Conclusion:** Graves' disease manifests with varied clinical manifestations, including the atypical ones, warranting careful clinical assessment to ensure an accurate diagnosis. Gender and orbitopathy are the independent risk factors predicting the relapse of the disease.

Keywords: Anti-thyroid drugs; diffuse toxic goiter; hyperthyroidism; total thyroidectomy; thyrotoxicosis

Özet

Amaç: Graves hastalığı (GD), çeşitli sonuçları olan yaygın bir otoimmün bozukluktur. İyodizasyon sonrasında GD'nin klinik belirtilerinin ve tedavi sonuçlarının incelenmesi. **Gereç ve Yöntemler:** Bu çalışma, merkezimize başvuran toplam 180 GD'li (127 erkek ve 53 kadın) hastanın retrospektif olarak incelendiği kesitsel bir çalışma olarak tasarlandı. Hastaların demografik verileri, tedavi şekilleri, komorbiditeleri, remisyon ve relaps oranları belirlendi. Tüm hastalar başlangıçta anti-tiroid ilaçlar (ATD'ler) ile tedavi edildi ve ardından hastalığın seyrine bağlı olarak yönetildi. **Bulgular:** Tanı anındaki ortalama (±SS) yaş 38,30 (10,73) idi, semptomların başlangıcı ile tanı arasındaki gecikme süresi 5,12 (2,69) aydı, erkek hastalar kadınlara göre anlamlı olarak daha kısa hastalık süresine sahipti (4,36 vs 5,44 ay; p=0,015). Hastaların çoğunluğu hipertiroidizm ve/veya guatr ile ilişkili tipik semptom ve bulgularla başvurdu, ancak atipik tablolar da nadir değildi. ATD'ler, klinik ve biyokimyasal remisyon sağlamak için en çok tercih edilen tedavi yöntemiydi. Ötiroidizme ulaşılması ve TSH düzeylerinin normalleşmesi için geçen ortalama süre sırasıyla 3,31±1,51 ve 7,45±3,35 aydı. Üç aylık takipte, hastaların %46,1'i ötiroid oldu ve %15,6'sında TSH seviyeleri normalleşti. Erken remisyon/hastalık kontrolü sağlanamaması erkeklerde (p=0,003) ve sigara içenlerde (p=0,036) anlamlı olarak daha yüksekti. Medikal tedaviyi tamamlayan 72 hastadan 49'unda remisyon sağlandı, bunlardan 20'sinde relaps izlendi, ilk yıl relaps oranı %20,4 idi. Hastalığın relapsı, başvuru sırasındaki daha yüksek başlangıç 99 mTechnetium (99^{m}Tc) alımı (p=0,022) ve daha yüksek guatr derecesi (p=0,026) ile anlamlı şekilde ilişkiliydi. Lojistik regresyon analizine göre, GD relapsını öngördüren bağımsız risk faktörleri erkek cinsiyet (p=0,048) ve orbitopati (p=0,036) idi. **Sonuç:** Graves hastalığı, atipik olanlar da dahil olmak üzere çeşitli klinik belirtilerle kendini gösterir ve doğru tanı için dikkatli bir klinik değerlendirme gereklidir. Cinsiyet ve orbitopati, hastalığın relapsını öngördüren bağımsız risk faktörleridir.

Anahtar kelimeler: Antitiroid ilaçlar; toksik diffüz guatr; hipertiroidizm; total tiroidektomi; tirotoksikoz

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Introduction

Graves' disease (GD) is a multi-systemic disorder of autoimmune etiology, which results from complex interactions between genetic and environmental factors (1,2). It is the most common cause of hyperthyroidism, accounting for 60% to 80% of the cases, with an annual incidence of 20 to 50 cases per 100,000 individuals (3). As with the other autoimmune diseases, GD affects women more than men, with a peak incidence occurring between the age of 30 and 50years, although people may be affected at any age. According to the data from Nurses' Health Study II (NHSII), the 12-year incidence of GD among women aged between 25 to 42years was as high as 4.6/1000 (4). Hyperthyroidism, diffuse goiter, and/or orbitopathy are the cardinal manifestations of GD, although other organ systems may also be affected, resulting in a plethora of clinical signs and symptoms. The severity and duration of the disease, as well as the patient's age at onset, determine the presentation and the course of the disease (5). The manifestation of the disease either results from hyperthyroidism (goiter in certain cases) or is a consequence of underlying autoimmunity (6). Impaired quality of life, resulting in an inability to work (7,8) and an increased risk of death (9) associated with GD render it imperative to understand the natural history of GD. While trying to study GD, there is a need to renew focus on epidemiology, pathogenesis, and the subsequent management to restore euthyroidism for a favorable outcome of the disease. Clinical and biochemical features of thyrotoxicosis, particularly those with a long duration and/or orbitopathy, along with a diffuse increase in radioactive iodine or technetium uptake scan, confirm the diagnosis of GD. The treatment for GD comprises rapid control of the symptoms, generally with a beta-adrenergic blocker, and reduction of thyroid hormone secretion using one of the several modalities available, including ATDs, radioactive iodine therapy (RAI), and surgery; the selection of the treatment modalities often varies according to different guidelines and local traditions.

The Kashmir Valley, located in the North-Indian Union territory of Jammu and Kashmir, has been a known iodine-deficient area (10). With the universal implementation of salt iodization, a marked improvement in the overall iodine nutrition was observed in the Kashmir Valley (11). It is well known that the incidence of hyperthyroidism, including GD, may increase after salt iodization (12). Therefore, it is imperative to study the clinical manifestations and the treatment outcome of GD in the Kashmir Valley in the post-iodization scenario.

Aims and Objectives

To have an in-depth discussion on GD through the study of clinical manifestations and treatment outcomes of GD in the post-iodization scenario.

Material and Methods

Study setting

The present study reports a two-year retrospective analysis of GD patients who presented to the Endocrine outpatient department of the super-speciality hospital of Government Medical College, Srinagar, between June 2017 and December 2019. The study was performed in accordance with the Declaration of Helsinki statement for medical research involving human subjects.

Study population and sample size

A total of 180 consecutive patients with GD were included in the present study.

Methods

The medical records of all patients were reviewed, and questionnaire sessions were conducted to obtain information regarding the demographics (age, gender, weight, and height), presentation (signs and symptoms), smoking status, duration of the disease, history of disease evolution and progression, routine laboratory investigations, thyroid function tests, treatment modality, outcomes, presence of comorbidities, and the last-available status of the disease. Various thyroid function tests were performed, including the estimation of Thyroid Stimulating Hormone (TSH), Total Thyroxine (T4), Total Triiodothyronine (T3), Free Thyroxine (FT4), Free Triiodothyronine (FT3), TSH Receptor Antibody (TRAb), and anti-thyroid peroxidase (anti-TPO). Informed consent was obtained from each pa-

tient, after which a general physical examination and a detailed systemic examination, was carried out and the relevant data were recorded and tabulated. The goiters were classified according to the World Health Organization (WHO) recommendations: Grade 0, no goiter is palpable or visible; Grade 1, palpable goiter not visible when the neck is held in normal position; Grade 2, goiter visible when the neck is held in normal position. The reference ranges for normal values for the various laboratory investigations included: 0-6.1 IU/mL for TPO, 0.35-5.5 mIU/L for TSH, 12-22 pmol/L for FT4, 3.2-12.6 mcg/dL for tT4, 0.6-1.81 ng/mL for tT3, and 3.1-6.8 pmol/L for FT3. Thyroid uptake evaluation and scintigraphy were performed twenty minutes after the administration of an intravenous injection of 370 MBq (10 mCi) of $^{99\,m}$Tc-pertechnetate. Images were captured in a supine position using a gamma camera with a pinhole collimator. ^{99m}Tc uptake between 0.4% and 7.1% was considered normal (13). The higher upper limit of ^{99m}Tc uptake was in accordance with the normal reference range results obtained for the thyroid uptake of ^{99m}Tc pertechnetate, which is used as a measure of the iodide-trapping function in the areas with iodine deficiency (14).

The diagnosis of GD was based on the clinical features of thyrotoxicosis and the biochemical evidence of hyperthyroxinemia (raised levels of FT3, FT4, tT4, and tT3) along with suppressed TSH complemented by diffuse uptake detection in thyroid imaging by ^{99m}Tc scintigraphy. In the absence of thyroid scintigraphy, a diagnosis of GD was considered in patients with a long duration of thyrotoxic symptoms, requirement of ATDs for a prolonged period, and the presence of an infiltrative ophthalmopathy.

Data collection

After obtaining written informed consent from the eligible patients, a detailed history from each patient was obtained from the patient and/or a reliable person well acquainted with the patient. The sociodemographic and clinical data were obtained from the patients and/or their relatives and recorded in semi-structured formats. Besides various thyroid function tests, different routine blood investigations like complete hemogram, ESR, blood glucose levels, serum urea/creatinine levels, lipid profile, liver function tests, levels of serum electrolytes, and imaging, were carried out as per need of the patient and were recorded in a structured proforma.

Statistical analysis

The obtained data were first entered into Microsoft Excel datasheets. All statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) version 25.0 software from IBM Corporation, NY, United States. The data were analyzed using the independent student's t-test and one-way ANOVA, along with multiple range tests. Appropriate non-parametric tests, such as Mann Whitney, Kruskal Wallis, and the other tests, were used wherever applicable. Multiple logistic regression analysis was performed using relapse as the dependent variable while age, gender, smoking status, goiter, the grade of goiter, dermopathy, orbitopathy, T3, T4, T3/T4 ratio, anti-TPO level, and the initial ^{99m}Tc uptake as the independent variables. A P-value of less than 0.05 was considered significant.

Results

The present study included 180 consecutive patients diagnosed with GD, comprising of 127 females and 53 males, with a female to male ratio of 2.4:1.0 (Table 1). The mean age at presentation was 38.30±10.73 years, with men relatively younger than the women. The anti-TPO antibody levels prior to the initiation of medical therapy were positive in 90.6% (n=145) of the patients with available levels (n=160). The mean lag period between the onset of symptoms and the diagnosis was 5.12 ±2.69 months, with males presenting earlier compared to females (p=0.015) (Table 2). The presenting signs and symptoms in decreasing order of frequency are listed in Table 3. The mean (±SD) pulse rate, systolic blood pressure, and diastolic blood pressure at presentation were 109.68 (±11.53), 118.36 (±11.20), and 77.64 (±7.21), respectively, with no significant differences between the genders. At the time of assessment, several patients had already been on anti-thyroid medication for variable periods of time. At the first assessment at three months, most of the pa-

Table 1. Baseline characteristics of the patients with Graves' disease and their treatment outcomes.

Variables	Total Cohort (n=180) Mean±SD	95% Confidence Interval (C.I)
Age Yrs.	38.30±10.73	36.72-39.87
BMI Kg/m^2	22.27±4.16	21.63-22.92
Smoker n (%)	43	23.9%
Duration of disease (months)	22.12±11.50	18.91-25.33
T3 ng/mL	3.71±1.72	3.46-3.97
T4 mcg/dL	19.15±5.18	19.14-20.62
T3/T4 ratio	18.42±6.57	17.44-19.41
^TSH microIU/mL	0.005	0.00-0.009
Anti-TPO level (IU/mL)	584.02±411.79	501.46-666.58
Anti-TPO Status n (%)	87	90.6%
TRABS (n=21)	4.80±3.59	1.47-8.12
Total 99mTechnetium uptake (Initial)%	38.03±24.34	34.06-42.00
Duration of symptoms prior to presentation (months)	5.12±2.69	4.73-5.52
Dose of ATD (mg)	32.25±11.01	30.63-33.87
Mean duration of treatment (months)	22.51±12.56	19.30-25.73
Mean duration on follow up (months)*	25.96±7.94	22.52-29.39
Active n (%)	69	38.3%
Control n (%)	83	46.1%
Duration to Euthyroidism months	3.31±1.51	3.06-3.55
Patients with normal TSH n (%)	28	15.6%
Duration to TSH normalization months	7.45±3.53	6.73-8.16
Hypothyroid episodes n (%)	32	17.8%

SD: Standard deviation; Yrs: Years; Anti-TPO: Anti-thyroid Peroxidase; BMI: Body mass index; TSH: Thyroid-stimulating hormone; T4: Total Thyroxine; T3: Total Triiodothyronine, TRABS: TSH receptor antibodies; ATD: Anti-thyroid drugs; median (minimum-maximum), *Follow up of patients post completion of the first course of ATDs.

Table 2. Comparative analysis of various parameters according to gender.

Variables	Males (n=53) Mean±SD (95% C.I)	Females (n=127) Mean±SD (95% C.I)	Sig
Age years	37.62±10.73 (34.66-40.58)	38.58±10.76 (36.69-40.47)	0.586
Smoker n (%)	37 (69.8)	6 (4.7)	0.001*
T4 mcg/dL	21.02±5.24 (19.56-22.49)	19.39±4.75 (18.53-20.24)	0.045*
Duration of symptoms before presentation (months)	4.36±2.73 (3.60-5.12)	5.44±2.62 (4.98-5.90)	0.015*
PR beats/minute	108.49±14.28 (104.55-112.42)	110.18±10.18 (108.38-111.97)	0.372
SBP mmHg	119.05±8.38 (116.74-121.36)	118.07±12.21 (115.92-120.23)	0.596
DBP mmHg	78.71±5.92 (77.08-80.35)	77.19±7.66 (75.84-78.55)	0.199
Active n (%)	27 (50.9)	42 (33.1)	0.003*
Control n (%)	24 (45.3)	59 (46.5)	0.003*
Patients with normal TSH n (%)	2 (3.8)	26 (20.5)	0.007*

SD: Standard deviation; *- Significant; Anti-TPO: Anti-thyroid peroxidase; TSH: Thyroid-stimulating hormone; T4: Total Thyroxine; T3: Total Triiodothyronine; M: Male; F: Female.

Table 3. Frequency of signs and symptoms.

Symptoms	n (%)
Palpitation	155 (86.1)
Heat intolerance with sweating	153 (85.0)
Weight loss	118 (65.6)
Increased frequency of Stools	97 (53.9)
Sleep disturbance	96 (53.3)
Pruritus	71 (39.9)
Muscle weakness and fatigue	40 (22.2)
Hyperpigmentation	34 (18.9)
Dysphagia	32 (17.8)

Signs		n (%)
Goitre		173 (96)
Grade of Goitre	2	147 (85)
	1	26 (15)
Sinus Tachycardia		140 (78.2)
Peripheral Tremor		164 (91.1)
Warm Moist hands		118 (65.6)
Ophthalmopathy		104 (57.8)
Clinical activity score	0-2	50 (88)
	≥3	7 (12)
Proptosis		48 (26.7)
Bruit		15 (8.3)
Thrill		8 (4.4)
Dermopathy		01 (0.6)
Atrial Fibrillation		07 (3.9)

tients were controlled (46.1%), with normalization of the TSH levels in 15.6% of them. Although 53.9% of the patients were still having hyperthyroxinemia, the intensity of symptoms and the degree of hyperthyroxinemia had improved.

The mean duration to achieve euthyroidism and the normalization of the TSH levels was 3.31±1.51 months and 7.45±3.35 months, respectively. In the course of treatment, 17.8% (n=32) of the patients developed overt hypothyroidism requiring a transient reduction/termination of ATDs along with the reintroduction of the drug in several of these patients once the biochemical euthyroidism (euthyroxinemia) was achieved. Infiltrative ophthalmopathy occurred in 57.8% (n=104) of the patients, while proptosis occurred in 26.7% (n=48) of the patients. Detailed information regarding TAO obtained in the present study would be published in a separate manuscript. Atypical presentation in the form of Hashimoto's encephalopathy and recurrent acute pericarditis were observed in one patient each, atrial fibrillation occurred in seven patients, and pyrexia of unknown origin was the presenting manifestation in four patients. The other associated comorbid conditions included diabetes mellitus in seven patients and hypertension in ten patients. In regard to the treatment offered, almost all the patients received ATDs to achieve biochemical and clinical remission, with the majority receiving treatment with carbimazole. Beta-blockers were prescribed to all symptomatic patients after excluding contraindications. Thionamides were generally well-tolerated in our cohort, with few patients developing common and mild adverse effects, including rash, pruritus, and gastrointestinal symptoms in the form of gastric irritation, dysgeusia, and loose stools. Two patients developed transaminitis and required transient interruption of the ATDs. These adverse effects were managed with symptomatic measures only.

The remission rate among the patients who completed 18-24 months of the first-line treatment with ATDs was 68% (n=49/72), with the mean duration of remission being 12.87±6.88 months. Remission was considered when the period of euthyroidism lasted for over six months after the termination of ATDs following a standard duration of 18-24 months of drug therapy. A comparison of various characteristics between remitters and non-remitters is provided in Table 4. Higher initial levels of TSH (p=0.007), smoking status (p=0.026), and higher ^{99m}Tc uptake (p=0.05) were associated with remission. Similarly, the development of hypothyroidism during therapy was significantly (p=0.018) associated with remission. Among the 49 patients who achieved remission, 20 patients (41%) relapsed after a mean duration of 11.86±3.34

Table 4. Comparative analysis of the remission and non-remission groups.

Variables		Remission (n=49) Mean±SD	Non Remission (n=23) Mean±SD	Sig.
Age (Years)		38.49±10.96	35.73±8.36	0.123
T3 ng/mL		3.63±1.42	3.71±1.80	0.124
T4 mcg/dL		19.89±5.23	20.46±4.15	0.216
T3/T4 ratio		17.76±5.87	17.95±7.54	0.267
TSH microIU/mL		0.015±0.026	0.009±0.013	0.007*
Anti-TPO level IU/mL		686.28±395.12	557.00±398.98	0.993
Duration of symptoms (months)		5.24±2.31	5.52±3.21	0.069
Duration for Euthyroidism (months)		8.79±3.51	7.36±2.95	0.322
Total 99 mTechnetium uptake%		43.85±24.59	38.56±25.19	0.05*
Gender	M-n (%)	12 (24.5)	10 (43.5)	0.089
	F-n (%)	37 (75.5)	13 (56.5)	
Smoker n (%)		9 (18.4)	10 (43.5)	0.026*
Goitre n (%)		48 (98)	23 (100)	0.681
Goitre grade	1 n (%)	3 (6)	4 (17)	0.264
	2 n (%)	45 (92)	19 (83)	
Orbitopathy n (%)		19 (38.8)	5 (21.7)	0.122
Hypothyroid n (%)		19 (38.8)	3 (13)	0.023*

SD: Standard deviation; *- Significant; Anti-TPO: Anti-thyroid Peroxidase; TSH: Thyroid-stimulating hormone; T4:Total Thyroxine; T3: Total Triiodothyronine; M: Male; F: Female.

Table 5. Comparative analysis of the relapse and non-relapse groups.

Variables		Relapse (n=20) Mean±SD	Non-relapse (n=29) Mean±SD	Significance
Age (Years)		39.50±12.02	37.79±10.32	0.597
T3 ng/mL		4.00±1.72	3.37±1.13	0.131
T4 mcg/dL		21.29±4.43	19.88±3.92	0.246
T3/T4 ratio		18.85±7.32	17.02±4.61	0.287
TSH microIU/mL		0.016±0.026	0.015±0.026	0.910
Anti-TPO level IU/mL		803.76±403.02	607.95±379.12	0.154
Duration of symptoms (months)		5.65±2.71	4.96±1.99	0.314
Total duration of disease (months)		47.40±17.01	52.00±23.10	0.452
Duration for Euthyroidism (months)		8.71±2.94	8.83±3.80	0.923
Total 99 mTechnetium uptake		54.07±26.64	37.28±21.13	0.022*
Gender	M-n (%)	7 (35)	5 (17)	0.140
	F-n (%)	13 (65)	24 (83)	
Smoker n (%)		5 (25)	14 (27.5)	0.543
Goitre n (%)		19 (95)	29 (100)	0.408
Goitre grade	1-n (%)	0	11 (37.9)	0.026*
	2-n (%)	19 (95)	18 (62.1)	
Orbitopathy n (%)		9 (45)	15 (29.4)	0.166

SD: Standard deviation; *- Significant; Anti-TPO: Anti-thyroid Peroxidase; TSH: Thyroid-stimulating hormone; T4:Total Thyroxine; T3: Total Triiodothyronine; M: Male; F: Female.

months (Table 5). Relapse was defined as a reappearance of the signs and symptoms of thyrotoxicosis and the elevation of serum T3 and T4 levels, at least after six months of discontinuation of the ATDs. The relapse rate after the first and second year was

22.5% and 41%, respectively. A comparison between the patients with and without relapse is presented in Table 5. Higher initial ^{99m}Tc uptake (p=0.022) and higher grades of goiter (p=0.026) were significantly associated with the relapse of the disease. Several factors were evaluated in the logistic regression analysis for their potential to predict the relapse of GD, and therefore, the requirement for definitive treatment. Male gender [odds ratio (OR)=0.548, p=0.048] and orbitopathy (OR=0.393, p=0.036) were identified as the independent risk factors predicting the relapse of GD.

Among the patients who failed to achieve remission (n=23), seven patients (mostly young and unmarried patients) were radioablated, while three patients having a large goiter with compressive features underwent surgery. The remaining patients (n=13) preferred undergoing another course of ATDs over the other treatment modalities. The remission rate in both radioablated and surgically-managed patients was 100%. However, Levothyroxine replacement was required in 71.4% (n=5) of the RAI-treated (n=7) and 66.7% (n=2) of the surgically-treated patients, as well as in 17% (n=5) of the initially ATD-treated patients in remission (n=29).

Discussion

Patients with Graves' disease (GD) constitute a major proportion of the patients presenting to the endocrine clinics across the world. GD is a disease with varied clinical presentations, including typical and atypical, and a relatively prolonged course on account of higher rates of recurrences and relapse. Therefore, a proper understanding of the epidemiology and the clinical manifestations of the GD cases, including the atypical ones, is crucial for detecting these disorders at the preclinical stage and preventing the subsequent complications associated with the disease. The association of GD with morbidity and an increased risk of mortality add to the necessity of proper management of this disease and the associated complications. The present retrospective study reports the clinical data, investigative profile, management, and outcome of the GD patients who presented to the endocrine unit of a tertiary-care hospital.

The predominant involvement of women in the present study is in agreement with the findings documented in the literature (4,15,16). This is also consistent with the autoimmune nature of GD, as autoimmune diseases tend to affect females more than males (17). While the females outnumbered the males in the present study, the proportion was less than that reported in the other studies (4,16). This could be the result of the improved iodine status of the population (11), following the universal iodization program and a consequent increase in the tendency for autoimmune disorders (18), which might have ultimately resulted in a lower female to male ratio. Moreover, ethnic variation, regional differences, and/or poor accessibility to medical facilities among females due to various socio-religious factors might also explain the lower female to male ratio.

Although no age group is immune to GD, it most commonly occurs in the third and fourth decade of life. The results of the present study also supported the occurrence of GD in this age group. The mean age (±SD) of presentation of GD in our study cohort was 39.46 (±11.59) years, which is in line with the results reported by a previous study (14). While the youngest age of presentation of GD was 13 years, a 67 year male was also diagnosed with this condition. GD presents with a plethora of characteristic manifestations, resulting from hyperthyroidism, the associated goiter in certain cases, or the underlying autoimmunity. The signs and symptoms observed in our study cohort (Table 2) were consistent with the results documented in the literature (15). Sleep disturbances were present in a significant number of patients in the present study. The exact reason for the same is not known, although factors such as palpitations, heat intolerance, and pruritus could be contributors. The proportion of ophthalmopathy in the present study was 57.4%, which is close to the range of 25-50% reported in the literature (18). One of the highlights of the present study was the presence of pruritus in a significant number of patients (40%), as was reported in a previous study (20), although much higher than the percentage reported in another study (21). Most of our patients responded to antihis-

tamines, although they attained complete relief of their symptoms only upon achieving euthyroidism (21). It is postulated that cell-mediated immunity results in the lowering of the mast cell threshold and the subsequent release of histamine responsible for pruritus in these patients.

One of the important observations of our study was the higher values of ^{99m}Tc uptake in the study cohort notwithstanding the marked improvement in the overall iodine nutrition in the Kashmir Valley (11). However, despite much improvement in the iodization status of the population in the Kashmir region, 49% of the population continued to have urinary iodine excretion below 100 µg/day (11); the fact that under mild to moderate iodine deficiency conditions, the thyroid uptake of ^{99m}Tc-pertechnetate exhibits an inverse correlation with the urinary iodine excretion to compensate for the iodine supply (23) could explain the higher ^{99m}Tc uptake levels observed in our study.

Thionamides are invariably used as first-line medication to control hyperthyroidism and induce remission of the disease, thereby relieving the symptoms. In case of failure of the medical therapy, which is quite common, definitive treatment with surgery or RAI was considered and discussed with the patients. In our cohort, carbimazole was the most common thionamide, used in 98.2% of the patients. Titration regimen is preferred over the block and replace regimen in this part of the world owing to its reduced side effect profile (24,25), independence of the remission rates from the drug type and dosage (26), and non-inferiority to the block and replace regimen. As far as the preferred dosage of carbimazole was concerned no specific criteria was used. However, the dose of carbimazole to be used was influenced by the severity of the disease, the level of T4, and the presence or absence of goiter, orbitopathy, and underlying comorbid conditions.

At the first assessment at three months, most of the patients were controlled (46.1%), with normalization of the TSH levels in 15.6% of them. Although 53.9% of the patients continued to have hyperthyroxinemia, the intensity of its symptoms and the degree of hyperthyroxinemia had improved. Failure to achieve early remission/disease control was significantly higher in male gender (51.7% vs. 48.3%; p=0.003) and smokers (44% vs. 22.5%; p=0.036).

Another important observation in the present study was the development of overt hypothyroidism in 18% of the patients during treatment with anti-thyroid medication, requiring termination/tapering of the dosage, and a few patients requiring thyroxine replacement, as well as its significant association with remission (p=0.023). This could be the result of the progression of autoimmune thyroiditis and/or the development of thyroid-inhibiting immunoglobulins compared to thyroid-stimulating immunoglobulins. Moreover, poor follow-up among patients resulting in failure of drug tapering at the appropriate time could be another reason for the development of hypothyroidism. However, the significant association of drug-induced hypothyroidism with remission favors the possible role of the former in the progression of underlying autoimmune thyroiditis and/or the development of TBII, leading to the remission of the disease in the long run.

The ATDs used for the treatment of GD help to maintain the euthyroid state in the majority of the patients, although they were not curative. Therefore, the possibility of relapse after the discontinuation of ATD remained. In the present study, the remission rate of 68% was observed in the patients who had completed 18-24 months of treatment with ATDs. Those who were uncontrolled (31%) after the initial treatment with ATDs for 24 months, including those who relapsed while the tapering of the drug dose, were considered for definitive treatment modalities, which was consistent with the findings that a treatment duration greater than 12-18 months did not improve the remission rate (27).

The remission rate of 68% after the initial treatment with ATDs in our study was in the range of 61-74% remission rate reported by the other studies (4,26,27). Higher initial TSH (p=0.007), smoking status (0.026), higher ^{99m}Tc uptake (p=0.05), and pharmaceutical hypothyroidism (p=0.023) were significantly associated with remission. The level of ^{99m}Tc uptake at initial presentation

is reported to predict remission in a previous study as well (30). Similarly, smoking status and higher TSH levels are associated with remission (29). The goiter, ophthalmopathy, and dermopathy did not have any association with remission in the present study, similar to the findings of a previous study (30). Among the 49 patients who went into remission, 20 (41%) patients relapsed with a first-year relapse rate of 22.4% (n=11), which is lower than relapse rate values reported in most of the published literature (31,32). However, this observation was consistent with the findings of a study from India, which reported a relapse rate of 19% (30).

Higher ^{99m}Tc uptake (p=0.022) and the presence of goiter (p=0.026) were significantly higher in the relapse group. The association of relapse with higher ^{99m}Tc uptake (30) and the presence of goiter (30) was consistent with the existing literature. Several studies have attempted to identify the factors that might predict the outcome of the disease and assist in selecting the appropriate treatment modality, although with contradictory results. These contradictions are attributed to regional differences, differences in the iodine status of different populations, and different study designs (33). The present study revealed male gender [odds ratio (OR)=0.548, p=0.048] and orbitopathy (OR=0.393, p=0.036) at the onset of disease as the independent factors for predicting the relapse of the disease. Similar results have been documented in the literature, with the studies reporting gender (34,35) and orbitopathy (36) as the independent risk factors known to predict the relapse of the disease.

In this our part of the world, ATDs continue to be the basic treatment option in GD, despite the high frequency of relapse and the potential for adverse effects upon prolonged exposure to ATD. Development of hypothyroidism necessitating lifelong levothyroxine replacement and impaired quality of life limits the use of surgery and RAI as the preferred modalities of management, despite the better outcome of GD reported for them. However, given the unpredictable course of GD and the associated morbidity and increased risk of mortality, discussing the short and long-term risks and efficacy of the different treatment modalities available is crucial for patient satisfaction. Meanwhile, long-term studies with a larger cohort of patients aimed to understand further the natural course of GD and its outcome after the use of different treatment modalities are required to establish the treatment preference for a better prognosis.

Limitations

The limitations of the present study are the same as those of any retrospective cohort study. First and foremost is that selection bias may exist as the outcome had already occurred at the time of data collection. Second, the quality of the available data was not good as the records used were not designed for the present study. This study is further limited by the inability of the participants to undergo thyroid auto-antibody (TRABS) testing due to the absence of such facility at an affordable cost in public or private setup and the absence of ultrasonographic data on thyroid size, thereby preventing us from assessing their role in the prediction of remission and relapse of GD. The other limitation of the present study was a lack of consideration for non-autoimmune diffuse hyperthyroidism and Hashitoxicosis as differentials for GD in the presence of atypical features. A prospective study design would allow a better understanding of the association of the various risk factors with the treatment outcome Graves' disease.

Conclusion

GD is the most common cause of hyperthyroidism and is associated with significant morbidity and an increased risk of mortality. GD predominantly affects females and occurs commonly in the third and fourth decade of life. Varied non-specific clinical manifestations, particularly the atypical presentations, warrant early recognition and subsequent management to minimize the morbidity associated with an undiagnosed condition. ATDs are applied as the primary modality for the management of GD, despite the high frequency of disease relapse reported for them. The other treatment modalities, namely, RAI and surgical management of disease, despite their respective shortcomings, should be discussed with the

patients and offered at an appropriate time to avoid the side effects of ATDs and the anxiety associated with long-term drug intake. Long-term studies assessing the disease outcome after different treatment modalities should be conducted to better understand the course of the disease and establish a treatment preference for a better prognosis.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mohammad Hayat Bhat, Shariq Rashid Maoodi; Design: Mohammad Hayat Bhat, Javaid Ahmad Bhat, Sharig Rashid Maoodi; Control/Supervision: Mohammad Hayat Bhat, Sharig Rashid Maoodi, Waseem Qureshi; Data Collection and/or Processing: Javaid Ahmad Bhat, Junaid Rashid Dar, Moomin Hussain Bhat; Analysis and/or Interpretation: Mohammad Hayat Bhat, Javaid Ahmad Bhat, Sharig Rashid Maoodi, Waseem Qureshi, Junaid Rashid Dar, Moomin Hussain Bhat; Literature Review: Mohammad Hayat Bhat, Javaid Ahmad Bhat, Sharig Rashid Maoodi, Waseem Qureshi, Junaid Rashid Dar, Moomin Hussain Bhat; Writing the Article: Mohammad Hayat Bhat, Javaid Ahmad Bhat, Sharig Rashid Maoodi; Critical Review: Waseem Qureshi, Javaid Ahmad Bhat, Moomin Hussain Bhat, Junaid Rashid Dar; References and Fundings: Mohammad Hayat Bhat, Javaid Ahmad Bhat, Sharig Rashid Maoodi, Waseem Qureshi; Materials:Mohammad Hayat Bhat, Javaid Ahmad Bhat, Waseem Qureshi, Junaid Rashid Dar.

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Original Article
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Evaluation of Oxidative Stress with a New Method in Differentiated Thyroid Cancer Patients on Thyrotrophin Suppression Treatment

Tirotropin Süpresyon Tedavisi Alan Diferansiye Tiroid Kanserli Hastalarda Oksidatif Stresin Yeni Bir Metot ile Değerlendirilmesi

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Abstract

Objective: Thyroid hormone suppression treatment (THST) is an essential modality in treating differentiated thyroid cancer (DTC). This study aimed to evaluate thiol/disulfide homeostasis with a new method in patients on THST, which causes a state of subclinical hyperthyroidism. **Material and Methods:** Serum thyrotrophin (TSH), free triiodothyronine (fT3), free thyroxine (fT4), duration of disease, levothyroxine dose, and radioactive iodine (RAI) dose were evaluated along with native and total thiol and disulfide levels. **Results:** Data of 50 patients with DTC and 41 healthy subjects were analyzed. Though native thiol and total thiol were lower in patients with DTC, the difference was not statistically significant. Disulfide was found to be 18.25 μmol/L in DTC patients and 15.23 μmol/L in the control group. The ratios of native thiol to total thiol (N/T), disulfide to native thiol (D/N), and disulfide to total thiol (D/T) were similar in the 2 groups. Disulfide, D/N, and D/T were significantly higher, and N/T was lower in patients with overt thyrotoxicosis than patients with subclinical thyrotoxicosis and the control group. Disulfide, D/N, and D/T were both positively, and N/T was negatively correlated with fT4/fT3. **Conclusion:** Although the thiol/disulfide balance was maintained in patients with subclinical thyrotoxicosis, there was a shift of redox status toward disulfide in patients with overt thyrotoxicosis on THST. This suggests that the potency of oxidative stress is associated with the degree of thyrotoxicosis. Considering the potentially harmful effects of oxidative stress, overt thyrotoxicosis must be avoided in patients on THST.

Keywords: Differentiated thyroid cancer; thyroid hormone suppression treatment; thiol-disulfide homeostasis; oxidative stress; thyrotoxicosis

Özet

Amaç: Diferansiye tiroid kanserinin (DTK) tedavisinde, tiroid hormon süpresyon tedavisi (THST) önemli bir modalitedir. Bu çalışmada, bir subklinik hipertiroidi durumuna yol açan THST alan hastalarda, thiol/disülfid homeostazını yeni bir metot ile değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** Nativ ve total tiol ve disülfid seviyelerinin yanı sıra serum tirotropin (TSH), serbest triiyodotironin (sT3), serbest tiroksin (sT4), hastalık süresi, levotiroksin dozu ve radyoaktif iyot (RAI) dozu değerlendirildi. **Bulgular:** DTK'li 50 hasta ve 41 sağlıklı birey analiz edildi. Nativ tiol ve total tiol, DTK'li hastalarda daha düşüktü fakat farklılık istatistiksel olarak anlamlı değildi. Disülfid, DTK'li hastalarda 18,25 μmol/L ve kontrol grubunda 15,23 μmol/L'ydi. Nativ tiolün, total tiole (N/T); disülfidin, nativ tiole (D/N) ve disülfidin, total tiole (D/T) oranı her 2 grupta da benzerdi. Aşikâr tirotoksikozlu hastalarda, subklinik tirotoksikoz ve kontrol grubuna göre disülfid, D/N ve D/T anlamlı olarak daha yüksek ve N/T daha düşüktü. sT4/sT3 oranı disülfid, D/N ve D/T ile pozitif ve N/T ile negatif olarak koreleydi. **Sonuç:** THST alan ve subklinik tirotoksikozu olanlarda, her ne kadar tiol/disülfid dengesi korunmuş olsa da aşikâr tirotoksikozu olan hastalarda, redoks durumunda disülfid yönünde kayma vardı. Bu oksidatif stres potensinin, tirotoksikozun derecesiyle ilişkili olduğunu göstermektedir. Oksidatif stresin zararlı etkileri düşünüldüğünde, THST alan hastalarda aşikâr tirotoksikozdan kaçınılmalıdır.

Anahtar kelimeler: Diferansiye tiroid kanseri; tiroid hormon süpresyon tedavisi; tiol disülfid homeostazı; oksidatif stres; tirotoksikoz

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Introduction

Thyroid cancer is the most common endocrine malignancy (1). Total thyroidectomy followed by radioactive iodine (RAI) ablation and thyroid hormone suppression treatment (THST) forms the mainstay of the treatment for differentiated thyroid cancer (DTC) for years, in selected cases (2). It has been found that the suppression of serum thyrotrophin (TSH) reduces the recurrence rate and improves outcomes in some patients with DTC (3). This approach aims to create a state of subclinical thyrotoxicosis without significantly increasing serum-free triiodothyronine (fT3) and free thyroxine (fT4). Long-term suppression of TSH might cause untoward effects mainly related to the cardiovascular system and bone health (4). It is recommended to individualize THST, considering its potential benefits and harms (5).

Multiple intracellular adaptive mechanisms in the human body are activated with an increase in the intracellular reactive oxygen species (ROS). This primarily occurs as a defense mechanism to protect the tissues and prevent apoptosis. One of the agents that work against oxidative cell damage is thiols (6). The plasma thiols are composed of protein thiols, albumin thiols, and in small amounts, low molecular weight thiols such as cysteine, cysteinylglycine, glutathione, homocysteine, and γ-glutamylcysteine (7). Thiol-disulfide homeostasis is a dynamic process. Excessive electrons of the ROS are transferred to the thiols after oxidation, and disulfide bonds are formed. These disulfide bonds can, after that, become thiol groups again (8). They are involved in antioxidant protection, signal transduction, detoxification, and apoptosis. They also contribute to enzymatic activity, transcription factors, and cellular signaling mechanisms (7).

Thyroid hormones have an essential role in regulating mitochondrial respiration and oxidative metabolism, which further affects oxidative stress (OS). Therefore, OS levels might be affected by variations in thyroid hormones (9). Previous studies have shown that high serum fT3 and fT4 stimulate free radicals in mitochondria and cause an increase in OS (10,11). This study aimed to determine whether THST influences OS in DTC patients.

Material and Methods

This study was designed as a cross-sectional, case-control study. DTC patients on THST followed between January 2016 and December 2018 were recruited. Patients with unilateral resection, patients with TSH unresponsive thyroid neoplasms such as medullary and anaplastic thyroid cancer and thyroid lymphoma, and patients with a history of radiotherapy of the head and neck region were excluded from the study. Other exclusion criteria were defined as the presence of chronic diseases such as diabetes mellitus, hypertension, coronary heart disease, renal insufficiency, any chronic inflammatory disease, and smoking. Patients using antioxidant agents were excluded, as well. Thyroid functions were found to be normal in the control group. Ethical review board of Ankara Yıldırım Beyazıt University approved the study protocol. The local ethical committee approved the study based on Helsinki''s Declaration (Date of approval: 14/06/2017; decree no: 2017/132). All patients gave written informed consent.

