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ADDRESS FOR MANAGEMENT / YAYIN İDARE MERKEZİ
The Society of Endocrinology and Metabolism of Turkey
Meşrutiyet Caddesi No: 29/12 Kızılay, Ankara, TURKEY
Phone / Tel: +90 312 425 20 72
Fax / Faks: +90 312 425 20 98
web: www.turkjem.org
E-mail / E-posta: president@temd.org.tr

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Phone: +90 312 508 21 00
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Asena Gökcay Canpolat, Şule Canlar, Berna İmge Aydoğan, Sevim Güllü, Murat Faik Erdoğan
EDITORIAL

Dear esteemed readers of TurkJEM Family,

First coronavirus (2019-nCoV) incidence took place in Wuhan, China on December 2019. Research reveals that first coronavirus fatalities in Hong Kong and Guangdong province both suffered from diabetes. Having mortality rates for a number of diseases have dropped sharply among diabetics in recent years, those rates have remained stubbornly static for pneumonia. Prof Juliana Chan, one of the researchers on a new study that shows pneumonia death rates among Hong Kong’s diabetic community have failed to decline alongside rates for conditions like heart disease. Juliana Chan, director of the Institute of Diabetes and Obesity at the Chinese University of Hong Kong, said if a patient’s blood sugar remained high, it would affect the body’s functions such as immunity, and would also destroy blood vessels, increasing the possibility of death in critical situations. People of all ages can be infected by the new coronavirus (2019-nCoV). Older people, and people with pre-existing medical conditions (such as asthma, diabetes and heart disease) appear to be more vulnerable to becoming severely ill with the virus. According to WHO about 80% of those who died were over the age of 60, and 75% of them had pre-existing health conditions such as cardiovascular diseases and diabetes, according to the NHC. Lancet also confirms that, scenario based on the available evidence now is that the newly identified COVID-19 is causing, like seasonal influenza, mild and self-limiting disease in most people who are infected, with severe disease more likely among older people or those with comorbidities, such as diabetes, pulmonary disease, and other chronic conditions. Among alternative efforts towards diabetes, exertion of research towards strengthening the immune system is the academic priority areas given the 2019-nCoV.

Spring issue of TJEM has some very interesting studies: “Comparison of new and old Body Shape Indices to Estimate Body Fat in Obese and Morbid Obese Turkish Females”, “Obesity is Associated with Increased Thyroid Volume and Heterogeneity in Ultrasonography”, “Association of Serum Resistin Level and Resistin (RETN) Gene (-420 C>G) Polymorphism in Pakistani Women with Polycystic Ovarian Syndrome”, “The Relationship Between C-Peptide Index and Proteinuria in Patients with Type 2 Diabetes Mellitus”, “Fatigue is Related to Insulin Use by Acting Via Depressive Mood in Patients with Diabetes Mellitus”, “Turkish Adaptation of Michigan Diabetes Research and Training Center’s Revised Diabetes Knowledge Test and Determination of Factors Affecting the Knowledge Level of Diabetic Individuals”, “Diagnostic and Therapeutic Approaches to Thyroid Nodules in Turkey”, “Evaluation of Hyperandrogenemia in Women with Prolactinoma”, “Immunohistochemical Subtypes of Growth Hormone-Secreting Pituitary Adenoma and Association with the Clinical Course and Secondary Malignancy”, “Endocrine Effects of Coffee Consumption”, “A Rare Combination: Multiple Endocrine Neoplasia Type 1 and Follicular Thyroid Carcinoma”, “Coexistence of Primary Mucosa-Associated Lymphoid Tissue Lymphoma of Thyroid and Papillary Thyroid Microcarcinoma in a Background of Hashimoto’s Thyroiditis” “Granulomatosis Polyangitis Presented with Diabetes Insipidus”, “Steroid Secreting Dedifferentiated Liposarcoma: An Unique Presentation” and “Development of Hypocalcemia Due to Targeted Therapies”.

Wish you all a very healthy and pleasant spring.

With my best regards,

Nilgün Başkal MD
Editor-in-Chief
Comparison of New and Old Body Shape Indices to Estimate Body Fat in Obese and Morbid Obese Turkish Females

Obez ve Morbid Obez Türk Kadınlarında Vücut Yağ Oranını Tahmininde Yeni ve Eski Vücut Şekil İndekslerinin Karşılaştırılması

Merve MELİKOĞLU, Can ÖNER, Sabah TÜZÜN, Şule TEMİZKAN, Ekrem ORBAY

Kartal Dr. Lütfi Kirdar Training and Research Hospital, Department of Family Medicine, Istanbul, Turkey

Abstract

Objective: The estimation of fat mass with indirect techniques is beneficial in daily clinical practice. This study aimed to compare new and old body shape indices using bioimpedance analysis for the assessment of body fat mass in obese and morbid obese Turkish females. Material and Methods: Four hundred thirty-eight obese and morbid obese females were enrolled in the study. Anthropometric measurements of the study participants were completed using standard techniques. Body mass index (BMI), body adiposity index (BAI), a body shape index, waist to hip ratio and body roundness index (BRI) were calculated. The body fat ratio was evaluated using TANITA-48M. Results: All the anthropometric indices except waist circumference, waist-to-hip ratio, and ABSI correlated with body fat (%). BMI and BAI were the best predictors of body fat ratio derived from bioimpedance analysis for all participants. Only for obese females, BAI alone as well as BAI and BMI together were the best predictive methods of body fat (%). In the morbid obese group, BAI alone proved to be the best predictive method for body fat (%). Conclusion: In clinical practice, the determination of body fat ratio with indirect techniques may help physicians estimate the risk of diseases in obese and morbid obese patients. BAI can help estimate body fat ratio easily.

Keywords: Body fat; body mass index; body roundness index; body adiposity index; a body shape index; obesity

Anahtar kelimeler: Vücut yağ oranı; beden kitle indeksi; beden yuvarlaklık indeksi; beden adipozite indeksi; beden şekli indeksi; obezite

Address for Correspondence: Merve Melikoğlu, Kartal Dr. Lütfi Kirdar Training and Research Hospital, Department of Family Medicine, Istanbul, Turkey

Phone:: +90 216 3776554 E-mail: dr.mervemelikoglu@gmail.com

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Introduction
Obese and overweight individuals pose a significant and widespread health problem all over the world. Nearly 1.9 billion adults throughout the world are overweight or obese (1). According to the national statistics, 1 in every 3 adults is obese and this ratio reaches up to 35.8-44.0% in females (2). Body fat amounting to more than 25% of body weight in males and more than 35% in females is classified as obesity in adults. Many in-vivo techniques for the determination of body fat and diagnosis of obesity exist and many new indices are still being developed to obtain results that are more accurate. The most common and popularly used index is body mass index \([\text{BMI} = \text{weight (kg)/height (m}^2\text{)}]\). World Health Organization defines overweight as a BMI \(\geq\) 25 and obesity \(\geq\) 30 (kg/m\(^2\)) (3).

The body fat percentage and distribution are important because of their relationship with chronic diseases, especially cardiac diseases and diabetes. The techniques determining body fat percentage may be studied as direct and indirect ones. Several direct techniques are employed for the evaluation of body fat percentage and distribution including bioimpedance analysis (BIA), air displacement plethysmography (ADP) and dual-energy X-ray absorptiometry (DEXA) (4). DEXA and magnetic resonance imaging (MRI) are considered the gold standard for determining body fat; yet, the requirement of equipment, trained personnel, and the involved cost limits the use of these techniques. For that reason, bioimpedance analysis is more convenient and is employed more often.

Indirect techniques of determination of body fat percentage are easy to use, involve low cost and do not require auxiliary staff; thereby becoming the most recommended and widely used. The most popular indirect technique for body fat measurement that has been accepted as a tool for defining obesity is Body Mass Index (BMI). Although BMI is very easy to calculate and use, the limitations of this index are well documented; it includes the inability to distinguish the level of fat-free mass and scarce information about fat distribution (5). In order to overcome these limitations, new indices have been developed. Waist circumference (WC) and waist to hip ratio (WtHR) have been introduced as an alternative to BMI, and many studies have confirmed that these indices are superior to BMI in indicating cardio-metabolic diseases (6). In 2011, Body Adiposity Index (BAI) was developed, which use hip circumference and height as the basic anthropometric measures and estimates the body adipose tissue in percentage (7). BAI has been reported to be useful in both genders and in all ethnicities and is strongly correlated with adiposity, in contrast to BMI (4). In 2012, A Body Shape Index (ABSI) was developed in which waist circumference, body weight and height are used as basic measures (8). Although ABSI measurements correlate positively with abdominal adipose tissue deposition, but it is weaker in estimating cardiovascular diseases as compared to BMI (9).

In 2013, a new geometric model, Body Roundness Index (BRI) was developed (5). It is based on the assumption that the body is an ellipse which has two lengths; body height is accepted as major and the diameter of hip or waist circumference as minor axes. The degree of body roundness is characterized by a non-dimensional value called as “eccentricity”. BRI ranges between 1 and 16; a larger value is associated with rounder individuals whereas a value closer to 1 is related to more narrowly shaped individuals (5). It was shown that BRI can predict cardiovascular risk and diabetes mellitus, although it is not superior to BMI and WC (10,11).

Accurate estimation of body fat percentage and its distribution through a numerical value proves very advantageous for clinical practice. Therefore, the aim of this study was to assess the concordance of indirect techniques (BMI, BAI, ABSI, BRI, WC and WtHR) via measured body fat (%) using bioimpedance analysis in obese and morbid obese Turkish women to determine the best indicator index for body fat (%).

Material and Methods
This study was conducted in Kartal Dr. Lutfi Kirdar Education and Training Hospital obesity outpatient clinic between 1 January and 30 June 2016 retrospectively. Women with a BMI of 30 or above were included in the study. Pregnancy, age <18 years, secondary
obesity, thyroid and adrenal diseases were the exclusion criteria. The study was approved by the local Ethics Committee of Kartal Dr. Lutfi Kirdar Training and Research Hospital (No: 2016/514/83/6). Anthropometric measurements of the study participants were performed. Weight was measured in light clothing using calibrated electronic scales to the nearest 0.1 kg. Height was measured barefoot using a stadiometer to the nearest 0.1 cm. Waist circumference was measured over bare skin, midway between the lower rib margin and the iliac crest at the end of expiration while hip circumference was measured as the maximum circumference over the buttocks to the nearest 0.1 cm using a fiberglass measuring tapes. Using these dimensions, BMI, BAI, ABSI, WtHR and BRI were calculated using the formulas summarized in Table 1.

The body composition of the study participants was assessed after overnight fasting. BIA was used for the estimation of the body fat ratio. Bioimpedance measurements of all participants were performed using a TANITA-48M device. The whole body impedance measurements were made using standard positions of outer and inner electrodes on the right hand and right foot. Body fat percentage was obtained according to algorithms developed by the producer (TANITA). Statistical analyses were performed using the Software Statistical Package Sciences (SPSS) for Windows version 17.0. The data were presented as percentages and frequencies, mean±standard deviation. Pearson’s correlation or Spearman’s correlation test was used to find out the correlation between the variables. Significant indices in correlation tests were assessed via the step-wise linear regression to determine the best index/indices. Statistical analyses were two-sided and a p-value <0.05 was considered significant.

Results

The study sample comprised 438 obese and morbid obese females, out of which 272 (62.1%) were obese and 166 (37.9%) were morbid obese. Mean BMI and mean age of the study participants were 38.84±6.19 kg/m² and 42.92±10.78 years, respectively. Nearly 1 in 5 females had diabetes (n=87) and 97 (22.1%) females had hypertension. Anthropometric measures of the participants are presented in Table 2.

The correlation of different body fat (%) measurements were analyzed in all the study participants and the results have been depicted in Table 3. All the anthropometric indices except WC, WtHR and ABSI correlated with body fat (%) as assessed by BIA. Body Roundness Index showed a significant correlation with all the other indices (Table 2).

Stepwise linear regression analysis showed that BMI and BAI were the best predictors of body fat ratio derived from BIA for all the participants ($R^2=0.385$; $p=0.000$ for model 1 and $R^2=0.412$; $p=0.000$ for model 2) (Table 4).

The participants were divided into two groups: obese and morbid obese according to BMI. In the obese group, waist circumference ($r=0.349$; $p=0.000$), BRI ($r=0.437$; $p=0.000$), and BAI ($r=0.385$; $p=0.000$) were significant predictors of body fat ratio. In the morbid obese group, BMI, BAI, ABSI, and WtHR were significant predictors of body fat ratio ($R^2=0.412$; $p=0.000$ for model 1 and $R^2=0.454$; $p=0.000$ for model 2) (Table 5).
p=0.000), BAI (r=0.546; p=0.000), BMI (r=0.544; p=0.000) were correlated significantly with body fat (%). The stepwise linear regression analysis of only the obese group revealed that BAI alone (r²=0.298), as well as BAI and BMI together (r²=0.377), were the best predictive methods of body fat (%) in obese patients.

In the morbid obese group, BAI (r=0.268; p=0.000), BMI (r=0.191; p=0.000) and hip circumference (r=0.174; p=0.025) were significantly correlated with body fat (%). The stepwise linear regression analysis in the morbid obese group showed that BAI alone (r²=0.72) was the best predictive method for body fat (%).

**Discussion**

It is well known that higher body fat ratio (%) is associated with coronary artery diseases, cardiovascular events and mortality (12). The amount of adipose tissue forms an important part of the body weight and includes a large quantity of fluid in its interstitial space which plays a critical role in heart failure. The stroke volume, cardiac output and left ventricular mass are related to fat-free mass. Furthermore, fat tissue acts as an endocrine organ that synthesizes and releases a variety of peptidases and non-peptides playing a role in cardiovascular homeostasis (13). Recent studies have also established the relationship between insulin resistance, diabetes and body fat ratio (14,15). Lipolysis of adipose tissue produces free fatty acids which in turn increases lipid synthesis and gluconeogenesis, resulting in peripheral insulin resistance. This process ends with hyperlipidemia, glucose intoler-
ance, hypertension and atherosclerosis (16). Because of these relationships between obesity, fat ratio and chronic metabolic diseases, it is important for the physicians to determine body fat ratio. The direct methods of estimation of body fat involve higher costs and require equipment and personnel. Estimation of body fat ratio (%) using indirect measures is therefore feasible in daily clinical practice. World Health Organization recommends the use of BMI for the determination of obesity. However, as the ability of BMI to determine body fat percentage is debatable, new indices like WC, WtHR, BAI, ABSI and BRI have been developed. In this research, the anthropometric indices were compared with body fat ratio and all anthropometric indices except waist circumference, WtHR and ABSI showed a statistically significant correlation with body fat ratio (%) in both, obese and morbid obese females. The strongest correlation between these indices and body fat ratio was observed between BAI (r=0.621) and BMI (r=0.610). In the obese females, BAI and BMI were almost equally effective in predicting body fat; however, in morbid obese females, the value of BMI decreased and BAI was the only index that could determine body fat (%).

Bergman et al. established an easily measured anthropometric index; BAI, for determining body fat ratio. They reported a higher correlation of BAI with body fat ratio (r=0.790) than with BMI (r=0.569). The conformity of BAI and body fat ratio is poor at lower levels of adiposity. BAI predicts body fat optimally if the body adiposity is >20% (7). Sun et al. carried out a research on the concordance of BAI and BMI with DEXA. They found that BAI significantly correlated with body fat in the entire cohort (r=0.78). According to gender, BAI was more consistent with the body fat ratio in the females as compared to the males and the entire cohort (r=0.74 vs. r=0.67). Nevertheless, in obese females, such as those in the present study, the correlations of BMI and BAI with body fat were almost equal (r=0.58 vs. r=0.54) (17). On the other hand, one study showed that BAI overestimates body fat at lower levels of adiposity (<20%) and provides 5-10% underestimates at higher adiposity (>40%) (18). In morbid obese females, BMI has been observed to be the single best predictor of body fat (4). This difference could be due to the BAI formula that includes hip circumference and height, which are both related to bone structure. Turkish women are mostly short in stature and have a smaller waist, but larger hip circumference (19). Moreover, in some ethnic groups weight gain is not related to increased hip circumference, but waist circumference (20).