Demographical features, serum TSH, fT3, fT4, and albumin were recorded. Chemiluminescence methods were used to measure TSH, fT3, and fT4 (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA and UniCel DXI 800, Beckman Coulter, Brea, CA). The normal ranges were as follows- for TSH: 0.4-4 μIU/mL, for fT3: 1.57-4.71 pg/mL, and for fT4: 0.85-1.78 ng/d. Disease duration, levothyroxine dose per week, and RAI treatment and dose were determined in DTC patients. All patients with DTC underwent total thyroidectomy. Patients with a serum TSH ≤0.1 μIU/mL were defined to have suppressed TSH.

Dynamic thiol/disulfide homeostasis was determined in the patient and control group using a new automatic and spectrophotometric method, defined by Erel and Neselioglu. (7). With this method, disulfide bonds are reduced by sodium borohydride ($NaBH_4$), and free functional thiol groups are formed. The unused reductant $NaBH_4$ was removed by formaldehyde. The aim was to inhibit the reduction of 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Reduced and native thiol groups were determined after reaction with DTNB. When native thiol is subtracted from total thiol, and the result is divided by

two, some amount of dynamic disulfide is obtained. The ratios of disulfide/total thiol percent (D/T), disulfide/native thiol percent (D/N), and native thiol/total thiol percent (N/TS) were calculated. This study compares the parameters between patients on THST and the healthy control group. The THST group was then classified as overt and subclinical thyrotoxicosis and a further analysis was made to compare the OS parameters in all three groups. The authors also tried to determine whether there is any correlation between some clinical features and OS measurements.

Statistical Analysis

All statistical analyses were done with IBM SPSS Statistics 22.0. Shapiro-Wilk test was used to find out whether continuous variables were distributed normally. All continuous variables (except the ratio of fT4/fT3 in patient and control groups) were assessed by median (minimum-maximum), while gender was summarized by frequency and percentage. fT4/fT3 was reported as "Mean±Standard Deviation (Mean±SD)". Independent sample t-test, Mann-Whitney U test, and Yates chi-square test were used to compare groups. The patient group was classified as overt and subclinical thyrotoxicosis in terms of the degree of hyperthyroidism. These groups and the control group were compared for the ratio of fT4/fT3 and OS measurements by the Kruskal-Wallis test followed by a stepwise step-down procedure to obtain homogeneous subsets. Spearman's rho correlation analysis was used to investigate the correlations between OS measurements and other clinical features. Statistical significance was accepted when the p value was <0.05.

Results

The data of 50 patients in the THST group and 41 patients in the control group were analyzed. The frequency of male patients was 10.0% in the control group and 29.3% in the patient group (p=0.038) (Table 1). Among DTC patients, 12 (24.0%) had extrathyroidal extension, 7 (14.0%) had lymph node metastasis, and 4 (8.0%) had vascular invasion. Median age, serum albumin, and fT3 levels did not differ in the two groups (p=0.088, p=0.125, and p=0.185,

Table 1. Demographic and clinical characteristics of patients on thyroid hormone suppression treatment and control group.

	Patients on THST (n=50)	Control (n=41)	
	Median (minimum-maximum)	Median (minimum-maximum)	p value
Age (year)	45 (24-66)	42 (18-68)	0.088
Gender* (Male)	5 (10.0)	12 (29.3)	**0.038**
Albumin (g/dL)	4.65 (4.07-5.20)	4.70 (4.20-5.90)	0.125
TSH (µIU/mL)	0.035 (0.001-0.101)	2.100 (0.439-4.000)	**<0.001**
fT4 (ng/dL)	1.69 (1.31-2.88)	1.28 (0.90-1.70)	**<0.001**
fT3 (pg/mL)	3.05 (2.30-4.97)	3.30 (2.20-4.20)	0.185
fT4/fT3** (ng/dL/pg/mL)	0.567±0.080	0.392±0.073	**<0.001**
Duration of treatment (years)	4 (1-18)	-	-
Levothyroxine dose (mcg/week)	875 (525-1,400)	-	-
Radioactive iodine	48 (96%)	-	-
Radioactive iodine dose (mCi)	100 (75-175)	-	-
Native thiol (µmol/L)	435.30 (347.30-554.80)	439.80 (333.20-836.70)	0.604
Disulfide (µmol/L)	18.25 (1.20-33.0)	15.30 (0.45-31.10)	0.096
Total thiol (µmol/L)	465.10 (353.00-601.60)	471.60 (353.60-859.80)	0.820
Disulfide/native thiol	0.042 (0.003-0.080)	0.033 (0.001-0.076)	0.068
Disulfide/total thiol	0.038 (0.003-0.069)	0.031 (0.001-0.066)	0.069
Native thiol/total thiol	0.922 (0.862-0.993)	0.938 (0.868-0.998)	0.067

THST: Thyroid hormone suppression treatment; TSH: Thyrotrophin; fT3: Free triiodothyronine; fT4: Free thyroxine.
*n (%); **Mean±SD.

respectively). Serum TSH was lower, and fT4 and fT4/fT3 were higher in the THST group than in the control group (p<0.001, for each). The median duration of levothyroxine treatment was four years (minimum 1-18) with a median dose of 875 mcg/week (minimum-maximum: 525-1,400 mcg/week). Forty-eight (96%) patients with DTC had received RAI treatment, and the median dose was 100 mCi (minimum-maximum: 75-175 mCi). THST group demonstrated higher disulfide and lower native and total thiol levels than the control group; however, the differences were not statistically significant. D/N, D/T, and N/T were similar in the control and THST groups (p=0.068, p=0.69, and p=0.067, respectively).

Patients on THST were further classified into overt and subclinical thyrotoxicosis groups (Table 2). Compared with each other and the control group, the lowest fT4/fT3 was observed in the control group, and the highest value was observed in the overt thyrotoxicosis group. The three groups differed significantly from each other concerning fT4/fT3 (p<0.001). Patients with overt and subclinical thyrotoxicosis had similar native and total thiol concentration (p=0.590 and p=0.421, respectively). Disulfide, D/N, and D/T were similar in subclinical thyrotoxicosis and control groups. However, all these parameters were significantly higher in patients with overt thyrotoxicosis than in the subclinical thyrotoxicosis and control groups. Patients with overt thyrotoxicosis presented lower N/T levels than the control group.

The correlation analysis revealed that OS parameters were not correlated with RAI dose, duration, and levothyroxine treatment dosage. fT4/fT3 was positively correlated with disulfide, D/N, and D/T, and was negatively correlated with N/T (Table 3).

Discussion

Stimulation of TSH receptors present on the membrane of DTC cells increases thyroid proteins such as thyroglobulin (Tg) and sodium-iodide symporter. This further increases the rate of cell growth. The current guidelines recommend suppressing TSH by levothyroxine treatment in some patients with DTC to prevent recurrence (12). However, this approach is also associated with some side effects, primarily related to the cardiovascular and skeletal system and cognitive and psychological status, quality of life, glucose metabolism, coagulation, and immunological function (13). Thus, the potential benefits and the possible side effects of THST should be interpreted carefully during the management of these patients (5).

Thyroid hormones play a role in regulating mitochondrial respiration and oxidative metabolism, affecting free radical production and OS. Accelerated basal metabolic rate and oxidative metabolism in hyperthyroidism lead to an increased free radical generation and OS (9). The literature re-

Table 2. Comparison of fT4/fT3 and oxidative stress measurements in overt thyrotoxicosis, subclinical thyrotoxicosis, and control groups.

	Overt thyrotoxicosis (n=21) Median (minimum-maximum)	Subclinical thyrotoxicosis (n=29) Median (minimum-maximum)	Control (n=41) Median (minimum-maximum)	p value
fT4/fT3 (ng/dL/pg/mL)	0.596 (0.49-0.78)[a]	0.535 (0.37-0.65)[b]	0.387 (0.22-0.59)[c]	**<0.001**
Native thiol (µmol/L)	445.9 (369.0-530.3)	432.9 (347.3-554.8)	439.8 (333.2-836,7)	0.590
Disulfide (µmol/L)	23.2 (2.55-33.0)[a]	15.1 (1.5-31.05)[b]	15.3 (0.45-31.1)[b]	**0.029**
Total thiol (µmol/L)	491.1 (393.4-353.0)	456.0 (353.0-601.6)	471.6 (353.6-859.8)	0.421
Disulfide/native thiol	0.052 (0.005-0.080)[a]	0.035 (0.003-0.065)[b]	0.033 (0.001-0.076)[b]	**0.029**
Disulfide/total thiol	0.047 (0.005-0.069)[a]	0.033 (0.003-0.058)[b]	0.031 (0.001-0.066)[b]	**0.030**
Native thiol/total thiol	0.906 (0.862-0.989)[a]	0.935 (0.885-0.993)[a,b]	0.938 (0.868-0.998)[b]	**0.031**

fT3: Free triiodothyronine; fT4: Free thyroxine.
Each superscript indicates a subset of the measurement.

Table 3. Correlation between oxidative stress measurements and clinical features.

	RAI Dose (mCI)	Duration of levothyroxine use (years)	Levothyroxine dose (mcg/week)	fT4/fT3
Native thiol (µmol/L)				
rS	0.127	-0.073	0.086	-0.043
p value	0.380	0.613	0.551	0.686
Disulfide (µmol/L)				
rS	0.072	-0.085	-0.026	0.247
p value	0.621	0.556	0.858	**0.018**
Total thiol (µmol/L)				
rS	0.156	-0.030	0.035	0.023
p value	0.279	0.837	0.809	0.831
Disulfide/native thiol				
rS	0.060	-0.082	-0.053	0.286
p value	0.681	0.573	0.713	**0.006**
Disulfide/total thiol				
rS	0.060	-0.082	-0.053	0.285
p value	0.681	0.573	0.713	**0.006**
Native thiol/total thiol				
rS	-0.061	0.080	0.054	-0.285
p value	0.673	0.580	0.707	**0.006**

RAI: Radioactive iodine treatment; fT3: Free triiodothyronine; fT4: Free thyroxine; rS: Spearman's correlation coefficient.

ports controversial results about the effects of thyrotoxicosis on the antioxidant defense system (14). Komosinska-Vassev et al. reported a marked increase in erythrocyte superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activities that are responsible for intracellular antioxidant activity in hyperthyroid patients, as compared to the healthy subjects (15). Another study reported lower erythrocyte SOD and catalase activities in patients with Graves' disease than in the control group (16); though, erythrocyte GPx and total reactive antioxidant potential were similar. Andryskowski and Owczarek showed that sulfhydryl (SH) groups were 15% lesser in hyperthyroid patients than in euthyroid subjects (11).

The SH group in thiol plays a role in maintaining the level of the intracellular OS. Thiol concentration can differ at the cellular level during proliferation or apoptosis. SH groups of sulfur-containing amino acids are the main targets of oxygen radicals. Oxidation of these groups causes the formation of reversible disulfide bonds. The loss of these thiol groups is related to structural and functional changes in cellular proteins. Plasma and tissue levels of thiol groups can decrease during these processes to overcome the destructive effects of free radicals (6). Erel first introduced a method that could measure dynamic thiol/disulfide automatically (7). Only a small fraction of thiols-thiol and disulfide in low molecular weight compounds could be measured until this approach was developed. However, thiols of albumin and other proteins make up the major thiols in the body. The previous methods were insufficient in precisely measuring the body's thiol and disulfide levels (17).

The previous studies evaluating OS in thyrotoxicosis usually included patients with Graves' disease, with high or normalized thyroid hormones. The present study evaluated OS in DTC patients on THST with the help of a relatively new method. Patients on THST had lower native and total thiol and higher disulfide. Still, the differences were not statistically significant; thus, suggesting that the thiol/disulfide balance was maintained in these patients. A subgroup analy-

sis revealed that patients with overt thyrotoxicosis had higher disulfide levels than patients with subclinical thyrotoxicosis and control group; however, native and total thiols were similar. Additionally, D/T and D/N were increased, and N/T was decreased in the patients, demonstrating a shift of the thiol/disulfide status to the part of disulfide bond generation. Disease duration and levothyroxine dose did not affect these parameters. These findings suggest that oxidative balance is disturbed in relation to the severity of thyrotoxicosis in patients on THST. There is an association between the degree of thyrotoxicosis and the development and severity of adverse effects. In the study by Selmer et al., the risk of all-cause mortality was increased by 23% and 25% in subclinical and overt hyperthyroidism, respectively (18). They also found that patients with subclinical and overt hyperthyroidism were at an increased risk of major adverse cardiovascular events (9% and 16%, respectively). In another study, the risk of hip fractures was increased by 22% to 25% in euthyroid postmenopausal women with lower TSH and higher fT4, although within the reference range (19). In one more study involving 5,860 subjects over 65 years, fT4 was higher in patients with atrial fibrillation (20).

It is unclear whether there are differences in the cellular effects of exogenous and endogenous subclinical thyrotoxicosis. fT3 is generally higher in endogenous compared to exogenous thyrotoxicosis. In patients on THST, high serum fT4 rather than fT3 is more commonly encountered. Patients using levothyroxine after total thyroidectomy usually have significantly higher serum fT4 as compared to the presurgical levels. This increase is particularly prominent in patients on THST. It is known that 20% of circulating T3 is usually secreted directly by the thyroid gland. However, since this portion of T3 lacks in patients without a thyroid gland, higher serum T4 levels are required to maintain normal serum T3 levels (21). In the present study, fT3 was similar to the control group, while fT4 was significantly higher in patients on THST. The study also found that fT4/fT3 was the only parameter correlated with OS (positively with disulfide, D/T, and D/N and negatively with N/T).

This study also has some limitations. Designed as a single-center study, it involved a small sample size. Another limitation of the study was the lack of thiol/disulfide evaluation before thyroidectomy. Comparison of OS before surgery in euthyroid status and after surgery on THST would probably lead to more valuable inferences about OS in these patients.

Conclusion

In conclusion, to the best of the author's knowledge, this study was the first to evaluate thiol/disulfide balance in patients on THST. Although the thiol/disulfide balance seems to be preserved in these patients, redox status has shifted toward disulfide in patients with overt thyrotoxicosis. The OS potency in patients with THST appears to be related to the degree of thyrotoxicosis. Disturbed OS can be added to the well-known long-term complications of THST, and these findings can be considered a piece of evidence that fT4 levels should be maintained within normal limits in patients receiving THST.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Concept: Abbas Ali Tam, Bekir Çakır, Özcan Erel; Design: Didem Özdemir, Reyhan Ersoy; Data Collection and Processing: Afra Alkan, Sevgül Fakı, Nagihan Beştepe; Analysis or Interpretetion: Abbas Ali Tam, Didem Özdemir; Literature Search: Abbas Ali Tam, Didem Özdemir; Writing: Abbas Ali Tam, Didem Özdemir, Bekir Çakır, Özcan Erel.

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Patients with Ectopic Posterior Pituitary: Report of Six Cases

Ektopik Posterior Hipofizi Olan Hastalar: Altı Olgunun Sunumu

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Abstract

Objective: Ectopic posterior pituitary (EPP) can occur because of a migration defect or neurodegeneration of the hypothalamic nuclei. EPP is typically rarely diagnosed. Therefore, we aimed to report our patients with EPP. **Material and Methods:** This is a retrospective study (approved by the Ege University Ethical Committee, protocol 20-7T/49) that included 6 patients with EPP who were followed up between 2012 and 2019. We collected information on age, sex, height, weight, body mass index, age at the diagnosis, history of traumatic delivery, consanguinity, multiple hormone deficiency and treatment. We examined laboratory levels and medical records, and, magnetic resonance imaging (MRI) reports. **Results:** The mean age of patients was 25.83 years, and the age at diagnosis was 11.16 years. One patient was female, and the others were male. They were receiving hormone replacement treatment. The patients were diagnosed with EPP during their childhood. All patients, except 2, were taking growth hormone replacement therapy. Only one patient had a history of consanguinity. Additional information about the patients is described in the patient sections. **Conclusion:** Patients with EPP are rarely seen, and this rare condition should be considered when a patient has panhypopituitarism. MRI is the gold standard imaging modality for hypophysis to identify this condition. In addition, patients who have EPP in MRI should be screened for hypopituitarism.

Keywords: Hypopituitarism; combined pituitary hormone deficiency; posterior pituitary gland; ectopic neurohypophysis

Özet

Amaç: Ektopik posterior hipofiz [ectopic posterior pituitary (EPP)] migrasyon defektinden ya da hipotalamik nükleusun nörodejenerasyonundan kaynaklanabilir. EPP, genellikle nadiren teşhis edilir. Bu nedenle EPP'li hastalarımızı paylaşmak istedik. **Gereç ve Yöntemler:** Bu çalışma, (Ege Üniversitesi Etik Komitesi tarafından onaylanan, protokol 20-7T/49) 2012 ile 2019 arasında takip edilen 6 EPP hastasını içeren retrospektif bir çalışmadır. Yaş, cinsiyet, boy, kilo, beden kitle indeksi, tanı yaşı, travmatik doğum öyküsü, akrabalık, çoklu hormon eksikliği ve tedavisi hakkında bilgi topladık. Laboratuvar değerlerini ve tıbbi kayıtları, manyetik rezonans görüntüleme (MRG) raporlarını inceledik. **Bulgular:** Hastalarımızın yaş ortalaması 25,83 ve tanı yaşı ortaması ise 11,16'ydı. Hastalarımızdan 1'i kadın, diğerleri erkekti. Hipofizer yetmezlikleri olması nedeniyle hormon replasman tedavisi alıyorlardı ve çocukluk çağında tanı almışlardı. İki hasta dışında diğerleri büyüme hormonu tedavisi almaktaydı. Sadece 1 hastamızın öyküsünde akraba evliliği vardı. Hastaların yarısında, travmatik doğum öyküsü vardı. Hastalar hakkında ek bilgiler, hasta bölümlerinde anlatılmıştır. **Sonuç:** EPP'li hastalar nadiren görülür ve bir hastada panhipopituitarizm olduğunda bu nadir durumu dikkate almalıyız. MRG, bu durumu tanımlamak için hipofiz için altın standart görüntüleme yöntemidir. Ek olarak, MRG'de EPP'si olan hastalar hipopituitarizm açısından taranmalıdır.

Anahtar kelimeler: Hipopituitarizm; kombine hipofizer hormon eksikliği; pituiter bez, posterior; ektopik nörohipofiz

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Introduction

Ectopic posterior pituitary (EPP) can occur because of a migration defect or neurodegeneration of the hypothalamic nuclei (1). EPP can be identified by magnetic resonance imaging (MRI) and found at the median eminence or along the pituitary stalk, with complete or partial pituitary stalk agenesis and anterior pituitary hypoplasia (2).

Although the etiology is uncertain, traumatic birth, breech delivery and genetic factors may lead to EPP. Antenatal environmental factors such as birth trauma may cause hypopituitarism and EPP (3,4). Genetic factors may result in these developmental abnormalities and EPP (5,6). The formation and differentiation of the pituitary gland are regulated by specific transcription factors such as *Prop-1, Pit-1, HESX1, Pitx1, Pitx2, LHX3,* and *LHX4* (7). Mutations in the *HESX1, LHX4,* and *SOX3* genes may cause EPP (8-10).

The clinical presentation of EPP is variable and may range from isolated growth hormone (GH) deficiency (IGHD) to multiple pituitary hormone deficiency (MPHD) because of the normal posterior pituitary functions (2,11,12). The pathogenesis of hormone deficiency associated with EPP is not well understood. Previous studies have demonstrated the relationship between EPP and the severity of hormone dysfunction (13).

EPP is typically diagnosed in childhood by pediatric endocrinologists, and adult endocrinologists come across these patients rarely. Therefore, we aimed to report our patients who were transferred to our department from the pediatric endocrinology of our university.

Material and Methods

The study was conducted in accordance with the Declaration of Helsinki Principles. Six patients were enrolled who were transferred to our department from the pediatric endocrinology of our university between 2012 and 2019. All patients were included after signing the informed consent. Ethical approval was obtained from Ege University (approved by the ethical committee, protocol 20-7T/49, 08.07.2020).

We collected information on age, sex, height, weight, body mass index (BMI), age at the diagnosis, history of traumatic delivery, consanguinity, multiple hormone deficiency and treatment. We examined laboratory levels, and medical records, and, MRI reports. MRIs were performed on 1.5 Tesla (Siemens Amira, Erlangen, Germany) or 3 Tesla (Siemens Verio, Erlangen, Germany) scanners. Fast spin-echo, pre-contrast and dynamic post-contrast multiplanar T1 and T2-weighted sequences were obtained from all the patients.

Results

Table 1 demonstrated the baseline characteristics of the patients. Table 2 shows the laboratory levels of the patients at the last appointment. Additional information about the patients is described in the patient sections.

Patient 1

A 19 year-old male patient was diagnosed at the age of nine years. He had a history of breech presentation. Pituitary MRI revealed a hypoplastic pituitary gland, complete pituitary stalk agenesis and ectopic posterior pituitary (Figure 1). He was receiving hormone replacement treatment when he started to follow up at our department, and we continued his treatment.

Patient 2

She had applied to the pediatric endocrinology department because of her short stature when she was ten years old. MRI revealed ectopic posterior pituitary. She initiated hormone replacement treatment because of multiple hormone deficiency. In addition, in the follow up period, she was first initiated with estrogen treatment to provide thelarche, and after one year, she continued with combined estrogen and progesterone. We continued her replacement treatment.

Patient 3

He was diagnosed with EPP when he was ten years old**.** Pituitary MRI demonstrated pituitary stalk agenesis, ectopic neurohypophysis, corpus callosum dysgenesis and neurohypophysis located in the hypothalamic region (Figure 2). He had multiple hormone deficiency and was taking hormone replacement treatment. Although he had received GH treatment for eight years, he de-

Table 1. Baseline characteristics of the patients with ectopic posterior pituitary.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (y)	19	35	19	32	25	25
Sex (F/M)	M	F	M	M	M	M
Height (cm)	174	165	172	168	170	176
Weight (kg)	62	54	60	115	63	65
BMI (kg/m^2)	20.48	19.83	20.28	40.75	21.8	20.98
Age of the diagnosis (y)	9	10	10	19	9	10
History of traumatic delivery	Yes	No	No	Yes	Yes	No
Consanguity	Yes	No	No	No	No	No
Multiple Hormone deficiency	Yes	Yes	Yes	Yes	Yes	Yes
Treatment	GH, T4, hydrocortisone, testesterone	GH, T4, hydrocortisone Estrogen+ progesterone	T4, hydrocortisoneestosterone	GH, T4, hydrocortisone testosterone, desmopressin	GH, T4, hydrocortisonetestosterone	T4, hydrocortisone testosterone

BMI: Body mass index; GH: Growth hormone.

veloped epileptic seizures in the follow-up period. Therefore, the neurology department suggested stopping GH treatment. After stopping GH, he never developed epileptic seizures and his insulin-like growth factor-1 (IGF-1) level was normal. Furthermore, we did not initiate GH but continued the other hormone replacement treatments.

Patient 4

He was diagnosed with tuberculosis meningitis when he was three years old. He developed hydrocephalus and had ventriculoperitoneal shunt insertion. When he was six years old, he had diabetes insipidus and initiated desmopressin treatment. At the age of 19 years, a pituitary MRI revealed hypoplastic pituitary gland and, EPP. He was started with hormone replacement treatment because of multiple hormone deficiency. In the follow-up period, we continued his treatment.

Patient 5

He presented with short stature when he was nine years old. Cranial MRI showed ectopic neurohypophysis and incision of the corpus callosum. We continued hormone replacement treatment in the follow-up period.

Patient 6

At the age of ten years, he was diagnosed with EPP. Cranial MRI revealed ectopic neurohypophysis, located in the hypothalamic region. He did not want to continue GH replacement treatment, thus he only took other hormone replacement treatments. In the follow-up period, although he did not take GH, he had no hypoglycemia and central adiposity.

Discussion

A summary of the patients with EPP is provided. The EPP can occur because of defective embryogenesis during neuronal migration. EPP may be isolated or be associated with stalk anomalies. The pituitary stalk interruption syndrome consists of stalk hypoplasia, absence or interruption of the stalk, hypoplastic anterior pituitary, and EPP (14,15). Antenatal factors such as breech deliveries, neonatal hypoxia, and hypoglycemia play significant roles in the

Table 2. Laboratory levels of the patients at the last appointment.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
ALT (U/L)	10	16	13	23	14	6
FPG(mg/dL)	72	83	71	80	84	65
GH (µg/L)	0.109	0.265	0.311	0.224	0.730	0.068
IGF-1(µg/L)	74.1	160	204	115	82.5	68.5
TSH (mU/L)	0.01	0.01	0.93	0.42	0.53	0.27
FT4 (ng/dL)	1.27	1.11	0.77	1.64	1.22	1.08
Cortisol (µg/dL)	1.31	3.38	1.63	1.65	1.31	2.99
T. testosterone (ng/dL)	631		400	580	316	800
Estradiol (ng/L)		38.34				

ALT: Alanine aminotransferase; FPG: Fasting plasma glucose; GH: Growth hormone (normal range: <3); IGF-1: Insulin-like growth factor 1 (normal range: 117-323); TSH: Thyroid stimulating hormone (normal range: 0.27-4.2); FT4: Free thyroxine (normal range: 0.89-1.76); T. testosterone: Total testosterone (normal range: 280-800).

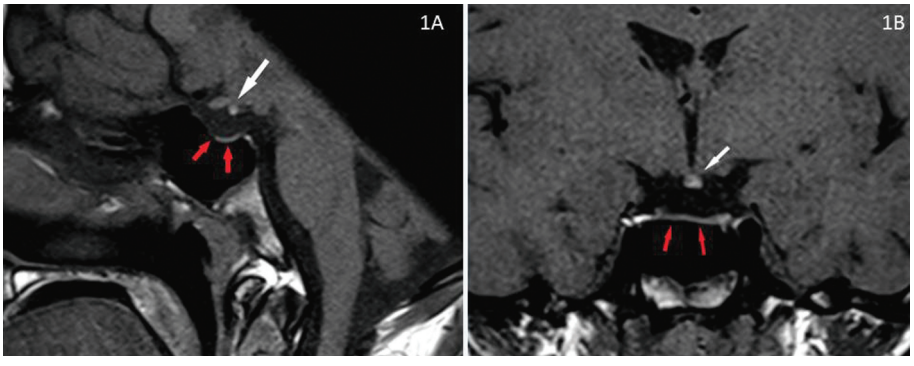

Figure 1: The non-contrast T1-weighted sagittal **(A)** and coronal **(B)** magnetic resonance (MR) images demonstrate ectopic neurohypophysis as a bright spot (white arrow) that is located adjacent to the optic chiasm and the hypothalamus. In addition, shallow sellae turcica and thin/hypoplastic adenohypophysis (red arrows) are seen on the same MR images.

development of EPP and stalk anomalies (15). In this study, three out of six patients had a history of a traumatic delivery. Genetic factors may cause EPP, and several genes are reported to be involved in the EPP development such *as PROP1, IFT172, LHX4, HESX1, OTX2* and *SOX3* (5,10,16,17). Phenotypes of these mutations are variable and affect patients in different ways. Some patients had only pituitary hormone deficiencies in their adulthood (18).

All our patients could reach their estimated height when they became adults. In their follow-up, they did not have any osteoporotic fracture. Although Pubarche was normal, male patients did not have normal testes volumes. In additon, they had received gonadotropins in their chidhood but still did not have normal testes volumes and had azoospermia. All our patients were not mentally retarded.

Although some patients with an EPP may have a normal pituitary function, EPP typically presents with IGHD or multiple anterior MPHD. It depends on the severity of the structural abnormality (19,20). Murray et al. (1) demonstrated that small EPP surface area was predictive of MPHD development. In addition, hypothalamic sited EPP was predictive of MPHD (1,21). The absence of the stalk was also reported as a risk factor (2,22). All of our patients had MPHD and were receiving hormone replacement treatments. Although receiving GH treatment in adulthood is controversial, the 2019 guideline suggests it in patients with genetic defects affecting the hypothalamic-pituitary

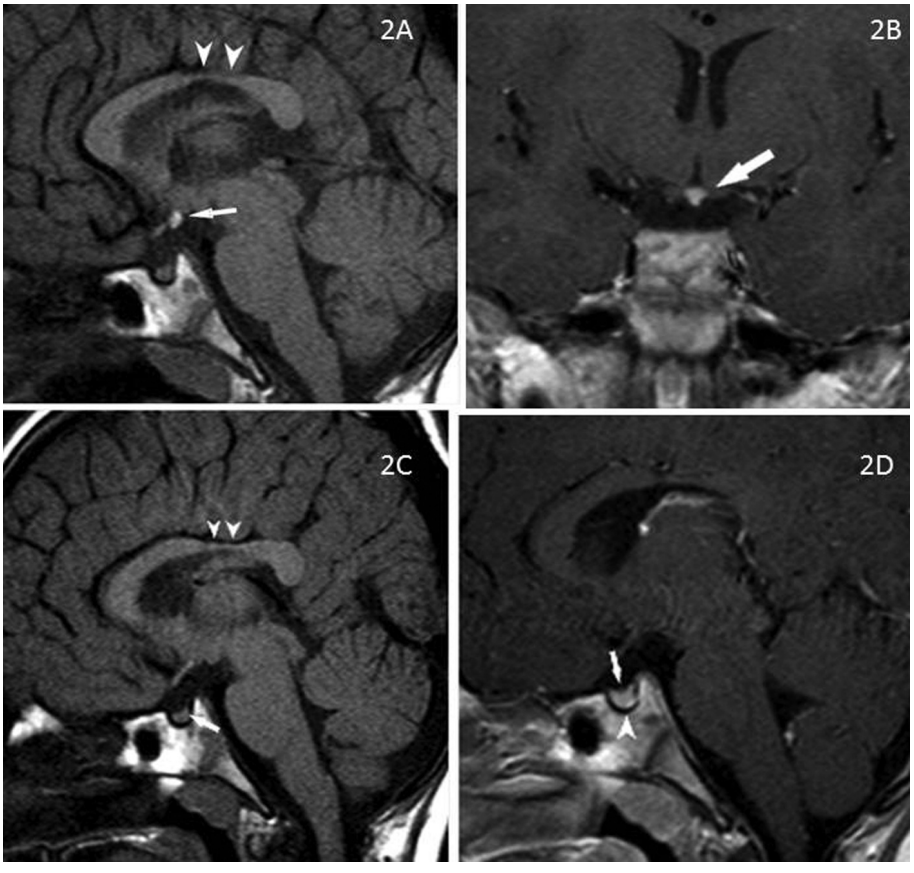

Figure 2: A, B) Turbo spin-echo T1-weighted images show hyperintense dot (arrow) in the hypothalamic region that is consistent with ectopic neurohypophysis. There is also thinning of the corpus callosum (arrow heads). **C)** The non-contrast T1 weighted sagittal image, which is next slice to Fig2A, well depicts the corpus callosum dysgenesis (arrow heads) and lack of normal posterior pituitary bright spot (arrow). **D)** The size of the sellae turcica and adenohypophysis (arrow) is normal.

axes, and hypothalamic-pituitary structural brain defects without performing GH stimulation tests in adults.(23) On the contrary, Leger et al.(21) demonstrated that 22 % of patients with EPP and childhood -onset GH deficiency presented normal GH secretion after GH withdrawal.

Although the roles of GH in the brain, including cognitive functions, neural development and neuroprotection were reported, Kato et al. demonstrated that GH enhances epilepsy progression by increasing and signaling the hormone itself in neural circuits. (24-26) In our third patient, we stopped GH because of epileptic seizures. In the follow-up period, he never developed epileptic seizures and his IGF-1 level was normal despite stopping GH treatment.

Our study was limited because of the small number of patients. Furthermore, no pathology proof was found, and genetic testing was not available for any of our patients.

In conclusion, patients with EPP are rarely seen and this rare condition should be considered when a patient has panhypopituitarism. MRI is the gold standard imaging modality for hypophysis to identify this condition. In addition, patients who have EPP in MRI should be screened for hypopituitarism.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Hatice Özışık, Banu Sarer Yürekli; Design: Hatice Özışık, Banu Sarer Yürekli; Control/Supervision: Füsun Saygılı; Data Collection and/or Processing: Hatice Özışık, Banu Sarer Yürekli, Ömer Kitiş, Mehmet Erdoğan; Analysis and/or Interpretation: Hatice Özışık; Literature Review: Hatice Özışık, Banu Sarer Yürekli; Writing the Article: Hatice Özışık; Critical Review: Mehmet Erdoğan, Füsun Saygılı; Materials: Hatice Özışık.

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Original Article
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Investigation of Survivin Promoter -31 G/C Polymorphism and Survivin Levels in Acromegaly

Akromegalide Survivin Promoterinin -31 G/C Polimorfizmi ve Survivin Düzeylerinin Araştırılması

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Abstract

Objevtive: Acromegaly is a rare disease characterized by growth hormone hypersecretion generally arising from pituitary adenomas. Survivin, an apoptosis inhibitor protein, plays an important role in cell cycle regulation and possibly involves hypophysis gland proliferation mechanisms. However, the underlying causes of somatotroph adenomas with different behaviors and useful prognostic markers are still not fully understood. We investigated possible associations between survivin gene promoter -31 G\C genotypes and serum survivin level and clinical prognostic factors in acromegaly. **Material and Methods:** Sixty-eight acromegaly patients and 171 age-sex matched control subjects were enrolled in the study. Survivin -31 G\C polymorphism was performed by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Blood GH and IGF1 levels were assayed using a chemiluminescence immunometric assay. Serum survivin levels were determined by ELISA. **Results:** Acromegaly patients had significantly higher serum survivin levels than controls (p=0.001). We found no significant association between acromegaly patients and controls in terms of survivin gene promoter -31 G\C genotype distribution and allele frequencies. No correlation was found between disease characteristics and survivin gene polymorphisms. **Conclusion:** Our study suggests that serum survivin levels might be associated with acromegaly, but survivin -31 G\C polymorphisms do not modify individual susceptibility to acromegaly in the Turkish population.

Keywords: Acromegaly; polymorphism; survivin

Özet

Amaç: Akromegali, genellikle hipofiz adenomlarından kaynaklanan büyüme hormonu hipersekresyonu ile karakterize nadir görülen bir hastalıktır. Apoptozun bir inhibitör proteini olan survivin, hücre döngüsü düzenlemesinde önemli bir rol oynar ve hipofiz bezi proliferasyon mekanizmalarında yer alabilir. Farklı davranışlara sahip somatotrof adenomlarının gelişme mekanizmaları tam olarak anlaşılamamış ve kullanışlı prognostik faktörler saptanamamıştır. Bu çalışmada amacımız, survivin gen promotörü -31 G\C genotipleri ve ayrıca serum survivin düzeyi ile akromegalide klinik prognostik faktörler arasındaki olası ilişkiyi araştırmaktı. **Gereç ve Yöntemler:** Çalışmaya 68 akromegali hastası ve 171 yaş-cinsiyet uyumlu kontrol hastası dâhil edildi. Survivin -31 G\C polimorfizmi, bir polimeraz zincir reaksiyonu sınırlama fragmanı uzunluk polimorfizmi [polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)] kullanılarak gerçekleştirildi. Kan GH ve IGF1 seviyeleri, bir kemiluminesans immünometrik test kullanılarak analiz edildi. Serum survivin düzeyleri ELISA ile belirlenmiştir. **Bulgular:** Akromegali hastalarında serum survivin düzeyleri kontrol grubuna göre anlamlı derecede yüksekti (p=0,001). Akromegali hastaları ile survivin gen promotörü -31 G\C genotipi ve allel frekanslarının dağılımı için kontroller arasında anlamlı bir ilişki bulunamadı. Hastalık özellikleri ile survivin gen polimorfizmleri arasında korelasyon bulunmadı. **Sonuç:** Çalışmamız, serum survivin düzeylerinin akromegali ile ilişkili olabileceğini, ancak survivin -31 G\C polimorfizmlerinin Türklerden oluşan bir popülasyonda akromegali açısından bireysel duyarlılığı değiştirmediğini göstermiştir.

Anahtar kelimeler: Akromegali; polimorfizm; survivin

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Introduction

Acromegaly is a rare disease with a prevalence of 40-70 cases per million population and an annual incidence of 3-4 new cases per million population (1). Hypersecretion of growth hormone (GH), which is usually caused by pituitary adenoma, leads to progressive disfigurement mainly involving the face and extremities, especially in the soft tissue. Except for familial pituitary syndromes, limited studies have observed the association between genetic structure and sporadic pituitary tumors secreting GH. Sporadic tumors secreting GH generally arise from a somatotrope cell allowing clonal expansion (2,3). About one-third of somatotroph tumors have somatic mutations in the guanine nucleotide-binding α-subunit 1 gene, leading to cellular proliferation and GH hyperfunction (4,5). Several studies have also determined single nucleotide polymorphisms such as exon-3 deleted GH receptor and aryl hydrocarbon receptor-interacting protein gene and attributed to long term complications of acromegaly (6-8). Although familial pituitary syndromes such as multiple endocrine neoplasia type 1 have been widely studied, the genetic basis of the sporadic somatotrophs (9).

Survivin is regarded as an apoptosis inhibitor protein, which plays an important role in cell cycle regulation (10,11). Survivin causes negative regulation of programmed cell death by acting as an inhibitor of caspase activation (12). The overexpression of survivin is associated with several malignancies and poor prognosis in colorectal cancer, lung cancer, pancreatic cancer, and hepatocellular carcinoma (13-15), and can play a crucial role in tumorigenesis. Although survivin was recently shown to be expressed in normal pituitary tissue and overexpressed in pituitary adenomas, its role in the development and course of acromegaly is yet unexplored (16,17).

This study investigated whether survivin polymorphism and serum survivin levels have a role in the development of acromegaly in the Turkish population. In addition, correlations between survivin gene polymorphisms, serum survivin levels, and tumor size and their influence on the remission of acromegaly were studied.