The authors observed that BMI is the second-best index to determine the body fat ratio in obese women. A study including 12901 adults showed that BMI significantly correlates with body fat, both in men and women, although body fat does not increase linearly with weight. Moreover, in females, body fat percentage correlated with WC in a better way (21). A study comparing BAI, BMI, HC, WC and WtHR for determination of body fat (%) revealed that the highest correlation was 0.78 (r²=0.60) for BMI and 0.67 for BAI (r²=0.45) (22). In another study, BMI was significantly correlated with body fat ratio in women (r=0.76) as well as in obese women (r=0.54) (17). Ehrampo et al. speculated that BMI showed the strongest relation with body fat ratio (r=0.868) (23). Similarly, Geliebter et al. reported BMI as the single best predictor of body fat that significantly correlated with BIA (r=0.90) in severely obese women (4).

The literature also reports studies presenting significant correlations between BMI and body fat ratio which is similar to the results of the present study (24,25). Lopez et al. compared BAI and BMI in the determination of body fat ratio and found that BAI is advantageous over BMI, as it does not use weight; however, in general, the BAI does not overcome the limitation of BMI (26).

Some researchers suggest that BMI is well correlated with body fat percentage which is similar to the results of the present study (24,25). Lopez et al. compared BAI and BMI in the determination of body fat ratio and found that BAI is advantageous over BMI, as it does not use weight; however, in general, the BAI does not overcome the limitation of BMI (26). The relationship between BMI and body fat percentage varies according to age, sex and ethnicity (21). Moreover, its weight dependence and inability to determine the distribution of body fat could be the reasons for this discordance observed in the literature.
ABSI is weakly correlated with height and weight, so its correlation with BMI is poor but on the other hand, it shows a strong correlation with mortality rates (8). In the present study, ABSI was unable to predict body fat (r=-0.052, p>0.05). Ehrampoush et al. found a weak correlation between body fat and ABSI, which is in accordance with the results of the present study (23). The height differences between varied ethnic groups may decrease the ability of ABSI in determining body fat.

The authors observed that after BAI and BMI, the new index, BRI was the third-best method that correlated with body fat ratio. It was also strongly correlated with BAI and BMI. BRI could determine body fat ratio and it was possible to obtain a better correlation coefficient when hip circumference rather than waist circumference was used for the calculation of the eccentricity coefficient. Santos et al. reported that BRI could be used for estimating body fat ratio although it has a lower correlation than BMI (29).

Waist circumference and WtHR were not correlated with body fat ratio in the present study. Jablonowska et al. also found that WtHR and WC have a non-significant correlation with body fat, as assessed by BIA (30). On the other hand, literature reports studies indicating WC and WtHR as effective methods for determining body fat indirectly (21,23). The authors deliberate that variations between study groups could be the reason for this discrepancy. The study group in the present research includes obese and morbidly obese, middle-aged Turkish women. The amount and distribution of body fat have been found to be directly related to age and ethnicity.

This study poses some limitations. A cross-sectional design and small sample size are the major limitations. The study group included only those patients who were followed up in the author’s hospital-based outpatient clinics (only obese/morbid obese women), thereby rendering it impossible to use these results when evaluating the entire population. Another limitation of this study is the non-homogenous distribution of age in the study group. Menopause is an important factor in body fat distribution. Postmenopausal women have higher amounts of body fat and reduced muscle mass. BAI underestimates body fat ratio measured by DXA in white postmenopausal women (31).

Conclusion
In clinical practice, the determination of body fat ratio with indirect techniques could help physicians estimate the risk of diseases in obese and morbid obese patients. In conclusion, it was found that BAI and BMI are the best indicators of body fat in obese women. On the other hand, BAI is the best predictive method for body fat estimation in morbid obese females. Body Adiposity Index can be easily used in situations when the weight of the patient is not known.

Source of Finance
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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: Merve Melikoğlu, Can Öner; Design: Can Öner, Sabah Tüzün; Control/Supervision: Merve Melikoğlu, Can Öner, Sabah Tüzün, Şule Temizkan, Ekrem Orbay; Data Collection and/or Processing: Merve Melikoğlu, Şule Temizkan; Analysis and/or Interpretation: Can Öner, Sabah Tüzün; Literature Review: Merve Melikoğlu, Can Öner, Sabah Tüzün, Şule Temizkan; Writing the Article: Merve Melikoğlu, Can Öner, Sabah Tüzün; Critical Review: Ekrem Orbay; References and Fundings: Merve Melikoğlu; Materials: Merve Melikoğlu, Can Öner, Sabah Tüzün, Şule Temizkan, Ekrem Orbay.
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Obesity is Associated with Increased Thyroid Volume and Heterogeneity in Ultrasonography

Abstract

**Objective:** The aim of the present study was to investigate the possible association between obesity and thyroid functions and thyroid morphology. **Material and Methods:** A total of 674 subjects-121 obese and 553 nonobese-were included in the study. Body mass index, serum thyrotrophic, free triiodothyronine (FT3), free thyroxine (FT4), antithyroid peroxidase antibody, and antithyroglobulin antibody (antiTg) were evaluated in each subject. The subjects with thyroid nodules in ultrasonography (USG) were excluded. The thyroid volume of each subject was calculated and analyzed. **Results:** Obesity was significantly associated with increased age and low FT4 in univariate analysis (p<0.05). With multivariate analysis, the odds of obesity was found to increase by 21.8% (95% CI: 12.4-77.9%) for each 5-year increase in age and decrease by 53.1% (95% CI: 0.4-77.9%) for each 1 ng/dL increase in sT4. The odds of obesity in patients with positive antiTg was 1.603 (95% CI: 1.047-2.454) times higher than the odds of obesity in patients with negative antiTg. The median total thyroid volume was significantly higher in obese as compared to nonobese subjects (12.11 mL vs. 10.77 mL, p<0.001). Heterogeneous gland with negative thyroid antibodies was observed in 17 (14%) obese and 40 (7.2%) nonobese subjects (p=0.024). **Conclusion:** Obesity was positively associated with antiTg and age, whereas negatively associated with FT4. Approximately, in every seven obese subjects, one showed heterogeneity in US despite negative thyroid autoantibodies. This suggests that the value of US in the diagnosis of autoimmune thyroiditis might decrease in obese patients.

**Keywords:** Obesity; thyroid functions; thyroid volume; heterogeneity; thyroid morphology

**Anahtar kelimeler:** Obezite; tiroid fonksiyonları; tiroid volümü; heterojenite; tiroid morfolojisi

**Address for Correspondence:** Abbas Ali TAM, Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey

**Phone:** +90 312 2912525 **E-mail:** endoali@hotmail.com

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Introduction
Obesity is a major public health problem with increasing prevalence all over the world. Excess body weight is a predisposing factor for several diseases including diabetes, hypertension, cardiovascular disease, and various cancers (1). Although the complex pathophysiology of obesity could not be elucidated completely, many factors including genetic, environmental, behavioral, and psychological are known to play a significant role. Identification of thyroid hormone targets might provide insight for optimal management of the obese patients (2,3). Thyroid hormones play a key role in the regulation of body metabolism at hunger and fed state. These hormones are also found to regulate the basal metabolic rate and thermogenesis. They significantly affect energy expenditure and body weight. While thyrotoxicosis is associated with increased energy expenditure, proteolysis, lipolysis, and weight loss, hypothyroidism affects these processes in the opposite direction (4,5). The literature reveals controversial results about thyrotropin (TSH) and thyroid hormone levels in obese patients. However, changes in thyroid functions are generally accepted as reversible, since they mostly return to normal ranges after weight loss obtained through diet or surgical intervention (4,6).

Although the current data provide evidence for an association between thyroid hormones and obesity, the mechanism is not explained clearly (4). A limited number of studies are available regarding thyroid morphology in obesity. Preliminary studies reported a correlation between thyroid volume and body weight and body mass index (BMI) (6-8). Radetti et al. evaluated thyroid ultrasonography (US) in obese children and revealed that obesity was associated with structural changes in thyroid morphology which was not related to autoimmunity (6). In this study, we aimed to investigate the possible association between obesity and thyroid functions and thyroid morphology.

Material and Methods
This retrospective study was conducted in a single center and 674 subjects between the ages of 18-75 were included. Patients using thyroid hormone preparations, antithyroid drugs or drugs that can affect thyroid hormones (steroids, amiodarone, etc.), patients with a history of thyroid surgery or radiotherapy to head and neck region, and pregnant or lactating women were excluded from the study. Patients with thyroid nodules were also excluded owing to possible effects on thyroid volume. Demographical features, BMI, serum TSH, free triiodothyronine (fT3), free thyroxine (fT4), anti-thyroid peroxidase antibody (antiTPO), antithyroglobulin antibody (antiTg), and thyroid US findings were recorded in each subject. Weight and height were measured with light clothes and after taking off shoes. BMI was calculated as weight (kg) divided by the square of height (m²). The classification of the World Health Organization was used to define obesity (BMI ≥30 kg/m²).

Serum TSH, fT3, and fT4 and thyroid autoantibodies were measured by chemiluminescence methods (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA and UniCel DXI 800, Beckman Coulter, Brea, CA). The normal levels for TSH, fT3, fT4, antiTPO, and antiTg were 0.4-4 µIU/mL, 1.57-4.71 pg/mL, 0.85-1.78 ng/dL, 0-35 IU/mL, and 0-40 IU/mL, respectively. Serum TSH lower than 0.4 µIU/mL was defined as low, serum TSH in normal ranges was defined as normal and serum TSH higher than 4 µIU/mL was defined as high TSH. The thyroid antibody levels over the upper range were accepted as positive.

Thyroid US was performed by two experienced endocrinologists using an Esaote color Doppler US (Model 796FDII; MAG Technology Co. Ltd., Yung-Ho City, Taipei, Taiwan) and a superficial probe (Model LA523 13-4, 5.5-12.5 MHz). The echogenicity of the thyroid parenchyma was evaluated in a longitudinal position and classified as normal and hypoechoic after comparison with the echogenicity of the adjacent sternohyoideus, sternothyroideus, and sternocleidomas-toides muscles (9). The thyroid gland that is not uniform in echogenicity with hypoechoic areas was defined as heterogeneous. The volume of each lobe was calculated with the formula (maximal length x width x depth x π/6). Thyroid volume was determined by the sum of the volume of two lobes. The isthmus was not taken into account in volume calculation. Written informed con-
sent was acquired from all the patients, included in the study. An approval from the local ethics committee was obtained in accordance with the ethical standards of the Helsinki Declaration.

**Statistical Analysis**

The distributions of the continuous variables were examined by Shapiro-Wilk’s test and normality graphs. All continuous and categorical variables were summarized by median (min-max) and frequency (%), respectively. Mann-Whitney U test and Chi-square tests were used to compare obese and non-obese groups with respect to the continuous and categorical variables, respectively. All possible risk factors for obesity, having a p-value <0.250 in the univariate analysis, were investigated by multiple logistic regression analysis with backward likelihood ratio procedure. Odds ratio (OR) and 95% confidence interval (CI) of the estimates were reported. Simple and multiple linear regression analyses were performed for thyroid total volume in the same manner with automatic linear modeling using best subset variable selection method and automatic data preparation for outliers and influential data points. Regression coefficients of the predictors were provided with their standard errors (SE).

A p-value<0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

**Results**

The obese group comprised of 22 (18.2%) male and 99 (81.8%) female subjects, whereas, there were 105 (19.0%) male and 448 (81.0%) female subjects in nonobese group (p=0.939). The median ages were 32 years (min-max:18-68) in obese and 41 years (min-max:18-68) in nonobese groups (p<0.001). Differences in the levels of TSH, fT3, and fT4 among the two groups were statistically insignificant. AntiTPO and antiTg positivities were also similar in the obese and nonobese subjects. However, total thyroid volume was significantly higher in obese compared to nonobese subjects [median:12.11 mL (min-max: 3.65-25.88) vs. median:10.77 mL (min-max: 2.39-71.15), p<0.001]. Ultrasonographically, the thyroid parenchyma was heterogeneous in 70 (57.9%) of the obese and 305 (55.2%) of the nonobese subjects (p=0.589). There were 71 (58.7%), 2 (1.6%), and 48 (39.7%) obese patients with normal, low, and high TSH, respectively. In the nonobese group, 319 (57.7%) patients had normal, 14 (2.5%) had low and 220 (39.8%) were diagnosed with high serum TSH. The proportion of the patients with heterogeneous gland and negative antibodies was higher in the obese group than that in the nonobese group (14.0% vs. 7.2%, p=0.024). 16 (13.2%) obese and 55 (9.9%) nonobese patients had high TSH despite negative antibodies (p=0.368) (Table 1).

Univariate analyses revealed that age and fT4 were significantly related to obesity (p<0.05, Table 2). The effects of age, fT4, and antiTg positivity on obesity were significant in multivariate analyses. Although the effect of total thyroid volume was nonsignificant (p=0.056), the contribution of it to the model was significant. The odds of obesity was increased by 21.8% (95% CI: 12.4-31.9%) for each 5-years increase in age, adjusting for fT4, antiTg positivity and total volume. The odds of obesity was decreased by 53.1% (95% CI: 0.4-77.9%) for each one-unit increase in fT4. The odds of obesity in patients with positive antiTg was 1.603 times (95%CI: 1.047-2.454) higher than the odds of obesity in patients with negative antiTg (Table 2).

The factors that might affect thyroid volume were also analyzed. In univariate analyses, age, male sex, BMI, fT4, fT3, AntiTPO, and antiTg positivities had an increasing effect on total thyroid volume (Table 3). The multivariate analysis resulted in a model with the best subset of these variables as predictors; gender, antiTg positivity, BMI, and fT3, each having an increasing effect on total volume (F=24.359, p<0.001, R²=12.2%). Based on the model, male patients had a larger volume with a mean of 3.855 mL (95% CI:2.693-5.018) than females. The total volume was 2.323 mL (95% CI:1.434-3.212) higher in patients with positive as compared to negative antiTg. The per unit increase in BMI and fT3 was associated with 0.186 mL (95% CI:0.097-0.276) and 1.098 mL (95% CI:0.239-1.957) increase in the total volume, respectively (Table 3).
Discussion

The association between obesity and thyroid hormones and the possible mechanisms of this association have yet to be elucidated. One of the hypotheses is the increased deiodinase activity as a defense mechanism. This stimulates the conversion of T4 to T3 in obesity. This causes increased energy expenditure which might counteract the accumulation of fat (4,10,11). It was also suggested that high serum levels of leptin in obese patients stimulate thyrotropin-releasing hormone (TRH) production in the hypothalamus, which in turn might affect thyroid hormones and thermogenesis (12). Some inflammatory cytokines secreted from the adipose tissue such as TNF-alpha, IL-1, and IL-6 might inhibit the expression of sodium/iodine symporter mRNA and iodine uptake activity which results in an increase in TSH levels. Tissue resistance to TSH that returns to normal after weight loss can partly be explained by this mechanism (6). There are contradictory results in thyroid functions in obesity. In many studies, high TSH, low fT4, and high fT3 were reported, while some others found normal or reduced TSH and fT3 and normal or increased fT4 (13). The variations in results reported in different studies might be related to the differences in patient selection, BMI, presence of insulin resistance, and timing of samplings.