Material and Methods

Patients and Hormone Assays

Sixty-eight acromegaly patients and 171 control subjects were recruited for this study in the Endocrinology Clinic of Bezmiâlem University Hospital between 2012 and 2015. The mean age of the acromegaly group was 45±1.73 years, and that of control was 42.92±2.11 years. The mean age at diagnosis was 39.88±1.7 years.

Acromegaly was diagnosed using serum GH, which could not be suppressed to <1 ng/mL during oral glucose tolerance test and age- and gender-matched high serum IGF1 levels (18). Hypophysis magnetic resonance imaging was performed on all acromegalic patients, and the maximum diameter obtained from the data was determined as the tumor size. Nine patients were diagnosed in other centers, and tumor size before treatment of these patients was lacking. Patients with acromegaly, controlled under disease-specific treatment (lanreotide or octreotide), and those with post-operative cure were included in the controlled patient group. Serum IGF1 values were adjusted according to the percentage of the upper limit of normal (ULN) using the formula ($100*C_{IGF1}/ULN_{IGF1}$). Acromegaly patients are considered to be in biochemical remission when basal GH is under 1 ng/mL and IGF1 level 1.2 x ULN.

Healthy volunteers between 18-65 years of age and without a history of chronic disease or medication use were included in the age- and gender-matched control group.

Blood GH and GF1 levels were assayed using chemiluminescence immunometric assay (Siemens Advia-Centaur USA). Age-related reference ranges for IGF1 were: 18-20 y: 197-956; 20-23y: 215-628; 23-25y: 169-591; 25-30y: 119-476; 30-40y: 100-494; 40-50y: 101-303; >50y: 78-258 (ng/mL).

Bezmialem Vakıf University Clinical Research Ethics Committee approved this study with the number of 71306642/050-01-04/64 and dated 11.3.2013, and all procedures were conducted in accordance with the Helsinki Declaration.

DNA isolation

Blood samples were collected in tubes containing EDTA. A standard salting procedure was used to isolate genomic DNA (19).

Genotyping

Genotyping studies were performed by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The -31G\C polymorphisms in the promoter region of the survivin gene were examined using 0.25 µM of each primer shown in Table 1 for the reaction in a volume of 25 µL containing, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl, pH 8.4, 0.16 mM of each deoxyribonucleotide triphosphate (MBI Fermentas), and 1 U Taq polymerase (MBI Fermentas). Amplification was carried out using the following protocol: initial denaturation at 94 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s, and a cycle final extension at 72 °C for 10 min (19). The PCR product exhibited a 329-bp fragment indicating the -31G/C region polymorphism. PCR product (10 µL) was digested with *Bcn*I (MBI Fermentas). Samples were repeated if a conflict occurred. The expected results after the restriction digestion for each gene fragment are given in Table 1.

Survivin Assay

Fresh-blood samples were immediately centrifuged at 3000 rpm for 5 min to separate serum, and samples were kept frozen at -20 °C until the study. Serum survivin levels were determined with a commercially available sandwich ELISA kit (Platinum ELISA, Bender MedSystems GmbH, Vienna).

Statistical Analysis

Statistical analyses were performed using SPSS version 11.0 for Windows (SPSS Inc. Chicago, IL, USA). Differences in the frequency of the survivin -31 G/C polymorphism between acromegaly patients versus the control group and clinical data within the acromegaly subgroup were analyzed using the Chi-square test. The Hardy-Weinberg equilibrium was tested for all polymorphisms. Numeric values were evaluated by the Student's t-test and Mann-Whitney U test. The relative associations between acromegaly patients and controls were assessed by calculating odds ratios (ORs) and 95% confidence intervals (95% CIs). The threshold for significance was $p<0.05$.

Results

Table 2 shows the demographic characteristics of acromegaly patients and the control group. In the acromegaly group, mean GH, IGF1 levels, and IGF1 norm were 16.0±2.3 ng/mL, 842±48.9 ng/mL, and 321.1±17.3% before treatment, respectively. Forty-six (78%) patients had macroadenoma at the time of diagnosis. After treatment, mean GH, IGF1 levels, and IGF1 norm decreased to 1.3±0.2 ng/mL, 247±19.8 ng/mL, and 104.2±9.2%, respectively. Mean tumor size after treatment was <1 cm in 41 (80.4%) acromegaly patients, and 43 (78.2%) patients were in biochemical remission. The mean survivin level was slightly higher at 34.5±0.6 in acromegaly patients and 32.4±1.3 in control subjects ($p=0.001$).

Genotypes and allele frequencies for survivin -31G\C polymorphism in acromegaly patients and controls were listed in <Table 3. Genotype distributions for survivin -31 G/C polymorphism in control and patient

Table 1. PCR-RFLP Producers and Products of Survivin-31 G\C Polymorphism.

	Primers	PCR product	Restriction Enzyme	Restriction Product
-31 G\C (rs9904341)	F:5′ -CGTTCTTTGAAAGCAGTCGAG-3′ R:5′ -TGTAGAGATGCGGTGGTCCT-3′	329 bp	Bcnl	CC: 329 bp CG: 329/234/92 bp GG:234/92 bp

bp: Base pair; F: Forward; R: Reverse.

Table 2. Demographic characteristics of acromegaly patients and control group.

	Acromegaly	Control
Gender (n/%)		
Female	40 (%59)	110 (%64.3)
Male	28 (%41)	61 (%35.7)
Age (y)	45.0±1.7	42.9 ±2.1
Age Onset (y)	39.8±1.7	
Tumor Size Before Treatment	n=59	
Macroadenoma	46 (%78)	
Microadenoma	13 (%22)	
Number of Resection		
0	16 (%23.5)	
1	42 (%61.8)	
≥2	10 (%14.7)	
IGF1 (ng/mL)		
IGF1 Before Treatment	842.9±48.9	
IGF1 After Treatment	247.7±19.8	
IGF1 norm		
%ULN Before Treatment	321.1±17.3	
%ULN After Treatment	104.2±9.2	
GH		
GH Before Treatment	16.0±2.3	
GH After Treatment	1.3±0.2	
Remission Status	n=55	
Controlled	43 (%78.2)	
Uncontrolled	12 (%21.8)	
Tumor Size After Treatment	n=51	
≥1 cm (n/%)	10 (%19.6)	
<1 cm (n/%)	41 (%80.4)	
Survivin Level (pg/mL)*	34.5±0.6	32.4 ±1.3

Mean values±standard error; *p=0.001 (Mann-Whitney U test); IGF1: Insulin-like growth factor 1; GH: Growth hormone; ULN: Upper Limit of Normal Range.

groups were in agreement with the Hardy Weinberg equilibrium (p=0.197; p=0.335, respectively). Survivin -31 G\C genotypes and allele frequencies between acromegaly patients and controls were not statistically significant (p=0.73; p=0.46, respectively).

Table 4 shows the comparison of the characteristics of acromegaly patients according to survivin genotypes. No significant difference in genotype distribution was observed between patients with microadenomas (<1 cm) and macroadenomas (≥1 cm) (p=0.32). All 26 patients who had 3-fold higher IGF1 levels than ULN before treatment were carrying the G allele, but the difference was not statistically significant (p=0.068) (Data not shown). All ten patients operated at least two times, and 91.7% of the patients with uncontrolled disease activity were carrying the G allele (p>0.05). No association was found be-

Table 3. Genotypes and allele frequencies for survivin genotypes in acromegaly patients.

Genotype	Patients n (%)	Controls n (%)
GG	28 (41.2%)	63 (36.8%)
CG	34 (50%)	88 (51.5%)
CC	6 (8.8%)	20 (11.7%)
p value >0.05		
Alleles		
G	90 (66.2%)	214 (62.6%)
C	46 (33.8%)	128 (37.4%)
p value >0.05		

Table 4. Characteristics of acromegaly patients according to survivin genotype.

	GG	CG	CC
Gender (n)			
Female	15	19	6
Male	13	15	0
Age onset (y)	37.5±12.1	43±16.1	33.5±9.9
GH (ng/mL)			
Before Treatment	19.6±15.6	13.5±15.4	10.6
After Treatment	1.5±1.5	1.3±1.4	0.9±0.5
IGF1 (ng/mL)			
Before Treatment	831.3±295.5	858.7±373.4	780.9±229.9
After Treatment	225.3±107.9	279.0±183.2	207.0±105.1
IGF1 norm			
%ULN Before Treatment	305.3±119.4	340.9±119.5	257.5±23.7
%ULN After Treatment	95.0±40.2	116.2±87.8	89.1±54.3
Tumor Size Before Treatment (cm)			
<1	4	7	2
≥1	20	24	2
Number of Adenoma Resections (n)			
0	3	6	0
≤1	16	14	6
≥2	5	5	0
Tumor Size After Treatment (cm)			
≤1	18	18	5
≥1	5	5	0
Remission			
+	20	18	5
-	4	7	1
Survivin Level (pg/mL)	35.0±4.2	34.0±4.8	34.3±5.4

Mean values±standard error; *p=0.001 (Mann-Whitney U test); IGF1: Insulin-like growth factor 1; GH: Growth hormone; ULN: Upper Limit of Normal Range.

tween the distribution of survivin genotypes and pre-post treatment GH, IGF1 levels, and remission status. Additionally, there was no significant relationship between treatment options and survivin polymorphism and levels.

Discussion

Survivin has attracted much interest in cancer research studies due to its essential role in tumorigenesis initiation and progression. It is an apoptotic inhibitor protein, which regulates caspases and programmed cell death (20). The survivin gene is located on chromosome 17q25, and the most widely studied polymorphism of the survivin gene is the G to C substitution at position -31 (survivin -31G>C, rs9904341) (21). To our knowledge, this is the first study to investigate the distribution of survivin -31 G/C polymorphism and survivin levels in acromegaly.

In the present study, no difference was observed in the distribution of survivin -31G/C genotypes between acromegaly and control subjects. We found similar results for the distribution of -31G/C genotypes to those reported by Bayram et al. in the Turkish population (22). Our results were consistent with previous findings wherein there was no association between the -31G/C polymorphism and development risk for several cancer types such as renal cell and esophageal carcinoma (23,24). In patients with overexpressed survivin mRNA, no significant difference might be found in the distribution of -31G/C genotypes (25). On the other hand, survivin -31 G/C polymorphism was associ-

ated with an increased risk of developing many tumors (26-28). This situation may be explained in terms of differences in the study population, environmental, and ethnic factors (22).

To date, several chromosomal lesions have been reported in sporadic somatotroph adenomas. Loss of heterogeneity in chromosomes 11q13, 13, and 9 have been associated with 20% of sporadic acromegaly cases (29,30). Additionally, Gsp, Ras, and pituitary tumor transforming gene (PTTG) mutations were shown to be responsible for the development and possible behaviors of pituitary tumors in a subset of cases (31-33). However, genetic mechanisms involving disease development and course are still not known in most acromegaly cases. Besides, the survivin transcription level is possibly modified positively by the presence of survivin genotype C allele (26). Its prognostic value in human neoplasms has not been clarified yet. In a study from Turkey, Ademoglu et al. observed higher serum survivin levels in the acromegaly patients than in the control group, but the difference was not statistically significant (17). Likewise, we found that serum survivin levels were higher in acromegaly patients than in control subjects. Survivin expression could play an essential role in regulating hypophysis gland proliferation (34) and delay cell death (21). Jankowska et al. demonstrated that survivin expression was six-fold higher in tumor tissue than in normal pituitary (16). Overexpression of survivin has also been demonstrated in solid tumors and is related to higher proliferative markers and poor prognosis (35,36).

Our study showed that survivin -31 G/C polymorphism and survivin levels were not associated with clinical characteristics of acromegaly such as tumor size, hormone levels, and acromegaly disease phase.

The study's limitation was that it was a cross-sectional and hospital-based case-control study. Therefore, patients were included at a single center and may not represent acromegaly patients in the general population. However, it must be noted that acromegaly is a rare disease, and our center is a reference hospital that admits patients from all over the country.

In summary, it can be suggested that survivin -31G\C polymorphisms do not modify individual susceptibility to the acromegaly. In this study, no association was observed between disease characteristics (such as tumor size, hormone levels, and remission status) and survivin polymorphism in surviving gene, although increased survivin levels were observed in acromegaly patients compared to that in healthy subjects. However, the molecular effects of these different single nucleotide polymorphisms on the functional mechanism of surviving in tumorigenesis have not yet been clarified, and the involved mechanisms need further studies.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Muzaffer İlhan, İlhan Yaylım, Ertuğrul Taşan; Design: Seda Turgut, Muzaffer İlhan, Gurbet Korkmaz; Control/Supervision: Özcan Karaman, Ertuğrul Taşan, İlhan Yaylım; Data Collection and/or Processing: Seda Turgut, Gurbet Korkmaz, Nazlı Ezgi Özkan, Muzaffer İlhan, Saime Turan; Analysis and/or Interpretation: Nazlı Ezgi Özkan, İlhan Yaylım, Muzaffer İlhan; Literature Review: Seda Turgut, Saime Turan; Writing the Article: Muzaffer İlhan, Seda Turgut, İlhan Yaylım; Critical Review: Özcan Karaman, Muzaffer İlhan, Gurbet Korkmaz, Seda Turgut; References and Fundings: Ertuğrul Taşan; İlhan Yaylım; Materials: Muzaffer İlhan, İlhan Yaylım, Ertuğrul Taşan.

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Original Article
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Pheochromocytoma: 16 Years of Experience in a Single Center

Feokromasitoma: Tek Merkezde 16 Yıllık Deneyim

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Abstract

Objective: Reviewing the 16-year experience of pheochromocytoma in a tertiary referral center. **Material and Methods:** The demographics data and the results of clinical, biochemical, and radiological evaluations of 67 patients who received a diagnosis of pheochromocytoma between the years 2004 and 2020 were obtained retrospectively. **Results:** The mean age (±SD) of the patients at the time of diagnosis was 46 years (±16.1) with a slight female predominance. The percentage of patients diagnosed due to complaints was 50.8%, while 31.2% were diagnosed during the adrenal incidentaloma screening, and 18% were diagnosed during screening for hereditary conditions. Pre-existing hypertension was detected in 56.7% of the patients, while 11.9% of the patients were diagnosed to have hypertension at the time of diagnosis. Paroxysmal pattern was observed in 53.7% of the patients and was accompanied by the classical triad of palpitation (32.8%), headache (20.9%), and sweating (14.9%) as the leading symptoms. Median tumor size was 40 mm (range: 9-90 mm) and the lesion size correlated significantly ($p<0.001$) with the urinary catecholamine metabolite levels. The overall rate of hemodynamic instability in both perioperative and postoperative periods was 6%. Hereditary syndromes, including multiple endocrine neoplasia type 2A (MEN 2A), MEN 2B, von Hippel-Lindau (VHL), and neurofibromatosis type 1 (NF1), were diagnosed in 24% of these patients. Hereditary pheochromocytomas were diagnosed at younger ages, and bilateral lesions were more prevalent in hereditary pheochromocytomas ($p=0.003$ and $p<0.001$, respectively). In addition, patients with hereditary pheochromocytomas were more asymptomatic rather than sporadic ($p=0.016$). Metastasis was detected in 3% of these patients. **Conclusion:** Pheochromocytoma is a rare, life-threatening condition, and therefore, it is important to suspect and test for pheochromocytoma in patients with clinical suspicion. In addition, hereditary syndromes associated with pheochromocytomas should be considered while evaluating patients with pheochromocytoma. A life-long annual follow-up is recommended for the detection of recurrent or metastatic disease, and its evaluation, treatment, and follow-up should involve a multidisciplinary approach in experienced centers.

Keywords: Hereditary pheochromocytoma; sporadic pheochromocytoma; paroxysmal hypertension; multiple endocrine neoplasia type 2; von Hippel-Lindau disease

Özet

Amaç: Üçüncü basamak bir üniversite hastanesinde, 16 yıllık feokromasitoma deneyiminin gözden geçirilmesi. **Gereç ve Yöntemler:** Bu araştırma, 2004-2020 yılları arasında feokromasitoma tanısı almış 67 hastanın değerlendirildiği retrospektif bir çalışma olup, hastaların demografik özellikleri ve klinik, biyokimyasal ve radyolojik değerlendirmelerine ilişkin sonuçlar araştırılmıştır. **Bulgular:** Araştırmada, feokromositoma tanısı konulan hastaların yaş ortalaması 46 (±16,1), kadın/erkek oranı 1,2, feokromositoma ile ilişkili yakınmalarla başvurup, bu tanının konulduğu hasta oranı ise %50,8'dir. Hastalara konulan tanıların %31,2'si rastlantısal adrenal kitle araştırılması sırasında, %18'i ise genetik geçişli sendromlar araştırılırken gerçekleştirilmiştir. Hastaların %56,7'sinde tanıdan önce de hipertansiyonun bilindiği, %11.9'unda ise hipertansiyonun tanı sırasında belirlendiği görülmüştür. Hastaların %53.7'sinde tansiyon yüksekliğinin ataklar halinde ortaya çıktığı saptanmıştır. Hastalarda ataklara en sık eşlik eden belirtilerin çarpıntı (%32,8), baş ağrısı (%20,9) ve terleme (%14,9) olduğu dikkati çekmektedir. Araştırmada, ortanca tümör boyutu 40 mm (9-90) saptanmış ve tümör boyutu ile idrar katekolamin metabolit düzeyleri arasında anlamlı korelasyon saptanmıştır ($p<0,001$). Cerrahi uygulanan hastalarda operasyon sırasında ve sonrasındaki takiplerde hemodinamik dengesizlik görülme sıklığı %6 olarak bulunmuştur. Hastaların %24'üne genetik geçişli sendromlar eşlik etmekte olup, bu sendromlar arasında multipl endokrin neoplazi tip 2A (MEN 2A), MEN 2B, von Hippel-Lindau (VHL) ve nörofibromatozis tip 1 (NF1) yer almaktadır. Öte yandan, kalıtsal feokromositomalı hastaların daha erken yaşlarda tanı aldıkları ve bu hastalarda adrenal lezyonların iki taraflı olma eğilimi gösterdikleri belirlenmiştir ($p=0,003$ ve $p<0,001$). Ayrıca, kalıtsal feokromositomalı hastaların, sporadik vakalara göre bulgu vermeden tanı aldıkları görülmüştür ($p=0,016$). Çalışmamızda, feokromositoma tanılı hastalarda metastaz oranının %3 olduğu saptanmıştır. **Sonuç:** Feokromositoma, ender görülmesine karşın yaşamı tehdit edebilen bir durumdur. Bu nedenle, klinik açıdan kuşku duyulan hastalarda feokromasitoma tanısını akla getirmek ve hastaları bu açıdan irdelemek büyük önem taşımaktadır. Ayrıca bu tanının konulduğu hastalarda altta yatan genetik sendromların da olabileceği özellikle dikkate alınmalıdır. Hastaların ömür boyu yıllık olarak takip edilmesi, nüks ve metastaz belirlemesi açısından ayrı bir önem taşımaktadır. Öte yandan, bu hastalıkla ilgili olarak değerlendirme, tedavi ve takip çalışmalarının, multidisipliner bir yaklaşımla deneyimli merkezlerde yapılmasına ayrı bir özen gösterilmelidir.

Anahtar kelimeler: Kalıtsal feokromasitoma; sporadik feokromasitoma; paroksismal hipertansiyon; multipl endokrin neoplazi tip 2; von Hippel-Lindau hastalığı

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Introduction

Pheochromocytomas are rare tumors arising from the adrenomedullary chromaffin cells that originate from the neural crest (1). The reported annual incidence of pheochromocytomas is 2%-9.1% per million adults (2). Pheochromocytomas may either be sporadic or a component of hereditary syndromes such as multiple endocrine neoplasia type 2A and 2B (MEN 2A and MEN 2B), von Hippel-Lindau disease (VHL), and neurofibromatosis type 1 (NF-1); it may also be associated with the succinate dehydrogenase (SDHx) mutations (3,4). Sporadic pheochromocytomas are more common in the 3rd to 5th decades of life, while hereditary pheochromocytomas tend to occur at younger ages (3,5). The prevalence of pheochromocytomas in patients with hypertension is 0.1%-0.6%, and the incidence of pheochromocytomas in patients with adrenal incidentalomas is 5% (6-9).

Plasma free metanephrine or urinary fractioned metanephrine measurement is recommended to the patients for diagnosis in case of pheochromocytoma suspicion (10). The patients with biochemical evidence of pheochromocytoma should be subjected to computer tomography (CT) or magnetic resonance imaging (MRI) for localization (10). The nuclear imaging methods are useful for the functional evaluation and detection of metastatic disease. Laparoscopic adrenalectomy is suggested to most of the patients with adrenal pheochromocytomas after achieving adequate alpha blockage (2,10). Despite the availability of multiple histological algorithms for the prediction of the biological behavior and malignant potential of pheochromocytomas, no gold standard grading system exists to date. Even now, malignancy is being diagnosed through the detection of local invasion of the surrounding tissues and organs or distant metastases (11). Since all kinds of pheochromocytomas have a potential for malignancy, life-long annual biochemical evaluation is recommended for the detection of recurrent or metastatic disease in the patients (10).

The present retrospective study was aimed to present our experiences related to pheochromocytomas in 16 years of practice. The patients diagnosed with pheochromocytoma were investigated and the concomitance with genetic syndromes, their symptoms, signs, and biochemical characteristics was evaluated at the time of application as well as during follow-up. In addition, the treatment methods applied, the success rates of the applied treatment, and the recurrence rates in the patients were studied.

Material and Methods

A total of 67 patients diagnosed with pheochromocytoma between January 2004 and July 2020 were included in the present study. Data regarding the demographic properties (age and gender), clinical history (presentation, medical history, complications, and family history), laboratory tests (24-hour urinary metanephrines and normetanephrines or plasma fractionated metanephrines and normetanephrines), imaging studies, surgical approaches, and pathological reports were obtained using paper charts and electronic records. Pheochromocytoma was diagnosed by measuring the 24-hour urine metabolites of catecholamines and searching for the detection of adrenal lesions in CT or MRI in the suspected patients. The 24-hour urine metanephrines and normetanephrines were measured using high-performance liquid chromatography (HPLC). CT without contrast and contrast-enhanced MRI were used as the primary imaging modalities. Lesion size was measured considering the largest dimension, and in bilateral cases, the size of the largest side was considered. In selected patients, nuclear imaging methods such as MIBG or PET CT were used. The patients with typical clinical signs and symptoms of pheochromocytoma, the patients with a history or a family history of genetic syndromes associated with pheochromocytoma, and those with adrenal incidentalomas were investigated for pheochromocytoma. Genetic tests were performed for the patients. Pheochromocytoma in the patients with negative genetic mutations and the patients with no suspicion of genetic syndromes and no available genetic test results were defined as sporadic. Laparoscopic adrenalectomy or adrenalectomy with open surgery was performed as the primary treatment approach, while other treatment options were considered in case of recurrent or metastatic disease. An 81-year-old female patient

who was clinically, biochemically, and radiologically diagnosed with pheochromocytoma refused to undergo surgery and is being followed-up with antihypertensive therapy, including the alpha blockers. The pathological specimens were examined by the experts in this field. All patients received at least 21 days of alpha blockage therapy (doxazosin), and a few patients received beta blockage after adequate alpha blockage therapy preoperatively. Beta blockage therapy was provided additionally in cases of persistent tachycardia, tachyarrhythmias, and persistent hypertension, despite adequate alpha blockage. Adequate fluid and salt intake were recommended to all patients during the preoperative period.

The study followed the ethical standards and adhered to the study protocol according to the international agreements (Helsinki Declaration revised in 2013). The study was approved by the Clinical Research Ethical Committee of Dokuz Eylül University on 21.10.2019; 2019/26-32.

Statistical Analysis

Statistical analyses were performed using IBM SPSS for Mac Version 20 (IBM Corp. Released 2011, Armonk, NY). Numeric variables were expressed either as mean ±SD or as median (minimum-maximum) based on their distribution features. Categorical variables were evaluated with cross-table analysis and expressed numerically as a percentage. Mann-Whitney U test was used for pairwise comparison of the data that was not normally distributed, while Student's t-test was used for the pairwise comparison of normally distributed data. Spearman's correlation analysis was performed. $p<0.05$ was considered statistically significant.

Results

Demographic Information and Pheochromocytoma Evaluation

The present retrospective study included 67 patients with an initial diagnosis of pheochromocytoma, among which 66 patients had undergone one or more adrenal surgeries. The mean age (±SD) of the patients at the time of diagnosis of pheochromocytoma was 46 years (±16.1), and the female to male ratio was 1.2. The urinary catecholamine metabolite levels, biochemical parameters, and lesion characteristics in the patients are summarized in Table 1.

The median urinary metanephrine level was 633.9 ug/24 h (range: 28-18642), and the median urinary normetanephrine level was 1001 ug/24 h (range: 37.9-10071). Among the 50 patients with available urinary catecholamine levels, 18 patients (36%) exhibited predominantly increased urinary normetanephrine levels, ten patients (20%) had predominantly increased urinary metanephrine levels, 14 (28%) exhibited an increase in both urinary metanephrine and normetanephrine levels, and eight patients (16%) had normal levels. These urinary catecholamine metabolite levels correlated positively with the tumor size ($p<0.001$).

Table 1. Demographic and anthropometric characteristics, urinary catecholamine metabolite levels, biochemical parameters, localization, and lesion sizes of the patients at the time of diagnosis of pheochromocytoma.

Parameter (reference range)	Value
Age (y)	46 (±16.1)
Gender (F/M)	37/30 (55.2%/44.8%)
Weight (kg)	67.8 (±12.8)
BMI (kg/m^2)	25.2 (±6.3)
Urinary Metanephrines (52-341ug/24 h)	633.9 (28.2-18642)
Urinary Normetanephrines (88-444 ug/24 h)	1001 (37.9-10071)
Size (mm)	40 (9-90)
Localization (Left/Right/Bilateral)	23/34/10 (34.3%/50.7%/14.9%)

BMI: Body mass index; F: Female; M: Male; y: years.
Mean values (±SD) are provided for equally distributed variables while median (minimum-maximum) values are provided for unequally distributed variables. Nominal parameters are provided as number (percentage).

In all patients, CT and/or MRI were used for diagnosis, while MIBG and PET CT were used additionally for the lesions that required further characterization (29.9% and 9%, respectively). Imaging analysis of the patients revealed lesions other than adenomas that were suspects for pheochromocytomas. The median size of the adrenal lesions was 40 mm (range: 9-90 mm), with 57 patients (85.1%) having unilateral adrenal lesion and ten patients (14.9%) having bilateral pheochromocytoma (Table 1).

Presentation Characteristics

Among the 61 patients whose presentation details could be obtained, pheochromocytoma diagnosis was provided on the basis of the clinical signs and symptoms in 31 patients (50.8%), while diagnosed during adrenal incidentaloma evaluation happened in 19 patients (31.2%). The remaining 11 patients (18%) were investigated either due to family histories of MEN, medullary thyroid cancer, VHL, and pheochromocytoma or due to their personal histories of familial syndromes or the diagnosis of diseases associated with the familial syndromes such as MTC (Table 2). Among these, eight patients had a family history of pheochromocytoma (7 had MEN2A and 1 had VHL), one patient had a family history of paraganglioma (her genetic analysis is non-applicable), and one patient with a family history of adrenal surgery died in a cerebrovascular event (his genetic analysis for hereditary syndromes was negative).
Hypertension pre-existed in 38 patients (56.7%), while eight patients (11.9%) received a diagnosis of hypertension at the

Table 2. Pheochromocytoma diagnosis histories of the patients.

Detection history	Number of patients (%)
Investigation due to symptoms and signs	31 (50.8%)
Incidentaloma screening	19 (31.2%)
Screening due to family history of MEN	4 (6.6%)
Screening due to diagnosis of MTC	3 (4.9%)
Investigating due to history of NF1	2 (3.3%)
Screening due to family history of VHL	1 (1.6%)
Screening due to family history of pheochromocytoma	1 (1.6%)
Accompanying diseases and symptoms	
Existing hypertension	38 (56.7%)
Preeclampsia/abortus	2 (3%)
Hypertensive retinopathy	2 (3%)
Hypertension diagnosed at the time of diagnosis	8 (11.9%)
Paroxysmal pattern	36 (53.7%)
Asymptomatic	17 (25.4%)
Type 2 diabetes	15 (22.4%)
Impaired fasting glucose and/or impaired glucose tolerance	5 (7.5%)
Cardiovascular disease (angina, PTCA, MI, medical treatment)	12 (17.9%)
Cardiomyopathy	2 (3%)
Palpitation	22 (32.8%)
Headache	14 (20.9%)
Sweating	10 (14.9%)
Weight loss	4 (6%)
Pallor	4 (6%)
Flushing	4 (6%)
Fainting	3 (4.5%)
Vomiting	2 (3%)
Nervousness	1 (1.5%)
Anxiety	1 (1.5%)
Hematemesis	1 (1.5%)
Chest pain	1 (1.5%)

MEN: Multiple endocrine neoplasia; MTC: Medullary thyroid cancer; NF1: Neurofibromatosis type 1; VHL: von Hippel-Lindau; PTCA: Percutaneous transluminal coronary angioplasty; MI: Myocardial infarction.

time of pheochromocytoma diagnosis. A paroxysmal pattern was observed in 36 patients (53.7%). The most common symptoms accompanying paroxysmal hypertension were palpitation, headache, and sweating. Detailed information regarding the symptoms at the initial presentation is summarized in Table 2. The accompanying malignancies in the patients with sporadic pheochromocytoma were papillary thyroid cancer in one patient and breast cancer in one patient at the time of diagnosis. Two patients were diagnosed with malignancies during follow-up (bladder cancer in one patient; papillary thyroid cancer and basal cell carcinoma in one patient).

Accompanying Adrenal Cortex Evaluation

In eight patients (11.9%), low-dose dexamethasone suppression test (DST) was unable to suppress cortisol (>1.8 mcg/dL), while three patients with cortisol levels higher than 5 mcg/dL after 1 mg DST received steroid coverage during the surgery as well as for a short time after the surgery. We have previously reported a patient's case with the diagnosis of CRH producing pheochromocytoma (12). Postoperative ACTH levels and cortisol values after DST were normalized in these three patients.

Surgical Approach and Complications

All patients had successful surgery and were discharged from the hospital. Among these patients, one patient experienced intraoperative hypertension approaching 200 mmHg systolic pressure, and another experienced severe hypotension in the postoperative period; both were treated successfully. Moreover, one patient was readmitted to the endocrinology clinic on postoperative Day 7 with a complaint of hypotension and a history of syncope. Her blood pressure was 70/40 mmHg. She was hospitalized, treated with intravenous isotonic fluid and increased oral salt intake, and discharged from the hospital after three days. Adrenal insufficiency was excluded, and she did not report any complaints afterward.

Ten patients had bilateral adrenalectomy, either in one session or in separate surgeries during follow-up. Nine patients had genetic syndromes associated with pheochromocytomas, while the genetic test results of one patient (with a family history of paraganglioma) were non-applicable (Table 3). Two

Table 3. Pathological diagnosis, surgery types, and outcomes of the patients.

Operation features	
Type of surgery Laparoscopic/Open surgery*	45/11 (80.4%/19.6%)
Perioperative/Postoperative hemodynamic instability**	4 (6%)
Pathological diagnosis (n=66)	Number of patients (%)
Pheochromocytoma	60 (91%)
Composite pheochromocytoma (+ganglioneuroma)	3 (4.5%)
Adrenal medullary hyperplasia	2 (3%)
Necrosis	1 (1.5%)
PASS score***	4 (0-17)
PASS <4	15 (42.9%)
PASS ≥4	20 (57.1%)
Outcome	
Median follow-up (y)	3 (0.3-14)
Remission	47 (71.2%)
Missed follow-ups	15 (22.7%)
Metastatic disease	2 (3%)
Recent operation	2 (3%)
Deceased	1 (1.5%)
Being followed-up without surgery	1 (1.5%)

PASS: Pheochromocytoma of the Adrenal gland Scaled Score.
*n=56; **n=50; ***n=35.

patients with unilateral pheochromocytoma had non-functional stable, benign adrenal lesions on the contralateral adrenal gland.

Pathological Evaluation

Pathological diagnoses of the patients are summarized in Table 3. Among the patients who underwent surgery, 60 patients had pheochromocytoma, three patients had composite pheochromocytoma (+ganglioneuroma), two patients had medullary hyperplasia, and one patient had necrosis. The median PASS score of the patients was 4 (range: 0-17). Laparoscopic surgery was preferred over open surgery (Table 3).

Outcomes of the Patients

Our median follow-up time after the surgeries performed in our hospital was three years (range: 0.3-14). The follow-up characteristics of the patients are summarized in Table 3. The detailed information regarding the hereditary syndromes of the patients and their families, along with the outcomes, is provided in Table 4. Forty-seven patients are being followed-up in remission, while 15 patients missed their follow-ups. Among the two patients with metastatic disease, one patient had MEN2A, and the metastasis was detected one year after the surgery (Table 4, MEN2A, Family 2, patient 1). She had multiple metastatic lesions in the lung, liver, and bone, as well as retroperitoneal implants and local recurrence. She is currently being followed-up by the medical oncology and nuclear medicine teams. The other patient with metastatic pheochromocytoma had no family history or diagnosis of a hereditary syndrome. He was diagnosed with lytic bone metastasis four years after the surgery with no local recurrence and is currently receiving chemotherapy. One patient with the diagnosis of MEN2A died one year after bilateral adrenalectomy due to severe upper respiratory infection and adrenal insufficiency despite receiving adequate steroid replacement therapy and warnings regarding stress conditions (Table 4, MEN2A, Family 5, patient 1).

Characteristics of Hereditary and Sporadic Pheochromocytomas

Sixteen of the patients had accompanying hereditary syndromes, including MEN2A, MEN2B, VHL, and NF1. None of our patients had SDHx mutations. Among the 67 patients included in the present study, ten patients had MEN2A (14.9%), one patient had MEN2B (1.5%), three patients had VHL (4.5%), and two patients had NF1 (3%). The genetic analysis of one patient with possible hereditary syndromes was non-applicable; she was, therefore, excluded from the comparative analyses. The mean age of the patients with hereditary syndromes at the time of diagnosis (36±11.3 years) was significantly less ($p=0.003$) than the mean age of those with sporadic pheochromocytomas (49.4±16.3 years). The patients with sporadic pheochromocytomas tended to be more asymptomatic ($p=0.016$) rather than sporadic. Hereditary pheochromocytomas tended to be bilateral ($p<0.001$) (Table 5). Although statistically insignificant ($p=0.064$), the median lesion size of sporadic pheochromocytomas was observed to be larger than that of hereditary pheochromocytomas (Table 5).

Discussion

In the present study, we have comprehensively reviewed our experience in the diagnosis, treatment, and follow-up of pheochromocytomas in a tertiary center during the period between 2004 and 2020. Our results concerning the initial presentations of the patients are rather different from those in the literature. Falhammar et al. (13) reviewed 98 cases in their study, in which diagnoses of pheochromocytomas were obtained mostly due to the investigation for incidentalomas (64%), followed by clinical suspicion of pheochromocytoma (32%) and screening for a family history of MEN2A (4%), respectively. In the present study, most of the patients (50.8%) were diagnosed due to suspicious symptoms of pheochromocytoma, while 31.2% of the patients were diagnosed during the incidentaloma screening. The remaining 18% of the patients were diagnosed while investigating for possible hereditary syndromes. This difference might be due to the use of more aggressive approaches for identifying the cause of secondary hypertension and the increased awareness of pheochromocytoma during the past years.

CT and MRI are generally accepted to be sufficient for the diagnosis of pheochromo-

Table 4. Detailed information regarding the patients and their families with hereditary syndromes.

Age at diagnosis/Current age (y)/ Genetic mutation	HT and symptoms	Concomitant diseases	Urine catecholamine metabolites Size and localization	Treatment and outcome for pheochromocytoma
		MEN 2A		
Family 1				
Patient 1 (index)	Paroxysmal HT	MTC PHPT	M: N/A	Bilateral adrenalectomy
33/43	Palpitation Sweating		NM: N/A	Bilateral pheochromocytoma
			Right: 20 mm	PASS: N/A
			Left: 65 mm	Remission (11y follow-up)
Patient 2 47/58	Screening due to family history	MTC	M:2800 ug/24 h	Bilateral adrenalectomy
No symptoms		PHPT	NM: 2629 ug/24 h	Bilateral pheochromocytoma
			Right: 43 mm	PASS: N/A
			Left: 46 mm	Remission (11y follow-up)
Patient 3	HT	MTC	M: 462 ug/24 h	Bilateral adrenalectomy
53/60	Paroxysmal HT	PHPT	NM: 231 ug/24 h	Bilateral pheochromocytoma
	Palpitation Nervousness	Type 2 DM	Right: 15 mm	PASS: N/A
			Left: 9 mm	Remission (8y follow-up)
Patient 4	Paroxysmal HT Palpitation	MTC	M: 673 ug/24 h	Unilateral adrenalectomy
27/29			NM: 1916 ug/24 h	Pheochromocytoma
			Left: 30 mm	PASS: 5
				Remission (2y follow-up)
Family 2				
Patient 1 (index)	Paroxysmal HT	MTC	M: N/A	Unilateral adrenalectomy
46/58	Pallor	Type 2 DM	NM: N/A	Pheochromocytoma
	Fainting		Right: N/A	PASS: N/A
	Headache			Metastatic disease (1y after surgery)
	Palpitation			Total follow-up 12 years
	High glucose			Medical oncology and nuclear medicine
Patient 2	Screening due to family history	MTC	M: 708	Bilateral adrenalectomy
30/38	Hypertension	PHPT	NM: 1186	(2 years apart)
RET c.1901G>A	Pallor		Left: 25 mm	Bilateral pheochromocytoma
	Fainting		MIBG: left	PASS: N/A
	Headache		2 years later	Remission (9y after second surgery)
			Right: 25 mm	
			MIBG: right	

continued →

Table 4. Detailed information regarding the patients and their families with hereditary syndromes *(contunied)*.