### Table 1. Demographic and clinical characteristics of obese and non-obese patients.

|                      | Non-obese [n=553] | Obese [n=121] |
|----------------------|-------------------|---------------|
|                      | Median (min-max)  | Median (min-max) | Test Statistics | p   |
| Age [years]          | 32 (18–68)        | 41 (18–68)    | 5.462          | <0.001 |
| Gender (Male)        | 105 (19.0)        | 22 (18.2)     | 0.006          | 0.939  |
| TSH [µIU/mL]         | 3.24 (0.01–100.00)| 3.22 (0.01–100.0) | 0.348          | 0.728  |
| fT3 [pg/mL]          | 3.27 (0.93–22.67) | 3.15 (0.384.78)| 1.262          | 0.207  |
| fT4 [ng/dL]          | 1.18 (0.19–7.77)  | 1.15 (0.21–2.03)| 1.908          | 0.056  |
| AntiTg positivity    | 259 (46.8)        | 49 (40.5)     | 1.608          | 0.205  |
| AntiTPO positivity   | 250 (45.2)        | 51 (42.1)     | 0.376          | 0.540  |
| Total volume of thyroid [mL] | 10.77 (2.39-71.15) | 12.11 (3.65-25.88) | 3.481          | <0.001 |
| Heterogenous US      | 305 (55.2)        | 70 (57.9)     | 0.293          | 0.589  |
| TSH                   |                   |               | 0.340          | 0.844  |
| High                 | 220 (39.8)        | 48 (39.7)     |               |       |
| Normal               | 319 (57.7)        | 71 (58.7)     |               |       |
| Low                  | 14 (2.5)          | 2 (1.6)       |               |       |
| Heterogeneous USG & negative antibodies | 40 (7.2) | 17 (14.0) | 5.110 | 0.024 |
| High TSH level & negative antibodies | 55 (9.9) | 16 (13.2) | 0.810 | 0.368 |

### Table 2. The possible risk factors of obesity.

| Independent variables | OR (95% CI) | p  |
|----------------------|-------------|----|
| **Univariate**       |             |    |
| Age [per five years] | 1.226 (1.133-1.325) | <0.001 |
| Gender (F vs. M)     | 1.055 (0.634-1.754) | 0.837 |
| TSH [per µIU/mL]     | 1.009 (0.991-1.028) | 0.310 |
| fT3 [per pg/mL]      | 0.810 (0.607-1.083) | 0.155 |
| fT4 [per ng/dL]      | 0.451 (0.211-0.964) | 0.040 |
| AntiTg positivity    | 1.294 (0.868-1.930) | 0.206 |
| AntiTPO positivity   | 1.132 (0.761-1.686) | 0.540 |
| Total volume [per mL] | 1.025 (0.996-1.054) | 0.093 |
| **Multivariate**     |             |    |
| Age [per five years] | 1.218 (1.124-1.319) | <0.001 |
| fT4 [per ng/dL]      | 0.469 (0.221-0.996) | 0.049 |
| AntiTg positivity    | 1.603 (1.047-2.454) | 0.030 |
| Total volume [per mL, increase] | 1.032 (0.999-1.066) | 0.056 |

OR: Odds ratio, CI: Confidence interval.
TSH: Thyrotropin; fT3: Free triiodothyronine; fT4: Free thyroxine; Anti TPOAb: Anti-thyroid peroxidase antibody; Anti TgAb: Anti-thyroglobulin antibody; US: Thyroid ultrasonography.
such as without any intervention or when the patient was on a weight loss program including diet or strenuous exercise (14). In the present study, obesity was negatively associated with the level of fT4; however, there was not any association between obesity and TSH and fT3. In a frequently cited study about this subject -The Dan Thyr Study- BMI was also negatively correlated with fT4 and there was no correlation with fT3. This result was in accordance with our study. However, a positive correlation observed between BMI and serum TSH in The Dan Thyr Study was in contradiction with the present finding (15).

Autoimmune thyroid dysfunctions and morphological changes in the thyroid gland in obesity are less studied subjects. In a study on morbidly obese patients, autoimmune was observed in a minority of patients with hypoechoic thyroid glands in US. Hypoechoic appearance in the absence of any thyroid abnormality was observed in 1.9% of nonobese patients, while it was noted in 64.8% of morbidly obese patients (16). Radetti et al. also reported differences in thyroid structure and functions that cannot be explained by autoimmune involvement in obese children (6). Although thyroid US was suggestive for Hashimoto thyroiditis, thyroid autoantibodies were negative in 37.6% of obese children. Some of these children were evaluated by thyroid fine-needle aspiration biopsy and results indicated normal thyrocytes without evidence of autoimmune thyroid disease. It is difficult to explain the hypoechoic appearance in obese subjects, but there are few hypotheses. A possible mechanism behind this might be fat accumulation in the thyroid (6,16). However, this would result in hyperchoic instead of hypoechoic appearance in US (17). Another hypothesis predicts that secretion of cytokines and inflammatory cells from adipose tissue induce vasodilatation and increased permeability of thyroid vessels, which in turn result in plasma exudation to the thyroid parenchyma (13,14). In our study, the frequency of patients diagnosed with heterogeneous thyroid parenchyma in US which was suggestive for chronic thyroiditis and negative thyroid autoantibodies, comprised of 14% in the obese group, whereas, 7.2% in nonobese group. In a recent study with 10 morbidly obese and euthyroid patients, a 25% increase in the echogenicity of thyroid US was reported after >5% weight loss achieved by bariatric surgery (17). The authors, therefore, concluded that morphological changes in the thyroid in case of obesity were reversible with weight loss. Whatever the cause is, it seems that obesity affects thyroid morphology and the value of US in the diagnosis of autoimmune thyroiditis might decrease in obese patients. A significant association between obesity and antiTg antibody was also demonstrated in our study. However, the relationship between thyroid autoimmunity and obesity is a controversial issue. Marzullo et al. suggested an attractive hypothesis about the link between obesity, leptin, and autoimmunity. They showed that leptin was higher in obese patients when compared to those without autoimmune thyroid disease (AITD). This association between AITD and leptin was irrespective of body fat mass or BMI. Multiple logistic regression analysis indicated female sex and leptin to be significant predictors of AITD. The mechanism underneath was explained by possible induction of autoimmune thyroid injury by high leptin levels in subjects, genetically or environmentally prone to Th-1 immune response (18).

Table 3. The possible related factors of thyroid volume.

| Independent variables | b±se (95% CI) | p      |
|-----------------------|--------------|--------|
| **Univariate**        |              |        |
| Age [per year]        | 0.040±0.019  | 0.034  |
| Gender (M vs. F)      | 4.193±4.589  | <0.001 |
| BMI [per kg/m²]       | 0.18±0.046   | <0.001 |
| TSH [per µIU/mL]      | -0.08±0.003  | 0.335  |
| fT3 [per pg/mL]       | 1.41±0.466   | 0.002  |
| fT4 [per ng/dL]       | 2.27±1.047   | 0.030  |
| AntiTg positivity     | 1.98±0.477   | <0.001 |
| AntiTPO positivity    | 1.55±0.477   | 0.001  |
| **Multivariate**      |              |        |
| Constant              | 8.67±2.078   | <0.001 |
| Gender (M vs. F)      | 3.85±0.592   | <0.001 |
| AntiTg positivity     | 2.33±0.453   | <0.001 |
| BMI [per kg/m²]       | 0.18±0.046   | <0.001 |
| fT3 [per pg/mL]       | 1.09±0.437   | 0.012  |
In the study by Rotondi et al., although the prevalence of high TSH was higher in morbidly obese patients, thyroid autoantibodies were negative in most of them. They underlined that the diagnosis of subclinical hypothyroidism should be questioned in morbidly obese patients with negative thyroid autoantibodies since high TSH might not always indicate true hypothyroidism in these patients (2). In our study, though the frequency of patients with high TSH and negative autoantibodies was higher in the obese than the nonobese group, the difference was not significant statistically. However, the small sample size in these subgroups might be a limitation in this case.

The published reports have documented increasing thyroid volume with increasing BMI (7,8,19). Changes in body composition might affect thyroid volume. Sarı et al. reported that thyroid volume and TSH decreased in obese women who had lost more than 10% weight in six months (8). The thyroid volume was also found to be positively correlated with BMI, leptin, and TSH in obese women in the study undertaken by Eray et al. (7). Eray et al. also showed the change in thyroid volume with weight loss was affected only by BMI and leptin. In another study including 268 patients, there was a positive correlation between leptin and thyroid volume (20). These were in line with the present study where we observed a significant correlation between thyroid volume and BMI. These findings suggest a possible role of leptin in the relation between weight loss and decreased thyroid volume.

Cellular dysfunction induced by steatosis in nonadipose tissue of patients with obesity was shown in previous studies (21). Increased thyroid volume in obesity might also be a consequence of increased adipocyte in the thyroid gland. Lee et al. did not find any difference in TSH and fT4 between obese and nonobese patients. However, TSH was reported to be higher in those with interfolicular adipose depot or steatosis in thyroid follicular cells (22). Paracrine factors secreted from these adipocytes and thyroid steatosis due to interfolicular fat accumulation might cause changes in thyroid hormone levels (23).

The main limitation of our study was its retrospective design. Moreover, the serum leptin levels in patients were not evaluated. It is known that thyroid functions and volume might be influenced by some other factors such as TSH receptor antibody levels, iodine status of the patient which can be assessed by urinary iodine content, glucose metabolism disorders, and insulin resistance. Unfortunately, we did not have data about these parameters. As another limitation, thyroid US was performed by two clinicians. The smoking status of the patients was also not taken into consideration. Thiocyanate, which is a potential goitrogen and 2,3-hydroxypyridine, which inhibits thyroxine deiodination, might affect thyroid functions (24).

In conclusion, obesity was negatively associated with fT4 and positively associated with antiTg positivity and age in our study. Additionally, there was a significant association between obesity and morphological changes in the thyroid gland. Approximately, in every seven obese patients, one had heterogeneous thyroid gland despite negative thyroid autoantibodies. This suggests that the diagnostic value of US in autoimmune thyroiditis might reduce in obesity. These patients should be evaluated with clinical and biochemical findings and thyroid autoantibody results. Obesity also affects thyroid volume, possibly through changes in leptin levels. Further prospective studies might exhibit whether there is a causal relationship between obesity and changes in the thyroid gland.

Ethics
Ethics Committee Approval and Informed Consent: Ethical review board of Yıldırım Beyazıt University Ataturk Training and Research Hospital approved the study protocol.

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: Abbas Ali Tam, Bekir Çakir; Design: Didem Özdemir, Reyhan Ersoy; Data Collection and/or Processing: Berna Evranos Öğmen, Fatma Dilek Dellal; Analysis and/or Interpretation: Abbas Ali Tam, Afra Alkan; Literature Review: Abbas Ali Tam, Didem Özdemir; Writing the Article: Abbas Ali Tam, Didem Özdemir, Oya Topaloğlu.

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Association of Serum Resistin Level and Resistin (RETN) Gene (-420 C>G) Polymorphism in Pakistani Women with Polycystic Ovarian Syndrome

Polikistik Over Sendromlu Pakistanlarda Resistin (RETN) Gen (-420 C>G) Polimorfizmi ve Serum Resistin Düzeyi İlişkisi

Yasar NAWAZ, Sumbla GHAZANVI, Nadia RASHEED, Shah JAHAN*, Muhammad Ikram ULLAH**

University of Health Sciences, Department of Clinical Pathology, Lahore, Pakistan
*University of Health Sciences, Department of Cell Biology and Physiology, Lahore, Pakistan
**Jouf University, Department of Clinical Laboratory Sciences, Sakaka, Aljouf, Kingdom of Saudi Arabia

Abstract

Objective: The objective of the present study was to investigate the association of altered serum resistin levels to RETN gene (−420 C>G) polymorphism in women with polycystic ovarian syndrome (PCOS) and in healthy controls. Material and Methods: Eighty (40 PCOS cases and 40 healthy controls) individuals were included. Whole blood and serum samples were taken from all participants. Enzyme linked immunosorbent (ELISA) was performed for measuring the levels of serum resistin. Whole blood was used for extracting total genomic DNA by the phenol-chloroform method. Polymerase chain reaction with fragment length polymorphism was performed for detecting single nucleotide polymorphism (SNP) in the promoter region (−420 C>G) of the resistin (RETN) gene by amplifying the oligonucleotide sequence of the SNP. The amplified products were first confirmed on 2.0% agarose gel for product size, and then restriction digestion of these products was performed by using the Bpil restriction enzyme. After completion of digestion, the products were resolved on 2.5% agarose gel with a 100 bp DNA ladder, and the bands were inspected to infer genotype. Data analysis was done using SPSS software and the association between serum resistin levels and RETN genotypes was analyzed. Results: There was no significant difference (p=0.125) observed in serum resistin levels between PCOS cases (mean±SD=19.33±3.50) and healthy controls (mean±SD=13.48±1.31). The frequency of the G allele was high in PCOS cases (65%) than in controls (53.7%). The GG genotype frequency of SNP (−420 C>G) was high in PCOS cases (40%) than in controls (20%), but no association was found (p=0.148). The high serum resistin levels were significantly associated with the GG genotype in PCOS cases (p=0.027). Conclusion: High serum resistin levels are not associated with the genotypes of RETN (−420 C>G) polymorphism in PCOS women and controls, although women with PCOS had high GG genotype levels of serum resistin. Further studies with large sample size should be conducted to explore the mechanism of genetic factors in complex diseases like PCOS.

Keywords: Resistin; polycystic ovarian syndrome; genetic polymorphism; association; Pakistan

Amaç: Polikistik öorusu (PKOS) olan kadınlarda ve sağlıklı kontrollere değişmiş serum resistin düzeylerinin RETN geni (−420 C>G) polimorfizmi ile ilişkisini araştırmaktır. Gereç ve Yöntemler: Çalışmaya, seksen kişilik (40 PKOS hastası ve 40 sağlıklı kontrol) dâhil edildi. Tüm katımların tam kan ve serum örnekleri alındı. Serum resistin düzeylerini ölçmek için enzime bağlı immunosorbent (ELISA) yapıldı. Tam kan, fenol-kloroform yöntemiyle total genom DNA’nın ekstrakt edildiği kullanıldı. Tek nükleotit polimorfizminin (SNP) oligonukleotid sekansını amplifye ederek resistin (RETN) geninin promotor bölgesinde (−420 C>G) SNP saptanması için polimeraz zincir reaksiyonu ile parça uzunluk polimorfizmi yapıldı. Amplifi edilen ürünler önce ürün boyutunu %2,0 agaroz jel üzerinde incelemekte, daha sonra ürün örneklerini Bpil restriksiyon enzimi kullanarak incelemekte. Sınırlı veri analizinde olduğu görüldüğü için, SPSS yazılım kullanılarak veri analizi yapıldı ve serum resistin düzeyleri ile RETN genotipleri arasındaki ilişki analizi yapıldı. Bulgular: PKOS hastalar (ortalama±SD=19,33±3,50) ile sağlıklı kontroller (ortalama±SD=13,48±1,31) arasında serum resistin düzeylerinde anlamlı bir fark izlenmedi (p=0,125). G alelininin sıkılığı PKOS (%65) hastalarında kontrollerden (%53,7) yüksek. SNP’nin (−420 C>G) GG genotip frekansı, PKOS (%40) vakalardında kontrollerden (%20) yüksekti, ancak ilişki bulunmamı (p=0,148). Yüksek serum resistin düzeyleri PKOS hastalarında GG genotipi ile anlamlı şekilde ilişkilidir (p=0,027). Sonuç: PKOS’lu hastaların serum resistin GG genotip düzeyleri yüksek olmak PKOS’lu kadınlarda ve kontrollerde yüksek serum resistin düzeyleri ile RETN (−420 C>G) polimorfizm genotipleri ilişkili değildir. PKOS gibi karmaşık hastalıklarda genetik faktörlerin mekanizması araştırılmak için büyük ölçüde daha ileri çalışmalara yapılmalıdır.

Anahtar kelimeler: Resistin; polikistik over sendromu; genetik polimorfizm; ilişki; Pakistan

Address for Correspondence: Nadia RASHEED, University of Health Sciences, Department of Clinical Pathology, Lahore, Pakistan Phone: 92 3211019577 E-mail: naddiya24@gmail.com

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Introduction

Polycystic ovarian syndrome (PCOS) is an endocrine defect commonly affecting women of reproductive age with a prevalence of 5-13%. The characteristic features of PCOS include infrequent or absent menstruation, polycystic ovaries, and high blood levels of androgen (1,2). PCOS is characterized by oligomenorrhea, hirsutism, infertility, insulin resistance, obesity, and acanthosis nigricans with polycystic ovaries (3). In Pakistan, the frequency of PCOS in fertile women is about 17.6% (4). The syndrome occurs in all races and geographical locations and is the most common disorder and cause of infertility (5). The prevalence of PCOS in infertile Pakistani women is 40.9% (6), and the prevalence of PCOS patients among first-degree relatives is 25-50%, suggesting a high-risk inheritance (7). Published literature has described that genetic factors strongly contribute to the development of PCOS. Although various studies have investigated the variable changes in the genes regarding the complex biological mechanism, the role of genetic predisposition on PCOS pathophysiology is not elucidated remarkably (8). Several candidate genes of metabolic defects have a role in PCOS, although the contributing genes remain to be elucidated (9,10). Some recent studies have identified various single nucleotide polymorphism (SNP) sites to be associated with PCOS in different populations (11-13).

Resistin is a cysteine-rich hormone belonging to the resistin-like molecule, and it acts as a macrophage in multiple inflammatory disorders (14). Resistin is involved in various metabolic defects like metabolic syndrome and diabetes (15), atherosclerosis and coronary artery diseases (16), and osteoarthritis (17). Various SNPs have been reported in the resistin (RETN) gene and an important promoter region SNP (-420 C>G; rs1862513) is associated with various diseases and with variable serum resistin levels (18,19). Previous genetic association studies have demonstrated a link between RETN polymorphism and metabolic syndrome (20).

However, some studies did not establish the role, while in other studies the disease susceptibility to the RETN gene was heterogeneous and conflicting. Several SNPs of the RETN gene have been reported for the association with variable serum or plasma levels of resistin in different pathologies like the development of insulin resistance and dyslipidemia (21). The genetic association of RETN polymorphisms has been not determined in PCOS women. We aimed to assess the role of SNPs (-420 C>G; rs1862513) in PCOS predisposition in Pakistani women. The association between serum resistin levels and PCOS was also explored. According to our knowledge, this is the first study ever to investigate the relation between serum resistin levels and RETN genetic variants in PCOS susceptibility.

Material and Methods

Ethical permission was obtained from Advance Studies & Research Board (AS&RB) of the University of Health Sciences, Lahore, Pakistan. Written informed consent was obtained from all the participants and 2013 modified Helsinki guidelines were followed for human subjects. This was a case-control study and PCOS cases were retrieved from a teaching hospital of Lahore (Jinnah Hospital) and age- and sex-matched healthy controls of similar ethnicity were recruited. Family history was obtained and clinical examination of PCOS women was performed and demographic data were recorded. The diagnosed cases of PCOS according to the criteria were included. Patients with diabetes and other metabolic defects were excluded. The Rotterdam diagnostic criterion (22) was used to establish the PCOS cases (presence of at least two contributing factors from the following; a. oligo/anovulation, b. hyperandrogenism [clinical: hirsutism; biochemical: raised androgen levels], c. polycystic ovaries on ultrasound). The controls were also screened for these criteria, and some control participants carried a single feature at the time of recruitment like irregular menstruation, hirsutism, acne without raised androgen levels and absence of other factors.

After obtaining the written informed consent, about 5 mL of venous blood was drawn from the participants under aseptic conditions, which was divided into two different vacutainers: 2 mL in a serum-separating
tube for serum resistin hormone analysis; after clot formation, the sample was centrifuged at 3000 rpm for 10 min to separate serum and stored at -20 °C until resistin assay was performed. About 3 mL of blood was collected in an EDTA tube for genomic DNA extraction and stored at 4 °C until further process.

Serum resistin levels were determined using a commercially available ELISA kit, which was based on the sandwich principle (Glory Science Co. Ltd, USA), according to the kit manual. The absorbance of samples was taken by reading the micro-plate on a semi-automated micro-plate reader (Bio-Rad, Germany) at a wavelength of 450 nm. Resistin standards were also tested on the plate along with the samples and a standard curve was generated to measure serum resistin levels.

Genomic DNA extraction was performed by the phenol-chloroform standard method (23). The primer sequence of the RETN gene (-420C>G) was used as described previously (24). The oligonucleotide sequences of SNP (forward primer 5’-TGTCATTCTCAC-CCAGAGACA–3’ and reverse primer 5’-TGGGCTCAGCTAACCAAATC–3’) were amplified by PCR. The reaction was performed in a 25-µL reaction tube containing 14 µL deionized water, 8 µL PCR Master Mix (2X GreenTaq), 0.5 µL forward and reverse primers (10 µM), and 2.0 µL DNA template. Thermal cycler conditions were the following: first strand denaturation (one cycle at 95 °C for 5 min), then 35 cycles of denaturation (95 °C), annealing (64 °C), extension (72 °C) for 30 s of each steps, and the final extension at 72 °C for 5 min. Amplification was verified on 2.0% agarose gel electrophoresis with a 100-bp DNA ladder and the amplicon size was 534 bp for the oligonucleotides. The PCR products were digested by restriction endonuclease BpiI enzyme, also known as Bbs1 (Fermentas, USA), and the digested PCR products were digested at 37 °C for 16 h. Enzyme inactivation was performed by incubating at 65 °C for 20 min. The digested PCR products were resolved on 2.5% agarose gel and band resolution was observed on the Gel Doc system (Bio-Rad) to interpret the genotype.

The data were analyzed using SPSS for Windows, version 21. Quantitative variables such as age and BMI were presented as mean±standard deviation. Serum resistin levels were presented as mean±standard error of the mean. An independent t-test was used to determine the mean difference in serum resistin levels between the groups. Categorical variables such as polymorphism were calculated in frequencies and percentages. In order to calculate differences in genotypes and allele frequencies, Fisher’s exact test was used. The effect of SNP on the risk of developing PCOS was estimated with an odds ratio (OR) by the chi-square test. A p-value of less than 0.05 was considered statistically significant.

Results

The study included 80 participants: 40 PCOS cases and 40 healthy controls without a history of PCOS. The comparison of different clinical parameters between cases and controls and their demographic data are given in Table 1. There were significant differences in clinical features (irregular menstrual cycle, weight gain, and hirsutism) between PCOS women and healthy controls (p<0.05). The quantitative variables are presented in Table 2. In PCOS cases, the mean±SD age was 24.20±4.76 years, while in controls it was 22.30±3.52 years. The mean BMI was 27.44±7.855 and 20.69±3.982 in cases and controls, respectively. The mean serum level of resistin was higher in PCOS women (19.33±3.50 ng/mL) than in controls (13.48±1.31 ng/mL), but there was no significant difference (p=0.125).

Genotype and allele distribution of RETN (-420C>G) SNP showed a single band of 534 bp for CC genotype (wild, homozygous), double bands of 327 and 207 bp for GG genotype (rare, homozygous), and triple bands of 534, 327, and 207 bands for CG (heterozygous) (Figure 1). The frequency of the RETN genotype in PCOS cases was 10.0% (n=4) for CC, 50.0% (n=20) for CG, 40.0% (n=16) for GG genotypes, and in controls was 12.5% (n=5) for CC, 50.0% (n=20) for CG, 40.0% (n=16) for GG genotypes, and in controls was 12.5% (n=5) for CC, 67.5% (n=27) for CG, and 20% (n=8) for GG genotypes. The allele distribution frequency shows that the G allele was present in 52 (65%) PCOS cases, which is higher compared with that in controls [43 (53.7%)]. The association determined by using chi-
square ($X^2$) test was not significant ($p=0.148$) between genetic polymorphism and disease (Table 3).

The association between serum resistin levels and $RTEN$ gene polymorphism was analyzed for genotype carriers. GG genotype carriers had the highest resistin levels in PCOS cases than in controls and a significant association was noted ($p=0.027$), while CG and CC genotypes were not associated between cases and controls (Table 4).

**Discussion**

The prevalence of PCOS is alarmingly increasing and PCOS is becoming a health issue for women of reproductive age. Several genetic and environmental factors are responsible for PCOS. Resistin, an adipokine, is considered a risk factor of metabolic syndrome, PCOS, osteoarthritis, type 2 diabetes, insulin resistance, and obesity (14-17). Several SNPs of the resistin ($RTEN$) gene have been reported for complex diseases, but the promoter region SNP -420C>G has a potential influence on circulating resistin levels and $RTEN$ gene expression (17).
In the present study, the allele distribution frequency of the RETN gene (-420C>G) SNP was not different in PCOS patients than in controls (p=0.148). The GG genotype distribution of the RETN gene was higher in PCOS cases than in controls [16 (40%) and 8 (20%), respectively], but no statistically significant difference was observed. The findings of the present study are consistent with those of the previous studies, in which no association was reported between RETN polymorphism and PCOS (25, 26). A study of Spanish women with PCOS showed a high frequency of the G allele of the RETN gene (420 C>G; rs1862513) polymorphism, but no association was found (27). Similarly, the frequency of the G allele variant of the RETN gene is common in Pakistani women. A study of South Indian women reported a high frequency of G allele of the RETN promoter region (28), which is consistent with the results of the present study. In contrast to the current study, some previous studies have demonstrated the association of RETN polymorphism in PCOS women (29). The exact molecular mechanism of -420C>G polymorphism is still unclear, but the polymorphism may be a disease predisposing factor in the combination of complex genetic and environmental contributors. In the present study, serum resistin levels were higher in PCOS patients but there was no association in the levels between PCOS cases and controls. Previous studies have described high serum resistin levels in PCOS cases, but the association was not significant (28, 30, 31). On the other hand, some studies did not find higher serum resistin levels in PCOS women compared to healthy controls (31, 32).

The variants of the RETN gene affect mRNA expression and circulating serum levels of resistin. Our results showed that women with the GG genotype had higher serum resistin levels compared with control GG carriers. A previous study found elevated levels of resistin mRNA in the adipocyte cells of PCOS patients and reduced adipocyte-resistin mRNA expression in laparoscopic ovarian drilling (33). Many studies on genetic polymorphism have described RETN gene involvement in PCOS pathogenicity. These studies were inconsistent and non-conclusive, which may be due to the variabilities in sample size, disease status, and ethnicity (18, 24, 34, 35). PCOS is a complex syndrome that depends on the interaction of genetic and environmental factors. Variants in proteins acting functionally upstream of the resistin gene could modulate the expression of serum resistin levels and affect the disease phenotype.

Table 3. Polymorphism of RETN Gene promoter region (-420C>G) in the cases and controls.

| Genotype/Allele | PCOS Cases | Controls | OR (CI) | p-value |
|-----------------|------------|----------|---------|---------|
| CC              | 4 (10.0%)  | 5 (12.5%)|         | 0.148*  |
| CG              | 20 (50.0%) | 27 (67.5%)|         |         |
| GG              | 16 (40.0%) | 8 (20.0%)|         |         |
| C               | 28 (35%)   | 37 (46.3%)| 1.59 (0.846-3.017) | 0.148*  |
| G               | 52 (65%)   | 43 (53.7%)|         |         |

*p-value was calculated by Fisher’s exact test, *calculated by chi-square test.

Table 4. Association of RETN (-420C>G) polymorphism and serum resistin levels in PCOS cases and controls.

| Genotype | Serum resistin |
|----------|----------------|
|          | PCOS Cases     | Controls     | p-value* |
| CC       | 11.88±1.008    | 12.92±2.009  | 0.659    |
| CG       | 13.49±2.726    | 14.72±1.867  | 0.703    |
| GG       | 28.48±7.671    | 9.66±0.527   | 0.027    |

*p-value was calculated by Student’s t-test.
Conclusion
The association of RETN (-420C>G) polymorphism genotype frequencies and serum resistin levels are not associated with disease susceptibility of PCOS in Pakistani women. Although GG genotype carriers with PCOS had higher serum levels of resistin than control GG phenotype carriers did, there is no exact role of the genotype in disease pathogenicity. Furthermore, future studies including large sample size and various ethnic or language groups are needed to understand the molecular mechanism of disease progression.

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Ethical Consideration
Ethical approval for this study was obtained from the Ethical Committee and IRB of the University of Health Sciences, Lahore.

Author Contributions
Idea/Concept: Sumbla Ghazanvi, Shah Jahan; Design: Sumbla Ghazanvi; Control/Supervision: Sumbla Ghazanvi; Data Collection and/or Processing: Yasar Nawaz, Nadia Rasheed; Analysis and/or Interpretation: Shah Jahan, Muhammad Ikram Ullah; Literature Review: Yasar Nawaz, Nadia Rasheed; Writing the Article: Yasar Nawaz, Nadia Rasheed, Muhammad Ikram Ullah; Critical Review: Sumbla Ghazanvi, Shah Jahan, Muhammad Ikram Ullah.
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The Relationship Between C-Peptide Index and Proteinuria in Patients with Type 2 Diabetes Mellitus

Tip 2 Diabetes Mellitus Hastalarında C-Peptid İndeks ile Diyabetik Nefropati Arasındaki İlişki

Bilal KATİPOĞLU, Mustafa ÇOMOĞLU, İhsan ATEŞ, Nisbet YILMAZ, Dilek BERKER*

Ankara Numune Training and Research Hospital, Department of Endocrinology and Metabolism, Ankara, Turkey

*Ankara Numune Training and Research Hospital, Department of Endocrinology and Metabolism, Ankara, Turkey

Abstract

**Amaç:** C-peptid indeksi (CPI), beta hücre rezervinin daha güvenilir bir markanın kabul edildiği, ancak Tip 2 diabetes mellitus (DM) hastalarında CPI’nin mikrovasküler komplikasyonlara ilişkisi net olarak aydınlatılamamıştır. Bu nedenle, bu çalışmada, Tip 2 DM hastalarda C-peptid düzeyleri ile mikrovasküler komplikasyonların arasındaki ilişkiyi araştırmayı amaçladık. **Gereç ve Yöntemler:** 2014-2018 yılları arasında endokrinoloji ve dahiliyede Tip 2 DM tanısı ile takip, C-peptid seviyeleri analiz edilen, normal böbrek fonksiyonlar (glomerüler filtre hizi >60 mL/dak) olan ve insulin sekretagog türevi oral antidiabetik kullanmayan (sülfonilüre vb.) 18 yaş üstü hastalar çalışmaya dahil edildi. Oral ilaçların alımı veya insulin kullanım olmayan hastaların 12 saat açık sonrası serum örnekleri alınarak, hemogram, hemoglobin A1c (HbA1c), lipid, glukoz, C-peptid parametreleri analiz edildi. **Sonuç:** Bu çalışma, Tip 2 DM hastalarında C-peptid, CPI ve diyabetik nefropati arasındaki ilişkiyi işk tutmaktadır.

Keywords: C-peptide index; diabetes mellitus; diabetic nephropathy

Anahtar kelimeler: C-peptid indeks; diabetes mellitus; diyabetik nefropati

Address for Correspondence: Bilal KATİPOĞLU, Sağlık Bilimleri University Konya Training and Research Hospital, Department of Internal Medicine, Konya, Turkey

Phone: +90 332 323 67 09 E-mail: drbilial07@gmail.com

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Introduction

C-Peptide levels are generally evaluated in clinical practice as a marker for the demonstration of beta-cell reserves. Recent studies have emphasized the role of C-peptide as an active hormonal molecule (1). It plays an important role in blood glucose regulation by activation of many intracellular pathways. Inflammation and intracellular reactive oxygen molecules may cause the progression of diabetic microvascular complications. C-peptide exerts a cytoprotective effect against oxidative damage and inflammation induced by hyperglycemia (2). For instance, type 1 DM patients have a negative correlation between diabetic nephropathy and C-peptide levels (3). On the other hand, the relationship between C-peptide level and microvascular or macrovascular complications has not been clearly elucidated in non-insulin-dependent (type 2) DM patients (4-6).

Previous studies assert that C-peptide levels may be associated with microvascular complications in patients with type 2 DM. C-peptide levels can be affected by fasting. For that reason, the C-peptide index (CPI) may prove to be a useful indicator of beta-cell function. Previous studies have shown that CPI is a more reliable marker in demonstrating beta-cell reserves (7). The function of CPI as a more valuable marker for demonstration of microvascular complications is not yet clear. The authors assume that CPI may be associated with microvascular complications in type 2 DM patients. Therefore, this study was performed with an aim to determine the relationship between CPI and diabetic nephropathy in type 2 DM patients.

Material and Methods

The study is in accordance with the patient rights regulation of the Helsinki Declaration and has been approved by the Scientific Research Evaluation Commission of Ankara Numune Training and Research Hospital on 25/05/2018 (decision number 1990/2018).