Age at diagnosis/Current age (y)/ Genetic mutation	HT and symptoms	Concomitant diseases	Urine catecholamine metabolites Size and localization	Treatment and outcome for pheochromocytoma
Family 3				
Patient 1 40/46 RETc611y (index)	Investigated for MEN due to MTC No symptoms	MTC	M: 79 ug/24 h NM: 77 ug/24 h Left: 16 mm	Unilateral adrenalectomy Pheochromocytoma PASS: 4 Remission (6y follow-up)
Family 4				
Patient 1 45/49 RETp.cys634arg (index)	Investigated for MEN due to MTC No symptoms	MTC PHPT	M: 537 ug/24 h NM: 109 ug/24 h Right: 35 mm Left: 25 mm	Bilateral adrenalectomy Pheochromocytoma/composite pheochromocytoma (+ganglioneuroma) PASS: 1/4 Remission (4y follow-up)
Family 5				
Patient 1 34/35 ex N/A	HT Paroxysmal HT Palpitation	MTC PHPT	M: 3018 ug/24 h N: 929 ug/24 h Right: 60 mm Left: 65 mm	Bilateral adrenalectomy Bilateral pheochromocytoma PASS: 6 Deceased (1 y after surgery)
Family 6				
Patient 1 42/55	Screening due to family history No symptoms	MTC	N/A Right: 25 mm Left: 50 mm	Bilateral adrenalectomy Bilateral pheochromocytoma PASS: N/A Remission (13y follow-up)
		MEN 2B		
Family 1				
Patient 1 26/29 RETM918T (index)	Investigated for MEN due to MTC No symptoms	MTC Marfanoid habitus Lingual ganglioneuroma	M: 129 ug/24 h NM: 62 ug/24 h Right: 9 mm	Unilateral adrenalectomy Pheochromocytoma PASS: 0 Remission (3y follow-up)

continued →

Table 4. Detailed information regarding the patients and their families with hereditary syndromes *(contunied)*.

Age at diagnosis/Current age (y)/ Genetic mutation	HT and symptoms	Concomitant diseases	Urine catecholamine metabolites Size and localization	Treatment and outcome for pheochromocytoma
		VHL		
Family 1				
Patient 1 22/49	Detected during pregnancy (Intrauterine ex) Paroxysmal HT	Autoimmune thyroid disease	M: 28.19 ug/24 h NM: 333 ug/24 h (before second op) R: 25 mm L: 18 mm MIBG: left adrenal active	Bilateral partial adrenalectomy Steroid replacement: only during pregnancy Local recurrence (24y after) Left unilateral adrenalectomy Pheochromocytoma PASS:2 3y follow-up after second surgery
Patient 2 10y/24y VHL VHL c.695 G>A (index)	Screening due to history of retinal angioma and family history of pheochromocytoma No symptoms	Retinal angioma	N/A Bilateral Size: N/A	Bilateral adrenalectomy Invasive pheochromocytoma PASS: N/A Remission (14y follow-up)
Family 2				
Patient 1 32/34	Screening due to family history No symptoms	Intracranial lesion Spinal hemangioblastoma Clear cell RCC Pancreatic serous cystadenoma	M: 42 ug/24 h NM: 505 ug/24 h Left: 33 mm MIBG: Left	Unilateral adrenalectomy Hematoma and medullary hyperplasia PASS: N/A Remission (2y follow-up)
		NF1		
Family 1				
Patient 1 46/52	Incidentaloma No symptoms	Subcutaneous neurofibromas	M: 766 ug/24 h NM:1547 ug/24 h Right: 70 mm 1y after op. Left: 20	Unilateral adrenalectomy Adrenal medullary tumor, composite pheochromocytoma (+ganglioneuroma) PASS: 7 Remission (6y follow-up) Left adrenal lesion: stable
Family 2				
Patient 1 43/49	Paroxysmal HT Headache Diagnosis of NF	Schwannoma (mediastinal and in the extremity) Neurofibromas Café-au -lait	M: 709 ug/24 h NM: 718 ug/24 h Left: 44 mm	Unilateral adrenalectomy Pheochromocytoma PASS: 4 Remission (7y follow-up)

HT: Hypertension; M: Metanephrines; MEN 2A: Multiple endocrine neoplasia type 2A; MEN 2B: Multiple endocrine neoplasia type 2B; MIBG: Metaiodobenzylguanidine; MTC: Medullary thyroid cancer; N/A: Non-applicable; NF: Neurofibromatosis; NM: Normetanephrines; PASS: Pheochromocytoma of the adrenal gland scaled score; PHPT: Primary hyperparathyroidism; RCC: Renal cell carcinoma; VHL: von Hippel-Lindau; y: Year.

Table 5. Comparison of the characteristics of hereditary and sporadic pheochromocytomas.**

	Hereditary pheochromocytoma	Sporadic Pheochromocytoma	p value
Age (y)	36 (±11.3)	49.4 (±16.3)	0.003
F/M	9/7	27/23	1
Asymptomatic/Symptomatic	8/8	9/41	0.016
Bilateral/Unilateral adrenalectomy	9/7	0/49	p<0.001
Urinary Metanephrines	605 (28-3018)	600.4 (34.8-18642)	0.588
Urinary Normetanephrines	612 (62-2629)	1200 (37.9-10071)	0.117
Lesion size	31.5 (9-70)	40 (18-90)	0.064

F: Female; M: Male; y: Years.

cytomas owing to characteristic appearances and contrast-enhancement profiles (14). These typical findings were observed in all our patients who had radiological imaging reported to be suspicious for pheochromocytoma or consistent with the non-adenoma adrenal lesion. Although MIBG and PET CT are not recommended for diagnosis in routine use, they are used for the evaluation of functionality and metastatic disease, respectively (14). In the present study, CT and/or MRI were sufficient for diagnosis for most of the patients, while nuclear imaging methods had to be used additionally for 37.3% of the patients. In our study, tumor size was significantly correlated with the urinary catecholamine levels, similar to other studies, while the median size of the lesions in our study (40 mm) was slightly smaller than that reported (49 mm) in previous studies (13,15). The smaller tumor size in our study could be attributed to the relatively higher number of patients investigated for genetic syndromes.

As the patients with clinical suspicion constituted the dominant portion of clinical presentation, 56.7% of the patients had pre-existing hypertension, while 11.9% of the patients were diagnosed with hypertension at the time of pheochromocytoma diagnosis. Two patients were diagnosed with pheochromocytoma while being investigated for preeclampsia and abortus. Hypertension exhibited a paroxysmal pattern in 53.7% of the patients. The major accompanying symptoms of the hypertension episodes were palpitation, headache, and sweating, consistent with the classical triad of pheochromocytoma (16).

Pheochromocytoma incidence during pregnancy was <0.2 per 10,000 pregnancies (17). It is generally stated that the diagnosis of pheochromocytoma during pregnancies might go unnoticed due to the rarity of this disease and because its symptoms generally mimic the other forms of hypertension observed during pregnancy, such as preeclampsia and gestational hypertension (17). Two of our patients had a history of abortus and preeclampsia at the time of presentation. It is important to consider pheochromocytoma in pregnant women with hypertension as this disease may cause significant morbidity and mortality to both fetus and mother. Since the screening of all pregnant women with hypertension is not a cost-effective approach, it is recommended to screen the pregnant women with resistant hypertension, those having adrenal mass, and the ones with classical signs and symptoms of pheochromocytoma (17).

Despite improvements in preoperative preparation with alpha blockage (and subsequent beta blockage if required) and adequate fluid intake, both perioperative and postoperative complications can occur in patients with pheochromocytoma. Laparoscopic surgery is our preferred surgical approach, and the intraoperative and postoperative complications observed are low in our institution. A study concerning 100 patients with pheochromocytoma reported 27.3% hemodynamic instability in the perioperative period (18). The overall rate of he-

modynamic instability in perioperative and postoperative periods in our patients was 6%. General anesthesia, along with sympathetic blockage, is the preferred method for anesthesia. Adequate preoperative preparation, appropriate anesthesia administration, laparoscopic approach, and the experienced team could be the possible reasons for a low frequency of intraoperative severe hypertension. Tumor manipulation during the resection of pheochromocytoma is thought to be the most probable reason for perioperative hypertension (19). Prolonged hypotension after tumor removal might be due to chronically low circulating levels of plasma volume, an abrupt decrease in the plasma catecholamine levels, downregulation of adrenoreceptors, increased blood loss, and cardiogenic or septic shock (19,20). Larger tumor size and higher urinary catecholamine metabolite levels are reported as the predictors of prolonged hypotension requiring postoperative catecholamine support (20).

Low dose DST was unable to suppress cortisol levels in 11.9% of the patients, while three patients had cortisol levels higher than 5 mcg/dL after 1 mg DST. Among the latter, one patient had accompanying obvious Cushing syndrome and was diagnosed with ectopic CRH secretion from the pheochromocytoma, as previously reported (12). In addition, the ACTH levels of the remaining two patients were not suppressed. Despite normalization of both ACTH levels and cortisol suppression after DST, we were unable to explain the causality as ACTH, and CRH staining of the pathology specimens could not be performed. When considering the patients with DST values compatible with subclinical Cushing syndrome, there are possible reasons for such results. First, the abnormal test results, particularly in patients with subclinical Cushing, may be interpreted as false-positive results as there are several common sources of error for DST's. Acute stress and illness, conditions elevating the serum corticosteroid-binding globulin (CBG) levels, and drugs causing variations in dexamethasone metabolism through cytochrome 3A4 (CYP3A4) are reported to cause false-positive results (21). In addition, increased cortisol secretion could accompany the pheochromocytomas via several different mechanisms, one of which is the increased catecholamine secretion that causes increased cortisol secretion via activation of aberrant adrenal beta-adrenergic receptors (22). Furthermore, cytokines such as tumor necrosis factor-alpha, interleukin-1 (IL-1), and interleukin-6 (IL-6) are reported to activate the hypothalamic-pituitary-adrenal (HPA) axis (23). There are also studies demonstrating cytokine production from pheochromocytomas resulting in increased cortisol production from the adrenal cells (24,25). Corticomedullary mixed tumors causing both pheochromocytoma and subclinical Cushing syndrome are also reported (26).

The pathological reports of two patients stated adrenal medullary hyperplasia, and three patients stated composite pheochromocytoma, both of which are rare conditions reported to be associated with hereditary conditions, although sporadic cases are also reported (27-31). One of our patients with adrenal medullary hyperplasia was diagnosed with VHL, and the other patient was a sporadic case with negative genetic test results. To the best of our knowledge, our patient is the first one to have been diagnosed with both VHL and adrenal medullary hyperplasia. On the other hand, among the three patients with composite pheochromocytoma, two had hereditary syndromes (MEN2A and NF1). Medullary hyperplasia is considered a precursor of pheochromocytoma, while composite pheochromocytoma is clinically and radiologically indistinguishable from pheochromocytoma and these cases are, therefore, recommended to be managed similar to pheochromocytomas (32-34).

The risk of pheochromocytoma was reported to be 50% in MEN2A and MEN2B, 10-20% in VHL, and 1-3% in NF-1 (35,36). Although pheochromocytomas in NF-1 are generally benign and unilateral, bilateral, recurrent, or malignant pheochromocytomas may also be detected (36). Our patients with NF-1 had unilateral lesions and are being followed-up regularly after adrenalectomy in remission. Pheochromocytomas in MEN2 and VHL tend to be bilateral and generally occur at younger ages compared to sporadic pheochromocytomas (37,38). In our study, most of the patients with bilateral adrenal lesions had hereditary syndromes. Heredi-

tary pheochromocytomas in our study tended to occur at younger ages, and the adrenal lesions tended to be smaller. The mean age of our patients with hereditary syndromes at the time of diagnosis was significantly lower than that of the sporadic cases. Although the median lesion size in our patients with hereditary syndromes tended to be smaller than that in the sporadic cases, the difference was not statistically significant. Hereditary pheochromocytomas were more asymptomatic rather than sporadic. These observations could be attributed to the early detection of the lesions because of the screening in hereditary syndrome-diagnosed families. Pheochromocytomas are generally diagnosed after medullary thyroid cancer in patients with MEN2, as in our study (38). Pheochromocytomas in VHL are generally asymptomatic, while the pheochromocytomas in MEN are generally associated with paroxysmal hypertension, which was partially true for our study as well (38).

Study Limitations

The retrospective pattern of our study generated multiple limitations. Missing data for certain patients, such as symptom durations, urinary catecholamine metabolite levels, PASS scores, and types of surgeries, and the patients missing their follow-ups were the main limitations. In addition, not all patients had genetic testing results, and the lack of routine genetic screening reduced the reliability of sporadic cases as the sporadic case definition was generally based on no clinical suspicion for a hereditary syndrome. In addition, the sample size was relatively small, and the data were obtained from a single institution.

CONCLUSION

In conclusion, our study group may be defined as a small cohort of pheochromocytoma as it represents the general features and the accompanying hereditary syndromes of the disease. Pheochromocytoma should be suspected in the hypertensive patients with resistant hypertension, hypertension with a paroxysmal pattern, or secondary hypertension. In addition, as generally observed in hereditary syndrome cases, investigating the patients diagnosed with pheochromocytoma for possible hereditary diseases and screening for pheochromocytoma in the patients with hereditary diseases are important. Owing to the lesser understanding of the malignant potential of these tumors, life-long annual follow-up is recommended for the detection of recurrent or metastatic disease. Since pheochromocytoma is a rare condition that could be life-threatening, its evaluation, treatment, and follow-up should involve a multidisciplinary approach in experienced centers.

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Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Serkan Yener, Mustafa Seçil, Ömer Demir, Kutsal Yörükoğlu, Mehmet Ali Koçdor; Design: Serkan Yener, Mustafa Seçil, Ömer Demir, Kutsal Yörükoğlu, Mehmet Ali Koçdor; Control/Supervision: Serkan Yener, Mustafa Seçil, Ömer Demir, Kutsal Yörükoğlu, Mehmet Ali Koçdor; Data Collection and/or Processing: Başak Özgen Saydam, Süleyman Cem Adıyaman, Ozan Bozkurt; Analysis and/or Interpretation: Başak Özgen Saydam, Süleyman Cem Adıyaman, Ozan Bozkurt, Serkan Yener; Literature Review: Başak Özgen Saydam, Süleyman Cem Adıyaman, Ozan Bozkurt; Writing the Article: Başak Özgen Saydam, Süleyman Cem Adıyaman, Ozan Bozkurt; Critical Review: Başak Özgen Saydam, Süleyman Cem Adıyaman; Materials:Süleyman Cem Adıyaman, Başak Özgen Saydam.

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Original Article
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Evaluation of Influenza, Pneumococcus, Zoster, Measles, Diphtheria, and Pertussis Vaccination Rates in Patients with Type 1 and Type 2 Diabetes Mellitus; a Single-Center Experience from Turkey

Diabetes Mellitus Tanılı Hastalarda İnfluenza, Pnömokok, Zoster, Kızamık, Difteri ve Boğmaca Aşılama Oranlarının Değerlendirilmesi; Türkiye'den Tek Merkez Deneyimi

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Abstract

Objective: Several vaccines have been recommended for adults with diabetes. This study aimed to determine the rates of uptake of recommended vaccines in diabetic adults and estimate their association with sociodemographic and clinical factors. **Material and Methods:** This was a cross-sectional study conducted on patients with either type 1 or type 2 diabetes, who had attended the outpatient clinics of internal medicine and endocrinology. The patients were inquired about their immunization status against influenza, pneumococcus, zoster, measles, pertussis, and diphtheria. **Results:** Among the 350 diabetic patients, 38 (10.8%) had received a vaccine against pneumococcus, 90 (26%) against seasonal influenza, and only one patient had been administered the zoster vaccine. None of the patients had been vaccinated against measles, diphtheria, and pertussis. The rate of pneumococcal vaccination (PV) increased with age (65.5±9.7 vs. 57±9.1 [OR 2.9 (95% CI=14.3-2.67], p=0.005), although there was no such association between influenza vaccination (IV) and age (p=0.456). The rate of PV increased with the number of routine follow-up visits per year (10/38 vs. 28/38 [OR 4 (95% CI=0.994-16.096], (p=0.039). The rates of PV and IV were significantly higher in diabetic patients with chronic pulmonary disease (21/38 vs. 14/312 [OR 52.80 (95% CI 8.4-333.1], p=0.005 and 31/90 vs. 4/260 [OR 29.15 (95% CI 3.37-252.28], p=0.001) respectively. The rates of IV in diabetic patients with chronic renal failure were also significantly different from those without (27/90 vs. 8/260, [OR 14.28 95% CI 1.51-133.74], p=0.013). **Conclusion:** We observed low rates of vaccination against influenza, pneumococcus, and zoster in patients with diabetes, which were below the targets recommended by the World Health Organization.

Keywords: Diabetes; vaccination; influenza; pneumococcal vaccines; zoster; pertussis

Özet

Amaç: Diyabetik hastalarda çeşitli aşıların yapılması önerilmektedir. Bu çalışmada diyabetik hastalarda aşılanma oranlarının belirlenmesi ve sosyodemografik ve klinik faktörlerle ilişkilerinin saptanması amaçlanmaktadır. **Gereç ve Yöntemler:** Çalışma, İç Hastalıkları ve Endokrinoloji Kliniğine başvuran tip 1 ve tip 2 DM'li hastalar üzerinde kesitsel-gözlemsel olarak yapıldı. Hastaların influenza, pnömokok, zoster, kızamık, boğmaca ve difteri enfeksiyonlarına karşı aşılanma durumları sorgulandı. **Bulgular:** Çalışmaya tip 1 ve tip 2 diabetli, toplam 350 hasta dahil edildi. Hastaların 38 (%10,8)'i pnömokok, 90 (%26) mevsimsel grip ve sadece bir hasta zoster enfeksiyonuna karşı aşılanmıştı. Hastaların hiçbirinde kızamık, diphetria ve boğmaca aşısı yapılmamıştı. Pnömokok aşılanma (PA) oranı yaşla birlikte artmış bulundu (65,5±9,7 vs 57±9,1 [OR 2,9 (%95 CI-14,3-2,67], p=0,005). Ancak influenza aşısı (IA) ile yaş arasında böyle bir ilişki saptanmadı (p=0,456). PA oranı, yıllık rutin poliklinik vizit sayısı ile ilişkili olarak artmış görüldü (10/38 vs 28/38 [OR 4 (%95 CI -0,994-16,096], (p=0,039). PA ve IA oranları kronik akciğer hastalığı olan diabetes mellituslu hastalarda anlamlı olarak daha yüksekti (sırasıyla 21/38 vs 14/312 [veya 52,80 (%95 CI 8,4-333,1], p=0,005 ve 31/90 vs 4/260 [veya 29,15 (%95 CI 3,37-252,28], p=0,001). Kronik böbrek yetmezliği olan ve olmayan diyabetik hastalarda IV oranları arasında istatistiksel olarak anlamlı bir fark saptandı (27/90 vs 8/260, [OR14,28 %95 CI 1,51-133,74], p=0,013). **Sonuç:** Diyabetik hastalarda influenza, pnömokok ve zona aşılanma oranları Dünya Sağlık Örgütü tarafından belirlenen hedeflerin altında saptandı.

Anahtar kelimeler: Diabetes mellitus; aşılanma; influenza; pnömokok; zoster; boğmaca

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Introduction

Diabetes Mellitus (DM) is an important systemic disease with increasing incidence, complications, and economic burden on society (1). Patients with DM show a higher prevalence of comorbidities and are more susceptible to infectious disease, which in turn leads to increased morbidity and mortality risk via several mechanisms (2,3). Influenza runs a more severe course (e.g., higher incidence of complications such as cardiovascular events) in diabetic patients, with a 2–4-fold increased risk of pneumonia-related hospitalization and mortality, compared to non-diabetics (3-5). Numerous studies show an increased risk of pneumococcal pneumonia and invasive pneumococcal disease (IPD) in diabetic patients, with increased rates of mortality (6,7). Diabetics are also at higher risk of herpes zoster infection, including the risk of ophthalmic nerve involvement and/or debilitating postherpetic neuralgia (8). Although the risk of infection in diabetics is similar to that in a healthy population, diseases such as measles, diphtheria, and pertussis have a more serious course in diabetic patients. Therefore, vaccination in patients with DM is essential. The reported immune responses to vaccines in people with diabetes are variable. However, it is generally considered that immunization against influenza and pneumococcus in this risk group yields favorable results overall (9-13). Herpes zoster vaccine lowers the risk of disease by 51% and postherpetic neuralgia by 67% in immunocompetent individuals aged 60 and above (14). In pertussis, diphtheria, tetanus, and measles, which are known for their high incidence and contagiousness, natural or vaccination-induced immunity does not provide lifelong protection. Recent outbreaks of measles and pertussis in adult populations have suggested an epidemiological shift of such infections to an older age, where the initiating cause is unvaccinated people or those who have lost their immunity. Therefore, childhood vaccines for preventable diseases should also be administrated in adults (15).

The approach of international and national organizations to vaccination may differ in the type of recommended vaccine, patient's age, and the presence of chronic illness. All the organizations suggest the administration of IV annually before the flu season begins and PV for patients with diabetes of all ages, with some differences in vaccination regimens according to the age (16-19). A single dose of zoster vaccine (ZV) for all diabetic adults aged 60 years and above is recommended by the Advisory Committee on Immunization Practices (ACIP) and the Infectious Diseases and Clinical Microbiology Speciality Society of Turkey (EKMUD) (17,19). Pertussiscontaining vaccines have recently been started to be used for adults (15). Some organizations recommend that all adults should get a single dose of the tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine followed by Td booster every ten years, and at least one dose of MMR vaccine for adults born during or after 1957 who have no evidence of immunity (15,19).

Studies investigating the uptake of and response to recommended vaccines in adults with chronic diseases in Turkey are limited. Moreover, local epidemiological changes, the rise of immigration over the past years, along with an increased risk of re-emerging epidemics, and the aging of the population associated with an increased risk of comorbidities, require a systematic approach to vaccination and updating the immunization schedules. This study aimed to investigate the uptake of influenza, pneumococcus, zoster, measles, diphtheria, and pertussis vaccination in diabetic patients, in the context of proposed changes in vaccination and lack of consensus on the applicability of childhood vaccination in adults. Furthermore, we aimed to explore the clinical attributes associated with the actual vaccination rates (VR).

Material and Methods

This was a cross-sectional study conducted between January and August 2019 at the Istanbul Prof. Dr. Cemil Taşçıoğlu State Hospital, Department of Internal Medicine, İstanbul, Turkey.

Participants

Patients with type 1 and type 2 DM aged over 18-years-old, who attending the internal medicine and endocrinology outpatient clinics, gave informed consent for data col-

lection, and had no contraindications to vaccination were consecutively included in the study. The exclusion criteria were age below 18 years and inability to provide reliable information regarding vaccination.

Study design

Sociodemographic data, comorbid diseases, current medication, type of diabetes, disease duration, and the number of outpatient clinic visits during the previous years were recorded for all the patients included in the study. Medication data were obtained from the patient charts. The comorbid diseases recorded were hypertension (HT), asthma, chronic obstructive pulmonary disease (COPD), chronic kidney damage (CKD), coronary artery disease (CAD), and cirrhosis. Although asthma and COPD were treated as distinct diseases, we recorded them under the title "chronic pulmonary disease (CPD)". According to regular follow-up visits during the previous five years, we divided the patients into two groups: those with a maximum of two annual follow-up visits and those with at least three. Since the guidelines on diabetes management recommended patients to have their routine follow-up at least twice a year but with an optimal frequency of four times a year, we divided the patients into the above-defined groups, highlighting the patients' adherence to the diabetes management plan (18). History of vaccination against influenza, pneumococcus, zoster, Tdap, and measles of all the enrolled patients for the past five years was obtained from the physicians' records and/or based on the patient's statement. We attempted to ascertain the timing of the vaccination as much as possible. For seasonal IV, patients were categorized as 1) vaccinated during the previous year, 2) at least once in the last five years, and 3) annually for the last five years. For pneumococcus, pertussis-containing (Tdap/Td) combinations, and measles, we inquired about the time of vaccination in the past. The trade names of vaccines were not recorded since our objective was only to confirm the uptake and not the efficiency of vaccination.

Ethics statement

This study was conducted following the Declaration of Helsinki. The study was approved by the institutional ethics committee (07.02.2017/48670771) of the Health Sciences University, Okmeydani Training and Research Hospital. All patients and/or their caregivers were informed about the study, and their informed consent was obtained.

Statistical Analysis

The sociodemographic and clinical data were summarized using appropriate summary statistics. We used the Student's t-test to compare the continuous variables and the Chi-square test to compare the categorical variables. Continuous values were presented as mean ±SD. Spearman's correlation was used to assess the relationship between vaccination statuses (yes/no) and factors probably able to affect the VR. A p-value <0.05 was considered statistically significant. All the data were analyzed using the statistical software SPSS for Windows, version 15.0 (SPSS Inc, Chicago, IL).

Results

A total of 350 diabetic patients who fulfilled the inclusion criteria were enrolled in the study. The population in the study consisted of type 1 (n=35) and type 2 (n=315) diabetic patients. The demographic and clinical characteristics of all patients were recorded, as shown in Table 1.

Thirty-eight (10.8%) of the patients received pneumococcal vaccine all in the previous five years. Twenty-four (63%) of the patients receiving PV were 65 years of age or older. The number of older patients (≥65 years, n=103) receiving PV was higher than younger ones (<65 years) ($p<0.005$) (Table 2). No patient with type 1 DM received pneumococcal vaccine.

Analysis of distribution of influenza vaccination over the past five years for all patients revealed that 90 (26%) patients received an influenza vaccine during the last year, 108 (31%) had received it at least once during the last five years, and 60 (17%) had received IV annually. The seasonal IV rate of 65 years or older diabetic patients during the last year was 30%. The distribution of vaccination in type 1 DM patients was as follows: 10 (2.8%) patients had received an influenza vaccine during the last year, 16 (4.5%) had received it at least once during the last five years, and 3 (0.8%) had received IV annually.

Table 1. The demographic and clinical characteristics of the patients.

Variables	Patients n=350
Age (mean±SD; year)	57,9±10,7
Age of T1DM*	**48,5±6,3**
Age of T2DM**	**58,9±9,3**
Number of the patient over the age of 65 (n; %)	103 (29%)
Gender male/female (n)	97/253
Education status	
No education	142 (41%)
Primary school	165 (47%)
High school	42 (12%)
University	0
Diabetes duration (years; mean±SD)	8,9±6,7
Diabetes duration of T1DM*	**17,8±7,9**
Diabetes duration of T2DM**	**7,9±5,4**
Comorbidities (n; %)	
HT^{+} (n=232)	232 (66%)
CAD^{++} (n=59)	59 (17%)
CKD^{+++}(n=35)	35 (10%)
CPD^{++++} (n=35)	35 (10%)
Treatment options (n; %)	
Only diet	9 (3%)
$OAD^{\&}$	180 (51%)
OAD+basal Insulin	43 (12%)
Intensive insulin therapy	118 (34%)
Number of follow up visits per year (n; %)	
Less than one visit per year	43 (12%)
One regular visit per year	59 (%17)
Two regular visits per year	132 (%37)
Three regular visits per year	62 (%18)
Four and more regular visit per year	90 (%26)

T1DM*: Type 1 diabetes mellitus; T2DM**: Type 2 diabetes mellitus; HT^{+}: Hypertension; CAD^{++}: Coronary artery disease; CKD^{+++}: Chronic kidney damage; CPD^{++++}: Chronic pulmonary disease; $OAD^{\&}$: Oral antidiabetic drugs.

Only one patient with type 2 DM had been vaccinated against herpes zoster. No patient was vaccinated against measles, diphtheria, or pertussis.

The rates of PV and IV were similar between genders. We observed that the rate of PV (65.5±9.7 vs. 57±9.1 [OR 2.9 (95% CI–14.3 -2.67)]; p=0.005) increased with increasing age, whereas there was no such association between IV and age (Table-2). IV and PV rates increased with an increase in the number of regular DM follow-up visits, but it was only statistically significant for PV (10/38 vs. 28/38 [OR 4 (95% CI -0.994 - 16.096], (p=0.039) (Table 2). The rates of PV and IV were higher in diabetic patients with CPD with a high statistical significance (21/38 vs. 14/312 [OR 52.80 (95% CI 8.4-333,1], p=0.005 and 31/90 vs. 4/260 [OR 29.15 (95% CI 3.37-252.28], p=0.001), respectively. There was also a statistically significant difference between the rates of IV in diabetic patients with CKD than those without (27/90 vs. 8/260 [OR 14.28 95% CI 1.51-133.74], p=0.013). Although the rate of PV was higher in diabetic patients with CKD, we did not find a significant difference in the rate of PV between patients with CKD and without CKD (Table 2). Of the 38 patients who were vaccinated against pneumococcus, 34 also received influenza vaccination. There was a significant association between the rates of two vaccinations (P<0.001). Patient with zoster vaccine uptake had been simultaneously vaccinated against pneumococcus and influenza. There were no statistical differences between the rates of IV and PV regarding education status, treatment characteristics, duration of diabetes, HT, and CAD (Table 2).

Discussion

In this study, we observed lower rates of IV, PV, and ZV in type 1 and type 2 diabetic patients, which were below the targets recommended by the World Health Organization (WHO) (20,21). Childhood vaccines required to protect public health were not administered at all.

VRs, both in general and diabetic populations, vary depending on age, socio-economic status, or regional development. A seven-year retrospective study on the rates of IV among 124,503 diabetic patients in the United States, as an example of a developed country, has demonstrated that IV rates vary between 63% and 69% annually. Vaccinated diabetics were older and had more comorbidities than the non-vaccinated ones (11). According to the National Center for Health Statistics (NCHS) data of the last four years, the rates of IV and PV were 62% and 53%, respectively, with no variation between the years. The rates of both IV and PV in diabetic patients have been demonstrated to increase with income status and age and vary by race and ethnicity (22). In a Canadian study, the rate and trend of IV between 2006/07 and

Table 2. Association of influenza and pneumococcal vaccinations with demographic and clinical factors.

Variables	Influenza vaccination			Pneumococcal vaccination		
	Vaccinated (n=90)	Non vaccinated (n=260)	P value	Vaccinated (n=38)	Non vaccinated (n=312)	P value
Age	59.7±10.1	57.3±9.3	0.288	65.5±9.7	57±9.1	**0.005**
Age						
<65 (n=247)	59 (17%)	188 (54%)		14 (4%)	233 (67%)	
≥65 (n=103)	31(9%)	72 (20%)	0.456	24 (7%)	79 (22%)	0.015
Gender						
Male (n=97)	35 (10%)	62 (18%)		17 (5%)	80 (23%)	
Female (n=253)	55 (16%)	199 (57%)	0.118	21(6%)	232 (66%)	0.150
Education status						
No education	35 (10%)	107(31%)		14 (4%)	128 (36%)	
Primary school	48 (14%)	117 (33%)		20 (6%)	146 (42%)	
High school	7 (2%)	36 (10%)	0.326	4 (1%)	38 (11%)	0.877
University	-	-		-	-	
Diabetes duration (years; mean±SD)	10.4±6.5	8.4±6.3	0.184	8.2±4.9	9±6.5	0.708
Comorbidities (n;%)						
HT* (n=232)	69 (20%)	163(47%)	0.143	31 (9%)	201(57%)	0.212
CAD** (n=59)	20 (6%)	39 (11%)	0.085	10 (3%)	49 (14%)	0.390
CKD***(n=35)	27 (8%)	8 (2%)	**0.013**	12 (3%)	23(7%)	0.090
CPD**** (n=35)	31 (9%)	4 (1%)	**0.001**	21(6%)	14 (4%)	**0.005**
Treatment options						
Only diet (n=9)	2 (0.5%)	7 (2.5%)		0	9 (3%)	
OAD& (n=180)	34 (10%)	146 (42%)		24 (7%)	156 (45%)	
OAD+basal Insulin (n=43)	14 (4%)	29 (8%)		7(2%)	36 (10%)	
Intensive insulin therapy (n=118)	40 (11%)	78 (22%)	0.363	7(2%)	111 (32%)	0.570
Number of follow up visits per year						
Twice or less	36 (10%)	162 (46%)		10 (3%)	187 (53%)	
More than twice	54 (15%)	98 (28%)	0.056	28 (8%)	125 (36%)	**0.039**

HT*: Hypertension; **CAD: Coronary artery disease; ***CKD: Chronic kidney damage; **** CPD: Chronic pulmonary disease.

2013/14 were relatively stable, with an average rate of 40% (23).

Data from European countries have demonstrated that the rates of IV vary between 10-86% (24-28). Some member states of the WHO European Region have reported an increase in vaccination coverage rates in this population, while others have reported a decrease over time (26,28). Among these countries, the Netherlands (>75%) consistently had the highest coverage in the diabetic group, followed by England and Belarus (24,26). Greece had the lowest IV coverage in the diabetic group. Although there was a substantial gap in data on vaccination coverage, it was observed that IV coverage in diabetic patients differed considerably among European countries (25,26,28). The proportion of diabetic patients administered PV varied between 2% and 23% and was much lower than that of IV in these countries (29,30). Turkey is a developing country and a member state of the WHO

European Region. The IV rates observed in our study were lower compared to those in some developed countries such as the United States, Netherlands, and Canada. Similarly, some of the European countries had also reported unsatisfactory outcomes. Our rates of PV were lower in diabetic patients, as in most European developed countries.

However, most of the European countries are developed and have national policies for IV/PV, which allows for a predominant payment mechanism for the coverage of recommended vaccinations by national health insurance (26,31). Thus, differences in VRs among these countries cannot be explained only by financial reasons. They are also influenced by a lack of awareness that infectious diseases may trigger serious complications, inadequate vaccination recommen- dations by physicians, and community perception that vaccination is unnecessary (24).

In developing countries, data for uptake of the recommended vaccines in diabetic patients is insufficient. Rates of IV were reported to vary between 0.4% and 28%, and rates of PV varied between 0.7 and 26% (32-35). The rates of IV and PV in our study were slightly higher compared to the study results from other developing countries, including China, India, Morocco, and Thailand. In contrast to developed countries, the unsatisfactory results of vaccination in developing countries are due to a lack of access to vaccination services, absence of a national vaccination policy incorporating health insurance coverage, and insufficient investment in vaccine manufacturing (36).

Turkish healthcare organizations have precise recommendations for vaccinations in diabetic patients as adopted in developed countries. Although the payment for vaccination is covered by the national health insurance system, the decision on the vaccine uptake is the choice of the diabetic patient. The low VRs observed in our study are probably due to similar reasons, such as low awareness of vaccination-related benefits, which was indirectly supported by the fact that the VR increased with an increase in the number of regular follow-up visits. On the other hand, most of our patients who were vaccinated against one of the infectious agents were observed to have received other recommended vaccines, implying an enhancement in awareness for vaccination. Our observations on the associations between previous IV and PV, and more findings of several studies on prior PV and current PV, indicate that the patients who had received any kind of vaccination were likely to be more vigilant about disease prevention (37,38). The VRs in developed countries increased with the presence of CPD, a higher number of regular follow-up visits, and a longer duration of diabetes (25,38). Our findings regarding the relationship between comorbidities and vaccination status were consistent with the literature. A higher number of follow-up visits affected the PV rates in our population but did not affect the rates of IV. No relationship was observed between the duration of diabetes and VR. Older age was the most predictable factor for vaccination in most studies, although the strength of this association was variable. In our study, only the rate of PV increased with age. Achieving a relatively higher rate of IV than PV without any difference between the age groups could be partially explained by the recurrent influenza outbreaks, which caused an impact on increasing awareness on this infection, as well as encouraging vaccination in almost all age groups in Turkey in recent years. Development of geriatric medicine and initiation of a vaccination program for elderly patients, as part of a preventive arrangement, may have contributed to an increase in rates of PV in the elderly diabetic population, as observed in our study. The rates of IV and PV did not differ across educational level in contrast to the findings of other studies (39,40). The discrepancy between our results and those of others may be explained by the fact that much of our cohort comprised patients without or having a low level of education; thus, preventing a possible comparison.

In Turkey, data on the VRs of diabetic patients are also variable. In elderly diabetic patients living in the Izmir region (n=274; mean age=72±six years), rates of IV and PV were 38.1% and 13.4%, respectively, which were observed to be significantly higher than those in diabetic patients in

general (41). In a study from Antalya (2006, n=1494) revealed that only 111 (7.4%) patients had received influenza vaccine. Of those vaccinated, 13.4% were DM patients (42). Another study from Ankara (n=318; type 1 DM=6.9% and type 2 DM=93.1%; average age=54.7 years) reported that the rates for IV and PV were 14.6% and 3.8%, respectively, which were lower than our results. Similar to our results, there was no relationship between educational status and VRs (43). In a recent study (n=293) from Istanbul, the rates of IV and PV in diabetic patients were 34.1% and 9.9%, respectively (44). In 2013, a large (n=5682) study conducted by Satman reported the rates of IV and PV in Turkish diabetic patients as 27% and 9.8%, respectively (45). The age of patients and duration of diabetes of our cohort were similar to those in Satman's study that characterized the profile of the diabetic population in Turkey. The rates of IV and PV observed in our study were similar to those reported in other studies. In our study, regular follow-up visits were observed to positively influence PV, which was not observed in Satman's study. In Satman's study, diabetics with more severe health conditions were reported to be less likely to be vaccinated, whereas, in our study, certain diseases such as CPD and CKD were positively associated with VRs. Although we had a smaller cohort that represented a limited range of vaccination practice in diabetic patients in Turkey, when we compare our results with those in Satman's study, several important findings were highlighted. Firstly, although our work was conducted eight years after Satman's study, the rates of IV and PV in patients with diabetes were similar to the baseline data from this epidemiological study coming from 2013 (data collection in 2011). This suggested that the VRs did not significantly increase over time. In the referred study, explaining the importance of vaccination to the patients resulted in an increase in IV and PV rates from 27% to 63% and from 9.8% to 41%, respectively. Given the low VRs in our study, physicians who seemed to be the most important factors to enhance the rates of vaccination have not been actively involved in vaccination promotion initiatives over time. Finally, in Satman's study, diabetic patients with more comorbidities and/or familial risk factors had a lower rate of IV and PV uptake at the baseline. Our study revealed increased VRs in diabetic patients with certain conditions such CPD and CKD, compared to those without such conditions. This finding probably indicated an enhancement in the concern about the preventive measures in diabetic patients with comorbidities and showed an improvement in the multidisciplinary approach in preventive measures in high-risk patients.