Patient Selection

This retrospective study was performed at Ankara Numune Training and Research Hospital. A total of 359 type 2 DM patients who had C-peptide levels measured in the Endocrine and Internal Medicine clinics between 2014-2018 were enrolled in the study. Patients with a history of malignancy, infectious disease, chronic kidney disease and those using insulin secretagogue (such as sulfonylurea) were excluded from the study.

Parameters in the Study

Blood samples were collected from the antecubital vein after fasting for at least 12 h and without the consumption of any drug or insulin injection. All biochemical parameters were analyzed from the same serum sample. Laboratory parameters were recorded in the electronic files of each patient. Glucose was measured using the enzymatic UV Hexokinase method in Beckman Coulter AU 5800 (Beckman Coulter Inc., USA) autoanalyzer. Spot urine protein and microalbumin were measured via the turbidimetric method using Hitachi Modular P800 (Roche Diagnostic Corp., Indiana, Indianapolis, USA) autoanalyzer. Hemogram parameters were measured via the hematology analyzer Sysmex XE 2100 (Roche Diagnostic Corp., Indiana, Indianapolis, USA). HbA1c was measured via an automated glycohemoglobin analyzer (Tosoh HLC-723 7; Tosoh co., Tokyo, Japan) using a high-performance liquid chromatography method. Plasma C-peptide was assessed by the radioimmunoassay method (Coat-count RIA kit, Diagnostic Products Corporation, Los Angeles, California, USA).

C-peptide index (CPI): Fasting C-peptide as ng/mL and Fasting blood glucose as mg/dL×100.

Patients were classified into three groups according to spot urine albumin/creatinine ratio.

The value of the albumin-creatinine ratio in the spot urine <30 mg: normal, defined as Group 1; 30 to 300 mg: microalbuminuria, defined as Group 2, >300 mg: macroalbuminuria, defined as Group 3.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 22.0 (IBM SPSS Inc., Chicago, IL) software. Kolmogorov-Smirnov test was utilized to determine the distribution of data. Normally distributed numerical variables are expressed as mean±standard deviation,
While non-normally distributed variables are indicated as median (25th-75th percentile). Continuous variables were compared using independent sample t-test, ANOVA or Mann Whitney U test, and Kruskal Wallis H test, as appropriate. A comparison of categorical variables was done with the chi-square test. P<0.05 values were considered statistically significant. Logistic regression was used to determine the odds ratio (OR) for the independent predictors. A stepwise multiple logistic regression analysis was performed to identify independent determinants of proteinuria. Since C-peptide measurements were not normally distributed, the logarithmic transformation was applied in multivariate linear regression analysis.

**Results**

The demographic characteristics and laboratory findings of type 2 DM patients are summarized in Table 1. A statistically significant difference was noted between the age of patients in the three groups (p=0.014); the age of patients in Groups 2 and 3 was lower than that in Group 1 (p=0.004 and p=0.035). No statistically significant difference was observed between the other groups (p>0.05). A statistically significant difference was found between the gender of patients in the groups (p=0.018); the number of women in Groups 2 and 3 was lower than that in Group 1 (p=0.049 and p=0.031). No statistically significant difference was observed in the median DM duration and C-peptide between groups (p>0.05). A statistically significant difference was noted in the CPI level between the groups (p<0.001); the CPI level of Groups 2 and 3 was lower than that of Group 1 (p=0.007 and p<0.001). In addition, the CPI level of Group 3 was significantly lower than that of Group 2 (p=0.015). There was a statistically significant difference between the groups in terms of HbA1c levels (p<0.001); the HbA1c levels of Groups 2 and 3 were higher than that of Group 1 (p<0.001 and p<0.001). In addition, the HbA1c level of Group 3 was higher than that of Group 2 (p=0.04).

C-Peptide and CPI were compared with proteinuria using univariate logistic regression analysis (Table 2) and it was observed that C-peptide was not statistically significant in predicting proteinuria (p=0.112) and no association between C-peptide and proteinuria was observed (Odds ratio=0.898 (95% Confidence Interval: 0.787-1.025)). Instead, a statistically significant and inverse association between the CPI and proteinuria (Odds ratio=0.707 (95% confidence interval: 0.577-0.866) and p<0.001) was expressed. The multivariate regression model which included findings related to proteinuria (CPI, age, sex, DM duration, and HbA1c) showed that CPI did not predict proteinuria in a statistically significant form (p>0.05) (Table 3).

**Discussion**

This study highlights three important results. First, it was found that CPI was lower

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**Table 1. Demographic and clinical features of groups in Type 2 DM.**

|                      | Group 1 No Proteinuria (n=214) | Group 2 Microalbuminuria (n=102) | Group 3 Macroalbuminuria (n=43) | p-value |
|----------------------|---------------------------------|----------------------------------|---------------------------------|---------|
| Age (year)           | 55.0 (46.0-61.0) \(a\)\(^b\)    | 50.0 (39.0-57.0) \(a\)           | 46.0 (37.7-59.0) \(a\)          | 0.014 \(t\) |
| Gender, n (%)        |                                 |                                  |                                 | 0.018 \(t\) |
| Male                 | 86 (%40.2) \(a\)\(^b\)          | 53 (%52.0) \(a\)                | 26 (%60.4) \(a\)               |         |
| Female               | 128 (%59.8) \(a\)\(^b\)        | 49 (%48.0) \(a\)               | 17 (%39.6) \(a\)              |         |
| DM duration (year)   | 5 (2-10)                        | 5 (2-8)                         | 8 (4-15)                       | 0.236 \(t\) |
| C-peptide (ng/mL)    | 2.2 (1.5-3.4)                   | 2.0 (1.3-3.4)                   | 1.7 (1.1-2.7)                  | 0.125 \(t\) |
| C-peptide index      | 1.5 (0.8-2.5) \(a\)\(^b\)      | 1.2 (0.6-1.8) \(a\)\(^c\)      | 0.8 (0.5-1.1) \(a\)\(^c\)     | <0.001 \(t\) |
| HbA1c (%)            | 7.6 (6.5-10.0) \(a\)\(^b\)     | 9.3 (7.2-12.0) \(a\)\(^c\)     | 10.0 (8.8-12.3) \(a\)\(^c\)   | <0.001 \(t\) |

Descriptive statistics; median (25th-75th) percentile for continuous numerical variables, and number of cases (%) for categorical variables, \(t\)Kruskal Wallis test, \(\sim\)Likelihood Ratio test, \(^a\) The difference between Group 1 vs. Group 2 (p<0.05), \(^b\) The difference between Group 1 vs. Group 3 (p<0.05), \(^c\) The difference between Group 2 vs. Group 3 (p<0.05).
and HbA1c was higher in patients with proteinuria. In the second place, a statistically significant and inverse association between the CPI and proteinuria was observed. Since CPI is altered depending on fasting, it has been assumed to be a more precious marker. At the third place, it was found that HbA1c is the most important factor in the relationship with proteinuria.

C-peptide levels may also have direct molecular effects, which act as protective factors against diabetic microvascular and macrovascular complications. In light of recent studies, C-peptide has been shown to inhibit the formation of endothelial cell reactive oxygen species (8,9). The C-peptide also downregulates adhesion molecules on leukocytic cells. Furthermore, it prevents atherosclerotic plaque formation (10).

Besides, since glucose itself is a great stimulus for pancreatic cells, insulin secretion is increased by higher glucose levels. For that reason, the C-peptide level may also be higher than normal (11). Therefore, for the assessment of beta-cell function, the level of C-peptide should be adjusted by glucose (7). CPI is obtained by dividing the C-peptide level by fasting blood glucose. A recent study pointed out that CPI and beta-cell function were more closely associated with insulin requirements, though its association with microvascular complications could not be clarified completely (12,13).

Type 2 is the most common type of diabetes in adults (14). In the past, insulin resistance was accepted as the main problem in the pathogenesis of type 2 DM. However, Butler et al. established that beta-cell reserve decreases in both, obese and non-obese type 2 DM patients (15). Nowadays, the lower beta-cell reserve is considered to play a role in the etiopathogenesis of both, type 1 and type 2 diabetes (16). Therefore, evaluation of beta-cell function is important in either type of diabetes.

Several studies have investigated the relationship between C-peptide levels and diabetic complications in type 2 DM patients. The low C-peptide level has been associated with the progression of diabetic microangiopathy (4). Shin et al. showed that C-peptide measurement using glucagon

| C-peptide (ng/mL) | No Proteinuria (n=214) | Proteinuria (n=145) | p-value | Odds ratio (%95 Confidence interval) |
|-------------------|------------------------|---------------------|---------|-------------------------------------|
| 2.2 (1.5-3.4)     | 1.9 (1.2-3.0)          | 0.112               | 0.898 (0.787-1.025) |
| C-peptide index   | 1.0 (0.6-1.6)          | <0.001              | 0.707 (0.577-0.866) |

| Microalbuminuria (Group 2) | Odds ratio | %95 Confident Interval | Lower bound | Upper bound | Wald | p-value |
|-----------------------------|------------|------------------------|-------------|-------------|------|---------|
| Age                         | 0.997      | 0.972                  | 1.024       |            | 0.039 | 0.844   |
| Male factor                 | 1.142      | 0.612                  | 2.130       |            | 0.175 | 0.676   |
| DM duration                 | 0.943      | 0.889                  | 1.000       |            | 3.782 | 0.052   |
| HbA1c                       | 1.283      | 1.108                  | 1.484       | 11.155     | <0.001 |         |
| C-peptide index             | 0.920      | 0.667                  | 1.269       |            | 0.261 | 0.610   |

| Macroalbuminuria (Group 3)  | Odds ratio | %95 Confident Interval | Lower bound | Upper bound | Wald | p-value |
|-----------------------------|------------|------------------------|-------------|-------------|------|---------|
| Age                         | 0.951      | 0.907                  | 0.998       | 4.138       | 0.042 |         |
| Male factor                 | 1.333      | 0.487                  | 3.644       | 0.313       | 0.576 |         |
| DM duration                 | 1.039      | 0.963                  | 1.120       | 0.977       | 0.323 |         |
| HbA1c                       | 1.273      | 1.023                  | 1.582       | 4.702       | 0.030 |         |
| C-peptide index             | 0.543      | 0.259                  | 1.140       | 2.605       | 0.107 |         |
stimulation test has a negative correlation with albuminuria and diabetes duration (6). Several recent studies have reported that there is an important link between low C-peptide levels and diabetic nephropathy (5). Moreover, C-peptide has been shown to exert protective effects against renal impairment, which was demonstrated in patients with combined renal-pancreatic islet transplantation. The survival rate of renal allograft was found to be increased in patients with successful islet cell transplantation (17).
Lower levels of C-peptide and decreased beta-cell reserve have been correlated to greater levels of glucose variability (18). Moreover, glucose variability is known to be associated with diabetic complications (19). It is, therefore, possible that C-peptide may be a predictor for diabetic complications. In fact, it has long been known that the HbA1c level is a manifestation of blood glucose regulation. It can be assumed that patients with higher HbA1c levels also have more glucose toxicity than patients with lower HbA1c levels. For that reason, higher HbA1c levels have an increased risk of nephropathy.
On the other hand, some studies have shown that there is no relationship between the C-peptide level and diabetic microvasculopathy. Klein et al. demonstrated that there was no link between C-peptide level and the incidence or progression of diabetic retinopathy (20). Chowta et al. found that serum C-peptide level has a weak correlation with microalbuminuria and creatinine clearance (21).

Conclusion
In conclusion, a reverse relationship between CPI and nephropathy was observed. However, HbA1c level is a more valuable marker than others, for the prediction of diabetic microvascular complications like nephropathy. However, further studies with longer observation periods are needed to address this issue. The limitations of the study include the retrospective design and the fact that it was performed in a single center. Also, the results could not be generalized to the common population. There is a limitation in cross-sectional studies especially in terms of causality, and randomized controlled studies are required to overcome this weakness. Patients' history relating to drugs, duration of diabetes, treatment regimens, history of chronic diseases were recorded from the file and there may be missing or incorrect data.

Ethical Approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee that Ankara Numune Training and Research Hospital Scientific Studies Evaluation Commission on the date of 25/05/2018 with the decision number 1990/2018 and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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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: Bilal Katipoğlu, Dilek Berker; Design: Bilal Katipoğlu, Mustafa Çomoğlu, İhsan Ateş; Control/Supervision: Nisbet Yılmaz, Dilek Berker, Mustafa Çomoğlu; Data Collection and/or Processing: Bilal Katipoğlu, Mustafa Çomoğlu, İhsan Ateş, Nisbet Yılmaz; Analysis and/or Interpretation: Bilal Katipoğlu, İhsan Ateş, Dilek Berker; Literature Review: Bilal Katipoğlu, Mustafa Çomoğlu; Writing the Article: Bilal Katipoğlu, İhsan Ateş; Critical Review: Nisbet Yılmaz, Dilek Berker; References and Fundings: Nisbet Yılmaz, Dilek Berker; Materials: Bilal Katipoğlu, Mustafa Çomoğlu.
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Fatigue is Related to Insulin Use by Acting Via Depressive Mood in Patients with Diabetes Mellitus

Diyabet Hastalarında Görülen Yorgunluk, İnsulin Kullanımına Bağlı Oluşan Depresif Duygudurum ile İlişkilidir

Özlem HALİLOĞLU, Mesude TÜTÜNCÜ*, Serdar ŞAHİN, Özge POLAT KORKMAZ, Melis Dila ÖZER**, Zeynep OŞAR SİVA

Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Division of Endocrinology-Metabolism and Diabetes, Istanbul, Turkey

*Bakırköy Prof. Dr. Mazhar Osman Training and Research Hospital, Department of Neurology, Istanbul, Turkey

**Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Internal Medicine, Istanbul, Turkey

Abstract

Objective: Fatigue is a common symptom in diabetes mellitus. The aim of this study was to determine the factors leading to fatigue and to investigate the effect of insulin use on fatigue among the diabetic population. Material and Methods: One-hundred diabetic patients attending the diabetes clinic of Cerrahpaşa Medical Faculty between October 2017-January 2018 and 42 healthy controls were evaluated in this cross-sectional study. Questionnaires including demographic and disease characteristics, Fatigue Impact Scale (FIS), Fatigue Severity Scale (FSS), Beck Depression Inventory (BDI), quality of life scale (SF-36), Epworth Sleepiness Scale (ESS), and Pittsburgh Sleep Quality Index (PSQI) were used. Results: Ages (47.6±14.8 and 45.7±14.1 years; p=0.47) and body mass indices (26.6±4.1 and 25.3±3.5 kg/m²; p=0.08) of 100 patients with diabetes (Type 1/Diabetes Type 2 Diabetes= 29/71) and 42 healthy volunteers were similar. The diabetic group had worse FIS total (p=0.05), FIS psychological (p=0.04) scores and SF-36 scores compared to the healthy controls. When the patients with diabetes were divided into two groups according to insulin use and compared with healthy controls, the ESS and PSQI were similar but all FIS parameters (total p=0.005, cognitive p=0.007, physical p=0.01, psychological p=0.009) and BDI (p=0.05) were significantly worse in patients with insulin use than non-insulin and control groups. The relationship between fatigue and insulin use was independent of glycemic control and duration of diabetes but was affected by the BDI (p=0.001). Conclusion: Insulin use leads to fatigue in patients with diabetes, regardless of diabetes type, and this effect is influenced by depressive mood. Psychotherapeutic approaches prior to insulin treatment might yield fruitful results.

Keywords: Depression; diabetes mellitus; fatigue; quality of life; sleep

Anıta kelimeler: Depresyon; diabetes mellitus; yorgunluk; hayat kalitesi; uykudan etkilenmektedir.
Introduction

Diabetes mellitus (DM) is a chronic disease, increasing at an alarming rate. It is a serious public health problem all over the world. Apart from the most widely known microvascular and macrovascular complications associated with diabetes, comorbidities such as fatigue, depression, and sleep disturbances affect the quality of life and the compliance to treatments of patients with diabetes (1,2).