There are little data on VRs on zona, measles, diphtheria, and pertussis, where the vaccines have been recommended in the context of community protection in patients with diabetes. According to the data from NCHS, the rate of diabetic patients over 60 years of age vaccinated against herpes zoster was 27.9% and 27.2%, respectively. Low uptake of herpes zoster vaccination (4%) was generally observed, with variations based on age, race, and low-income levels. Data on vaccination in diabetic adults against tetanus toxoid, reduced diphtheria toxoid, Tdap, and measles are lacking, but have been reported to range between 8 and 28% in all adult populations during the period from 2012 to2016 (46). In a Brazilian study addressing immunization against measles, mumps, and rubella associated with younger patients, 14.9% of the patients with DM had been reported to have undertaken at least one dose of MMR. But Td vaccine in those diabetic patients was administered at a high rate of 65.5% (47). There were no data on vaccination against measles and Tdap/Td in Turkish diabetic adults.

The limitations of this study were the cross-sectional design and collection of the data based on self-reports, which may pose a challenge in recalling whether or when the vaccination was administered, especially in older patients.

Conclusion

Vaccination rates among adults with DM were below the targets recommended by the WHO. Specific policies are needed to improve the vaccination rates in this risk group of patients.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Müge Bilge, Aylia Yeşilova, Neslihan Özsoy; Design: Aylia Yeşilova, Müge Bilge, Neslihan Özsoy; Mine Adaş; Control/Supervision: Aylia Yeşilova, Müge Bilge, Mine Adaş; Data Collection and/or Processing: Neslihan Özsoy, Aylia Yeşilova, Müge Bilge; Analysis and/or Interpretation: Aylia Yeşilova, Müge Bilge, Mine Adaş, Neslihan Özsoy; Literature Review: Aylia Yeşilova, Müge Bilge, Mine Adaş; Writing the Article: Aylia Yeşilova, Müge Bilge, Mine Adaş; Critical Review: Mine Adaş, Müge Bilge, Aylia Yeşilova; References and Fundings: Müge Bilge, Aylia Yeşilova, Neslihan Özsoy; Materials: Neslihan Özsoy, Müge Bilge, Aylia Yeşilova.

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Metabolic Age: A New Predictor for Metabolic Syndrome

Metabolik Yaş: Metabolik Sendrom İçin Yeni Bir Öngördürücü

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Abstract

Objective: This study aimed to investigate the prevalence of metabolic syndrome (MetS) among the employees of the Tehran University of Medical Sciences, along with presenting a predictor for its identification. **Material and Methods:** 1583 employees from the Tehran University of Medical Sciences (TUMS) participated in our cross-sectional study, who were originally a part of the enrollment phase in the TUMS Employees' Cohort study (TEC). Their basic information, physical activity questionnaire, biochemical blood test, and body composition were obtained through the Bioelectrical Impedance Analysis (BIA), blood pressure, anthropometric measurements, and history of diseases and medication. The prevalence of MetS was determined according to the criteria of the International Diabetes Federation (IDF) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel-III (ATP-III). **Result:** According to the criteria of the IDF, the prevalence of MetS among total participants was 22.2%, where 21.9% were men and 22.4% were women. According to the criteria of ATP-III, the prevalence of MetS was found to be 15%. The prevalence of obesity (BMI≥30) and hyperglycemia (FBS ≥100 mg/dL) among the study participants was 23.4% and 9.7%, respectively. The prevalence of hypertension (SBP ≥130, DBP ≥85 mmHg) and high triglyceride level (TG ≥150 mg/dL) was found to be 14.6% and 19.6%, respectively, while the prevalence of reduced high-density lipoprotein in men and women was found to be 40.3% and 74.7%, respectively.Logistic regression analysis revealed that the predictors of metabolic syndrome were age, sex, physique rate (the evaluated levels of muscle and body fat), and metabolic age (where the BMR of a person was compared to the mean of the BMR of the same age group). **Conclusion:** This study introduces metabolic age as a new predictor of MetS. However, more studies are needed to confirm this association.

Keywords: Metabolic syndrome; body composition; body mass index; physical activity

Özet

Amaç: Bu çalışmada, Tahran Tıp Bilimleri Üniversitesi çalışanları arasında metabolik sendrom (MetS) prevalansını araştırmak ve tanımlanması için bir öngördürücü sunmak amaçlanmıştır. **Gereç ve Yöntemler:** Bu kesitsel çalışmaya, Tahran Tıp Bilimleri Üniversitesi [Tehran University of Medical Sciences (TUMS)]'nden 1583 çalışan katıldı, bu kişiler aslında TUMS Çalışanları Kohort çalışması [TUMS Employees' Cohort study (TEC)]nın kayıt aşamasına dâhildi. Temel bilgileri, fiziksel aktivite anketi, biyokimyasal kan testi ve vücut kompozisyonu Bioelektrik Empedans Analizi [Bioelectrical Impedance Analysis (BIA)], kan basıncı, antropometrik ölçümler ve hastalık ve ilaç öyküsü aracılığıyla elde edildi. MetS prevalansı, Uluslararası Diyabet Federasyonu [International Diabetes Federation (IDF)] ve Ulusal Kolesterol Eğitim Programı Yetişkin Tedavi Paneli-III [National Cholesterol Education Program Adult Treatment Panel-III (NCEP ATP-III)] kriterlerine göre belirlendi. **Bulgular:** IDF kriterlerine göre toplam katılımcılar arasında MetS prevalansı %22,2 idi ve %21,9'u erkek, %22,4'ü kadındı. ATP-III kriterlerine göre MetS prevalansı %15 olarak bulundu. Çalışma katılımcıları arasında obezite (BKİ ≥30) ve hiperglisemi (AKŞ ≥100 mg/dL) prevalansı sırasıyla %23,4 ve %9,7 idi. Hipertansiyon (SKB ≥130 mmHg, DKB ≥85 mmHg) ve yüksek trigliserid düzeyi (TG ≥150 mg/dL) prevalansı sırasıyla %14,6 ve %19,6 olarak bulunurken, erkeklerde ve kadınlarda düşük yoğunluklu lipoprotein prevalansı sırasıyla %40,3 ve %74,7 bulundu. Lojistik regresyon analizi, Mets'in öngördürücülerinin yaş, cinsiyet, vücut oranı (değerlendirilen kas ve vücut yağı seviyeleri) ve metabolik yaş [kişinin bazal metabolizma hızının (BMR) aynı yaş grubundakilerin BMR ortalamasıyla karşılaştırılması] olduğunu ortaya koydu. **Sonuç:** Bu çalışma, metabolik yaşın MetS için yeni bir öngördürücü olduğunu ortaya koymaktadır. Ancak, bu ilişkiyi doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Metabolik sendrom; vücut kompozisyonu; beden kitle indeksi; fiziksel aktivite

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Introduction

Metabolic syndrome is a complex and multi-risk factor in atherosclerotic cardiovascular disease (ASCVD) and type 2 diabetes (1). Metabolic syndrome consists of five risk factors, including atherogenic dyslipidemia, increased blood pressure, dysglycemia, a pro-thrombotic and pro-inflammatory state (2). Metabolic syndrome also multiplies the potential danger of ASCVD and type 2 diabetes by five times (3). About a quarter of the world's population i.e., more than one billion people, are estimated to have metabolic syndrome (4). According to various epidemiological studies, the incidence rate of metabolic syndrome is reported between 20% and 45% (5). Also, many other studies around the world have been reporting the incidence of metabolic syndrome. In 2017, a research work conducted in Iran revealed that the incidence of metabolic syndrome according to the IDF and ATP III criteria was 30% and 25%, respectively (4). In another meta-analysis study in Iran, the incidence of metabolic syndrome in 2017 was found to be 31% (6). A study among Brazilian healthcare providers in 2019 revealed a metabolic syndrome incidence of 24.4% (7), while in the same year, another study found that the incidence rate of metabolic syndrome among the Japanese white-collar workers was 23.1% (8).

Predisposing factors of metabolic syndrome can be categorized into two groups. The first group is based on the metabolic syndrome criteria presented by the IDF, ATP III, and WHO and consists of hypertension, high blood sugar, low HDL, high TG and waist circumference, along with obesity. The second group is reported in various studies and consists of the predictors of metabolic syndrome such as inactivity, age, sex, BMI, smoking, alcohol, TG/HDL ratio, WHR, body shape, and serum uric acid (7,9-17).

New predictors for metabolic syndrome are an important concern for the researchers in this field. And, body composition analysis has been introduced as a new predictor for metabolic syndrome. However, our knowledge in this field needs to improve further. The purpose of this study was to investigate the incidence of metabolic syndrome among the staff of the Tehran University of Medical Sciences and also to find if some components of the body composition analysis such as metabolic age and physique rating could act as the predictors of metabolic syndrome.

Material and Methods

Procedure

The data for this cross-sectional study was taken from the TEC study (Tehran university of medical sciences Employees' Cohort study) during its enrollment phase, which was collected between January 2017 and September 2018. The TEC study further intended to enroll 5500 people from their staff between January 2017 and March 2021 (18). It is designed as a longitudinal study to track the long-term health of their employees. Participants were recruited from different departments. They voluntarily enrolled for the research project upon completing the informed consent form. All the examinations were performed, and the information was collected from the participants in a single day. This study was carried out according to the Helsinki Declaration Principles.

Participants

A total of 1583 participants (1012 women and 571 men), all being TUMS employees from different divisions (clinical, research, service, technical, etc.), were enrolled in this study.

Data collection

The participants were asked about their sex, age, marital status, ethnicity, age of marriage, education level, occupational group, shift work, and tobacco usage. Also, to file a record of diseases in the participants, their current illnesses were examined through general medical examinations. The records included hypertension, type 2 diabetes, hyperlipidemia, thyroid diseases, and medications.

Blood pressure measurement

The blood pressure of the participants was measured thrice, and the average was reported with a precision of one mmHg. The participant was made to sit for a 15 min break, and then the measurements were taken. An interval of 30 minutes was provided between the first and second round of measurements, while a 2-hour interval was

given between the second and third rounds. Blood pressure was measured using a standard and calibrated clinical mercury manometer.

Anthropometry

The weight and height of the participants were measured with a precision of 0.1 kg and 0.1 cm, respectively. They were wearing light clothes with no shoes while the measurements were being taken. The waist circumference was calculated with a precision of 0.1 cm at the anatomical landmarks such as the middle of the lower rib margin and the iliac crest, and the widest portion of the hip. The BMI was measured as the weight (kg) of the participant divided by the square of their height (m).

Blood samples

After 12-hours of fasting, blood samples were taken between 7 and 9 am. The measured parameters in the blood samples included Fasting Blood Sugar (FBS), Triglyceride (TG), total cholesterol (CHO), and high-density and low-density lipoprotein cholesterol (HDL, LDL).

Body composition

One of the most common methods to study and analyze the body shape is the body composition analysis method, which can be performed using different technologies such as using of a Caliper, anthropometry, tracer dilution, densitometry, air displacement plethysmography, dual-energy X-ray absorptiometry, bioelectrical impedance analyzer, computed tomography, magnetic resonance imaging, and 3D body scanning. In the BIA method, the impedance from different tissues of the body is analyzed to predict the composition of the body. A very weak electrical current of 800 microamperes with a frequency of 50 kHz is passed through the body, and the impedance from the tissues is measured against this current. Due to the presence of electrolytes, water demonstrates high conductivity. However, adipose tissues show low conductivity (19,20). The body composition provides quantitative and qualitative information on various tissues such as fat-free mass, fat mass, total water content, bone mineral density and its content, metabolic age, and the physique rate. At the time of measurement, all the metal accessories such as watches, rings, and other jewelry were removed, and all the measurements were performed by the same trained personnel based on the same protocol. The body composition of participants was measured and reported using the bioelectrical impedance analysis (BIA) by the *Tanita® MC-780U Body Composition Analyzer.*

Metabolic age is determined by comparing a person's basal metabolic rate with the average basal metabolic rate that corresponds to a similar age group. It is now emerging as a marker for metabolic health. If the metabolic age is less than the actual age, it means that the body is healthy, but if it is higher than the actual age, it may indicate that the person is not in good health and needs to change their eating and exercising habits and also maybe their lifestyle.

The Physique rate evaluates the levels of muscle and body fat in an individual. It can assess which of the nine body types does one belongs to. The Body Composition Analyzer can be used to assess whether a person is healthy. It is used to measure the fat percentage of the body, muscle mass, and even water and bone content, along with the physique rating. The nine body types, according to the physique rating is as follows:

Hidden Obese: high-fat percentage with a low level of muscle mass.

Obese: high level of fat percentage with a standard level of muscle mass.

Solidly-built: high body fat percentage with a high level of muscle mass.

Under exercised: an average body fat with a low level of muscle mass.

Standard: an average level of body fat with average muscle mass.

Standard Muscular: an average amount of fat percentage with a high level of muscle mass.

Thin: a low amount of body fat with a low level of muscle mass.

Thin and Muscular: a low amount of body fat with a standard level of muscle mass.

Very Muscular: a low amount of body fat with a high level of muscle mass.

International Physical Activity Questionnaire- short form (IPAQ-SF)

The physical activity was calculated using the short form of the IPAQ (International

Physical Activity Questionnaire) along with the MET (the tasks that are equivalent to metabolic activity) hours per week (MET-hours/week). The validity of IPAQ has already been reported (21). Considering the frequency of participation in the activities mentioned over the past week, the MET scores for intense, medium, and hiking activities (for at least 10 min) were multiplied by the time each participant spent on the activity. The scores for the various activities were then summarized as MET-mins/week. Finally, they were categorized into three groups: low, medium, and high activity.

A HIGH-level of activity was scored upon the participant's engagement in vigorously intense activity for at least three days to achieve a minimum total physical activity of at least 1500 MET minutes a week OR 7 or more days of any combination of walking with moderately intense or vigorously intense activities to achieve a minimum total physical activity of at least 3000 MET minutes a week.

A MODERATE level of physical activity was scored upon engagement in 3 or more days of vigorously intense activity and/or walking at least 30 min per day OR 5 or more days of moderately intense activity and/or walking at least 30 min per day OR 5 or more days of any combination of walking with moderately intense or vigorously intense activities to achieve a minimum total physical activity of at least 600 MET minutes a week.

Scoring a LOW level of physical activity on the IPAQ indicated that the participant was not meeting any of the criteria for either MODERATE or HIGH levels of physical activity.

Metabolic syndrome and its components

In this study, the metabolic syndrome was diagnosed according to the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel-III (ATP- III) and the International Diabetes Federation (IDF).

The criteria to diagnose the metabolic syndrome based on ATP III had to fulfill three or more of the following:

1. Waist circumference >=102 cm for men and greater than 88 cm for women,
2. Blood triglycerides >=150 mg/dL or if a person is on high triglyceride medication,
3. HDL cholesterol level of <40 mg/dL for men and <50 mg/dL for women,
4. Fasting blood glucose >=100 mg/dL or if a person is on medication for high blood sugar,
5. Systolic blood pressure (SBP) >130 mmHg or diastolic blood pressure (DBP) >=85 mmHg or if an individual is on medication for hypertension.

The diagnostic criteria for the metabolic syndrome based on the IDF criteria are:

Obesity that is based on the abdominal circumference, which is >94 cm in men and >80 cm in women,

And any two of the following:

Blood triglyceride (TG) more than 150 mg/dL or if a person is on high blood triglyceride therapy,

HDL cholesterol levels of <40 mg/dL in males and < 50 mg/dL in females,

Fasting blood glucose greater than 100 mg/dL or if a person is on high blood sugar medication,

Systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg or if a person is on medication for hypertension.

Metabolic syndrome was assessed based on the above-mentioned criteria.

Statistical analysis

For descriptive statistics, means and standard deviations were used as quantitative variables, and frequency and percentage were used as qualitative variables. For univariate analysis, the Chi-square test was utilized for qualitative variables, while for the quantitative variables, the t-test was used to compare between the two groups, with and without metabolic syndrome. The logistic regression analysis was used to realize which one of our variables were the predictors of metabolic syndrome. The statistical analyses were done using the IBM SPSS®, version 24. P-values of less than 0.05 were considered statistically significant.

Ethical issues

This research was approved by the Ethics Committee for Research at Tehran University of Medical Sciences using the code of ethics IR.TUMS.VCR.REC.1398.246 (10 Jun 2019). We explained the details of the study, including the processes and procedures, to all the participants just before their enrollment, and

they signed and approved the informed consent form. The information of the participants was coded anonymously and kept confidential.

Results

The incidence of metabolic syndrome based on the IDF criteria was equivalent to 22.2% whereas it was 15% based on the ATP-III criteria. We used the IDF criteria in this study since it was stricter (it identifies a higher percentage of individuals having metabolic syndrome). In our study, the incidence rate of metabolic syndrome according to the IDF definition was found to be 21.9% and 22.4% in males and females, respectively.

Physical activity was also classified into three categories, namely high, medium, and low physical activity. According to this study, 70.7% of the overall total population had low physical activity, where 68.4% were women, and 74.8% were men. Table 2 shows the details of the physical activity performed by the participants based on their gender.

Table 3 presents the variables other than the IDF criteria, which includes age, sex, physical activity, metabolic age, and physique rating in both the groups, with and without metabolic syndrome.

In the next step, logistic regression was employed to find the predictors of the metabolic syndrome among the participants. In this model, Nagelkerke R Square was found to be 0.176. Table 4 here exhibits the results.

Discussion

This research study aimed to review the incidence rate of metabolic syndrome and its predictors among the employees of the Tehran University of Medical Sciences, resulting in an incidence rate of 22.2% and 15.0% based on the IDF and ATP-III criteria, respectively.

das Merces et al. in 2019 reported the incidence of metabolic syndrome in Brazilian healthcare providers as 24.4% based on the ATP-III criteria, which is about 10% more than our results (7). However, in another study, Brazilian healthcare providers showed a much lower incidence rate of 4.5% (22). This discrepancy may be due to different categorizations of the participants in both the studies based on the existence of metabolic syndrome. Moreover, the results of Mango et al. were closer to ours. In a 2019 study, the incidence of metabolic syndrome in the Japanese white-collar employees was found to be 19.5% (8). In the same year, the incidence rate of metabolic syndrome in Iranian petrochemical workers was revealed as 18.4% as per the ATP-III criteria (23), and in Korean staff, the incidence of metabolic syndrome was found to be 19.8% according to the criteria of ATP-III (9). In a study close to our research, the incidence of

Table 1. Basic information on the data and elements of metabolic syndrome and their incidence.

	Total **n=1583** Mean (SD)	**Male** **n=571** Mean (SD)	**Female** **n=1012** Mean (SD)	**p value**	**Incidence*** n (%)
Age (year)	43.0 (8.7)	44.0 (9.2)	42.4 (8.4)	0.002	-
TG (mg/dL)	119.1 (58.7)	137.4 (73.0)	108.7 (45.8)	<0.001	311 (19.6)
HDL (mg/dL)	43.3 (9.3)	42.2 (8.0)	43.9 (9. 9)	0.007	986 (62.3)
FBS (mg/dL)	86.0 (20.8)	89.7 (26.2)	84.1 (16.7)	<0.001	153 (9.7)
BP systolic (mmHg)	115.7 (12.8)	120.7 (12.7)	112.9 (12.0)	<0.001	230 (14.5)
BP diastolic (mmHg)	77.4 (8.2)	79.8 (8.6)	76.1 (7.8)	<0.001	
BMI (kg/m^2)	27.2 (4.5)	27.6 (4.3)	27.0 (4.7)	<0.001	371 (23.4)
Waist circumference (cm)	88.7 (11.6)	96.1(9.6)	84.5 (10.4)	<0.001	985 (62.2)
Metabolic Age (year)	41.1 (12.5)	42.9 (11.8)	40.0 (12.8)	<0.001	-

*According to IDF criteria.
TG: Triglyceride; HDL: High-density lipoprotein; FBS: Fasting Blood Sugar; BP: Blood pressure; BMI: Body mass index.

Table 2. Levels of physical activity among the participants based on their gender.

Physical activity status	Total n (%)	Male n (%)	Female n (%)	p value
Low	1119 (70.7)	427 (74.8)	692 (68.4)	
Moderate	366 (23.1)	114 (20)	252 (24.9)	0.027
High	98 (6.2)	30 (5.3)	68 (6.7)	

Table 3. Descriptive statistics of the study variables in both the groups, with and without metabolic syndrome.

	With Metabolic Syndrome			Without Metabolic Syndrome			
	Total	Male	Female	Total	Male	Female	
	n=352	n=125	n=227	n=1231	n=446	n=785	p value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age (year)	47.0 (7.9)	47.3 (8.8)	46.8 (7.3)	41.8 (8.6)	43.0 (9.1)	41.1 (8.2)	<0.001
Physical Activity met/min/week	481.1 (619.2)	401.5 (484.2)	524.9 (679.2)	544.4 (799.4)	533.7 (884.2)	550.3 (747.5)	<0.001
Metabolic Age (year)	48.6 (11.2)	50.0 (10.9)	47.8 (11.3)	38.9 (12.0)	40.9 (11.3)	37.8 (12.3)	<0.001
Physique Rating	29.3 (8.1)	26.1 (7.1)	31.1 (8.0)	34.8 (9.9)	31.1 (9.6)	36.1(9.7)	<0.001

SD: Standard deviation.

Table 4. Metabolic syndrome predicting the elements.

				95% C. I for EXP(B)	
Variable	B	Odds Ratio	Sig	Lower	Upper
Metabolic age	0.037	1.037	0.002	1.014	1.061
Physique rating	0.028	0.972	0.039	0.946	0.999
Age	0.050	1.051	<0.001	1.028	1.074
Sex	0.299	1.349	0.034	1.023	1.780
Constant	-4.648	0.010	<0.001		

metabolic syndrome in Iranian health workers was found to be 22.4% (24). Also, the incidence of metabolic syndrome among hospital health workers in Nigeria and Kenya were 24.2% and 34%, respectively (25, 26). The incidence was also 21.2% in developing countries such as Ghana, as per the IDF criteria, where the incidence was proven to be greater in females than in males (11). These results were similar to our study, as well. In 2016, the incidence of metabolic syndrome in Japanese healthcare workers was 8.7% (27), which was very different from our results and could be due to the differences in the lifestyle. A meta-analysis of the metabolic syndrome among the Chinese people was reported to be 24.5% (28), while the incidence among employees who participated in the Aragon Workers' Health Study (AWHS) was reported as 27.1% (29). Many studies have compared the incidence of metabolic syndrome between the employees with sedentary jobs and the ones with active jobs. The difference can be seen in some studies; for example, a study reported an incidence of metabolic syndrome in office workers to be 33% but found only a 14% incidence rate in firefighters (30). Various studies have also reported the incidence of the metabolic syndrome within different societies, which ranges from 20% to 35%, especially in developing countries. This incidence accounts for at least one-fifth of the population and could be an alarming

signal for ASCVD and type 2 diabetes (31). Abdominal obesity is the most common part of metabolic syndrome, and many studies have shown a direct relationship between abdominal obesity and the incidence of metabolic syndrome. These studies also validate our results (32-34).

Some studies argue that metabolic syndrome occurs at an older age. For example, at the age of 29 years, the incidence of metabolic syndrome accounted for as much as one-third of the total population, while at the age of 50 years, half of the population was having metabolic syndrome.

In our study, the mean±SD of the age in the group with metabolic syndrome was found to be 47±7.9 years, whereas the mean ±SD of the age in the non-metabolic syndrome group was shown as 41.8±8.6 years.

Many studies have explored the predictive components of metabolic syndrome, such as levels of HDL, TG, FBS, waist circumference, and elevated blood pressure (35).

In addition to the components of MetS described by the criteria of the IDF, NCEP ATP-III, and WHO, other predictors have also been introduced in various studies, such as inactivity, age, sex, BMI, smoking, alcohol usage, TG to HDL ratio, waist to hip ratio, serum uric acid, and leptin (7,9-16,36). However, in our study, age and sex were identified as predictors of metabolic syndrome based on the logistic regression analysis.

Metabolic age is a new term used to describe the overall fitness and the metabolic activity of an individual and is obtained by comparing the basal metabolism of a person with the mean basal metabolism of the same age group. If the metabolic age of a person was found higher than their chronological age, it indicated a level of basic metabolism with low physical activity. Metabolic age can be a useful tool for assessing the metabolic status of individuals. A study by the European Society of Cardiology (ESC) used metabolic age as one of the predictors for cardiovascular disorders in people having a higher metabolic age than their chronological age (37).

In addition to the known variables of metabolic syndrome, we also used results of body composition analysis as probable predictors of metabolic syndrome. However, our main target was to confirm if the body composition results could predict metabolic syndrome. The results indicated that metabolic age and physique rating could be considered as independent predictors of metabolic syndrome.

We also investigated if metabolic age could predict metabolic disorders in individuals.

Basal Metabolic Rate (BMR) changes with age (38). Metabolic age is the comparison of the BMR of a person with the mean BMR of the same age group (39). According to the logistic regression analysis, metabolic age can be a new predictor for metabolic syndrome. Also, physique rating, which indicates the body-type (40), can be a simple predictor of metabolic syndrome. In 2016, a study found that type 2 diabetic individuals were significantly different from the control in terms of physique rating (41).

Our results might open the door to the world of metabolic syndrome.

The R-square of the model of regression in our study reached 0.176, which is not good enough, and the odds ratios were also small. Hence, we recommend other researchers to consider studies with more variables that could affect the metabolic syndrome. This can help in creating a better picture of metabolic syndrome along with its anticipating factors.

Conclusion

This study introduces metabolic age as a new predictor of metabolic syndrome. However, more studies are needed to confirm this association.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Ramin Mehrdad, Mohadeseh Vesal; Design: Zahra Banafsheh Alemohammad; Control/Supervision: Mohadeseh Vesal; Data Collection and/or Processing: Gholamreza Pouryaghoub, Mohadeseh Vesal; Analysis and/or Interpretation: Ramin Mehrdad; Literature Review: Writing the Article: Hamidreza Pouragha, Mohadeseh Vesal; Critical Review: Ramin Mehrdad.

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Does Blood Glucose Regulation in Adults with Type 2 Diabetes Affect Exocrine Pancreatic Functions?

Tip 2 Diyabetli Erişkinlerde Kan Şekeri Regülasyonu Ekzokrin Pankreas Fonksiyonlarını Etkiler mi?

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Abstract

Objective: The purpose of this study was to assess the possible effects of blood glucose regulation on pancreatic exocrine functions in type 2 diabetes mellitus (T2DM) patients with poor glycemic control. **Material and Methods:** This prospective clinical study was performed with 20 patients with poorly controlled T2DM (HbA1c >10%) and age- and sex-matched 20 healthy controls. At the beginning of the study, metabolic parameters and fecal elastase-1 (FE-1) levels, one of the markers of pancreatic exocrine insufficiency (PEI), were compared between the patient and control groups. In addition, after blood glucose regulation was achieved with at least three months of intensive insulin therapy in the patient group, FE-1 levels and metabolic parameters were compared with pre-treatment. PEI was defined as FE-1 levels lower than 200 µg/g. **Results:** FE-1 levels were significantly lower in the T2DM group than the control group (median values for patients=333.1 µg/g and controls= 508.5 µg/g; p=0.013). PEI was detected in three patients (15%) but none in the control group. After intensive insulin therapy, T2DM patients FE-1 levels significantly increased compared to their pre-treatment (pre-treatment median: 333.15 (192.60) µg/g, post-treatment median: 415.40 (300.77) µg/g; p=0.044). The major factors impacting this increase were the duration of diabetes and the change in HbA1c levels. **Conclusions:** FE-1 levels in patients with poorly controlled T2DM were lower than the healthy control group, which significantly increased with blood glucose regulation.

Keywords: Type 2 diabetes mellitus; exocrine pancreatic insufficiency; pancreatic elastase 1

Özet

Amaç: Bu çalışmada, glisemik kontrolü zayıf olan Tip 2 diabetes mellitus hastalarında kan şekeri regülasyonunun pankreas ekzokrin fonksiyonları üzerine etkisini değerlendirmek amaçlandı. **Gereç ve Yöntemler:** Bu prospektif klinik çalışma, kan şekeri regülasyonu bozuk (HBA1c >10) Tip 2 diyabetes mellituslu 20 hasta ile cinsiyet ve yaş uyumlu 20 sağlıklı kontrol grubundan oluşan bir popülasyonda gerçekleştirilmiştir. Çalışmanın başlangıcında pankreas ekzokrin yetmezliği belirteçlerinden biri olan fekal elastaz 1 (FE-1) düzeyleri ve metabolik parametreler hasta ve kontrol grubu arasında karşılaştırıldı. Ayrıca hasta grubunda en az 3 ay yoğun insülin tedavisi ile kan şekeri regülasyonu sağlandıktan sonra FE-1 düzeyleri ve metabolik parametreler tedavi öncesi ile karşılaştırıldı. Fekal elastaz 1 düzeylerinin 200 µg/g'ın altında olması pankreas ekzokrin yetmezliği olarak tanımlandı. **Bulgular:** Diyabetik hasta grubunda kontrol grubuna göre FE-1 düzeyleri anlamlı olarak daha düşük bulundu (Medyan: Hasta grubu=333,1 µg/g; Kontrol grubu= 508,5 µg/g; P=0,013). Hasta grubunda 3 (%15) hastada ekzokrin pankreas yetmezliği görülürken kontrol grubunda ekzokrin pankreas yetmezliği görülmedi. Hasta grubunda FE-1 düzeyleri kan şekeri regülasyonu öncesine göre anlamlı olarak arttı (tedavi öncesi medyan=333,15 (192,60) µg/g, tedavi sonrası medyan=415,40 (300,77) µg/g; P=0,044) ve bu artışa etki eden majör faktörler diyabet süresi ve HbA1c düzeyindeki değişimdi. **Sonuç:** Çalışmamızda kan şekeri regülasyonu bozuk Tip 2 diabetes mellitus hastalarında fekal elastaz 1 düzeyleri sağlıklı kontrol grubuna göre daha düşüktü ve bu hastalarda kan şekeri regülasyonunun sağlanması FE-1 düzeylerini anlamlı olarak artırdı.

Anahtar kelimeler: Tip 2 diabetes mellitus; ekzokrin pankreas yetersizliği; pankreatik elastaz 1

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Introduction

The pancreas is a retroperitoneal organ with exocrine and endocrine functions. It is postulated that pancreatic exocrine insufficiency (PEI) could occur in diabetic patients or vice versa due to the close anatomical and physiological relation between the pancreatic exocrine and endocrine components (1-3). The increased global prevalence of diabetes and PEI's coexistence has drawn attention in recent years. Diabetes mellitus can develop due to decreased numbers and functional capacity of islet cells in patients developing PEI secondary to diseases of the pancreas or pancreatic resection (2). Moreover, PEI can also develop in diabetic patients through several mechanisms, including changes in the regulatory effect of hormones secreted by the pancreatic islets on exocrine pancreatic functions, pancreatic fibrosis and atrophy due to diabetic microangiopathy, and impairment of entero-pancreatic reflexes due to diabetic neuropathy and gastroparesis (3,4). It was reported that acute hyperglycemia might play a role in PEI development by inhibiting exocrine pancreatic enzyme secretion and increasing the proliferation and activation of pancreatic stellate cells causing pancreatic fibrosis (3).

Traditionally, direct stimulation tests like the secretin stimulation test, an invasive test, are accepted as the gold standard for evaluating pancreatic exocrine functions. However, recently, pancreatic fecal elastase-1 (FE-1), a non-invasive procedure, is validated as the diagnostic test used progressively. FE-1 is secreted into the intestine among the pancreatic exocrine secretions and excreted unaltered in the stool. FE-1 measurement is relatively inexpensive, reliable, and easy to use (5).

Studies have reported inconsistent effects of pancreatic enzyme replacement therapy on blood glucose regulation in diabetes and PEI patients (6-9). However, to the best of our knowledge, no study has investigated glycemic control's effect on pancreatic exocrine functions in diabetic patients with poor glycemic control. Our study aimed to assess the impact of blood glucose regulation on pancreatic exocrine functions in type 2 diabetes mellitus (T2DM) patients with poor glycemic control.

Material and Methods

Patients and Study Design

This prospective study considered 28 patients with poor glycemic control (glycated hemoglobin, HbA1c >10) T2DM admitted to Karadeniz Technical University Medical Faculty Hospital Endocrinology Clinic between July 2017 to August 2018. The Control group consisted of age and sex that matched 20 healthy individuals selected among healthy volunteers presenting to the hospital's Family Medicine Clinic for screening purposes during June-August 2018. T2DM was diagnosed based on the American Diabetes Association criteria (10). Patients younger than 30 or older than 70, consuming more than 20 g alcohol a day, using Orlistat, Acarbose, or Gliptins, with a medical history of abdominal surgery or known pancreatic disorders, any other known reason for malabsorption, or with cancer, inflammatory bowel disease or autoimmune disease, and pregnant women were excluded from the study. None of the patients were receiving pancreatic enzyme replacement therapy. Detailed medical history was taken from patients eligible for the study and admitted to the hospital for blood glucose regulation following a thorough physical examination. Patients' metabolic parameters were assessed before the initiation of therapy. Stool specimens collected for quantifying FE-1 levels were stored at -80 °C for further analysis. Patients who previously received only oral anti-diabetic therapy were started on insulin therapy, adjusted for those who previously received such therapy. Fasting and postprandial blood glucose targets were <130 mg/dL and <180 mg/dL, respectively. Basal plus bolus insulin regimen was started at 0.5 units per kg patients who previously were on only oral anti-diabetic therapy. In patients receiving basal insulin previously, the insulin doses were adjusted to at least 0.5 units per kg body weight, and the bolus insulin regimen was initiated. Insulin doses were adjusted by doing two visits per week until the target blood glucose was reached. Subsequently, patients' metabolic parameters were re-investigated at clinical follow-ups at least three months, and stool specimens were again collected and stored

at -80 °C. Blood specimens from the control group collected at the time of presentation were also studied, and stool specimens were stored at -80°C for the study. The study was carried out based on the guidelines of the Declaration of Helsinki. Informed consent forms were obtained from the patient and control groups and approved by the Ethics Committee of Karadeniz Technical University (No:2017/126).

Laboratory Examinations

Biochemical parameters such as fasting blood glucose, amylase, triglyceride, total cholesterol, C-reactive protein, alanine aminotransferase, blood urea, nitrogen, creatinine, albumin, total protein, calcium, phosphorus, magnesium were assayed using a Beckman Coulter AU 5800 autoanalyzer (Shizuoka, Japan) at the time of recruitment. Serum C-peptide levels were studied using the chemiluminescence immunoassay method on a Siemens Immulite 2000 XPi analyzer (Walpole, USA) and HbA1c levels using HPLC (Boronate affinity) on a Trinity Biotech Premier Hb9210 device (Kansas City, USA). Serum total 25 (OH)-vitamin D3 levels were studied using the chemiluminescence immunoassay method on a Beckman Coulter Unicel DXI 800 device (Minnesota, USA). At the end of the study, FE-1 levels in stools were quantified using a commercial kit to detect PEI's presence using enzyme-linked immunosorbent assay (Pancreatic Elastase ELISA, Bioserv Diagnostics GmbH, United Kingdom) and expressed as µg/g of stool. PEI was divided into three groups according to FE-1 levels (normal pancreatic exocrine function: FE-1 ≥200 µg/g stool, mild to moderate PEI: FE-1 ≥100 but <200 µg/g stool, or severe PEI: FE-1 <100 µg/g stool) (4).

Statistical Analysis

All statistical analyses were performed on the Statistical Program for Social Sciences software (SPSS 23.0) for Windows (Chicago, IL, USA). The normality of data distribution was assessed by the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean±standard deviation, and non-normally distributed data as median (IQR). Student's t-test, the paired samples test, and Wilcoxon, chi-square, and Mann-Whitney tests were applied to assess the significance of inter-group differences. Linear regression analysis was performed, taking into account the interactions between the variables. In the first model, the initial fecal elastase level was used as the dependent variable, and age, body mass index (BMI), initial HbA1c, 25 (OH)-Vitamin D3, magnesium, triglyceride, blood fasting glucose, and total cholesterol levels as independent variables. In the second model, fecal elastase differences were the dependent variable, whereas age, BMI, differences of HbA1c, 25 (OH)-Vitamin D3, triglyceride, fasting glucose, and duration of diabetes mellitus were independent variables. The Enter method was used in the analysis (11). Statistical significance was accepted as $p<0.05$.

Results

Five of the 28 participants included in the study subsequently dropped out due to non-compliance with treatment, and three due to failure to attend control examinations. Therefore, twenty patients with T2DM and 20 healthy controls were included in the analysis. The patient group consisted of 12 (60%) men and 8 (40%) women, and the control group of 9 (45%) men and 11 (55%) women. Mean age was 50.7±10.0 and 52.1±9.3 years in the patient and control groups, respectively. The patient and control groups were comparable in terms of age and gender. Demographic characteristics and laboratory parameters of participants are tabulated (Table 1). The mean follow-up duration of the patients in the study was 94.50 days (IQR:11.50). Five patients received only insulin therapy before the study, while 15 received combination therapy (oral antidiabetics and insulin). The median duration of diabetes was 5.00 years (IQR:4.75). At the beginning of the study, fasting blood glucose, HbA1c, and blood urea nitrogen levels were significantly higher in the patients than in the control group. In contrast, 25 (OH)-Vitamin D3, magnesium, and FE-1 levels were significantly lower (Table 1). In three (15%) patients, PEI was observed (one severe and two mild cases), but none from the control

Table 1. Baseline socio-demographic and laboratory findings among type 2 diabetic patients and healthy control groups.

Sociodemographics & laboratory findings	Patient group (n=20)	Control group (n=20)	P value
Age (years)*	50.7±10.0	52.1±9.3	0.650
Sex (n, %)			
Female	8 (40)	11 (55)	0.527
Male	12 (60)	9 (45)	
BMI (kg/m^2)**	31.5 (6.3)	29.1 (7.4)	0.200
Hemoglobin (g/dL)*	14.2±1.7	13.8±0.9	0.377
Fasting blood glucose (mg/dL)**	179.00 (145.00)	87.0 (13)	<0.001
HbA1c (%)*	11.5±1.3	5.6±0.3	<0.001
Total cholesterol (mg/dL)*	218.0±49.3	216.1±34.7	0.851
Triglycerides(mg/dL)**	143.5 (70.0)	112.0 (69.2)	0.072
25 OH Vitamin D3(µg/L)**	14.0 (6.9)	21.9 (12.0)	0.001
Albumin (g/L)**	4.15 (0.6)	4.2 (0.4)	0.162
Total Protein (g/L)*	7.0±0.5	7.3±0.5	0.147
Blood urea nitrogen (mg/dL)**	15.0 (9.2)	13.0 (2.0)	0.037
Creatine (mg/dL)**	0.7 (0.3)	0.8 (0.3)	0.850
Alanine aminotransferase (U/L)**	25.5 (30.7)	20.0 (9.0)	0.303
Amylase (U/L)**	59.5 (36.7)	81.0 (52.5)	0.106
Calcium (mg/dL)**	9.4 (0.8)	9.8 (0.7)	0.063
Phosphorus (mg/dL)**	3.6 (0.9)	3.1 (0.8)	0.183
Magnesium (mg/dL)**	1.9 (0.2)	2.1 (0.1)	0.003
C-reactive protein (mg/L)*	0.8±0.9	0.5±0.4	0.267
Fecal elastase 1(µg/g of stool)**	333.1 (192.60)	508.5 (130.13)	0.013

*Mean±standard deviation; **Median (interquartile range).
BMI: Body mass index; HbA1c: Glycated hemoglobin.

group. Analysis of the patient group revealed a significant decrease in fasting blood glucose, HbA1c, triglyceride, and alanine aminotransferase levels following at least three months of intensive insulin therapy compared to pre-treatment levels. In contrast, 25 (OH)-Vitamin D3, albumin, total protein, calcium, and FE-1 levels significantly increased (Table 2). Linear regression analysis was performed to examine the factors affecting the change in FE-1 levels after blood glucose regulation in the patient group. It was found that the significant factors were HbA1c levels and duration of diabetes mellitus (p=0.047 and 0.036, respectively, Table 3).

Discussion

The present study investigated fecal elastase levels and various metabolic parameters in T2DM patients with poor glycemic control and a healthy control group to evaluate PEI. The study's analysis revealed significantly lower fecal elastase levels in the T2DM patients than the control group (case group, median: 333.1 µg/g; control group, median: 508.5 µg/g; p=0.013). PEI was detected in 15% of the diabetic cases (prevalence of FE-1 <200 µg/g and <100 µg/g were 10% and 5%, respectively), but none in the control group.

Although in earlier studies employing invasive tests, meta-analysis evaluating the PEI prevalence in diabetic patients reported an average of 52.4% (18-100%), the mean prevalences of PEI using non-invasive tests was about 40% (26-74%) in type 1 diabetes patients and 27% (10-56%) in T2DM patients (3). Although patients with poor glycemic control were included in our study, PEI prevalence was lower than in other studies. In recent studies, a lower prevalence (between 9.2% and 13%) of PEI in diabetic patients was reported, concordant

Table 2. Laboratory findings and fecal elestasis-1 levels in patients with Type 2 diabetes mellitus before and after insulin treatment.

	Baseline	After the third month	p value
Hemoglobin (g/dL)*	14.2±1.6	14.1±1.5	0.523
Fasting blood glucose (mg/dL)**	179.0 (145.0)	121.0 (72.0)	0.003
C peptide (µg/L)**	2.3 (2.2)	1.6 (1.4)	0.076
HbA1c (%)*	11.5±1.3	7.1±1.0	<0.001
Total cholesterol (mg/dL)*	218±49.3	209±52.6	0.252
Triglycerides (mg/dL)**	143.5 (70.0)	99.0 (80.2)	0.003
25 OH Vitamin D3(µg/L)**	14.0 (6.9)	15.6 (9.4)	0.033
Prothrombin time (sec)	11.6±1.2	11.6±1.0	0.694
INR*	1.0±0.1	1.0±0.9	0.659
Albumin (g/L)**	4.1 (0.6)	4.3 (0.6)	0.020
Total Protein (g/L)*	7.0±0.5	7.3±0.4	0.009
Blood urea nitrogen (mg/dL) *	15.0 (9.2)	14.0 (6.7)	0.061
Creatine (mg/dL)**	0.7 (0.3)	0.7 (0.2)	0.615
Alanine aminotransferase (U/L)**	25.5 (30.7)	16.0 (17.7)	0.014
Amylase (U/L)**	59.5 (36.7)	63.0 (40.0)	0.057
Calcium (mg/dL)**	9.4 (0.8)	9.6 (0.6)	0.036
Phosphorus (mg/dL)**	3.5 (0.9)	3.4 (0.8)	0.723
Magnesium (mg/dL)**	1.9 (0.2)	2.0 (0.2)	0.355
C-reactive protein (mg/L)*	0.8±0.9	0.8±1.1	0.834
Fecal elastase 1 (µg/g of stools)**	333.1 (192.6)	415.4 (300.8)	0.044

* Mean±standard deviation, **Median (IQR).
HbA1c: Glycated hemoglobin.

Table 3. Linear regression analysis of factors affecting differences in fecal elastase in patients with type 2 diabetes mellitus.

	Coefficients[a]			
	Unstandardized coefficients		95.0% confidence interval for B	
Model	B	p value	Lower bound	Upper bound
(Constant)	152.55	0.600	-464.68	769.78
Difference at HbA1c	-59.34	0.047	-117.63	-1.04
Duration of diabetes mellitus	15.90	0.036	1.26	30.53
Age (years)	-0.07	0.982	-6.92	6.77
Body Mass Index (kg/m^2)	-12.41	0.101	-27.62	2.80
Difference in fasting glucose	-0.55	0.084	-1.17	0.08
Difference in 25 OH Vitamin D3	-0.33	0.956	-13.10	12.44
Difference in triglycerides	0.52	0.224	-0.36	1.40

a. Dependent Variable: Difference in fecal elastase

to our study (9,12). Vujasinovic et al. reported a low prevalence of PEI (5.4%) in a study of 150 patients with type-1 and type-2 diabetes, which was attributed to the exclusion of alcohol use and other causes of malabsorption (13). The inconsistencies between studies may be due to differences in patient selection and diagnostic methods. Patients with pancreatogenic diabetes (Type 3c) emerging secondary to pancre-

atic diseases such as chronic pancreatitis were generally not excluded from older studies, reporting higher PEI prevalence. However, patients with pancreatic disease were generally excluded from subsequent studies.

The relationship between glycemic control levels and PEI in diabetic patients is still not understood. Some studies have reported lower FE-1 levels in diabetic patients with poor glycemic control than patients with good glycemic control (14-16), while others have reported no difference (17,18). PEI rate increases with the rise in blood glucose regulation impairment. In our study, blood glucose regulation in patients with poorly controlled diabetes mellitus was performed employing intensive insulin therapy lasting at least three months. Significantly increased FE-1 levels was obtained in these patients compared to pre-treatment levels (pre-treatment median 333.1 (192.6) µg/g, post-treatment median: 415.4 (300.8) µg/g; p=0.044) (Table 2).

To the best of our knowledge, the present study is the first to reveal the effect of glycemic control in poorly controlled T2DM patients on pancreatic exocrine functions. Blood glucose regulation in diabetic patients with uncontrolled blood glucose improves acute and chronic complications associated with hyperglycemia and improves pancreatic exocrine functions. Pancreatic exocrine enzymes are responsible for absorbing fat and fat-soluble vitamins and proteins (19). Regulation of exocrine functions in patients with PEI, independent of the etiology, significantly increases the absorption of fat and fat-soluble fats and proteins and alleviates clinical symptoms such as bloating and abdominal discomfort (19,20). Improvement of exocrine functions in diabetic patients can also help establish blood glucose regulation by improving the digestion and absorption of these foods (6,7,21,22). In our study, significant improvements in 25 (OH)-Vitamin D3, total protein, albumin, and calcium values were observed in patients' after intensive insulin treatment. Improvement in exocrine pancreatic functions and diabetes regulation may have a role in these nutritional markers' progress.

However, there are some limitations in this study. In particular, the sample size was small as our study investigated T2DM patients with poor glycemic control (HbA1c >10), resulting in low statistical power. Another limitation was that other nutritional markers, such as vitamin A and E for PEI, could not be studied. Also, in some patients, the pancreatic structure could not be evaluated using ultrasonography or other imaging techniques. Pancreatogenic diabetes (type 3c) emerging secondary to pancreatic diseases such as chronic pancreatitis is sometimes misdiagnosed as T2DM (10). However, none of our patients had a history of alcohol use, and thus we considered that all our patients had Type 2 diabetes.

In conclusion, in this study, FE-1 levels were lower in T2DM patients with poor glycemic control than in the healthy control group. Although our patients consisted of poor glycemic control, PEI prevalence was lower than in previous studies. The establishment of blood glucose regulation with intensive insulin therapy significantly increased FE-1 levels, and the main factors affecting these changes were the duration of diabetes and shift in HbA1c. Therefore, blood glucose regulation is crucial for micro and macrovascular complications and improved pancreatic exocrine functions. Thus, this study has some implications for better understanding the pathogenesis and treating pancreatic inflammation and chronic pancreatitis.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Sami Fidan, Savaş Volkan Kiioğlu, Halil Önder Ersöz, Orhan Özgür; Design: Sami Fidan, Savaş Volkan Kişioğlu, Celal Kurtuluş Buruk, Elif Ateş, Arif Mansur Coşar; Control/Supervision: Sami Fidan, Savaş Volkan Kişioğlu, Arif Mansur Coşar, Halil Önder Ersöz, Orhan Özgür; Data Collection and/or Processing: Sami Fidan, Savaş Volkan Kişioğlu, Celal Kurtuluş Buruk, Elif Ateş, Arif Mansur Coşar; Analysis and/or Interpretation: Sami Fidan, Savaş Volkan Kiioğlu, Halil Önder Ersöz, Orhan Özgür; Literature Review: Sami Fidan, Savaş Volkan Kişioğlu, Celal Kurtuluş Buruk, Elif Ateş; Writing the Article: Sami Fidan, Savaş Volkan Kişioğlu, Celal Kurtuluş Buruk, Elif Ateş, Arif Mansur Coşar, Halil Önder Ersöz; Critical Review: Sami Fidan,Savaş Volkan Kişioğlu, Arif Masur Cosar, Orhan Özgür; Materials: Sami Fidan, Savaş Volkan Kişioğlu, Celal Kurtuluş Buruk.

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Serum Irisin Levels in Cigarette Smokers

Sigara İçenlerde Serum İrisin Düzeyleri

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Abstract

Objective: Irisin, a newly discovered myokine, increases thermogenesis and energy consumption, especially after exercise. It has also been reported to stimulate the transformation of white fat cells into brown fat cells. Thus, it causes weight loss and increases the sensitivity to insulin. Nicotine affects the energy metabolism of the body through several mechanisms. Cigarette smokers are widely known to show decreased weight gain and increased weight loss. Since irisin and smoking/nicotine have similar effects on energy metabolism, this study proposed to determine the serum irisin levels in smokers. **Material and Methods:** Thirty-one healthy smokers aged 17-24 years and 31 age-matched healthy non-smokers were included in this study. Serum irisin levels were determined using ELISA. **Results:** The smokers had been smoking for an average of 31±19 (2-84) months and smoked an average of 13.7±0.4 (5-25) cigarettes daily. The smokers had higher height and body weight and levels of serum creatinine and triglyceride and lower levels of HDL-C than non-smokers ($p<0.05$). Serum irisin levels and body mass index were not different between the two groups ($p>0.05$). The serum irisin level was correlated only with serum creatinine level in the entire group ($r=-0.26$, $p<0.05$). The serum irisin levels were not correlated with the duration and amount of cigarette smoking, as well as levels of serum glucose, insulin, total cholesterol, triglyceride, LDL-C, HDL-C, alanine aminotransferase, free T4, and TSH in the entire group ($p>0.05$). **Conclusion:** Serum irisin level in cigarette smokers is not different to that of non-smokers. It is not related to the duration and amount of cigarette smoking. Further prospective dose and time-controlled studies are needed to investigate whether similar effects of smoking/nicotine exposure on energy metabolism are related to irisin metabolism.

Keywords: Cigarette; smoking; irisin; body weight; body mass index

Özet

Amaç: İrisin, özellikle egzersizden sonra termogenezisi ve enerji harcanmasını artıran yeni tanımlanmış bir miyokindir. Aynı zamanda beyaz yağ dokusu hücrelerine, kahverengi yağ dokusu hücresi özellikleri kazandırdığı rapor edilmiştir. Böylece kilo kaybına neden olur ve insülin duyarlılığının artışına neden olur. Nikotin, pek çok değişik mekanizma ile vücut enerji metabolizmasını etkilemektedir. Sigara içenlerde kilo alımının azaldığı ve kilo kaybının arttığı yaygın olarak bilinmektedir. İrisin ve sigara içiminin/nikotinin enerji metabolizmasında benzer etkileri olduğu görüldüğü için bu çalışmada sigara içenlerde serum irisin düzeylerinin tayini planlanmıştır. **Gereç ve Yöntemler:** Bu çalışmaya, yaşları 17-24 yıl arasında değişen 31 sağlıklı, sigara içen birey ile yaş açısından benzer 31 sağlıklı, sigara içmeyen birey dâhil edildi. Serum irisin düzeyleri ELISA yöntemi ile tayin edildi. **Bulgular:** Sigara içenlerde sigara içimi süresi ortalama 31±19 (2-84) ay ve içilen sigara adedi ortalama 13,7±0,4 (5-25) idi. Sigara içen bireylerin boy uzunluğu, vücut ağırlığı, serum kreatinin, trigliserid düzeyleri sigara içmeyenlerden yüksek iken, HDL-K düzeyleri daha düşük idi ($p<0,05$). Sigara içenlerin serum irisin düzeyleri ve beden kitle indeksi, sigara içmeyenlerden farklı değildi ($p>0,05$). Serum irisin düzeyleri, sadece tüm çalışma grubunda serum kreatinin düzeyleri ile korele idi ($r=-0,26$, $p<0,05$). Serum irisin düzeyleri ile sigara içim süresi, sigara miktarı, serum glukoz, insülin, total kolesterol, trigliserid, LDL-K, HDL-K, alanin transaminaz, serbest T4 ve TSH düzeyleri arasında bir korelasyon saptanmadı ($p>0,05$). **Sonuç:** Sigara içenlerde serum irisin düzeyleri, sigara içmeyenlerden farklı değildir. Serum irisin düzeyleri, sigara içiminin süresi ve miktarıyla ilişkili değildir. Sigara içiminin enerji metabolizmasındaki benzer etkilerinin, irisin metabolizmasıyla ilişkili olup olmadığı ileriye dönük, doz ve zaman kontrollü çalışmalarla araştırılmalıdır.

Anahtar kelimeler: Sigara içimi; irisin; vücut ağırlığı; beden kitle indeksi

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Introduction

The origin and histological structure of brown adipose tissue (BAT) varies from that of white adipose tissue (WAT). BAT, known to mainly exist in the body during the neonatal period, has been reported in the supraclavicular, paravertebral, mediastinal, and peri-renal regions during adulthood (1-3). In BAT, non-shivering thermogenesis and energy expenditure occur through ATP synthesis by oxidative phosphorylation via the uncoupling protein 1 (UCP1), present in the inner membrane of the mitochondria of BAT cells (4).

The WAT, located in the visceral and subcutaneous tissue, has different functions from BAT and acts as a store of adipose tissue in the body. Some pharmacological stimuli have been shown to induce UCP1 transcription in WAT cells and confer thermogenetic BAT cell properties (browning) to these cells; thus, achieving good metabolic effects (1). One of these stimuli is irisin (1,2,5,6). Irisin is a myokine that was recently described in 2012 by Boström et al. (5). It is a protein that is a cleaved and secreted fragment of FNDC5, a membrane protein found in mouse and human plasma (5). Irisin has been reported to stimulate the transformation of WAT cells into BAT cells and increase the expression of metabolic genes through UCP1, which is especially stimulated after exercise (4-6). Irisin has also been documented to increase thermogenesis, oxidative metabolism, total body energy expenditure, glucose tolerance, as well as cause weight loss and improve insulin resistance (2,6-10). Due to its effects on energy metabolism, the serum irisin levels are monitored in obese and diabetic patients. Conflicting results have been reported in this regard (8). Irisin has also been recommended for use in the treatment of these pathological conditions (6,7).

It has been hypothesized that nicotine may also induce the transformation of WAT into BAT (1,2,10-12). The role of nicotine in BAT metabolism has been investigated previously. The activity of UCP1 is stimulated when BAT is activated by the sympathetic nervous system (4). Acute and chronic smoking have been demonstrated to increase thermogenesis by stimulating the sympathetic system in BAT (4,10-12). On the other hand, the effects of smoking on energy metabolism are also well known. In humans, nicotine consumption through smoking is the main agent that affects energy metabolism. It has been documented that food intake and body weight (BW) are reduced in cigarette smokers with the amount of smoking. In contrast, appetite and weight gain increase after quitting smoking. Another effect of cigarette smoking/nicotine consumption is that it increases the visceral fat content and insulin resistance by changing the distribution of body fat without changing the amount of total body fat tissue in direct proportion to the dose. It has been reported that cigarette smoking is associated with the development of Type 2 diabetes and metabolic syndrome. Moreover, it was observed that insulin sensitivity improved after stopping smoking (4,13,14). Thus, the metabolic effects of cigarette smoking or nicotine intake on energy metabolism appear to be similar to those of irisin. In some animal studies, the effects of nicotine intake on both UCP proteins and WAT/BAT have been investigated, with contradictory results (10,11).

To the best of our knowledge, none of the existing studies in the literature have investigated serum irisin levels in cigarette smokers. In this study, it was planned to investigate whether smoking and serum irisin levels were related, and to contribute to the issue of smoking and obesity, which are common public health problems.

Material and Methods

The study group consisted of students of the Eskişehir Osmangazi University and workers in the Hospital of Eskişehir Osmangazi University Faculty of Medicine. Thirty-one healthy cigarette smokers (8 female, 23 male) aged between 17-24 yrs were included in this study. The control group consisted of 31 healthy non-smokers (17 female, 14 male) who had a similar age range as the smokers.

Subjects with any known chronic disease and undergoing any drug treatment were not included in the study. After laboratory analysis, those who were detected with impaired fasting glucose, elevated alanine aminotransferase (ALT) levels, and subclinical hypothyroidism were also excluded from the study.

All smokers were inquired about the duration of cigarette smoking, and the number of cigarettes smoked in a day.

The study protocol was approved by the local Ethics Committee of Eskişehir Osmangazi University. The study was carried out per the Declaration of Helsinki (Helsinki Declaration, revised 2013). Written informed consent was obtained from all the participants.

The body weights (kg) and heights (m) of all the participants were measured, and the body mass index (BMI) (kg/m^2) was calculated.

After overnight fasting, venous blood samples were collected from all the participants. Levels of serum glucose, total cholesterol, triglycerides (TG), low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, Alanine Aminotransferase (ALT), free T4, and thyroid-stimulating hormone (TSH) were determined by photometry using the Roche Cobas 8,000 analyzer c702 module (Roche Diagnostics GmbH, Penzburg, Germany). Insulin levels were determined by the electrochemiluminescence immunoassay using the Roche Cobas 8,000 c602 (Roche Diagnostics GmbH, Penzburg, Germany) autoanalyzer.

Serum samples from the venous blood, which were aliquoted for determining the irisin levels, were stored at -80 °C until further analysis. Serum irisin levels were measured using a commercially available ELISA kit (RAG018R, BioVendor Inc., Candler, NC, USA) on the VICTOR X3 analyzer (PerkinElmer, USA) at the Biochemistry Laboratory in our hospital. The sensitivity of the method was 1 ng/mL, while the intra-assay and inter-assay CVs were <8.2% and <9.7%, respectively.

Statistical Analysis

The statistical package SPSS version 26.0 was used for the statistical analysis. For the comparisons between groups, the independent samples t-test and Mann-Whitney U test were used. The correlation was analyzed using the analysis of Spearman's correlation. p value of <0.05 was considered to be statistically significant.

Results

The clinical characteristics of the study groups are presented in Table 1, and laboratory results are summarized in Table 2. Serum irisin levels and BMI, did not significantly differ between smokers and non-smokers (p>0.05). It was observed that BW, height, TG, and creatinine levels of the smokers were high, and HDL-C levels were low (p<0.05). Serum irisin level was negatively correlated with only the serum creatinine levels (r=-0.26, p<0.05). The serum irisin levels were not correlated with the duration and amount of smoking, height, BW, BMI, and other investigated parameters in the entire study group, as well as separately in the smoker and the non-smoker groups (p>0.05).

Serum irisin levels did not differ according to gender (median=7.36 (5.78-16) µg/mL in women and 6.39 (5.77-7.12) µg/mL in men) in the entire group (p=0.32), as well as separately in the smoking (median=6.43 (5.44-7.39) µg/mL in women and 6.62 (6.04-6.9) µg/mL in men) (p=0.7) and the non-smoking group (median=8.45 (5.97-17) µg/mL in women and 5.85 (5-9.9) µg/mL in men) (p=0.2).

Table 1. Clinical features of the study groups.

	Smokers (n)	Non-smokers (n)	p value
F/M	8/23	17/24	
Age (month)	237±20 (209-288)	243±18 (204-288)	0.25
BW (kg)	72±13 (45-95)	64±11 (46-92)	0.02
Height (cm)	174±8 (153-186)	170±8 (152-185)>	0.046
Body mass index (kg/m^2)	23.4±3.6 (17-32)	22±2.5 (17-22)	0.789
Duration of smoking (month)	31±19 (2-84)		
Number of cigarettes smoked daily	13.7±0.4 (5-25)		

Table 2. Results of the laboratory analysis.*

	Smokers	Non-smokers	p value
Irisin (µg/mL)	6.61 (5.9-7.1)	6.65 (5.6-15.3)	0.799
Glucose (mg/dL)	82±7.2	84±9	0.61
Insulin (mIU/mL)	10 (7.6-16)	9 (7.5-11)	0.3
Creatinine (mg/dL)	0.8±0.13	0.74±0.14	0.002
ALT (U/L)	16 (11-30)	13 (9-21)	0.79
Free T4 (ng/dL)	1.3±0.15	1.3±0.15	0.61
TSH (mIU/L)	1.86 (1.48-2.63)	2 (1.3-3.1)	0.61
Total cholesterol (mg/dL)	143 (130-164)	147 (129-176)	1.00
LDL-C (mg/dL)	89 (77-119)	89 (71-101)	1.00
HDL-C (mg/dL)	47 (36-59)	55 (46-64)	0.042
Triglyceride (mg/dL)	96 (61-113)	62 (49-91)	0.042

ALT: Alanine transaminase; TSH: Thyroid-stimulating hormone; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol.
*Normal- and not normally distributed data were shown with mean±standard deviation and median (25-75 p), respectively.

Discussion

It is widely known that there is a decrease in weight gain and an increase in weight loss in smokers, whereas weight gain increases after quitting smoking. Nicotine reduces appetite and calorie intake and increases energy expenditure, which explains the reduction in weight gain in smokers (4,13,14). Moreover, it has been demonstrated that nicotine causes weight loss through its lipolytic effect on WAT (4).

It has been reported that this effect of smoking is related to the degree of smoking. While a decrease in BW is observed in both light and moderate smokers, heavy smokers and chronic smokers are overweight and obese, develop insulin resistance, and increased central adiposity (13,14).

In our study, the smokers were taller and heavier than non-smokers. Rather, these individuals had more appropriate BW for their height than simply being heavier than non-smokers. This also suggests that smoking did not induce weight loss and/or decrease weight gain in our study group, which may be related to the relatively shorter duration of smoking.

Irisin, a newly discovered myokine, stimulates the transformation of WAT cells into BAT cells and increases the expression of metabolic genes through UCP1, especially after exercise (4-6). Thus, it increases thermogenesis, oxidative metabolism, total body energy expenditure, and glucose tolerance and induces weight loss and improvement in insulin resistance (2,6-10). In this study, serum irisin levels in smokers were determined because of the possible link between smoking and irisin metabolism, as have been suggested to have similar effects on energy metabolism. In our study, serum irisin levels among smokers were not different from those among non-smokers. The serum irisin levels were not associated with the duration or amount of smoking either. This result suggested that irisin may not be related to smoking, and this hypothesis should be re-investigated with another study model.

It has been observed that irisin levels increase in obese individuals. In several studies, irisin levels are positively correlated with BW, BMI, amount of adipose tissue, and muscle mass, and decrease with weight loss (8). In our study on non-obese individuals, serum irisin levels were not correlated with BW and BMI.

Before the discovery of the irisin, it has been investigated using animal models whether nicotine has a browning effect on WAT in animals exposed to different doses of nicotine for different durations. Some studies have also investigated the effect of nicotine on UCP proteins involved in irisin metabolism (1,2,4,10-12). Acute and chronic smoking

has been demonstrated to increase thermogenesis by stimulating the sympathetic system in BAT (4,10-12). Chen et al. demonstrated in mice exposed to smoking for four days that food intake decreased significantly from the first day of smoking and that weight loss occurred with a decrease in the BAT and retroperitoneal adipose tissue from the second day of smoking. In this study, it was also observed that plasma leptin levels decreased, neuropeptide Y concentrations remained unchanged in the sub-hypothalamic regions, while the UCP1 mRNA expression decreased in WAT and remained unchanged in BAT. Based on these results, these researchers suggested that thermogenesis was not affected by nicotine exposure over a short time. However, in this study, the mRNA expression of UCP3, which is homologous to UCP1, was increased in BAT. The results suggested that lipid utilization and energy expenditure were upregulated with the increase of UCP3 protein, which is known to affect basal metabolism by participating in mitochondrial fatty acid transport in mice exposed to smoking. Thus, smoking may have contributed to the loss of weight and adipose tissue (10).

Yoshida et al. observed that appetite, amount of retroperitoneal and subcutaneous WAT, and BW decreased in obese mice injected with nicotine for six months. Moreover, the protein and mRNA of UCP1 were activated in both BAT and WAT. The researchers demonstrated that features of BAT developed in WAT as observed by immunohistochemical examination, indicating that nicotine caused browning (11).

Arai et al. reported that after 14 days of nicotine injection in rats, the plasma leptin levels, leptin mRNA expression in the omentum, epididymal, and retroperitoneal adipose tissues, and UCP1 mRNA expression in BAT were higher than that in mice which did not receive nicotine. Although the calorific intake of rats decreased on the sixth day of nicotine intake, it was observed that there was no change in either calorific intake or BW after 14 days. In this study, it was observed that continuous nicotine infusion stimulated mRNA expression of UCP1 in BAT regardless of leptin. However, increased UCP1 mRNA expression was not related to the decrease in BW gain in this study (12).

The studies mentioned above suggested that nicotine treatment had similar effects on BAT and UCP1 protein as irisin. However, no study in the literature has investigated the relationship between irisin and smoking or nicotine intake.

Since UCP proteins that mediate the metabolic effects of the irisin were not determined in our study, it is not possible to comment or make a connection regarding this. Moreover, serum irisin levels were not observed to be associated with smoking in this study. On the other hand, in the above-mentioned animal studies, contrasting results were obtained on UCP1 proteins and adipose tissue with exposure to different doses of nicotine for different durations (4,10-12). Similarly, there may be a threshold for the effect of nicotine on irisin in terms of duration, dose, or other factors that have not been investigated in our study. Dose and time-controlled, detailed prospective studies should be conducted on smokers to further investigate this possibility.

In our study, high triglyceride and low HDL-C levels were observed in smokers, which was in line with the atherogenic lipid profile. Serum irisin levels were negatively correlated only with serum creatinine levels. This finding indicates that the kidneys may play a role in the metabolism of irisin.

Contrasting results have been reported regarding the relationship of circulating irisin levels according to gender (5-18). In one study, circulating irisin levels in young adults were not different between the two sexes; however, when lean body mass was adjusted, the levels were observed to be lower in men (15). Circulating irisin levels were lower in adult males with prediabetes (16). On the other hand, it has been reported that in obese children and adolescents, circulating irisin levels did not differ between genders; in the normal BW group, higher irisin levels were observed in girls than in boys (17). Zügel et al. demonstrated that during rest, circulating irisin levels were not different between the two sexes in both of subjects either obese or normal BW and increased significantly in women with normal BW after aerobic exercise (18). Obese subjects had higher irisin concentrations at rest than lean subjects and there was no significant change in irisin concentrations in

neither obese women or men after exercise. These studies indicate that variation in circulating irisin levels according to gender may be affected by factors such as adiposity and exercise.

In our study, the median serum irisin level in the non-smoking group was lower in men than in women, but the difference was not statistically significant. The fact that serum irisin levels were similar between genders in the smoking group may indicate a change in irisin levels with smoking that may nullify the difference in irisin levels between the two sexes. However, in our study, the number of participants was low, and the distribution by gender in the groups was not uniform. This trend in irisin levels between the two sexes was also observed in the non-smoker group. This issue should be investigated further in a larger study.

The limitations of our study are that is the study was a cross-sectional one, the sample size was small, and the participants were light smokers who had just started smoking, due to which the duration of smoking was short.

In conclusion, serum irisin levels in smokers are not different from those of non-smokers. Serum irisin levels are not associated with the duration and degree of smoking and anthropometric parameters. It would be useful to conduct more comprehensive, dose and time-controlled, prospective studies to determine whether there is a link between smoking and irisin metabolism.

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During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Birgül Kırel; Design: Birgül Kırel; Control/Supervision: Birgül Kırel; Data Collection and/or Processing: Birgül Kırel; Analysis and/or Interpretation: İbrahim Özkan Alataş, Birgül Kırel; Literature Review: Birgül Kırel; Writing the Article: Birgül Kırel; Critical Review: Birgül Kırel; References and Fundings: Birgül Kırel; Materials:Birgül Kırel.

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MTHFR C677T Polymorphism in Turkish Women with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Türk Kadınlarında MTHFR C677T Polimorfizmi

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is one of the most common endocrine-reproductive-metabolic disorders of women at reproductive age, affecting 5-15% of the women worldwide. Although the pathogenesis of PCOS is not well defined, it is associated with an increased risk of premature coronary artery disease (CAD). Hyperhomocysteinemia (HHcy) is associated with hyperlipidemia and is an independent risk factor for CAD. The most common cause of HHcy is related to the deficiency of methylenetetrahydrofolate reductase (MTHFR). This study aimed to investigate the relationship between different genotypes of MTHFR C677T and the risk of PCOS. **Material and Methods:** Two hundred twenty voluntary premenopausal women (110 healthy controls and 110 PCOS patients) were included in the study. All the volunteers underwent a physical examination along with biochemical hormonal evaluation and genetic analysis. **Results:** The genotyping analyses and genetic model of inheritance analyses revealed that the frequencies of CC, CT, and TT genotypes in the control and PCOS group to be 51.8%, 45.5%, and 2.7% and 51.8%, 48.2%, and 0%, respectively. The frequency of C and T alleles in the control and PCOS group was determined to be 74% (C: 0.74/155) and 26% (T: 0.26/53), and 75% (C: 0.75/167) and 25% (T: 0.25/53), respectively. The "T" additive, "T" dominant, and "C" recessive models it found that the CT vs. CC (OR:1.06 CI:0.62-1.83), CC vs. TC+TT (OR: 0.99 CI: 0.58-1.72), and TC+TT vs. CC (OR: 0.99 CI: 0.58-1.70), respectively, did not show an increase in the PCOS risk. **Conclusion:** Our findings indicated that the different genotypes of MTHFR C677T were not associated with the risk of PCOS in Turkish women from Central Anatolia.

Keywords: Polycystic ovary syndrome; methylenetetrahydrofolate reductase gene; polymorphism, risk assessment

Özet

Amaç: Üreme çağındaki kadınların en yaygın endokrin-üreme-metabolik bozukluklarından biri olan polikistik over sendromu (PKOS) dünya genelinde kadınların %5-15'ini etkilemektedir. PKOS patogenezi tam olarak tanımlanmamış olmasına rağmen, PKOS'un artmış erken koroner arter hastalığı (KAH) riski ile ilişkili olduğu bilinmektedir. Hiperhomosistenemi (HHcy), KAH için bağımsız bir risk faktörü olan hiperlipidemi ile ilişkili olup HHcy'in en yaygın nedeni metilentetrahidrofolat redüktaz (MTHFR) eksikliğidir. Bu çalışma ile MTHFR C677T polimorfizmi ve PKOS riski arasındaki ilişkinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmaya benzer yaşta iki yüz yirmi gönüllü premenopozal kadın (110 sağlıklı kontrol ve 110 PKOS hastası) dahil edilmiştir. Tüm gönüllülere fiziksel muayene, biyokimyasal hormon değerlendirme ve genetik analizler uygulanmıştır. **Bulgular:** Genotipleme ve genetik kalıtım model analizleri ile CC, CT ve TT genotip sıklığının kontrol ve PKOS gruplarında sırasıyla %51,8, %45,5, %2,7 ve %51,8, %48,2 ve %0 olarak bulunmuştur. C ve T allellerinin sıklığı sırasıyla kontrol ve PKOS gruplarında %74 (C: 0,74/155), %26 (T: 0,26/53) ve %75 (C: 0,75/167), %25 (T: 0,25/53) olarak belirlenmiştir. "T" editif modelde CT'ye göre CC (OR: 1,06 CI: 0,62-1,83), "T" baskın modelde CC'ye göre TC+TT (OR: 0,99 CI: 0,58-1,72) ve "C" çekinik modelde TC+TT'ye göre CC (OR: 0,99 CI: 0,58-1,70) genotipine sahip olmanın PKOS riskini arttırmadığı belirlenmiştir. **Sonuç:** Mevcut çalışma ile MTHFR C677T genotiplerinin iç anadolu bölgesinde yaşayan Türk kadınlarında PKOS riski ile ilişkili olmadığı gösterilmiştir.

Anahtar kelimeler: Polikistik over sendromu; metilentetrahidrofolat redüktaz geni; polimorfizm, risk değerlendirmesi

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Introduction

Polycystic ovary syndrome (PCOS) affects 5-15% of women worldwide. It is one of the most common endocrine- reproductive-metabolic disorders and a major cause for anovulatory infertility (1-3) PCOS is not a local disease; it is a syndrome and a chronic systemic disease. Although the pathogenesis of PCOS is not well defined, in most of the cases, it is related to hyperandrogenemia, insulin resistance (IR), elevated risk of type 2 diabetes (T2DM), dyslipidemia, chronic inflammation, endometrial cancer, and an elevated risk of premature coronary artery disease (CAD) (4-6). The pathogenesis of PCOS remains unknown since it is a complex disease with a multifactorial etiology derived from the interactions between diverse genetic and environmental factors (1,7-9). In the last years, many studies were performed to observe the contribution of single nucleotide polymorphisms (SNPs) in the PCOS etiology (10-15).

The C677T (rs1801133) polymorphism located on the methylenetetrahydrofolate reductase (MTHFR) gene encodes a variant, where cytosine (C) is replaced with thymine (T) at nucleotide 677, which leads to the substitution of alanine (A) instead of valine (V) at codon 222, resulting in the reduction of enzyme activity in 30% to 70% of heterozygous and homozygous genotypes, respectively (16). In the general population, hyperhomocysteinemia (HHcy) reduces the activity of MTHFR that results from the single nucleotide polymorphism (SNP) (16). However, another factor for this can be an insufficient dietary intake of folate.

MTHFR regulates the metabolism of homocysteine (Hcy) and methionine. It converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the main form of folate in the blood. The methyl group acts as a donor for the production of methionine from Hcy. HHcy is related to hyperlipidemia (17) and is an independent risk factor for CAD (18). Laboratory findings suggested that an elevated Hcy concentration is both atherogenic and thrombogenic (19). Several studies have shown that hyperinsulinemia and insulin resistance are positively associated with HHcy (20-22). The changes in the folate metabolism may disrupt the homocysteine-methionine equilibration and maintenance of the methyl pool since folate, and homocysteine-methionine cycles work in connection (23).

In the late 1960s, the partly prevented ovulation found in the immature super-ovulated rats by an insufficient amount of folate raised questions about the effects of folic acid on ovarian function (24). HHcy caused by MTHFR deficiency may lead to impairment of the DNA methylation process (25). Incorporating a higher amount of uracil into the DNA can activate repair mechanisms, resulting in an increased risk of chromosomal breakage (26), reduced nitric oxide formation (27), reduced reactive oxygen species elimination (28), and increased proinflammatory cytokine release (29). Also, folliculogenesis and oogenesis are quite susceptible to the above-mentioned environmental changes. As shown by several experimental data, the oocyte maturation, ovulation, proliferation, and differentiation of granulosa cell and steroid biosynthesis can be affected by HHcy (24,30,31). Moreover, folate and DNA methylation both are important in Alzheimer's disease, mental health, various cancers, and neural tube defects. Additionally, Hcy may play an important role in oxidative stress as well (25,32-35).

The contribution of the MTHFR C677T polymorphism in increasing the PCOS risk was investigated in various populations with contradictory results (36-38). However, there was no study carried out on Turkish women from the Central Anatolian region. Therefore, the goals of our study were to determine whether the C677T variant was related to increase the PCOS risk among Turkish women and also to evaluate any possible relationship between the variant and the clinical and biochemical parameters.

Material and Methods

Subjects

The subjects included in this study were 220 voluntary premenopausal women (110 healthy controls and 110 PCOS patients) aged between 18 and 50 years who were examined in the endocrine clinic at Kayseri Training and Research Hospital between January 2019 and January 2020. The study was approved by the Ethics Committee of Medical School at

Erzincan University. The ethics committee decision number and date were 33216249-604.01.02 E.3294 and 15 January 2019, respectively. All the volunteers provided written informed consent and underwent a physical examination along with biochemical hormonal evaluation. A medical history form was filled, which contained information on hirsutism, menstrual cycle, reproductive and gynecological history, carbohydrate intolerance, use of medication (oral contraceptive pills (OCP)), and arterial hypertension. Ovarian and adrenal ultrasonography was not applied to all volunteers of control subjects in the study designed on a voluntary basis.

Inclusion criteria

The PCOS group included women diagnosed with PCOS according to the criteria of the Androgen Excess-PCOS Society (39).
The Control group included women who were eligible for blood donation, had a normal menstrual cycle with no excess hair growth nor any signs of hyperandrogenemia.

Exclusion criteria

Women who used OCP and/or were diagnosed with acromegaly, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, ovarian tumors, or adrenal tumors were excluded from the PCOS group.
Women who did not have a normal menstrual cycle with excess hair growth and/or any sign of hyperandrogenemia were excluded from the control group.