Fatigue is a common symptom in the general population that is known to be associated with different etiologies, negatively affecting both physical and mental capacity. It is very often encountered in patients suffering from diabetes and seriously affects the quality of life (3-5). The literature studies concerning patients affected with type 1 (T1D) and 2 (T2D) diabetes mellitus suggest the duration of diabetes mellitus, glycemic control, the frequency and severity of hypoglycemic attacks, depression, sleep problems, microvascular complications, pain, and body mass indices are the major predisposing factors for developing fatigue (2,6,7). Fatigue is also known to impair the compliance of treatment and disturb the glycemic control of patients with diabetes (1,2).

Depression is frequent comorbidity associated with diabetes, as recent studies documented that depression affects up to 25% of patients and the risk of major depression is doubled in patients with T2D (8). Depression hampers the quality of life, diminishes self-care, and impairs the glycemic control of patients with diabetes, leading to enhanced risks of micro- and macrovascular complications (9).

Insulin is one of the cardinal anti-diabetic treatments worldwide, but patients are often reluctant to start and use this medication. Various studies have demonstrated that diabetic patients suffering from comorbid conditions of depression or anxiety disorders avoided insulin therapy more than patients without these comorbidities (10). In addition, a study from France showed that fatigue was more frequently witnessed in T2D patients, especially in the insulin-treated T2D patients as compared to the T1D patients, coupled with significant impairment of the motivation scale (11). To our knowledge, this study from France is unique in literature in terms of evaluation of the insulin-fatigue relationship. However, the factors affecting this association were not estimated in detail.

In light of these findings, we aimed to investigate whether the use of insulin caused fatigue and to determine the factors responsible for developing fatigue in insulin-treated patients. For this purpose, we assessed the frequency of fatigue, sleep disturbances, and depression and the impact of these comorbidities on the quality of life of both T1D and T2D patients. The relationship between these parameters and insulin use were also estimated.

Material and Methods

Subjects and Study Design

One hundred patients with T1D and T2D who attended the Diabetes outpatient clinic of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty between October 2017 and January 2018 and 42 age and sex-matched healthy volunteers were enrolled in the study. Patients suffering from cancer, end-stage kidney disease, rheumatologic diseases, fibromyalgia, depression, symptoms of snoring or diagnosis of obstructive sleep apnea (OSA), recent acute cardiovascular events, acute and chronic infections, those who were hospitalized in 3-months period prior to the start of the study or who were pregnant, were excluded from the study.

Clinical Assessments

The demographic characteristics of the participants were obtained from the patients themselves and the disease characteristics were evaluated from their medical files. A detailed medical history and physical examination were performed by an endocrinologist and hypoglycemia symptoms in the last month were evaluated and classified as mild, severe, and nocturnal. When the patient was able to treat the symptoms of hypoglycemic episodes unaided, it was considered minor. If patients needed help or medical intervention from others, then it was recorded as major. Any minor or major hypoglycemic episodes that occurred at night during sleeping was documented as
nocturnal hypoglycemia. The Douleur Neuropathique 4 (DN4) questionnaire was used to assess neuropathic pain. The patients were then asked to complete 6 questionnaires under the supervision of a diabetes nurse: the Fatigue Impact Scale (FIS), Fatigue Severity Scale (FSS), Beck Depression Inventory (BDI), 36-Item Short Form (SF-36) quality of life scale, Epworth Sleepiness Scale (ESS), and Pittsburgh Sleep Quality Index (PSQI).

Diabetic neuropathic pain assessment: Douleur neuropathique 4 (DN–4) questionnaire
DN–4 is a ten-item questionnaire prepared by the clinician. It contains seven items to estimate the pain quality and evaluated through self-reports of the patients. The rest three items are assessed in physical examinations made by a physician. Painful neuropathy is signified by a value of ≥4 points. It was developed in France and the Turkish validation was reported by Unal-Cevik et al. (12).

Fatigue impact scale (FIS)
The FIS is a multi-dimensional scale that comprises of 40 questions measuring the physical (10 questions), cognitive (10 questions), and social (20 questions) effects of fatigue. Every question is scored between 0-4 and total scoring is between 0-160. The score is proportional to the impact of fatigue. The Turkish validation of FIS was performed by Armutlu et al. (13).

Fatigue severity scale (FSS)
The FSS is a 9-item questionnaire that quantifies the effect of fatigue on functioning. Every question has a score of 1-7 points and the mean of the points is determined as the total score. A score of ≥4 points indicates ‘severe fatigue.’ The first Turkish validation of the FSS was performed by Gencay-Can et al. (14).

Beck depression inventory (BDI)
The BDI is a 21-item, self-reporting scale that identifies the symptoms of depression. Every question has a score of 0-3 points. Depression is reflected by a score of ≥17. The Turkish validation of BDI was conducted by Hisli N. in 1989 (15).

36-item short form (SF-36) quality of life scale
The SF-36 has 36 questions with 8 subscales based on role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, emotional well-being, physical functioning, social functioning, general health, and pain. Every subscale has a score of 0-100 and the score is proportional to the quality of life. The Turkish validation of SF-36 was performed by Kocyigit et al. (16).

Epworth sleepiness scale (ESS)
The daytime sleepiness is estimated by a simple and self-reported questionnaire namely ESS. It has 8 questions and a total score of 0-24. A score of ≥10 points directs patients to be examined using polysomnographic methods. The Turkish validation of ESS was made by Agargun et al. (17).

Pittsburgh sleep quality index (PSQI)
The PSQI is another self-reported questionnaire that evaluates sleep quality over a period of 1 month. It has 19 items that generate seven subscales: overall sleep quality, sleep disturbances, sleep latency, sleep duration, sleep efficiency, use of sleeping medication, and daytime dysfunction. A high total score indicates poor sleep quality. The Turkish validation of the PSQI was conducted by Agargun et al. (18). The study was approved by the local ethics committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty in accordance with the 1964 Helsinki Declaration. Written informed consent was obtained from all the participants prior to the study.

Statistical analysis
The data were statistically analyzed by the Statistical Package for the Social Sciences for Windows version 21.0 software package (SPSS, Chicago, IL). The results were expressed as mean±standard deviation (SD). In two group comparisons, the independent Student’s t-test was used for continuous variables and the Chi-square (χ²) test was used for categorical variables. One-way analysis of variance (followed by Tukey’s post-hoc multiple analyses) together with Bonferroni correction was performed to compare three or four groups. In order to compute the potential effects of age, gly-
cated hemoglobin (HbA1c), and BDI on the fatigue scales, the analysis was performed by using these parameters as covariates. Pearson’s and Spearman’s correlations were used for parametric and nonparametric values, respectively. Receiver operating characteristic (ROC) curve analyses were used and the cut-off for the FIS was found to be 22.5 points. A stepwise multiple regression analysis was performed to define the predictors of FIS. Statistical significance was established at p≤0.05.

**Results**

One hundred patients and 42 age and sex-matched healthy volunteers were enrolled in the study. The demographic characteristics of the study groups are represented in Table 1.

The mean age of the participants with diabetes (female/male: 51/49) was 47.6±14.8 years. The mean duration of diabetes was 12.0±7.8 years. The comorbidities as observed in the present study reflected 14% cases of retinopathy, 16% nephropathy, and 37% neuropathic pain according to the DN–4 criteria. Macrovascular complications comprised of ischemic heart disease in 14% of the patients, cerebrovascular disease in 1%, and peripheral arterial disease in 2% of the diabetic subjects. Hypoglycemic symptoms were manifested in 70 patients, 53 being mild symptoms, 9 severe symptoms, and 8 had nocturnal hypoglycemia. On estimating the questionnaires according to the complications, higher scores of FIS total (p<0.001) and all FIS subscales (p<0.001 for all), FSS (p=0.004), BDI (p<0.001) were revealed for the patients suffering from painful neuropathy. These patients also exhibited lower scores for physical functioning (p=0.001), social functioning (p=0.04), pain (p<0.001), and general health (p=0.04) subscales of the SF-36. The patients with nephropathy also showed a high FSS score (p=0.008). It is noteworthy that the FIS total (0.004), FIS cognitive (p=0.002), FIS physical (p=0.006), FIS psychosocial (p=0.01), and BDI (p=0.02) scores were elevated in patients suffering from hypoglycemic symptoms. In addition, the patients with BDI scores of >17 had higher FIS (cognitive: p<0.001, physical: p<0.001, psychosocial: p<0.001, total: p<0.001) and FSS (p=0.005) scores than those with BDI of <17. The scores of the questionnaires depicted a similar outcome for the patients suffering from other diabetic complications. However, no significant correlation between the scales and glycemic control (HbA1c and fasting plasma glucose) was noted. When the subjects with diabetes were compared with the healthy volunteers, the patients with DM had higher FIS total (p=0.05) and FIS psychological (p=0.04) subscale scores and worse physical functioning (p=0.005), social functioning (p=0.02), pain (p=0.01), and general health (p=0.04) sub-

### Table 1. Demographic characteristics of the study group.

|                             | Type 1 DM (n=29) | Type 2 DM with insulin use (n=35) | Type 2 DM without insulin use (n=36) | Healthy controls (n=42) |
|-----------------------------|------------------|-----------------------------------|---------------------------------------|-------------------------|
| Age (years)                 | 32.9±11.1*,αβ     | 53.5±10.9'                        | 53.8±12.5'                            | 45.7±14.1               |
| Sex (F/M)                   | 11/18            | 18/17                             | 22/14                                 | 28/14                   |
| Educational status          |                  |                                   |                                       |                         |
| Primary education           | 8                | 19                                | 18                                     | 10                      |
| Higher education            | 21               | 16                                | 18                                     | 32                      |
| Monthly income (TL)         | 2865±1519'       | 2938±1881'                        | 2556±1142'                            | 4342±2507               |
| Exercise                    |                  |                                   |                                       |                         |
| No exercise                 | 7                | 12                                | 11                                     | 11                      |
| Intermittent                | 11               | 14                                | 10                                     | 15                      |
| Regular                     | 11               | 9                                 | 15                                     | 16                      |
| BMI (kg/m²)                 | 23.4±3.3*,α       | 28.3±4.0'                         | 27.8±3.2'                             | 25.3±3.5                |
| HbA1c (%)                   | 8.7±1.2          | 8.2±2.1                           | 7.0±1.1*,γ                            | ND                      |

*p<0.001 vs. Type 2 DM with insulin use; **p<0.001 vs. Type 2 DM without insulin use; *p<0.001 vs. healthy controls; †p<0.05 vs. healthy controls; ‡p<0.05 vs. healthy controls; γp<0.001 vs. Type 1 DM.
scale scores of the SF-36 index. However, the FSS, BDI, ESS, and PSQ indices were statistically similar between the two groups. On classifying the diabetic patients based on the diabetes type and insulin usage, FIS total (p=0.03) and FIS cognitive (p=0.01) subscales were significantly lower and pain subscale (p=0.01) of SF–36 index were higher in T2D patients without insulin use than in those with T1D and T2D with insulin therapy.

When the patients with diabetes were divided into two groups based on the use of insulin, viz., patients with insulin therapy and patients without insulin therapy, and compared with the healthy volunteers, FIS total (p=0.005) and all FIS subscales (cognitive: p=0.007; physical: p=0.01; psychological: p=0.009) and BDI (p=0.05) scores were higher and physical functioning (p=0.008), social functioning (p=0.01), and pain (p=0.001) subscales of SF–36 were lower in the group comprising of diabetic patients under insulin therapy (Table 2). Similar results were obtained after controlling for the HbA1c and age of the participants. The parameters related to FIS were examined using correlation analysis, both in the entire diabetic population and in each diabetes type. FIS scores were positively associated with FSS (r=0.45; p<0.001), total PSQI (r=0.74, p<0.001) (Figure 1a-c). Linear regression analyses depicted a one-unit increase in BDI led to a two-and-a-half-point increase in FIS total score (F=12.2; Beta= 0.74; p<0.001). ROC curve analysis for FIS was performed with 60% sensitivity and 60% specificity and a cut-off value of 22.5 was found. The parameters considered for the multivariate logistic regression model included age, sex, BMI, BDI score, PSQI, duration of diabetes, presence of hypoglycemia, insulin use, and fasting plasma glucose. Insulin use (p=0.01) and BDI scores (p=0.001) were shown to significantly influence the FIS score. Moreover, the exemption of the BDI parameter from the model was found to withdraw the effect of insulin use on fatigue (Table 3).

**Discussion**

In our study, we demonstrated that fatigue was more prevalent among the diabetic population compared to the healthy volunteers and the impact of fatigue affected their quality of life. Interestingly, as far as fatigue severity and sleep disturbances were concerned, a significant difference was absent between the two groups. Patients with diabetes with frequent hypoglycemic attacks were more prone to be affected by fatigue but hypoglycemia itself failed to explain the relationship between fatigue and insulin use.

| Patients with diabetes | Patients with diabetes | Healthy volunteers |
|------------------------|------------------------|--------------------|
|                        | with insulin use        | without insulin use |                        |
|                        | (n=64)                  | (n=36)             | (n=42)               |
| Fatigue impact scale   | 45.2±34.3               | 28.5±22.9          | 28.1±30.0           |
| Fatigue severity scale | 3.9±1.7                 | 3.6±1.7            | 3.7±1.5             |
| Beck depression inventory | 13.9±9.8              | 9.9±7.9            | 10.1±9.4            |
| SF-36                  |                        |                    |                     |
| Physical functioning   | 66.9±21.3               | 72.7±21.1          | 80.1±20.7           |
| Role limitations due to physical health | 61.7±28.5               | 72.2±27.8          | 71.4±32.9           |
| Role limitations due to emotional problems | 58.5±24.3               | 67.5±23.2          | 61±30.2             |
| Energy/fatigue         | 54±20.9                 | 57.6±19.8          | 57±21.2             |
| Emotional well-being   | 64.2±15.9               | 69.7±16.7          | 70.1±14.2           |
| Social functioning      | 63.2±22.4               | 71.6±17.1          | 75.1±22.5           |
| Pain                   | 61.6±21.6               | 75±21.6            | 77.2±24.1           |
| General health          | 54.1±24.8               | 57.9±21.3          | 63.8±18             |
| Epworth Sleepiness Scale | 5±3.6                | 4.9±3.8            | 4.8±3.3             |
| Pittsburgh Sleep Quality Index | 7±3.9              | 6.5±3.4            | 6.6±5               |

**Table 2. The comparison of the fatigue, sleep quality, depression, and SF–36 scales of the patients with diabetes according to the usage of insulin with healthy volunteers.**
The impact of fatigue was not related to diabetes type, glycemic control or disease duration, but it was more prominently observed in diabetic patients treated with insulin. The most impressive finding of this study was the significant correlation between the impact of fatigue with insulin use and depression, which has not been demonstrated previously. The difference in the fatigue impact scale, which shows the fatigue perception of the patient rather than the FSS, which reflects objective fatigue parameters, was important evidence with regards to the influence of the psychological factors. Fatigue is a common clinical finding among patients suffering from T1D and T2D (2,4,5). Various studies published in the literature have dealt with the causes of fatigue. Age, disease duration, BMI, glycemic control, acute and chronic complications, depression, and sleeping problems were the most common predisposing factors associated with acute and chronic fatigue (1,4-6,19). However, there are different schools of thought regarding the association between glycemic control and fatigue. Few studies revealed no relationship between glycemic control and fatigue (4,5). In our study, we demonstrated that fatigue was associated with hypoglycemia, painful neuropathy and nephropathy, quality of sleep, and depression, but we observed no significant relationship between the other parameters listed above.

Hypoglycemia is one of the most frequently seen complications among diabetic populations, particularly, those associated with insulin treatment. Fatigue is one of the most frequent findings following hypoglycemic episodes and it impairs the quality of life (20). In our study, we reported that 70% of patients with diabetes had hypoglycemia and we also demonstrated that patients with hypoglycemia had higher FIS and BDI scores in all subscales. However, we failed to establish any effects of hypoglycemia on the SF-36 quality of life index.