Diagnostic Criteria

A modified Ferriman-Gallwey score (FGS) of >8 was considered as hirsutism (40), where FGS was evaluated by the same investigator (Y.S). The intermenstrual intervals of longer than 35 days and shorter than 21 days were diagnosed as oligomenorrhea and polymenorrhea, respectively. Androgen value greater than the normal range was identified as hyperandrogenemia. Serum hormone levels were measured in the early follicular phase of the menstrual cycle after an overnight fast.

Hormone Measurements

Prolactin, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), progesterone, luteinizing hormone (LH), estradiol (E2), dehydroepiandrosterone sulfate (DHEA-S), total testosterone (TT), and insulin levels were analyzed using the chemiluminescence immunoassay method by Roche Elecsys Cobas E601 immunoassay analyzer (Roche Diagnostics, Penzberg, Germany). Glucose was analyzed using an enzymatic method by Roche Elecsys Cobas C702 chemistry analyzer (Roche Diagnostics, Penzberg, Germany).
The reference ranges for the TT, DHEA-S, TSH, E2, LH, FSH, prolactin, progesterone, and glucose were 0.084-0.481 µg/L, 350-4070 (ng/mL), 0.27-4.2 µIU/mL, 12.4-233 pg/mL, 2.4-12.6 mIU/mL, 3.5-12.5 mIU/mL, 4.79-23.3ng/mL, 0.05-0.893 ng/mL, and 70-100 mg/dL, respectively. The homeostatic model assessment for insulin resistance (HOMA-IR) was used as insulin resistance index (41).

Genotyping Analysis

Genotyping analysis was applied to all the volunteers. The Genomic DNA isolation kit (Roche, Germany Genomic DNA) was used to isolate the DNA from leukocytes. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was used to analyze the MTHFR C677T variant. The primer pairs used to amplify the C677T variant of the MTHFR gene were 5'-GCTCAAGGCAGGACAGTG-3' as the forward primer and 5'-CTGGGAAGAACT CAGCGAAC-3' as the reverse primer. DreamTaq polymerase (5U/µL, ThermoFisher, USA) was used to perform the PCR reaction. The PCR conditions were 30 cycles of 94˚C for 1 min, 63˚C for 30 s, and 72˚C for 30 s. The obtained PCR fragment of 585 bp was digested using the Taq1 restriction enzyme (ThermoFisher, USA) at 37˚C for 3 h. Agarose gel electrophoresis (2%) was used to separate the digested PCR product, which was then visualized under UV illuminator with SafeRed staining (IntronBio, Korea). In the TT homozygous mutant genotype, two fragments were obtained with a size of 359 bp, and 226 bp whereas, in the CT heterozygous mutant genotype, three fragments with a size of 585 bp, 359 bp, and 226 bp were obtained while in the CC homozygous wild genotype, only one fragment with a size of 585 bp was obtained.

Table 1. Clinical and hormonal parameters in PCOS patients and the control women.

	Control	PCOS	p value
Age(year)	28 (18-49)	22 (18-40)	0.000*
Height (cm)	162.8±5.2	162.6 (±6.02)	0.808
Weight (kg)	65±11.9	71.3 (±15.9)	0.010*
BMI (kg/m2)	23.6 (17.6-45.4)	26.6 (17.1-41.2)	0.003*
FGS	2 (0-7)	10 (0-26)	0.000*
Glucose (mg/dL)	86.0 (70.3-110.0)	87.0 (58.0-107.0)	0.377
Insulin (µIU/mL)	6.7 (2-34.5)	10 (2-66.2)	0.000*
HOMA-IR	1.4 (0.36-7.92)	2.2 (0.3-14.1)	0.001*
TT (µg/L)	0.35 (0.12-0.47)	0.56 (0.09-1.51)	0.000*
Prolactin (ng/mL)	8.3 (2.3-33.62)	12.7 (4.6-67.9)	0.000*
DHEAS (ng/mL)	1781 (531.5-4048.2)	2898.0 (169.4-8799.0)	0.000*
LH (mIU/mL)	5.9 (1.8-30.0)	9.5 (2.0-86.8)	0.001*
FSH (mIU/mL)	6.76 (2.1-17.5)	6.0 (1.1-19.5)	0.027*
E2 (pg/mL)	63.5 (11.2-331.3)	57.9 (15.2-353.4)	0.924
TSH (µIU/mL)	1.5 (±0.82)	1.93 (±1.16)	0.002*
Progesterone (ng/mL)	0.36 (0.01-2.63)	0.6 (0.05-10.4)	0.005*

The results are presented as mean±standard deviation or median (minimum-maximum). * Shows a significant difference. BMI: Body mass index; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: Polycystic ovary syndrome.

Table 2. Frequencies of C677T genotypes and alleles in PCOS patients compared with the control women.

	Control n (%)	PCOS n (%)	p value
MTHFR C677T			
Genotype			
CC	57 (51.8)	57 (51.8)	0.194
CT	50 (45.5)	53 (48.2)	
TT	3 (2.7)	0 (0)	
Allele			
C	0.74 (164)	0.75 (167)	0.198
T	0.26 (56)	0.25 (53)	

PCOS: Polycystic ovary syndrome.

Statistical Analyses

Results for the continuous variables were reported as mean±standard deviation (SD) and median (minimum-maximum) while n (%) was used for the categorical variables. The normality of variables was confirmed using the Kolmogorov-Smirnov test. The comparisons between the study groups were performed using the Student's t -test. For the variables, which were not normally distributed, the Mann-Whitney U test was used. For determining genotype frequencies of alleles between the study groups, the Chi-square test was used. Logistic regression analysis was applied to calculate the odds ratios (OR) and to test the relative risk associated with the risk allele for PCOS, confidence intervals (95% CI) were used. The variables analysis of covariance (ANCOVA) test was used to estimate the differences between the study groups with age and body mass index correction. P-values less than 0.05 were accepted as significant for all the tests. IBM SPSS 22 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used to perform all statistical analyses.

Table 3. Genetic test model analyses of C677T genotypes.

MTHFR C677T		OR (95% CI)	p value
"T" Additive model	CC	Ref.	0.972
	TC	1.06(0.62-1.83)	
	TT	ND	
"T" Dominant Model	CC	Ref.	0.988
	TC+TT	0.99 (0.58-1.70)	
"C" Recessive Model	TC+TT	Ref.	0.988
	CC	0.99 (0.58-1.72)	

ND: Not determined.

Results

Clinical and Biochemical Properties

The clinical and biochemical characteristics of the control and patients groups are presented in Table 1. Compared to the control group, the body mass index (BMI), weight, Ferriman -Gallwey score (FGS), insulin, HOMA-IR, TT, prolactin, DHEA-S, LH, and TSH were found to be significantly higher in the PCOS group, with age being significantly reduced (p=<0.05).

Genotype Distribution and Allele Frequency

To study the relationship between the MTHFR C677T variant and the PCOS risk, the genotype distribution and allele frequency were determined for each group, which is presented in Table 2.

The frequencies of CC, CT, and TT genotypes were determined as 51.8%, 45.5%, and 2.7% in the control group, and 51.8%, 48.2%, and 0% in the PCOS group. The TT genotype was not determined in the PCOS group. The C and T allele frequencies were determined as 74% (C: 0.74/164) and 26% (T: 0.26/56) in the control group and 75% (C: 0.75/167) and 25% (T: 0.25/53) in the PCOS groups. Both the genotype distribution and allele frequencies were not significant between the study groups (p=<0.05).

Table 4. Clinical and hormonal parameters in PCOS patients and the control women having C677T genotypes.

MTHFR 677		Control			PCOS	
Genotypes	CC	CT	TT	CC	CT	TT
Age (year)	27 (18-49)	28 (18-49)	30 (28-32)	22 (18-40)	22 (18-37)	na
Height (cm)	161.5±5.3	162±5.2	167±1.4	161.5±5.5	161.7±6.6	na
Weight (kg)	64.9±12.3	63.4±11.8	57.5±0.7	71.7±15.4	68.8±16.3)	na
BMI (kg/m^2)	24 (17.6-45.4)	23.5 (18.8-34.3)	20.6 (20.2-21.1)	27 (17.8-41.2)	26.3 (17.1-39.1)	na
FGS	2 (0-7)	3 (0-8)	3 (0-6)	10 (0-22)	11 (0-26)	na
Glucose (mg/dL)	86 (70.3-110)	85 (73-110)	78 (78-78)	89 (58-107)	83 (59-106)	na
Insulin (µIU/mL)	6.9±3.9	9.2±6.7	3.2±1.7	13.6±11.2	10.4±9.1	na
	5.7 (2-20.7)	8.1 (2-34.5)	3.2 (2-4.5)	11.6 (2-66.2)	8.59 (2-51.9)	
HOMA-IR	1.4 (0.36-4.3)	1.6 (0.36-7.92)	0.86 (0.86-0.86)	2.4 (0.41-14.1)	1.75 (0.3-11.5)	na
TT (µg/L)	0.34 (0.12-0.47)	0.36 (0.19-0.70)	0.36 (0.34-0.38)	0.58 (0.18-1.27)	0.5 (0.09-1.51)	na
Prolactin (ng/mL)	7.3 (2.3-33.6)	8.8 (3.5-26.8)	8.2 (7.9-8.5)	13.7 (4.7-67.9)	10.7 (4.6-30.2)	na
DHEAS (ng/mL)	1825.8	1635	1567.4	2900.3	2889.3	
	(585.9-4048.2)	(531.5-3952.4)	(1207.0-1927.7)	(1001.0-8799.0)	(169.4-8050.0)	na
LH (mIU/mL)	4.9 (2.1-30.0)	6.9 (1.8-22)	10.5 (10.5-10.5)	10 (2.6-86.8)	8.1 (2.1-32.5)	na
FSH (mIU/mL)	6.7 (2.1-17.5)	6.8 (3.46-12.3)	7.8 (7.8-7.8)	6 (1.6-19.5)	6.1 (1.1-15.1)	na
E2 (pg/mL)	69.1 (19.6-331.3)	60.6 (11.2-244.5)	55.5 (55.5-55.5)	56.8 (15.2-300.1)	58.4 (18.7-353.4)	na
TSH (µIU/mL)	1.4 (±0.7)	1.4 (±0.9)	2.4±1.7	1.7±1.03	2.1±1.3	na
Progesterone (ng/mL)	0.35 (0.01-2.63)	0.39 (0.08-0.57)	0.34 (0.34-0.34)	0.58 (0.09-8.91)	0.63 (0.05-10.45)	na

The results are presented as mean±standard deviation or median (minimum-maximum). BMI: Body mass index; FGS: Ferriman-Gallwey score; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: polycystic ovary syndrome. NA: not applicable.

Table 5. Clinical and hormonal parameters of each C677T genotypes in the study group.

	Genotypes		
Genotypes	**CC**	**CT**	**TT**
Age (year)	23 (18-49)	24 (18-49)	30 (28-32)
Height (cm)	161.5±5.4	161.9±5.9	167±1.4
Weight (kg)	68.4±14.4	66.4±17.7	57.5±0.7
BMI (kg/m^2)	25.6 (17.6-45.4)	24 (17.1-39.1)	20.6 (20.2-21.1)
FGS	4 (0-22)	5 (0-26)	3 (0-6)
Glucose (mg/dL)	87 (58-110)	83 (59-110)	78 (78-78)
Insulin (µIU/mL)	7.9 (2-66.2)	8.5 (2-51.9)	3.2 (2-4.5)
HOMA-IR	1.67 (0.36-14.1)	1.7 (0.3-11.5)	0.86 (0.86-0.86)
TT (µg/L)	0.42 (0.12-1.27)	0.41 (0.09-1.51)	0.36 (0.34-0.38)
Prolactin (ng/mL)	11.28 (2.3-67.9)	10.3 (3.5-30.2)	8.2 (7.9-8.5)
DHEAS (ng/mL)	2253.5 (585.9-8799)	2151.0 (169.4-8050.0)	1567.4 (1207.0-1927.7)
LH (mIU/mL)	7.5 (2.1-86.8)	7.8 (1.8-32.5)	10.5 (10.5-10.5)
FSH (mIU/mL)	6.3 (1.6-19.5)	6.5 (1.1-15.1)	7.8 (7.8-7.8)
E2 (pg/mL)	59.1 (15.2-331.3)	58.4 (11.2-353.4)	55.5 (55.5-55.5)
TSH (µIU/mL)	1.5±0.89	1.7±1.1	2.4±1.7
Progesterone (ng/mL)	0.45 (0.05-8.91)	0.5 (0.05-10.45)	0.34 (0.34-0.34)

The results are presented as mean±standard deviation or median (minimum-maximum). BMI: Body mass index; FGS: Ferriman-Gallwey score; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: polycystic ovary syndrome. "TT" genotype was determined in only three volunteers (less than 5), so it is not included in the statistical analysis.

Genetic Model of Inheritance Analyses

C677T variant was further analyzed using genotyping test models "dominant/recessive/additive" to have a better understanding of the genotype-phenotype association between the genes and PCOS (Table 3). Since the "TT" genotype was not determined in the PCOS group, "C" additive, "C" dominant, and "T" recessive models could not be applied.

In the "T" additive, "T" dominant, and "C" recessive models, it was found that CT vs. CC (OR: 1.06 95% CI: 0.62-1.83), CC vs. TC+TT (OR: 0.99 95% CI: 0.58-1.72), and TC+TT vs. CC (OR: 0.99, 95% CI: 0.58-1.70), respectively, did not increase the PCOS risk.

Clinical and Hormonal Parameters in Each of the C677T Genotypes

The genotypes of C677T were compared with the hormonal and clinical data of the PCOS and control group. The data of the analysis is presented in Table 4. The "TT" genotype was determined in only three volunteers; therefore, it could not be included in the statistical analysis (due to the numbers being less than five).

Our results found no significant difference between the C677T genotypes and the clinical and hormonal data from each genotype (p=<0.05). Similarly, even when the volunteers were not separated based on groups and analyzed only based on genotype, no significant differences were found between the C677T genotypes and the clinical and hormonal data of the volunteers (Table 5).

Discussion

PCOS is not a local disease; it is a syndrome and chronic systemic disease with a highly strong inheritance of up to 70% (9). Moreover, it has a multifactorial and polygenic etiology. Multifactorial diseases are probably related to the effects of multiple genes (often the genes are larger in quantity but smaller in effect) in combination along with lifestyle and environmental factors.

To understand the etiology and genetic basis of PCOS, case-control studies were carried out, which were related to the genes involved in different mechanisms, including adrenal and ovarian steroidogenesis (STAR, CYP11B1, CYP21A2, CYP19A1, CYP17A1, and VDR), the function of steroid hormones (AR, SHBG), function and regulation of gonadotropin (FSHR, LHR, GnRHR), function and secretion of insulin (INS, INSR, IRS-1, IRS-2, INSL3, and CAPN10), energy homeostasis (FTO, PPARG), chronic inflammation (TNF-α, TNF-β1, IL6, IFN-γ, and IL10), oxidative stress (SOD1, SOD2, GPx, and CAT), and cardiovascular risk factors (PAI-1 and MTHFR) (9-15,42). However, incompatible results stemmed from these studies and also the populations from which the study groups were formed (9,37,43-46). A lack of globally accepted diagnostic criteria of PCOS and incomplete knowledge of its pathophysiology may be the additional reasons for the inconsistency.

In humans, the MTHFR gene is located on chromosome 1 and consists of 12 exons. It has three transcripts with the sizes of 2.2 kb, 7.5 kb, and 9.5 kb (47). This gene has fourteen nucleotide polymorphisms associated with the enzymatic deficiency (48), out of which, C677T and A1298C (rs1801131) are the most commonly reported variants that can reduce the MTHFR activity by various degrees. As opposed to the C677T variant, although the A1298C reduces the MTHFR activity, it is not related to HHcy or a lowered plasma folate concentration, neither in the homozygous nor heterozygous state (33). PCOS women are more susceptible to CAD, and HHcy is an independent risk factor for CAD. Insufficient activity of 5,10-MTHFR is one of the most common causes for a high amount of plasma levels of Hcy (16,49). The frequency of the variant differs significantly based on the population from different geographic regions (50,51). Almost 17% of the subjects with cardiovascular disease (CVD) (52) and 28% of the subjects with HHcy and premature vascular disease (53) were found to have C677T polymorphism.

The latest meta-analysis was performed with 21 articles published between the years 1995 and 2020, where the authors found that the T allele was related to an increased risk of PCOS compared to the C allele. The result indicated that C677T polymorphism was related to an elevated risk of PCOS in both homozygous and heterozygous mutant genotypes. Ethnicity dependent subgroup analyses showed that the C677T mutant in the Middle Eastern population showed greater PCOS risk (TT+CT vs. CC: OR: 2.66, 95% CI: 1.54-4.58; CT vs. CC+TT: OR: 2.64, 95% CI: 1.27-5.49; TT vs. CC: OR: 2.21, 95% CI: 1.16-4.21; T vs. C: OR: 1.82, 95% CI: 1.39-2.37). However, such risk was not found in the Asian or Caucasian population (34). The analysis also showed that ethnicity played a significant role in the etiology of PCOS in terms of genetics. The author concluded that further studies need to be performed to explore the contribution of MTHFR C677T polymorphism toward the PCOS risk in the Turkish population since only one study was performed on the Turkish population till then. In our study, we did not find any correlation between PCOS and C677T genotype (CT vs. CC OR: 1.06 95% CI: 0.62-1.83, CC vs. TC+TT OR: 0.99 95% CI: 0.58-1.72, TC+TT vs. CC OR: 0.99, 95% CI: 0.58-170). On the contrary to our findings, Karadeniz et al. found that women with PCOS had four times the higher CT genotype, statistically higher frequency of the T allele, and a statistically higher level of Hcy compared to the healthy control although the Hcy level was not correlated with the C677T genotype. Therefore, the authors concluded that the MTHFR genotypes did not affect the plasma Hcy levels in patients with PCOS in Turkey (54). Van der Put et al. found that the C677T genotypes were associated with reduced folate and higher Hcy levels (33). The previous meta-analysis by Zhu et al. performed using 21 articles, available from the inception till 17 June 2019, found that the T allele in MTHFR C677T polymorphism could be a genetic risk factor for PCOS, especially in the Asian population (TT+CT vs. CC OR: 1.47 95% CI: 1.12-1.94, TT vs. CT+CC OR: 1.50 95% CI: 1.25-1.81). However, the evidence did not support the same association in the Caucasians (TT+CT vs. CC OR: 1.30 95% CI: 0.78-2.61, TT vs. CT+CC OR: 1.01 95% CI: 0.68-1.50) (37). Another meta-analysis by Wang et al. performed using 16 studies published before December 2016 found that the T allele was not a risk factor for PCOS (OR: 1.08; 95%

CI: 0.96-1.21). Upon ethnicity-based analysis, an increased risk was found in the PCOS in the Asian population because of the T allele (OR: 1.31; 95% CI: 1.09-1.58), but such results were not found in the Middle Eastern population (OR:1.26; 95% CI: 0.96-1.67). The authors claimed that the T allele had a protective effect against PCOS in the Caucasian population (OR: 0.82; 95% CI: 0.68-0.99). Additionally, they concluded that MTHFR C677T polymorphism might be providing diverging effects on the PCOS etiology depending on the ethnicity. HHcy can not only be seen in the case of genetic defects, but it can also be due to nutritional deficiencies (insufficient dietary intake of folate), which can be the reason for the discordant results besides ethnicity.

PCOS women from different ethnicity were presented with different clinical appearances of the syndrome. Therefore, differences in ethnicity seem to have an important effect on the disease etiology. Different genetic components may contribute differently to the disease phenotype. For instance, Caucasian patients with PCOS were unlikely to have diabetes when compared to East Asian patients (55), or the European and Maori women were more prone to hirsutism compared to the other ethnic groups (56).

No correlation was observed between the MTHFR C677T genotype and the PCOS risk in our study. Moreover, even in the current literature, there were discordant results. Clinical studies showed that HHcy levels in PCOS patients returned to normal levels upon folic acid supplementation (21,57). Therefore, in the PCOS treatment, folic acid supplementation may be considered to lower the Hcy levels, considering the possibility of HHcy in PCOS patients. The interactions between gene-environment, gene-gene, and even different alleles of the same gene may modulate PCOS risk. Hence, the investigation of polymorphisms on other genes involved in the folate metabolism may be of great importance to understand the role of folate metabolism in PCOS etiology. On the other hand, to avoid dietary interference in women who participate in such studies, it might be better to consider folate intake while designing the study. Additionally, some genes are known to have altered expression levels in PCOS (9). Since the MTHFR deficiency may cause impairment in the DNA methylation process, which is also one of the most important mechanisms in the regulation of gene expression (25), MTHFR genotypes and folate mechanisms may be considered to have an indirect effect on PCOS etiology. However, laboratory studies are needed to support this hypothesis.

Limitations

The study has several limitations; firstly TT genotypes were identified only in three volunteers. Secondly, there was a lack of information about Hcy and folic acid levels in the blood. Thirdly, the study group was formed with volunteers from the Central Anatolia region of Turkey.

Conclusion

This study demonstrated that neither T nor C allele of MTHFR C677T polymorphism contributed to PCOS risk in our study population. Further studies are required to conclude the impact of folate metabolism on PCOS etiology more accurately using a larger study group and detailed individual data.

Ethical Approval

The study was approved by the Ethics Committee of Medical School at Erzincan University. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and

medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Seher Polat; Design: Seher Polat; Control/Supervision: Seher Polat; Data Collection and/or Processing: Yasin Şimşek; Analysis and/or Interpretation: Seher Polat Yasin Şimşek; Literature Review: Yasin Şimşek, Seher Polat; Writing the Article: Seher Polat; Critical Review: Seher Polat Yasin Şimşek; Materials:Yasin Şimşek, Seher Polat.

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Pharmacotherapy and Neoteric Dietary Approaches for Polycystic Ovary Syndrome: A Systematic Review

Polikistik Over Sendromu İçin Farmakoterapi ve Neoterik Diyet Yaklaşımları: Sistematik Bir Derleme

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Abstract

Polycystic ovary syndrome (PCOS) is an ovarian disorder secondary to the dysregulated hypothalamic-pituitary-adrenal axis leading to androgen excess. Numerous studies have documented that insulin resistance is the key pathophysiological element for the development of PCOS. Insulin acts synergistically with luteinizing hormone (LH) to increase androgen production in the theca of the follicles. PCOS is the most malignant endocrine disorder affecting females (7%; from adolescence to menopause). PCOS results in multi-organs derangements categorized by raised androgen levels, irregular menses, and infertility with microcysts formation. The manifestation of PCOS can be specified as polycystic ovaries (morphological) and hyperandrogenemia & hyperlipidemia (metabolic derangements). Clinical hallmarks in PCOS are dyslipidemia, impaired glucose tolerance, hyperandrogenism, microcysts in ovaries, menstrual irregularities, anovulation, and obesity. During clinical examination, a woman's identity is markedly threatened due to hirsutism, acne, alopecia, obesity, irregular menses, and infertility symptoms. Diagnosis is based on European Society for Human Reproduction and Embryology/The American Society for Reproductive Medicine or Rotterdam consensus criteria. In this article, we present a precise and comprehensible glimpse of updated and efficient patient management via pharmacotherapy and diet therapy with the most practicable type of diets and their positive outcomes. Nutrients (inositol, isoflavonoids, omega-3) and their dose regimens are discussed. A calorie deficit of 500-1,000 kcal based on the patient profile has proven effective in revamping biochemical values and weight loss.

Keywords: PCOS; metformin; clomiphene; genistein; dyslipidemia; androgen

Özet

Polikistik over sendromu (PKOS), androjen fazlalığına yol açan hipotalamus-hipofiz-adrenal aksındaki bozulmaya sekonder bir over hastalığıdır. Çok sayıda çalışma, insülin direncinin PKOS gelişiminde anahtar patofizyolojik unsur olduğunu ortaya koymuştur. İnsülin, foliküllerin teka hücrelerinde androjen üretimini artırmak için luteinize edici hormon (LH) ile sinerjik olarak hareket eder. PKOS, kadınları etkileyen (%7; ergenlikten menopoza kadar) en kötü huylu endokrin bozukluktur. PKOS, yüksek androjen seviyeleri, düzensiz adetler, mikrokist oluşumu ve infertilite ile karakterize olan çoklu organ bozukluklarına neden olur. PKOS'un manifestasyonu polikistik overler (morfolojik), hiperandrojenemi ve hiperlipidemi (metabolik bozukluklar) olarak belirtilebilir. PKOS'un klinik karakteristikleri dislipidemi, bozulmuş glukoz toleransı, hiperandrojenizm, overlerde mikrokistler, adet düzensizlikleri, anovülasyon ve obezitedir. Klinik muayenede, kadınlık özeliklerinin hirsutizm, akne, alopesi, obezite, düzensiz adet kanaması ve kısırlık semptomları nedeniyle önemli ölçüde tehdit altında olduğu görülebilir. Tanı, Avrupa İnsan Üremesi ve Embriyoloji Derneği (ESHRE)/Amerikan Üreme Tıbbı Derneği (ASRM) veya Rotterdam konsensüsü kriterlerine dayanmaktadır. Bu yazıda, en uygulanabilir diyet türleri ve olumlu sonuçları ile diyet tedavisi ve farmakoterapi üzerinden güncellenmiş ve etkili hasta yönetimine dair kesin ve anlaşılır bir bakış sunulmuştur. Besinler (inositol, izoflavonoidler, omega-3) ve bunların doz rejimleri tartışılmıştır. Hasta profiline göre 500-1.000 kcal'lik bir kalori açığının, biyokimyasal değerlerin düzeltilmesinde ve kilo kaybında etkili olduğu kanıtlanmıştır.

Anahtar kelimeler: PKOS; metformin; klomifen; genistein; dislipidemi; androjen

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Introduction

Polycystic ovary syndrome (PCOS) is the most malignant endocrine disorder affecting women (7%; from adolescence to menopause) (1). PCOS results in multi-organ derangements categorized by raised androgen levels, irregular menses, and infertility with microcysts formation. The manifestation of PCOS can be specified as polycystic ovaries (morphological), hyperlipidemia (metabolic derangements), and hyperandrogenemia (2). Clinical hallmarks in PCOS are dyslipidemia, impaired glucose tolerance, hyperandrogenism, microcysts in ovaries, menstrual irregularities, anovulation, and obesity. During clinical examination, a woman's identity is markedly threatened due to hirsutism, acne, alopecia, obesity, irregular menses, and infertility symptoms (3). Diagnosis is based on the European Society for Human Reproduction and Embryology/The American Society for Reproductive Medicine (ESHRE/ASRM) or the 2003 Rotterdam consensus criteria. In this article, our objective is to present a precise and comprehensible glimpse of updated and efficient patient management via pharmacotherapy and diet therapy with the most feasible types of diets and their positive outcomes. Nutrients (inositol, isoflavonoids, omega-3) are discussed along with their dose regimen. A calorie deficit of 500-1,000 kcal based on the patient profile is effective in revamping biochemical values and weight loss (4).

Methodology

We conducted a joint literature search using PubMed, Elsevier, and Google Scholar for the period 2000 to 2020. Keywords used were PCOS, infertility, metformin, omega-3, nutraceuticals, insulin resistance, and diet therapy. Papers in the English language alone were considered. Initially, over 100 articles were reviewed, but only 23 research papers were shortlisted based on their acceptability and our set criteria. PRISMA flow chart diagram describes the selection of studies (Figure 1). A literature review aimed to highlight the importance of potential nutrients and types of diets with the most positive outcomes in the early management of PCOS. The ability of nutraceuticals was found to significantly ameliorate complications of PCOS and the prognosis of medical nutrition therapy.

Presentation

National Institute of Health (NIH), in 1990, proposed a list of internationally accepted diagnostic criteria. According to these criteria, both hyperandrogenemia and oligo-anovulation, are mandatory to diagnose PCOS (5). However, NIH criteria were revisited by the ESHRE and ASRM in 2003. NIH 2012 and Androgen Excess Society 2006 guidelines are listed in Table 1. The revised criteria, internationally known as Rotterdam consensus criteria, are now widely used in the diagnosis and requires a minimum of two of the following three features to confirm PCOS:
(i) Clinical or biochemical hyperandrogenism, (ii) anovulation/oligomenorrhoea, and (iii) polycystic ovaries on ultrasound, with the exclusion of conditions having similar presentation (6). Polycystic and ovarian volume >10 mL in the absence of a dominant follicle or ovaries on ultrasound was defined as 12 or more follicles measuring 2-9 mm in at least one ovary. Nevertheless, as PCOS remains a diagnosis of exclusion, other endocrinopathies with similar presentation to PCOS should always be considered. PCOS is an ovarian disorder secondary to the dysregulated hypothalamic-pituitary-ovarianaxis leading to androgen excess (9). Numerous studies have also specified that insulin resistance is the key pathophysiological element for the development of PCOS. Insulin acts synergistically with luteinizing hormone (LH) to increase androgen release in the theca cells of the ovarian follicles (10). Raised levels of LH pulse frequency and an elevated ratio of LH to the follicle-stimulating hormone (FSH) in many women are reported. This abnormality of gonadotrophins is responsible for many of the ovarian features of PCOS, including increased androgen synthesis (11). Hyperandrogenism characteristically varies with race and ethnicity, but the most common manifestations include menstrual irregularities (predominantly oligomenorrhoea), hirsutism, central obesity, and even frontal alopecia (12-15). Furthermore, pregnant women with PCOS carry a greater risk of de-

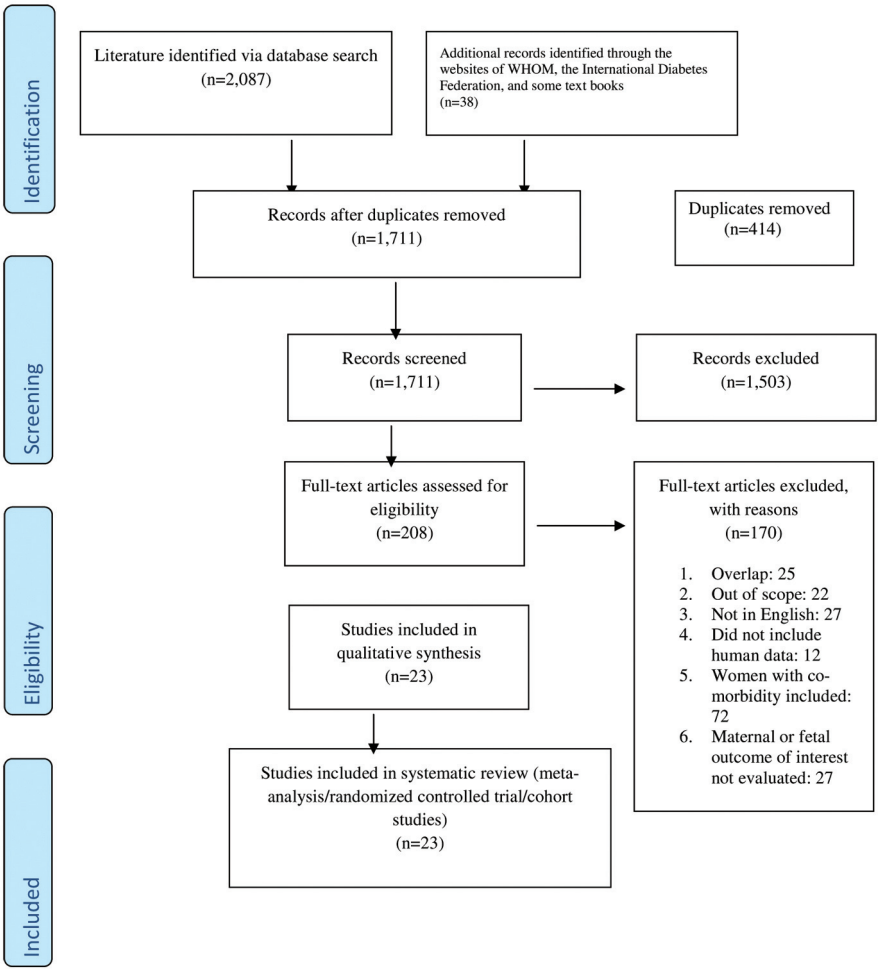

Figure 1. PRISMA flow diagram showing different stages in the systemic review in the selection of studies.

veloping complications such as hypertensive disorders, gestational diabetes, premature delivery, and congenital abnormalities in their neonates (16).

Due to underlying metabolic and hormonal disturbances associated with PCOS, women are more susceptible to cardiovascular diseases, particularly hypertension (HTN) (17,18). Wild et al. documented in a cohort study an increased prevalence of HTN in subjects with PCOS (19). Nevertheless, the association between hypertension and PCOS remains inconclusive as few studies have reported a counter link between systolic arterial pressure and insulin sensitivity in the subjects with PCOS (20).

Treatment

As the primary source of PCOS remains elusive, medication is usually directed at symptoms, such as menstrual irregularities, hirsutism, infertility, and psychological issues. The first step in management for PCOS is lifestyle modification, including diet and exercise, to reduce weight. Weight loss not only helps to decrease levels of androgen, LH, and insulin but also aids in regulating ovulation, thereby improving the chances of pregnancy (21). The treatment plan should be customized to individual patients. Bariatric surgery may be considered in obese patients and cases requiring lifestyle modifications.

Induction of Ovulation in Pcos

PCOS is the principal cause of 70% of all anovulatory-related types of infertility (22). Hart et al. reported that infertility was ten times more common among women with PCOS in comparison to healthy controls

Table 1. Diagnostic criteria for PCOS by different global health organizations.

Global health organizations	NICHD-1990 (5) (a)	ESHRE/ASRM or Rotterdam's criteria (6) (b)	AES criteria (7) (c)	NIH 2012 (8) (d)
Criteria	a)HA b)CA	a) HA b) OD c) Multi-cyst ovaries*	a) Hirsutism or HA b) OD	a) HA b) OD c) PCOM
No. of criteria required for diagnosis	2/2	2/3	2/2	2/3
Phenotype	-	-	-	a) HA+OD+PCOM b) HA+OD c) HA+PCOM d) OD+PCOM

HA: Hyperandrogenism; OD: Ovulatory dysfunction; CA; Chronic anovulation; PCOM: Polycystic ovarian morphology.
a) National Institute of Child Health and Human Development, 1990;
b) European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (Rotterdam), 2003;
c) Androgen Excess Society Guidelines, 2006;
d) National Institute of Health 2012-an extension of ESHRE/ASRM, 2003;
*12 follicles 2-9 mm in each ovary with a volume of 10 mL.

(23). For women without any plans for children, long term control can be achieved with oral contraceptive pills (OCPs). In individuals with reproductive desires, ovulation can be induced by several methods. Recently, clomiphene citrate (CC) has been universally acknowledged as the first-choice drug for inducing ovulation in PCOS individuals. After binding to estrogen receptors on the hypothalamus, the CC makes an antiestrogenic effect and stimulates a gonadotropin-releasing hormone pulse that induces gonadotropin secretion from the anterior pituitary gland. Although up to 15-40% of patients with PCOS show resistance to CC; anovulation persists despite treatment for three successive months, and such patients are considered to be "clomiphene-resistant" (24).

Interestingly, a recent study has documented an alternative therapy of gonadotrophins as standard second-line treatment (25). The study formulated a new CC treatment protocol, named "intermittent CC treatment (ICT) for non-responders to standard CC therapy (25). Under the protocol, the non-responders for five days were given 100 mg/day of CC for 1-3 months depending on follicular growth (size >10 mm), observed after completion of each phase of CC treatment. When the diameter of the follicle reached ≥18 mm, ovulation was induced by injecting 10,000 IU of human chorionic gonadotropin (hCG). Overall, ICT was effective in around 80% of the CC-resistant PCOS patients. However, gonadotrophins, letrozole, and laparoscopic ovarian diathermy therapies are also recommended in "clomiphene-resistant" subjects (24).

Role of Antiandrogens in the Treatment of Hirsutism

Hirsutism, a common manifestation in women with PCOS, is defined as an unnecessary growth of terminal hair at androgen-dependent areas in females analogous to male pattern. It can be managed in several ways, including by spironolactone, flutamide, finasteride, oral contraceptive pills, and laser beam. Souter et al. reported a diagnosis of PCOS on further evaluation in approximately 50% of women, who complained of unwanted excess facial hairs (26). Androgen excess is predominantly responsible for hirsutism, and thus, antiandrogens offer an excellent choice to counter hyperandrogenism effects. Competitive in-

hibition of androgen-binding receptors or 5-alpha-reductase inhibitors decreases androgen production.

Spironolactone is the most commonly used antiandrogen drug (standard dose, 25-100 mg/day), generally well-tolerated, and has shown more efficacy on hirsutism than by use of OCPs (27). Flutamide 250 mg/day and finasteride 5 mg/day are other antiandrogens but are inferior to spironolactone in terms of efficacy (28). Contraception is recommended when patients use antiandrogens for the treatment of PCOS as these drugs pose a risk to the developing male fetus (opposing genital formation). Hirsutic women usually show clinical improvement approximately six months after treatment with OCPs and also present an enhanced clinical effect when OCPs are combined with antiandrogens. Ezeh et al. found that combined treatment with OCP and spironolactone showed greater improvements than with either drug individually (29).

Role of Metformin in PCOS

To date, numerous studies have reported on the vital role of hyperinsulinemia in the development of metabolic abnormalities in PCOS, regardless of body weight index (30,31). Metformin, a biguanide, acts to improve insulin sensitivity and thus lowers free circulating insulin as well as androgens in the bloodstream, resulting in improvement of the clinical sequelae of PCOS (32). However, despite a well-established role in the management of PCOS, conflicting results regarding its efficacy are found in the literature.

Wahab et al. conducted a study (33) on 35 female patients with established PCOS, age 20-35 years. They were given metformin (850 mg twice a day). In order to improve compliance, at every follow-up visit, patients were educated properly regarding the use of metformin. A final assessment was completed two years later with repetition of the transvaginal scan and reevaluation of all laboratory values (random blood glucose, serum insulin, LH/FSH, testosterone, prolactin, etc.). Metformin therapy for two years has shown improvements in the laboratory values. Furthermore, metformin is an effective drug to improve menstrual irregularities, LH, FSH, and testosterone, as indicated in this study (33). Similarly, numerous studies have reported that insulin-sensitizing drugs and dietary/lifestyle modifications improve not only hyperandrogenism but also menstrual irregularities, rate of ovulation, fertility, hirsutism, and weight in patients suffering from PCOS (34-36); these findings are confirmed in the current study. However, two recently published meta-analyses and systematic reviews (in which metformin efficacy was evaluated in improving reproductive outcomes for women with PCOS) concluded no significant evidence of improved rates of live births and clinical pregnancy with metformin alone or in combination with clomiphene (37,38).