Painful neuropathy is one of the most common and debilitating complications of diabetes mellitus, seriously distressing the quality of life (21,22). Nocturnal pain in patients suffering from painful neuropathy is a major cause of sleep disturbances (23). Moreover, it has been shown that painful diabetic neuropathy enhances levels of anxiety and depression and is responsible for pain-induced disability (24). In line with the

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**Figure 1:** The correlation plot of the Fatigue Impact Scale with; Fatigue Severity Scale (a), Pittsburgh Sleep Quality Index (Total) (b), and the Beck Depression Inventory (c) in patients with diabetes mellitus.
published reports, our study also documented that 34% of patients had painful neuropathy and it was related to fatigue, depressive symptoms, and compromised quality of life. Importantly, we used a validated tool (DN-4) for diagnosing painful neuropathy in order to prevent bias due to the self-reporting of the patient. However, no relationship between sleep problems and neuropathy could be detected in the present study.

Published articles in the literature showed that the prevalence of minor and major depression was augmented in individuals with diabetes mellitus (7). Younger age, female sex, low income, poor glycemic control, and comorbidities and complications were factors associated with depression in diabetes mellitus (25,26). Interestingly, some studies in the literature demonstrated that insulin treatment might be associated with depression (27). Similar symptoms of depression between diabetic patients and healthy controls were recorded in our study. However, in the diabetes group, BDIs were found to be worse in patients with painful neuropathy and with hypoglycemia.

It is known that there is a negative appraisal regarding insulin treatment in patients with diabetes, the so-called ‘psychological insulin resistance’ (28,29). Makine et al. demonstrated that higher levels of depression and diabetes distress were associated with more negative beliefs about insulin in insulin-naive patients with T2D (10). Iversen et al. also showed that patients suffering from anxiety and depression were less likely to start insulin therapy (30). On the other hand, Lasselin et al. employed 21 T1D patients and 24 T2D patients and found that fatigue was more pronounced in the insulin-treated patients with T2D than in patients with T1D (11). In line with the literature, our study focused on the causes of fatigue and found that the positive correlation between insulin treatment and fatigue was mostly related to depressive symptoms.

Limitations
Our study has some limitations. The first is the cross-sectional design of the study. The cause-effect relationship could be more clearly identified if it was prospectively designed. A small sample size of the control group is another limitation of the present study. This limitation may reduce the statistical power of the study. Nevertheless, to our knowledge, this is the largest-scaled study in the literature to evaluate the relation between fatigue and insulin use.

Conclusion
The present study revealed that the use of insulin therapy in diabetic patients was associated with fatigue, regardless of the diabetes type and this effect was mostly related to depression. A significant difference in the FIS parameters, rather than FSS, proved the effect of psychological factors. The quality of life of diabetic patients was severely compromised as a result of fatigue. Before initiating insulin therapy in these patients, psychotherapeutic approaches may be an important intervention that may improve the compliance of the treatment and also the quality of life.

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Conflicts 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: Özlem Haliloğlu, Zeynep Oşar Siva; Design: Özlem Haliloğlu, Mesude Tütüncü, Zeynep Oşar Siva; Control/Supervision: Özlem Haliloğlu, Serdar Şahin, Özge Polat Korkmaz, Melis Dila Özer, Zeynep Oşar Siva; Data Collection and/or Processing: Özlem Haliloğlu, Mesude Tütüncü, Serdar Şahin, Zeynep Oşar Siva; Analysis and/or Interpretation: Özlem Haliloğlu, Mesude Tütüncü, Serdar Şahin, Zeynep Oşar Siva; Literature Review: Özlem Haliloğlu, Serdar Şahin, Özge Polat Korkmaz, Melis Dila Özer, Zeynep Oşar Siva; Writing the Article: Özlem Haliloğlu, Mesude Tütüncü, Zeynep Oşar Siva; Critical Review: Zeynep Oşar Siva; References and Fundings: Özlem Haliloğlu, Özge Polat Korkmaz, Melis Dila Özer, Mesude Tütüncü, Zeynep Oşar Siva; Writing the Article: Özlem Haliloğlu, Mesude Tütüncü, Zeynep Oşar Siva; Critical Review: Zeynep Oşar Siva; References and Fundings: Özlem Haliloğlu, Zeynep Oşar Siva; Materials: Özlem Haliloğlu, Özge Polat Korkmaz, Melis Dila Özer.

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Turkish Adaptation of Michigan Diabetes Research and Training Center’s Revised Diabetes Knowledge Test and Determination of Factors Affecting the Knowledge Level of Diabetic Individuals

Diyabet Araştırması ve Eğitim Merkezi Revize Diyabet Bilgi Testi’nin Türkçeye Adaptasyonu ve Diyabetli Bireylerin Bilgi Düzeyini Etkileyen Faktörlerin Belirlenmesi

*Cemile İDİZ*, **Selda ÇELİK**, **Elif BAĞDEMİR**, **Melike DIŞSİZ**, **İlhan SATMAN**

Istanbul University Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey

University of Health Sciences Hamidiye Faculty of Nursing, Istanbul, Turkey

Health Institutes of Turkey, Istanbul, Turkey

Abstract

Objective: Education is the cornerstone of diabetes management, and numerous educational studies used Diabetes Knowledge Level Tests to determine the effectiveness of education. Our study was planned to adopt the revised Diabetes Knowledge Test (DKT2) of the Michigan Diabetes Research and Training Center for the Turkish population.

Material and Methods: A total of 296 diabetic subjects using insulin were included in the study. After the determination of the validity of the language and content of the test, it was applied to the patients. The reliability of the study was assessed using Cronbach’s alpha coefficient. The results of the DKT2 demographic values, and laboratory tests of the patients were noted. Results: Cronbach’s alpha values were 0.60, 0.59, and 0.70 for the first part, second part, and complete test, respectively. The test-retest reliability values were 0.76 and 0.87 (p<0.001), respectively. The correct response rate to the first part was 32.68±2.47% in patients with Type 1 diabetes and 32.16±2.66% in patients with Type 2 diabetes using insulin. The correct response rate to the second part was 19.68±2.05% and 19.55±2.96%, respectively.

Keywords: Diabetes; knowledge level; reliability

Anahtar kelimeler: Diyabet; bilgi düzeyi; güvenilirlik

Keywords: Diabetes; knowledge level; reliability

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Address for Correspondence: Cemile Idiz, Istanbul University Istanbul Faculty of Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey
Phone: +90 212 4142000 E-mail: cemileidiz@gmail.com

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Introduction

There is a rise in the prevalence of diabetes in Turkey as well as globally. There was a rise in the incidence of diabetes, from 7.2% in the TURDEP-I data in 1998 to 13.7% in the TURDEP-II study in 2010 (1). Diabetes reduces the lifespan by 5-10 years (2). It is the 5th leading cause of death in many countries (3,4). Adult diabetics are at 2-4 times higher risk of cardiovascular events than their non-diabetic peers (5). Its complications present a high economic burden for individuals and society (2). In addition, diabetes expenditure constitutes 3-12% of total healthcare expenditure in various countries (6).

Education is the cornerstone of diabetes management (7). Diabetes knowledge can improve parameters such as blood glucose, HbA1c, blood pressure, and body weight (8). In many studies, the Diabetes Knowledge Level Test was used to determine the effectiveness of education (9,10). The revised Diabetes Knowledge Level Test (DKT2) is a quick and low-cost method of assessing general diabetes knowledge and associated self-care (11). In our country, tests measuring the level of diabetes knowledge are needed. In this study, we aimed to adapt DKT2 for the Turkish population.

Material and Methods

Setting and Samples

The study was carried out in the Diabetes outpatient clinic between June to October 2016.

In the present study, a test, which consisted of 23 questions, was applied to 296 diabetic individuals using insulin in order to adapt DKT2 for the Turkish population. The scale was applied to 42 cases twice at 15 days interval for the examination of invariance over time. All participants filled the patient identification form, which questioned their demographic characteristics and the medical treatment they had undergone.

The inclusion criteria for the volunteers were as follows; patients aged 18 years or older, with type 1 and 2 diabetes receiving insulin therapy, having an established diagnosis of diabetes since at least a year, under insulin therapy for at least six months, literate and without hearing, speaking or understanding disorders.

Instruments

Two instruments including the basic information form and Michigan Diabetes Research and Training Center’s Revised Diabetes Knowledge Test, were used.

Basic Information Form

The basic information form was developed by the authors and consisted of two parts. In the first part, the questions about sociodemographic data such as age, gender, and educational status were inquired. In the second part, type, duration and complications of diabetes, and levels of HbA1c were questioned. These values were used to determine the variables affecting the diabetes knowledge of the patients.

Michigan Diabetes Research and Training Center’s Revised Diabetes Knowledge Test

This test form consists of 23 questions. The English version of the test is given in Table 1. The first 14 questions measure the general level of diabetes knowledge. In the last 9 questions, the level of knowledge about the use of insulin is evaluated. While the first 14 questions can be applied to all adults with type 1 and 2 diabetes, the last 9 questions are relevant only to those using insulin. DKT2 is a reliable and valid tool for researchers, clinicians, and diabetes educators to evaluate the overall diabetes knowledge of a patient or population (11). There is no threshold value or passing level for the test. This test usually compares different patient groups or pre- and post-intervention.

Procedures and Data Collection

Instruments were administered in the hospital education room, which is located in the diabetes outpatient clinic. It is a quiet, well-lit room providing an atmosphere in which patients could concentrate on completing the questionnaires without being disturbed. The test was applied to 296 diabetic individuals and the subjects were invited to visit the outpatient clinic within two weeks of the first evaluation for test-retest stability. A total of 50 patients agreed to make a second visit to the outpatient clinic. Two days prior to the scheduled visit, a researcher called up the patients to remind them of their appointment. Of the 50 patients who
| Question                                                                 | Options                                                                 |
|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
| 1. The diabetes diet is:                                                 | a. The way most American people eat                                     |
|                                                                         | b. A healthy diet for most people                                       |
|                                                                         | c. Too high in carbohydrate for most people                            |
|                                                                         | d. Too high in protein for most people                                  |
| 2. Which of the following is highest in carbohydrate?                   | a. Baked chicken                                                       |
|                                                                         | b. Swiss cheese                                                        |
|                                                                         | c. Baked potato                                                        |
|                                                                         | d. Peanut butter                                                       |
| 3. Which of the following is highest in fat?                            | a. Low fat (2%) milk                                                   |
|                                                                         | b. Orange juice                                                        |
|                                                                         | c. Corn                                                                |
|                                                                         | d. Honey                                                               |
| 4. Which of the following is a “free food”?                             | a. Any unsweetened food                                                |
|                                                                         | b. Any food that has “fat free” on the label                           |
|                                                                         | c. Any food that has “sugar free” on the label                         |
|                                                                         | d. Any food that has less than 20 calories per serving                  |
| 5. A1C is a measure of your average blood glucose level for the past:   | a. Day                                                                  |
|                                                                         | b. Week                                                                |
|                                                                         | c. 6-12 weeks                                                          |
|                                                                         | d. 6 months                                                            |
| 6. Which is the best method for home glucose testing?                   | a. Urine testing                                                       |
|                                                                         | b. Blood testing                                                       |
|                                                                         | c. Both are equally good                                               |
| 7. What effect does unsweetened fruit juice have on blood glucose?      | a. Lowers it                                                           |
|                                                                         | b. Raises it                                                           |
|                                                                         | c. Has no effect                                                       |
| 8. Which should not be used to treat a low blood glucose?               | a. 3 hard candies                                                      |
|                                                                         | b. 1/2 cup orange juice                                                |
|                                                                         | c. 1 cup diet soft drink                                               |
|                                                                         | d. 1 cup skim milk                                                     |
| 9. For a person in good control, what effect does exercise have on blood glucose? | a. Lowers it | b. Raises it | c. Has no effect |
| 10. What effect will an infection most likely have on blood glucose?    | a. Lowers it                                                           |
|                                                                         | b. Raises it                                                           |
|                                                                         | c. Has no effect                                                       |
| 11. The best way to take care of your feet is to:                       | a. Look at and wash them each day                                      |
|                                                                         | b. Massage them with alcohol each day                                  |
|                                                                         | c. Soak them for 1 hour each day                                       |
|                                                                         | d. Buy shoes a size larger than usual                                  |
| 12. Eating foods lower in fat decreases your risk for:                  | a. Nerve disease                                                       |
|                                                                         | b. Kidney disease                                                      |
|                                                                         | c. Heart disease                                                       |
|                                                                         | d. Eye disease                                                         |
Table 1. The Original English version of the Michigan Diabetes Research and Training Center's Revised Diabetes Knowledge Test (continued).

| Question                                                                 | Option A                                       | Option B                                       | Option C                                       | Option D                                       |
|-------------------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 13. Numbness and tingling may be symptoms of:                           | a. Kidney disease                             | b. Nerve disease                              | c. Eye disease                                | d. Liver disease                              |
| 14. Which of the following is usually not associated with diabetes:     | a. Vision problems                            | b. Kidney problems                            | c. Nerve problems                             | d. Lung problems                              |
| 15. Signs of ketoacidosis (DKA) include:                                | a. Shakiness                                  | b. Sweating                                   | c. Vomiting                                   | d. Low blood glucose                          |
| 16. If you are sick with the flu, you should:                           | a. Take less insulin                          | b. Drink less liquids                         | c. Eat more proteins                          | d. Test blood glucose more often              |
| 17. If you have taken rapid-acting insulin, you are most likely to have | a. Less than 2 hours                          | b. 3-5 hours                                  | c. 6-12 hours                                 | d. More than 13 hours                         |
| 18. You realize just before lunch that you forgot to take your insulin  | a. Skip lunch to lower your blood glucose     | b. Take the insulin that you usually take at  | c. Take twice as much insulin as you usually  | d. Check your blood glucose level to decide   |
| 19. If you are beginning to have a low blood glucose reaction, you     | a. Exercise                                    | b. Lie down and rest                           | c. Drink some juice                           | how much insulin to take                      |
| 20. A low blood glucose reaction may be caused by:                      | a. Too much insulin                           | b. Too little insulin                         | c. Too much food                              | d. Too little exercise                        |
| 21. If you take your morning insulin but skip breakfast, your blood    | a. Increase                                    | b. Decrease                                   | c. Remain the same                            |                                               |
| 22. High blood glucose may be caused by:                                | a. Not enough insulin                         | b. Skipping meals                             | c. Delaying your snack                        |                                               |
| 23. A low blood glucose reaction may be caused by:                      | a. Heavy exercise                             | b. Infection                                  | c. Overeating                                 |                                               |
|                                                                         | d. Not taking your insulin                    |                                               |                                               |                                               |
agreed, 42 patients made the second visit to the clinic and again completed the DKT2. The patients completed the DKT2 in 8-20 min, with 95% completing the questionnaire in 15 min or less.

**Statistical Analysis**

The data were analyzed using SPSS 21.0 package software (SPSS Inc., Chicago, Illinois USA). The sociodemographic characteristics of the participants were evaluated by number, percentage, mean, standard deviation, and median values. The effect of socio-demographic variables on the subscale scores of DKT2 was analyzed by variance analysis and t-test. In the reliability analysis of the scale, Pearson’s Correlation coefficient was calculated by using the test-retest method to evaluate the invariance with respect to time, and the Cronbach alpha reliability coefficient was calculated for the internal consistency. Lawshe technique was used to evaluate the opinions of experts for the content validity of the scale. The statistical significance level was accepted as p<0.05.

**Ethical Issues**

The study was conducted in accordance with the Helsinki Declaration and approval was obtained from the local ethics committee Istanbul University Istanbul Medical Faculty Clinical Research Ethics Committee 31.05.2016, No: 690). Written consent was obtained from the individuals who met the inclusion criteria for the study and the purpose of the research and possible benefits were explained. They were ensured of not using the data outside the purpose of the research and non-disclosure of individual data. In the present study, written permission was obtained from James T. Fitzgerald by e-mail on behalf of the working group who owned the questionnaire in order to adapt the DKT2 for the Turkish population.