In summary, metformin is an appropriate choice and plays a positive role in improving menstrual irregularities and weight reduction in females with PCOS, but current findings do not suggest its use as a first-line drug for ovulation induction. For medical practitioners, we outlined a schematic representation of an efficient clinical approach for PCOS patients in Figure 2. This provides quick insight into the management of PCOS assessment and treatment protocols.

Role of Diet Therapy in Pcos

Though PCOS is associated with overweight, central obesity with insulin resistance is markedly prevalent. In this article, we focused on how macronutrients and micronutrients strongly influence PCOS management. We also highlight the relationship of the type of diet with PCOS and the importance of calorie deficit for the treatment of PCOS. Besides nutritional management and pharmacotherapy, genetics, lifestyle, and ethnicity have a strong influence on the outcomes. Increased insulin resistance causes the overproduction of androgens in response to LH in ovaries. Previous studies confirm the significant role of short term calorie deficit therapy in correcting LH levels and menstrual irregularities. Levels of leptin (energy expenditure hormone) and ghrelin (ligand-increase appetite) levels get deranged in PCOS and can be corrected via calorie deficit therapy (39). Nutritional assessment and biochemical lab findings, along with physical assessment conducted by registered dietitian/nutritionist (RDN), calculates patient BMI and BMR according to

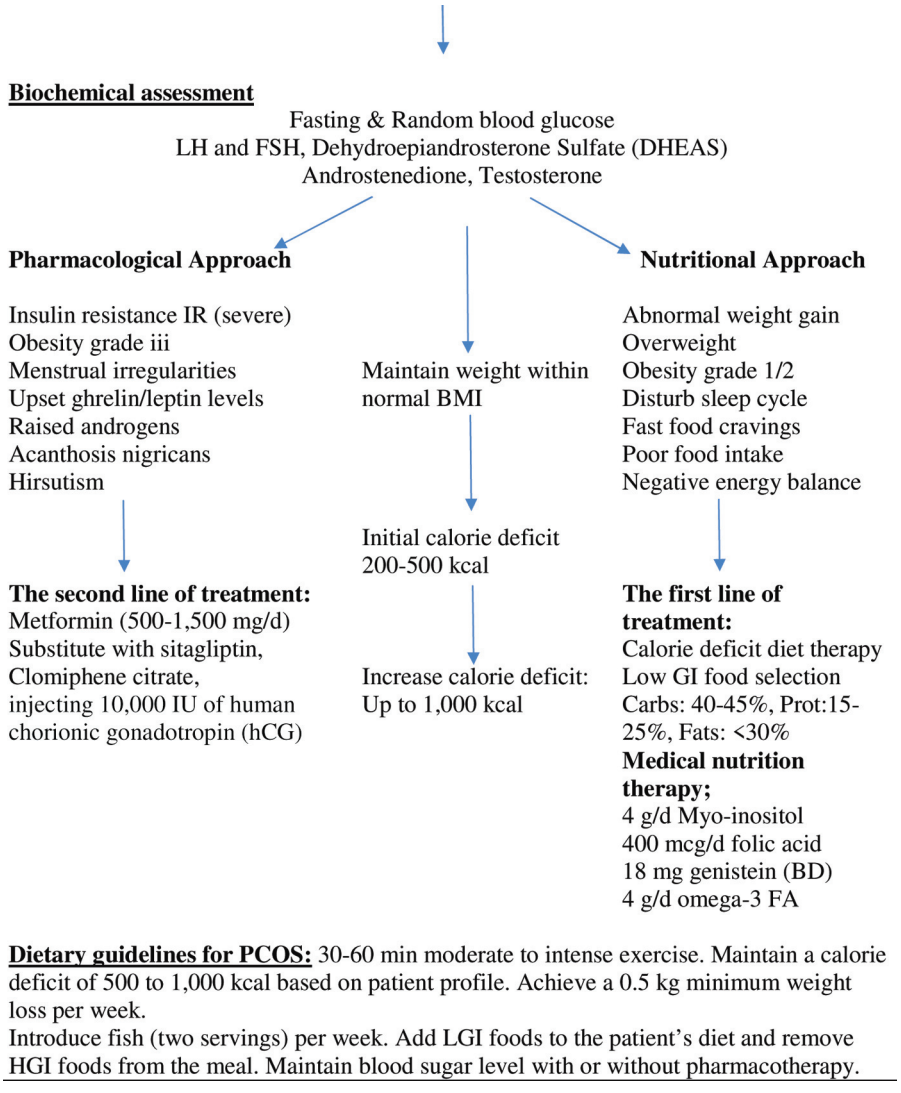

Figure 2. Schematic representation of an efficient and multipronged clinical approach for PCOS patients.
GI: Glycemic index; LGI: Low glycemic index; HGI: High glycemic index; FA: Fatty acid; FSH: Follicle stimulating hormone; IR: Insulin resistance; LH: Leutinizing hormone; BD: (bis in die in Latin) twice daily.

Harris-Benedict Equation. Based on the patient profile, the Dietitian (RDN) recommends a daily calorie deficit of 500-1,000 kcal.

Inositol present in whole grains, seeds, and fruits has two isomers D-chiro-inositol and Myo-inositol. Myo-inositol, a nutrient, belongs to the vitamin B complex. Studies have indicated the beneficial role of Myo-inositol in correcting hormonal profile, oxidative stress, and metabolic factors among PCOS patients. The dose of 4 g/d Myo-inositol along with 400 mcg/d folic acids has shown proven effects in diminishing serum androgen levels and improving glucose tolerance (40). Administration of 2-3 g/d myo-inositol plus 200 mcg/d folic acid has a beneficial role in the amelioration of plasma LH (41). D-chiro-inositol improves glucose levels and enhances uptake via post-receptor mediation of inositol phosphoglycans (IPGs), a mediator of insulin signaling pathways (42). Administration of 600-1,200 mg/dL D-chiro-inositol (DCI) for a period of 6 to 12 weeks in PCOS patients has shown positive outcomes with improving insulin resistance, serum androgen levels (43). **Isoflavonoids** (genistein and daidzein) found in soybean, chickpeas have promising effects on LDL-c levels. Administration of 18 mg genistein (twice a day) for three months significantly lowers LDL-c profile. Isoflavonoids also have

Table 2. Practice-able diets with the most positive outcomes observed in PCOS.

Type of diet/Author	Composition	Outcomes
CHCD & MHCD (46) Mehrabani et al. RCT (49 ss)	CHO: 55% & 40% Prot: 15% & 30% Fat: 30% & 30%	In comparison MHCD has more positive outcomes, BW (-4.1±0.58%), LDL-c (25.5±10.5%), DHEAS (-42.1±16.1) SHBG (8.8±2.8 nmol/L), insulin (-3.6±0.7 mIU/L) HOMA-IR (-o.8±0.2), hsCRP (-0.9±0.4 mg/L).
LGI Diet (47) Marsh et al. (12 months, 96 ss)	Carb: 50% Prot: 23% Fat: 27%	Post OGTT insulin sensitivity improves, LGI diet therapy plus metformin has better results, positive effect on menstrual cycle (95%), serum fibrinogen: (-0.2±0.1 g/L).
HP & SP (48) Sørensen et al. RCT (27 ss)	Carb: 30% & 55% Prot: 40% & 15% Fat: 30% & 30%	HP diet therapy significantly reduces BW (-4.4 kg), FM (-4.31 kg), WC (-3.7cm), BGL (HP: 5.2 mol/L, SP: 5.4 mmol/L). No significant effect over SHBG, Testosterone, C-peptide.
CRD & DASH Diet (49) Asemi et al. RCT (48 ss)	Carb: 52% & 52% Prot: 18% & 18% Fat: 30% & 30%	Calorie deficit: up to 700 kcal, DASH diet has significant effect compared to CRD. BW (-4.4 kg), BMI (-1.7 kg/m^2), serum TG's (-10.0 mg/dL), VLDL-c (-2.0 mg/dL), PTAC (+98.6 nmol/L), Total glutathione (+66.4 µmol/L).
LCKD (50) Mavropoulos (6 months NRCT)	CHO <20%	Significant reduction in; BW (12%), LH/FSH ratio 36%, FBS 54% from base line.

CHCD: Conventional hypocaloric diet; MHCD: Modified hypocaloric diet; LGI: Low glycemic index; HP: High protein; SP: Standard protein; CRD: Calorie deficit; DASH: Dietary approach to stop hypertension; LCKD: Low carbohydrate ketogenic diet; BW: Body weight; BMI: Body mass index; WC: Waist circumference; FM: Fat mass; LDL-c: Low density lipoproteins; DHEAS: Dehydroepiandrosterone; SHBG: Sex hormone-binding globulin; OGTT: Oral glucose tolerance test; BGL: Blood glucose level; VLDL-c: Very low density lipoprotein; FBS: Fasting blood glucose; CHO: Carbohydrate; RCT: Randomized control Trial; HOMA: Homeostatic model assessment for Insulin resistance; hsCRP: High-sensitivity C-reactive protein; TG: Triglyceride; PTAC: Plasma total antioxidant capacity.

a positive impact on reproductive hormones (44). Mohammadi et al. conducted an 8-week study on 61 subjects of PCOS (overweight/obese) and administered **omega-3** mcg/d in one group and compared placebo therapy in another group (45). A significant increase was reported in HDL. TC and LDL-c levels decreased, and insulin, glucose, and HOMA returned to optimum levels. Type of diets with the most feasible and positive outcomes are summarized in Table 2.

However, the type of diet to be prescribed is based on individual nutritional assessments and biochemical lab reports. We strongly recommend calorie deficit therapy (500-1,000 kcal) for PCOS patients with monitoring at regular intervals. Calories from carbohydrates should range from 40-45%, the protein starts with 15% and may increase as per patient profile. Fats should not exceed 30% of total calories. Moderate to an intense exercise of 30 min or more is effective. Exercises such as brisk walk, swimming, arm exercise while seated in a chair for 10 min have been shown to improve glycemic control.

Conclusion

There is an urgent need to conduct extensive research at a genomic and molecular level to understand the pathophysiology of PCOS and the development of metabolic and cardiovascular outcomes in women suffering from the disorder. PCOS is a complex disorder, and the pharmacological approach is limited to the presentation and concern of the patient since the etiology of the disorder remains poorly understood. Nutraceuticals have offered new opportunities for PCOS management and show promising results

(such as omega-3, myo-inositol, folic acid, vitamin-D, and calorie deficit diet therapy); all reduce weight and improve deranged reproductive hormones, insulin resistance, and lipid profile.

To sum up, this paper will be immensely useful for professionals and researchers and would offer a guidepost for future larger, multicentric, studies for the prevention and treatment of PCOS.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Tayyab Hamid Malik, Hussnain Ali; Design: Tayyab Hamid Malik, Hussnain Ali; Control/Supervision: Tayyab Hamid Malik; Data Collection and/or Processing: Tayyab Hamid Malik, Hussnain Ali, Amina Hassan, Junaid Quayyum, Arfah Naseem; Analysis and/or Interpretation: Tayyab Hamid Malik, Hussnain Ali, Amina Hassan, Junaid Quayyum, Arfah Naseem; Literature Review: Tayyab Hamid Malik, Hussnain Ali, Amina Hassan, Junaid Quayyum, Arfah Naseem; Writing the Article: Tayyab Hamid Malik, Hussnain Ali, Amina Hassan, Junaid Quayyum, Arfah Naseem; Critical Review: Tayyab Hamid Malik, Hussnain Ali.

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SRY-Positive 46XX Testicular Disorder of Sex Development as a Rare Cause of Male Hypergonadotropic Hypogonadism: A Case Report

Erkek Hipergonadotropik Hipogonadizmin Nadir Bir Sebebi Olarak SRY Pozitif 46XX Testiküler Cinsel Gelişim Bozukluğu: Olgu Sunumu

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Abstract

46XX testicular disorder of sex development (DSD) is a rare condition characterized by sexual differentiation disorder with testicular insufficiency. Normal sex development often complicates the diagnosis of this ailment in adults. Patients are usually diagnosed incidentally during infertility research. In this article, we aimed to highlight the hormonal, molecular, and cytogenetic results of an adult male patient diagnosed with 46XX testicular DSD suffering from hypergonadotropic hypogonadism.

Keywords: Disorder of sex development; hypergonadotropic; hypogonadism

Özet

46XX testiküler cinsel gelişim bozukluğu (CGB), testiküler yetmezliğin eşlik ettiği cinsiyet farklılaşma bozukluğu ile karakterize olan nadir bir durumdur. Normal cinsiyet gelişimi, genellikle erişkinlerde bu hastalığın teşhisini zorlaştırır. Hastalar, genellikle infertilite araştırılması sırasında tesadüfen teşhis edilir. Bu yazıda, hipergonadotropik hipogonadizmi olan, 46XX testiküler CGB tanısı almış bir yetişkin erkek hastanın hormonal, moleküler ve sitogenetik sonuçlarını vurgulamayı amaçladık.

Anahtar kelimeler: Cinsel gelişim bozukluğu; hipergonadotropik; hipogonadizm

Introduction

Disorders of sex development (DSD) are defined as situations where chromosome structure, gonads, or anatomical structure are incompatible with each other. One of these groups of disease, 46XX testicular DSD, was first reported in 1964 (1). The prevalence of 46XX testicular DSD is estimated to be 1 in 20,000 male births (2). Although pubic hair development and penis length post-puberty are normal, these patients have infertility associated with azoospermia (3). 46XX testicular DSD is diagnosed by evaluating clinical, endocrinological, and cytogenetic test results. The most important treatment method, testosterone replacement therapy, is necessary to improve sexual characteristics and sexual desire. In this report, we aimed to document a case of 46XX testicular DSD, who presented with complaints of small testes.

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Case Report

A 20-year-old male patient visited our outpatient clinic with a complaint of small testes. There was nothing remarkable in his family history and past medical history. Physical examination revealed ill-developed beard, pubic, and axillary hair. Testes were palpated in the scrotum and bilaterally small. Penis length was 10 cm that was smaller as compared to the normal. Patient's height: 168 cm, weight: 65 kg, body mass index: 23 kg/m^2, vertex-pubis/pubis-heel ratio <1, sexual development compatible with Tanner stage 3, and the patient had no gynecomastia. Laboratory examinations revealed fasting blood glucose, renal function tests, liver function tests, and thyroid function tests were within normal ranges. Endocrinological data were indicated as follows: a serum total testosterone (TT) levels of 275 ng/dL (normal range, 248-836 ng/dL); free testosterone (FT) levels of 5.07 pg/mL (normal range, 8.3-40.1 pg/mL); estradiol levels of 22.14 (normal range, 10-50 mcg/L); follicle-stimulating hormone (FSH) levels of 41.69 IU/L (normal range, 1.5-12.4 IU/L); luteinizing hormone (LH) levels of 38.72 IU/L (normal range, 1.7-8.6 IU/L); prolactin levels of 29.65 (normal range, 4.4-15.2 mcg/L); Adrenocorticotropic hormone (ACTH) levels of 20.86 ng/L (normal range, 7.2-63.3 ng/L); cortisol levels of 17.99 µg/dL (normal range, 6.02-18.4 µg/dL); Dehydroepiandrosterone sulfate (DHEAS) levels of 72.37 ug/dL (normal range, 19-407 ug/dL); Growth hormone (GH) levels of 1.08 ug/L (normal range, 0.03-2.47 ug/L); and Insulin-like growth factor 1 (IGF-1) levels of 152.8 ng/mL (normal range, 105-346 ng/mL). Scrotal ultrasonography reflected both testicles in the scrotum, but both were smaller than normal (right testicle: 18x12x9 mm, left testicle: 17x12x9 mm, testicular volume; right 3.9 cc and left 3.2 cc). Both epididymides were of normal size and structure with regular blood supply. Semen analysis indicated azoospermia. The clinical and laboratory findings of the patient are summarized in Table 1. Genetic examinations were conducted with the diagnosis of hypergonadotropic hypogonadism. Karyotype analysis of the patient confirmed a 46XX karyotype (Figure 1). Fluorescent in situ hybridization (FISH) analysis on the metaphase and interphase chromosomes of 100 cells from 2 peripheral blood cultures explored that the SRY gene was positive. Molecular analysis revealed AZFa SY84, AZFa SY86, AZFb SY127, AZFb SY134, AZFc SY160, AZFc SY254, AZFc SY255 loci deletions. The patient was diagnosed as a 46XX testicular DSD. Genetic counseling was provided. Testosterone replacement therapy was initiated.

Table 1. Clinical and laboratory findings of the patient.

	Patient
Age (year)	20
Height (cm)	168
Body weight (kg)	65
Body mass index (kg/m^2)	23
Secondary sexual characteristics	Tanner stage 3
Testicular volume	Right 3.9 cc and left 3.2 cc
Gynecomastia	No
Penile length (cm)	10 cm
FSH (mU/mL)	41.69 (normal range, 1,5-12,4 IU/L)
LH (mIU/mL)	38.72 (normal range, 1,7-8,6 IU/L)
TT (ng/mL)	275 (normal range, 248-836 ng/dL)
FT	5.07 (normal range, 8.3-40.1 pg/mL)
Semen analysis	Azoospermia

FSH: Folliclestimulating hormone; LH: Luteinizing hormone; TT: Total testosterone; FT: Free testosterone.

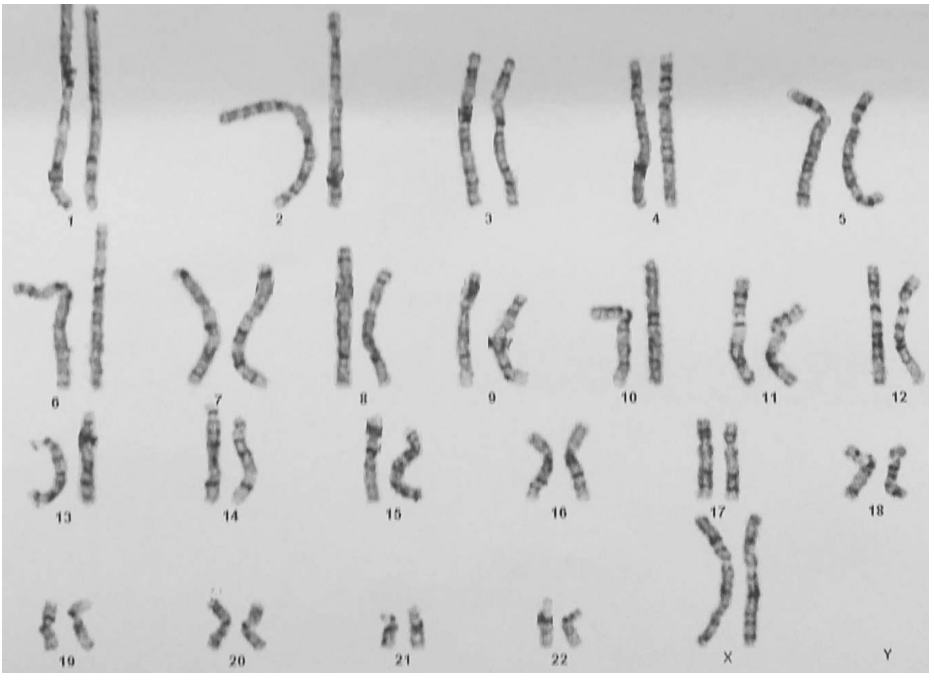

Figure 1: The karyotype confirms the presence of two X chromosomes and the absence of the Y chromosome in the patient.

Discussion

Several congenital and acquired causes are responsible for the onset of hypergonadotropic hypogonadism. Congenital causes include Klinefelter syndrome, 46XX testicular DSD, FSH-LH receptor gene mutations, mutations resulting in androgen synthesis disorder such as 3 beta-hydroxysteroid dehydrogenase, 17 alpha-hydroxylase, and 17 beta-hydroxysteroid dehydrogenase mutations, cryptorchidism, and myotonic dystrophy. Congenital causes of hypergonadotropic hypogonadism are rare in adults.

46XX testicular DSD is one of the rare causes of hypergonadotropic hypogonadism. Details etiology still remains unknown. However, 3 mechanisms have been proposed regarding pathophysiology. The first is the translocation of the Y chromosome containing the SRY gene on the X chromosome or the autosomal chromosomes, second is X chromosome-dependent mutation/overexpression in testis differentiation genes or mutation/overexpression in autosomal genes (such as the SOX9 gene that differentiates Sertoli cells), and thirdly, Y chromosome mosaicism and mutations in undefined genes may result in this disease for SRY negative individuals (3). For SRY positive individuals, usually, the appearance and masculinization of the external genitalia are normal. Clinical manifestation is absent, except for the undescended testicle before puberty. Following puberty, pubic hair development and penis size are normal (4). But their testicles are small, and a third of the affected individuals have gynecomastia (5). This form is diagnosed by chromosomal analysis, conducted while investigating infertility and/or small testicles during late adolescence or adulthood. Inadequate virilization of external genitalia immediately after birth help to detect SRY negative form. In most cases, signs of ambiguous genitalia such as micropenis, hypospadias, and cryptorchidism are witnessed (6). Patients with external genital organs with normal male appearance have rarely been reported in the literature.

A combination of clinical, endocrinological, and cytogenetic tests are employed to diagnose 46XX testicular DSD. In most cases, the diagnosis is made during genetic testing for infertility. Hormonal tests reveal hypergonadotropic hypogonadism secondary to

testicular insufficiency. In cytogenetic studies, 46XX karyotype is determined at 550th band level. SRY, the gene encoding the sex-determining domain on the Y chromosome, is known to be the most crucial gene for the 46XX testicular DSD. The SRY gene, located on the Y chromosome, plays a prominent role in sex determination. This gene activates the SOX-9 gene that enables the differentiation of Sertoli cells. The R-Spondin1 (RSPO1)-Wnt/β-catenin-FOXL2 signaling pathway, essential for ovarian development, is inhibited by both SRY and SOX-9 (7). SRY has been reported to be positive in approximately 80% of 46XX testicular DSD patients, whereas SRY is found to be negative in approximately 20% of these patients (8,9). The SRY-positive 46XX testicular DSD is generally not hereditary, whereas the SRY-negative 46XX testicular DSD is hereditary.

The differential diagnosis of 46XX testicular DSD includes sex chromosomal abnormalities such as Klinefelter syndrome, 46XX/46XY and 46X/46XY, and congenital adrenal hyperplasia (CAH). Klinefelter syndrome (47XXY) is the most common chromosomal abnormality in males. Classically, clinical features of Klinefelter syndrome include testicular atrophy, infertility, eunuchoidism, and gynecomastia. Contrary to the patients with 46XX testicular DSD, the patients with Klinefelter syndrome have longer stature, delayed speech, learning disorders, and behavioral problems (10). 46XX/46XY: The phenotypic spectrum of the patients with this karyotype varies from normal male or female genital regions to indistinct genital regions to varying degrees. 45X/46XY: Affected individuals are predominantly male and may be seen in short stature, depending on the 45X cell percentage. Chromosome analysis assists in the differential diagnosis between this chromosomal abnormality and 46XX testis DSD (11). Another disorder that should be considered in the differential diagnosis is Congenital Adrenal Hyperplasia (CAH). CAH comprises a group of autosomal recessive inherited disorders attributed to defects in one of the enzymes of steroidogenesis pathway in the adrenal cortex. The most common aberrant enzyme responsible for CAH is 21-hydroxylase (21-OH) deficiency. Signs of androgen excess during adolescence are evident in the non-classical form of CAH owing to 21-OH deficiency (12). In 46XY CAH patients, completely normal male-looking genitalia is observed, and virilizing type ambiguous genitalia can be perceived (13).

Our patient had normal male phenotype, sparse facial, pubic, and axillary hair, bilateral small testes, and penis size smaller than normal. Primary testicular insufficiency was considered following the consistency of results with hypergonadotropic hypogonadism in endocrinological examinations. Differential diagnosis from Klinefelter syndrome and other sex chromosomal abnormalities were inferred from karyotype analysis. Moreover, the presence of testicles, penis size smaller than normal, absence of ovary, and uterus ruled out the possibility of 46XX CAH.

In the literature, Sreejith et al. and Guneş et al. reported the cases who were admitted with infertility, small-sized testis, and azoospermia and subsequently diagnosed with 46XX testicular DSD and clinical characteristics were comparable to our case. Similar to our case, the SRY gene was also positive in these cases, and deletion was identified in AZFa, AZFb, and AZFc genes (14,15).

The testosterone replacement therapy holds the most prominent treatment modality for 46XX testicular DSD. Testosterone replacement is necessary to augment sexual characteristics and sexual desire. This treatment can develop secondary sex characteristics, including facial and body hair growth, deepening of the voice, muscle and bone accretion, penile enlargement, and pigmentation of the scrotum in patients with incomplete pubertal development. It is also used to rectify symptoms of testosterone deficiency, such as decreased libido, decreased sexual activity, and erectile dysfunction. Testosterone therapy enhances areal and volumetric vertebral and femoral BMD (Bone Mineral Density) and vertebral and femoral bone strength, but its impact on the risk of fracture is unknown. Testosterone is not an approved treatment to reduce the risk of osteoporosis or fractures. It has been documented that total testosterone levels in patients suffering

from cardiovascular disease (CVD) are significantly lower than those without CVD, and every 1 nmol/L increase in testosterone significantly lowers the relative risk for CVD (16). Compared to the control group, the total testosterone level was significantly lower in patients with CVD-related mortality (17). If the patients have symptoms such as psychological disorder, erectile dysfunction, and gynecomastia, a multidisciplinary approach can successfully reduce these problems.

In conclusion, karyotype analysis should be adopted for differential diagnosis of Klinefelter syndrome as well as rare syndromes such as 46XX testicular DSD in patients suffering from hypergonadotropic hypogonadism, presenting clinical symptoms like infertility, small testes, azoospermia, and gynecomastia.

Informed consent was obtained from the patient.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa Can, Melia Karaköse; Design: İlker Çordan; Control/Supervision: Feridun Karakurt, Mustafa Kulaksızoğlu; Data Collection and/or Processing: Mustafa Can, Muhammet Kocabaş; Analysis and/or Interpretation: Mustafa Can, Muhammet Kocabaş; Literature Review: Hatice Çalıskan Burgucu; Writing the Article: Muhammet Kocabaş; Critical Review: Melia Karaköse; References and Fundings: Mustafa Can; Materials: Mustafa Can, İlker Çordan.

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A Case Report of Dapagliflozin-Induced Nodular Vasculitis

Dapagliflozin İlişkili Nodüler Vaskülit Olgu Sunumu

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Abstract

Nodular vasculitis (NV), first described by Montgomery in 1945 for erythema induratum-like lesions, is a rare form of panniculitis that is particularly localized on the calves. Characterized by plaques and erythematous nodules, NV may often show ulceration and draining. It is known as a reactive disease associated with many causative factors. Several NV cases due to infectious or non-infectious causative factors have been reported, but no case of NV due to sodium-glucose cotransporter-2 inhibitors (SGLT2i) has yet been reported. In this case report, we presented a case diagnosed with NV, who presented with tender, erythematous, eroded plaques with hemorrhagic-purulent discharge on both legs during treatment with dapagliflozin (an SGLT2i).

Keywords: Nodular vasculitis; dapagliflozin; antinuclear antibodies; sodium-glucose cotransporter-2 inhibitors; diabetes mellitus

Özet

İlk olarak 1945 yılında Montgomery tarafından eritema induratum benzeri lezyonlar için tanımlanmış olan nodüler vaskülit (NV), özellikle bacaklarda lokalize olan ve nadir görülen bir pannikülit formudur. NV, plaklarla ve eritematöz nodüllerle karakterize olup, zaman zaman ülsere ve akıntılı hâle gelebilir. Birçok nedensel faktörlerle ilişkili reaktif bir hastalık olarak bilinmektedir. Enfeksiyöz ya da enfeksiyöz olmayan nedenlere bağlı birçok NV vakası bildirilmiştir, ancak sodyum glukoz ko-transporter 2 (SGLT2) inhibitörlerine bağlı bir NV vakası henüz bildirilmemiştir. Bu olgu sunumunda, dapagliflozin (bir SGLT2 inhibitörü) tedavisi esnasında her iki bacağında gelişen hassas, eritemli, erode ve hemorajik-pürülan akıntılı plaklarla başvuran ve NV tanısı konulan bir olguyu sunduk.

Anahtar kelimeler: Nodüler vaskülit; dapagliflozin; antinükleer antikorlar; sodyum glukoz ko-transporter 2 inhibitörleri; diabetes mellitus

Introduction

Nodular vasculitis (NV) was first mentioned by Montgomery in 1945 for erythema induratum-like lesions (1). NV is a rare form of panniculitis and is usually localized on the calves. Characterized by plaques and erythematous nodules, NV may occasionally show ulceration and draining. It is considered a reactive disease associated with many causative factors (2). Some of the infectious causereported in previous studies for NV are Tuberculosis, Nocardia, Fusarium, Pseudomonas, Chlamydia, and hepatitis C virus (3-5). Some cases of NV have also been found associated with non-infectious conditions such as drugs (6,7), inflammatory bowel diseases (8), several autoimmune diseases (9), and rare malignant diseases (10,11).

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are oral antidiabetic drugs that in-

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hibit glucose reabsorption by binding to SGLT2 channels of proximal tubules of the kidney. The fact that this mechanism is insulin-independent makes these drugs promising in the treatment of type 2 diabetes mellitus (T2DM) (12,13). Canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin are the SGLT2i approved until December 2017 by the United States Food and Drug Administration (FDA) (14). Several studies have revealed that SGLT2i drugs remarkably reduce the risk of cardiovascular events. Despite these promising results, some serious side effects and complications, such as increased amputation rates, ketoacidosis, and acute kidney injury associated with SGLT2i (15-17), have been reported. To the best of our knowledge, NV associated with SGLT2i has not been reported so far. In this paper, we present a case of nodular vasculitis associated with dapagliflozin, an SGLT2i.

Case Report

A 36-year-old male patient presented to our hospital with tender, erythematous lesions on both legs that appeared 15 days before. He stated that within a few days, the lesions became enlarged and draining. His previous medical history included T2DM and obesity. He was a smoker. While he was using only metformin 1,000 mg twice a day before, 20 days prior to his presentation, he started dapagliflozin 10 mg orally daily for T2DM. Followings were his vital signs recorded on his admission:

Blood pressure, 125/75 mmHg; pulse rate, 80 beats/min; respiratory rate, 16 breaths/min; pulse oximetry (SpO_2), 98% on room air. His height, weight, and BMI were 171 cm, 138 kg; 47.3 kg/m^2, respectively. Physical examination of the patient revealed eroded plaques with erythematous, violaceous edges measuring 8x9 cm on the medial aspect of the right leg and 10x15 cm on the posteromedial aspect of the left leg (Figure 1). The plaques showed central crusts, erosions, and a hemorrhagic-purulent discharge. All other physical examinations were found normal. Initial laboratory investigations revealed white blood cell (WBC) count of 17.9×10^9/L (4-11) with 82% neutrophils, hemoglobin of 12g/dL (13-18), platelets of 448×10^9/L (140-450), erythrocyte sedimentation rate (ESR) 66 mm/h (0-20), C-reactive protein (CRP) 189.28 mg/L (normal 0-5 mg/L), creatinine 0.7 mg/dL (0.7-1.2), normal partial thromboplastin time and prothrombin time, normal liver function tests, and normal electrolytes. Viral hepatitis and human immunodeficiency virus serological results were all negative. The patient was hospitalized; dapagliflozin treatment, started 20 days ago, was discontinued. From his wound cultures, *Staphylococcus aureus* (SA) was isolated, and he was started on cefuroxime and teicoplanin were started. Testing for antinuclear antibodies (ANA) and antiphospholipid antibodies (aPL) were negative in investigations with suspicion of vasculitis. Myeloperoxidase antibodies and proteinase 3 antibodies were both negative. C3 and C4 Complement levels were within normal limits. Histopathological findings of biopsy samples taken from the lesions showed compatibility with NV. Then we started 25

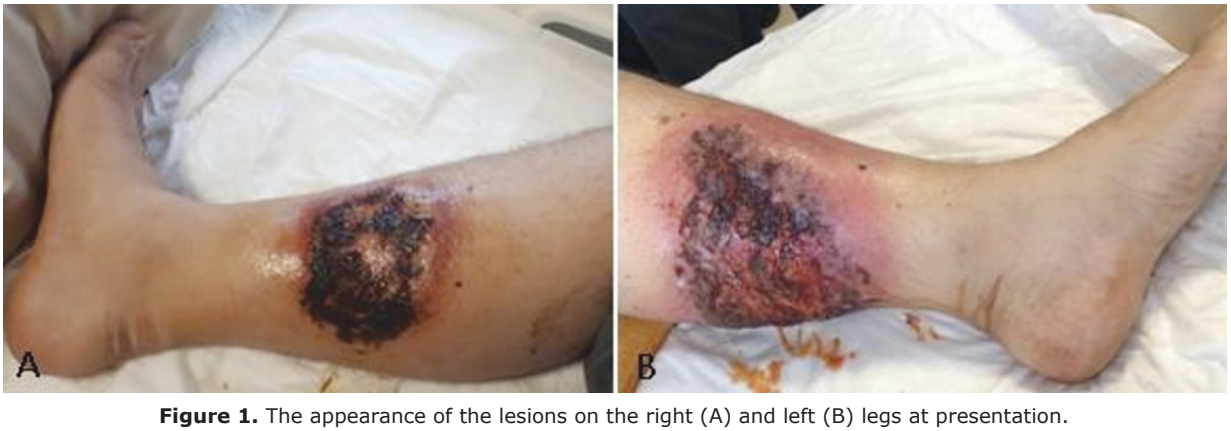

Figure 1. The appearance of the lesions on the right (A) and left (B) legs at presentation.

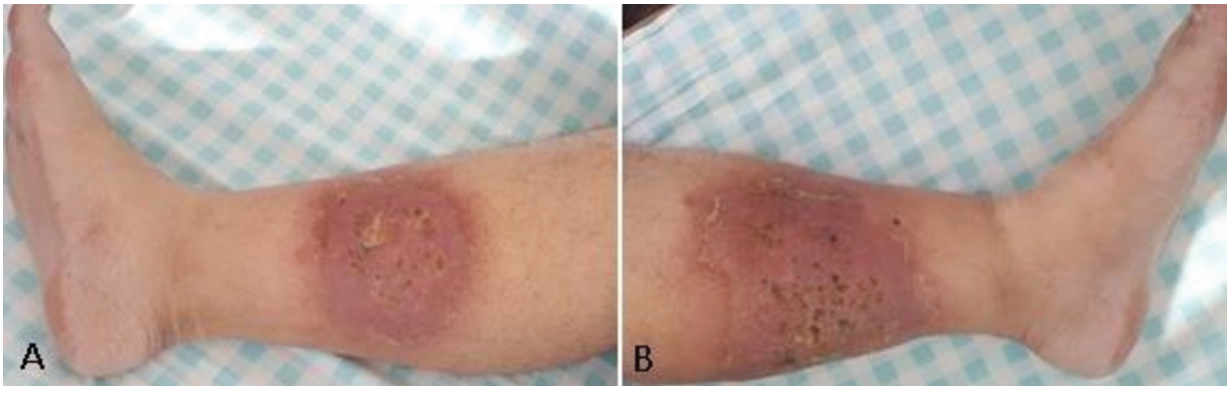

Figure 2. The appearance of the lesions on the right (A) and left (B) legs 20 days after dapagliflozin discontinuation.

mg of dexketoprofen trometamol orally twice a day. With the discontinuation of dapagliflozin treatment, the addition of dexketoprofen trometamol, and antibiotic treatment, his lesions reduced within days, and on the 20th day of his admission, his wounds were healed by leaving depressed scars (Figure 2). On the 20th day of the treatment, laboratory investigation showed a WBC count of 13.6×10^{9}/L (4-11) with 66% neutrophils, CRP of 19 mg/L (normal 0-5 mg/L), ESR of 60 mm/h (0-20). There was no recurrence within nine months after the patient's skin lesions were resolved.

Discussion

This is the first case of NV reported in the literature associated with dapagliflozin. Literature search to study the relationship between SGLT2i and vasculitis showed only one previously reported case of empagliflozin-associated cutaneous polyarteritis nodosa (PAN) (18). NV associated with any SGLT2i has never been previously reported.

Drug-induced NV has been rarely reported, and one case related to propylthiouracil and another case related to etanercept have been described (6,7). However, to date, there are no case reports of dapagliflozin as a cause of NV or other types of vasculitis. Hereby, we report the first case of dapagliflozin (even the first SGLT2i) as a possible cause of NV.

In the Canagliflozin cardioVascular Assessment Study (CANVAS), canagliflozin was shown to be associated with a significant reduction in the risk of cardiovascular events; however, it doubled the amputation risk in patients with T2DM in a study (15). Importantly, very rare side effects may remain undetected during studies before the drug approval and can only be detected in a large patient population after the drug has been approved. Also, phase IV studies are required to reveal these uncommon side effects and to understand their safety profile better.

This case initially presented with erythematous, tender nodules with central crusts, localized on the calves, and showed depressed scars during the recovery period. Also, the histopathological features of the biopsy samples taken from the lesions were found compatible with NV. Significant improvement was noticed in skin lesions within days following dapagliflozin discontinuation. Therefore, we believed that NV disease was caused by dapagliflozin in our case. This case suggests the possible relationship between dapagliflozin and NV and the immediate appearance of lesions after the initiation of dapagliflozin treatment and improvement within days after dapagliflozin discontinuation is the most notable feature. However, in the mechanism of NV that occurs during treatment with dapagliflozin, the role of an SGLT2i remains unknown. More case reports and clinical studies are needed in this context.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Muhammet Kocabaş; Design: Melia Karaköse; Control/Supervision: Mustafa Kulaksızoğlu, Feridun Karakurt; Data Collection and/or Processing: Zeliha Yarar; Analysis and/or Interpretation: İlker Çordan, Mustafa Can; Literature Review: Harun Aydemir; Writing the Article: Muhammet Kocabaş, Mustafa Can; Critical Review: Melia Karaköse; References and Fundings: Mustafa Can; Materials: Hatice Çalışkan Burgucu.

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