### Table 2. The age, height, weight, BMI, and A1c values of the patients.

| Features     | Mean  | ±Std  | Median | Min. | Max.  |
|--------------|-------|-------|--------|------|-------|
| Age (year)   | 52.98 | ±3.90 | 52     | 18   | 83    |
| Height (cm)  | 163.29| ±9.12 | 163    | 144  | 191   |
| Weight (kg)  | 79.48 | ±17.10| 78     | 40.70| 158.30|
| BMI (kg/m²)  | 29.89 | ±6.42 | 29     | 17.40| 53.50 |
| A1c%         | 8.91  | ±1.83 | 8.60   | 5    | 15.30 |

BMI: Body mass index.

### Results

**General Characteristics of Participants**

The general characteristics of the participants are shown in Table 2 and Table 3. The mean age of the participants was 52.98±3.90 years, with the majority of them having type-2 diabetes (70.6%). More than half of the participants were women (65.5%) and married (72.6%). Almost half of them (47.6%) were primary school graduates and having a job (48.6%), and a very large proportion was not using cigarettes (70.3%) and alcohol (90.5%). The complications observed were hypertension (60.8%), neuropathy (25.7%), retinopathy (25%), nephropathy (16.2%), diabetic foot (4.7%), and cardiovascular events (CVE) (1.4%), and 77% of the participants were educated for diabetes.

**Language Adaptation**

In order to evaluate the content and validity of the scale, the original English version was translated into Turkish by a faculty member of the Department of Foreign Languages and an English instructor. After the final Turkish version was examined by the literature teacher, the scale was translated into English by an Internal medicine specialist who had not seen the original scale and understood and spoke both languages (Turkish and English). The scale was then translated back to Turkish by two faculty members of the Department of Foreign Languages. The original version of the scale was compared with the English translation, and the necessary arrangements were made and presented to James T. Fitzgerald by e-mail on behalf of the working group. The final translation of the scale was presented to ten different Internal medicine specialists, and it was decided that there was no significant
Content Validity

After the validity of the scale, the Turkish version of the scale was given to ten experts to determine the scope of the scale. They were asked to score 1 to 4 items to assess the degree of measurement of each of them. The differences of opinion among the experts were examined by the Lawshe technique, and the data obtained from the experts were evaluated with the content validity index (CVI). The CVI of the items was calculated as 0.87.

As a result of the evaluations made by the experts, the final scale was evaluated by pilot application to a group of 30 people not included in the research, and necessary corrections were made.

Reliability Study

Internal consistency reliability coefficient: In the reliability analysis of DKT2, Cronbach’s alpha reliability coefficient ($\alpha$) was found to be $\alpha = 0.60$ for general test size; $\alpha = 0.59$ for insulin use size and $\alpha = 0.70$ for the complete scale (Table 4).

Test and Retest

In order to evaluate the invariance against time, 42 diabetic patients performed test-retest at 2 weeks interval and test-retest measurements evaluated Pearson’s product-moment correlation and t-test. The relationship between the scores obtained from the first and second applications of DKT2 and its sub-dimensions was examined by Pearson’s correlation analysis. The reliability coefficient was between 0.76 and 0.87 with positive and strong statistical significance ($p<0.001$) (Table 5). When the mean scores obtained from test and retest were compared with t-test independent groups, no statistically significant difference was found between them ($p>0.05$, Table 5).

There was no statistically significant difference between the groups in terms of type and duration of diabetes, HbA1c level, and education level of diabetes according to sub-scales of the level of knowledge DKT2 general test and insulin use of the participants ($p>0.05$, Table 6).

Discussion

The Diabetes Knowledge Level Test was validated and published in 1998, and later revised and published in 2016 by Fitzgerald et al. (11). In 2010, the Malaysian version of the first part of the Michigan Diabetes Knowledge Test (Questions 1 to 14) was made and $\alpha$ was found to be 0.702 (12). In 2016, Qah-
Cronbach’s alpha was 0.701 for the total scale with significant intra-class correlation coefficient (p<0.001).

Table 4. Reliability test of the 23-item Revised Diabetes Knowledge Test 2.

| DKT2 questions number | Mean±SD | Corrected item: Cronbach’s alfa if item deleted | Cronbach’s alfa General test (1-14) |
|-----------------------|---------|-----------------------------------------------|---------------------------------------|
| Question 1            | 1.24±0.42 | 0.162                                          | 0.703                                 |
| Question 2            | 1.21±0.41 | 0.212                                          | 0.699                                 |
| Question 3            | 1.26±0.44 | 0.125                                          | 0.707                                 |
| Question 4            | 1.78±0.41 | 0.364                                          | 0.686                                 |
| Question 5            | 1.31±0.46 | 0.338                                          | 0.687                                 |
| Question 6            | 1.06±0.25 | 0.164                                          | 0.701                                 |
| Question 7            | 1.18±0.39 | 0.207                                          | 0.699                                 |
| Question 8            | 1.65±0.47 | 0.318                                          | 0.689                                 |
| Question 9            | 1.11±0.31 | 0.232                                          | 0.697                                 |
| Question 10           | 1.23±0.42 | 0.232                                          | 0.697                                 |
| Question 11           | 1.12±0.33 | 0.216                                          | 0.698                                 |
| Question 12           | 1.15±0.36 | 0.238                                          | 0.637                                 |
| Question 13           | 1.22±0.41 | 0.294                                          | 0.692                                 |
| Question 14           | 1.19±0.39 | 0.286                                          | 0.693                                 |
| Question 15           | 1.80±0.40 | 0.309                                          | 0.691                                 |
| Question 16           | 1.43±0.49 | 0.319                                          | 0.689                                 |
| Question 17           | 1.16±0.36 | 0.132                                          | 0.704                                 |
| Question 18           | 1.51±0.50 | 0.349                                          | 0.686                                 |
| Question 19           | 1.18±0.38 | 0.216                                          | 0.698                                 |
| Question 20           | 1.19±0.40 | 0.310                                          | 0.691                                 |
| Question 21           | 1.15±0.35 | 0.255                                          | 0.695                                 |
| Question 22           | 1.50±0.50 | 0.263                                          | 0.695                                 |
| Question 23           | 1.33±0.47 | 0.311                                          | 0.690                                 |

Cronbach’s alpha was 0.701 for the total scale with significant intra-class correlation coefficient (p<0.001).

Table 5. Comparison and Correlation of Test and Retest Score Means of Revised Diabetes Knowledge Test Scale 2 and Sub-Dimensions (n=42).

| Scale and Sub-Dimensions | First Application | Second Application | t    | p    | r    | p     |
|--------------------------|-------------------|--------------------|------|------|------|-------|
| 1. General Test          | 32.92±2.16        | 32.64±1.84         | 1.284| 0.205| 0.76 | 0.000 |
| 2. Insulin usage         | 19.61±2.57        | 19.19±2.12         | 1.783| 0.085| 0.87 | 0.000 |

t: Paired Samples t-test, r: Pearson’s correlation test.

tani et al. translated the first part of the test (Questions 1 to 14) into the Arabic language and $\alpha$ was 0.60 (13). In this study, the reliability coefficient calculated by $\alpha$ was 0.60 for the first part of DKT2 in accordance with the literature; $\alpha$=0.59 for the second part and $\alpha$=0.70 for the complete scale. Considering $\alpha$ in a range of 0.50-0.70, which corresponds to moderate reliability, the Turkish version of DKT2 is a valid and reliable tool to measure patients’ knowledge of diabetes. In the validity studies of this test, individuals with diabetes have been evaluated in different countries. In the study by Al-Qazaz et al., the number of correct answers given to the 14 questions of the first part of the scale was 7.88±3.01 (12). In the study by Qahtani et al., the correct re-
response rate to all questions was approximately 54% (16-81%) (13). In the study by Fitzgerald et al., the correct response rate to the first part of the scale was found to be 84.7±20.0% in individuals with type 1 diabetes and 71.7±24.7% in patients with type 2 diabetes using insulin (11). In this study, rates were well below these values (32.68±2.47% and 32.16±2.66%, respectively). Similarly, in the same study by Fitzgerald et al., the correct response rate for the second part was 84.9±24.1% in patients with type 1 diabetes and 64.3±28.4% in patients with type 2 diabetes using insulin (11). In our study, no statistically significant difference was found between the groups in terms of type and duration of diabetes, type of education, HbA1c level, and the status of receiving education about diabetes (p>0.05). However, in accordance with the literature, it was found that individuals with type 1 diabetes had higher scores for both parts of the DKT2 scale than those with type 2 diabetes. Moreover, individuals with higher education level and diabetes education had higher scores than those without it, but these differences were not statistically significant.

In conclusion, DKT2 is a quick and low-cost method of assessing general knowledge of diabetes and self-care. However, the Turkish version is not yet available. Thus, we assessed the reliability of DKT2, and the Turkish version of DKT2 was observed to be a reliable tool to measure patients’ knowledge.

### Table 6. Comparison of Some Diabetes Characteristics According to Sub-dimensions of Revised Diabetes Knowledge Test 2 Scale.

| General Test % Correct | General Test % Correct |
|------------------------|------------------------|
| **Items 1-14** | **Items 15-23** |
| Mean±SD | n | Mean±SD | n |
| Diabetes Type | Type 1 | 32.68±2.47 | 87 | 19.68±2.05 | 87 |
| | Type 2 using insulin | 32.16±2.66 | 209 | 19.55±2.96 | 209 |
| | Difference (t,p) | t=1.566 | p=0.118 | t=0.461 | p=0.645 |
| Educational Level | Primary education | 32.16±2.84 | 141 | 19.62±2.08 | 141 |
| | High school | 32.41±2.56 | 87 | 19.44±2.74 | 87 |
| | University-doctorate | 32.52±2.18 | 68 | 19.69±1.89 | 68 |
| | Difference (F,p) | F=0.524 | p=0.592 | F=0.177 | p=0.838 |
| Diabetes duration | ≤10 years | 32.45±2.75 | 92 | 19.91±2.65 | 92 |
| | >10 years | 32.25±2.56 | 204 | 19.14±2.77 | 204 |
| | Difference (t,p) | t=0.597 | p=0.551 | t=1.357 | p=0.176 |
| A1c level | HbA1c ≤7% | 32.17±2.96 | 40 | 19.35±2.76 | 40 |
| | HbA1c >7% | 32.34±2.56 | 256 | 19.62±2.74 | 256 |
| | Difference (t,p) | t=0.378 | p=0.705 | t=0.593 | p=0.556 |
| Diabetes Education | Yes | 32.36±2.52 | 228 | 19.70±2.67 | 228 |
| | No | 32.16±2.93 | 68 | 19.22±2.95 | 68 |
| | Difference (t,p) | t=0.570 | p=0.569 | t=1.270 | p=0.205 |

* t: Student’s t-test, F: One-Way Anova.
of diabetes. However, the knowledge level of the patients can be increased by improving diabetes education.

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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: Cemile İdiz; Design: Cemile İdiz, İlhan Satman; Control/Supervision: Cemile İdiz, İlhan Satman; Data Collection and/or Processing: Cemile İdiz, Selda Çelik, Elif Bağdemir, Melike Dişiz; Analysis and/or Interpretation: Cemile İdiz, Selda Çelik, Elif Bağdemir, Melike Dişiz; Literature Review: Cemile İdiz, Selda Çelik, Elif Bağdemir, Melike Dişiz, İlhan Satman; Writing the Article: Cemile İdiz, Selda Çelik, Elif Bağdemir, Melike Dişiz, İlhan Satman; Critical Review: İlhan Satman.

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Diagnostic and Therapeutic Approaches to Thyroid Nodules in Turkey

Türkiye’de Tiroid Nodüllerine Tanı ve Tedavi Yaklaşımları

Berna İmge AYDOĞAN, Seher DEMİRER*, Yeşim ERBİL**, Murat Faik ERDOĞAN

Ankara University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey
*Ankara University Faculty of Medicine, Department of General Surgery, Ankara, Turkey
**Istanbul University Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

**Amaç:** Bu çalışmada, Türkiye’de farklı merkez ve uzmanlık alanlarından hekimlerin tiroid nodüllerine yaklaşımlarının değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Uluslararası Tiroid Kongresi (2010) için Ralf Paschke tarafından tasarlanan anket, Türkiye’de 400 (264 genel cerrah, 58 endokrin cerrah ve 78 endokrinolog) hekimle uçulan formülasyonu, üniversite hastaneler (n=110), devlet hastaneler (n=110), özel hastanelerdeki hekim sayısı (n=110), devlet hastaneler (n=84), üniversite hastaneler (n=122) ve özel hastaneler (n=84) dahil edildi. İndeks bir hasta verildi ve tanı, tedavi/takip stratejilerine ilişkin sorular soruldu. **Bulgular:** İndeks olgu 35 yaşında, yutma güçlü bir hasta idi. Tiroid Stimüle edici hormon (TSH) düzeyi 0,5 mIU/mL idi. Tiroid ultrasonografisi (USG)’sında sağda 13 mm ve solda 18 mm nodül mevcuttu. Uzmanlar hasta hakkında en sık öğrenmek istedigi bilgiler, ince iğne aspirasyon biyopsis sonucu (IIAB) (%38,5) ve sintigrafi+IIAB (%25,5) sonuçlarıyla. Rutin kalsitonin ölçümü uzmanların %33,5’i tarafından önerdi. USG ve sintigrafi; saş normoaktif solid nodüle mikrokalsifikasyon ve intra nodüler kanlanma ve sol hypoaktif nodüle hipoaktive nite olarak detaylandırdı. Her 2 nodül için IIAB, uzmanların %68,5’i tarafından önerdi. IIAB yapılmadan cerrahi, uzman hekimlerin %9’u (%n=36) tarafından ve çoğunlukla genel cerrahlarca (%n=32) önerdi. Özel hastanelerden uzmanlar, benign nodüller için cerrahi yaklaşımları, devlet ve üniversite hastanelerinde göre daha sık önerdi (%p<0.01). **Sonuç:** Türkiye’de hem girişimeller/girişimlerin olmayan tanısal testler hem de tedavi/takip stratejileri genel cerrahlar, endokrin cerrahlar ve endokrinologlar arasında değişiklik göstermektedir. Benign nodüllerin tedavisinde cerrahi yaklaşım özel hastanelerde daha sik tercih edilmektedir.

**Keywords:** Thyroid nodule; fine-needle aspiration biopsy; thyroid ultrasonography; surgery

**Anahat kelimeler:** Tiroid nodülü; ince iğne aspirasyon biyopsis; tiroid ultrasonografisi; cerrahi

**Address for Correspondence:** Berna İmge AYDOĞAN, Ankara University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey
**Phone:** +90 312 508 21 00  **E-mail** imeghalic@gmail.com

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Introduction
Thyroid nodules (TNs) are observed frequently, and the prevalence of palpable nodules reaches 1% in men and 5% in women in iodine-sufficient regions of the world (1,2). The detection of TNs has become more common with the widespread use of thyroid ultrasonography (US) and the prevalence is up to 68% using high-frequency (13 MHz) US examination (3). The differential diagnosis of thyroid malignancy and determination of functional status are essential in TN management. The frequency of malignancy among TNs is 5-15% (4). The diagnostic procedures include the evaluation of thyroid-stimulating hormone (TSH), thyroid autoantibodies, calcitonin, US, I\textsubscript{123}, and Tc–99m pertechnetate thyroid scintigraphy. Thyroid US followed by fine-needle aspiration biopsy (FNAB) is recommended for evaluating selected nodules (5). However, the overdiagnosis and overtreatment of TNs are topical issues (6).

The previous data from Europe and the United States demonstrated discrepancies among endocrinologists, general surgeons, and endocrine surgeons in the diagnostic and therapeutic approaches to TNs (7-10). The American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association medical (AACE/AME/ETA) guidelines 2010 and American Thyroid Association (ATA) guideline 2009 addressed several issues on the management of TNs (4,11). However, surveys conducted after the publication of guidelines showed that there were controversies among physicians from different specialties and centers, despite the presence of updated recommendations (12).

This study aimed to evaluate the management strategies of TNs among different specialties and medical centers in Turkey.

Material and Methods
Structure of the Questionnaire
This cross-sectional study was conducted in 2010. The questionnaire was adapted from that designed for International Thyroid Congress 2010 by Ralf Paschke with permission. A total of 400 physicians from different disciplines and institutions and 264 general surgeons (58 endocrine surgeons and 78 endocrinologists) answered the questions. Institutions were state hospitals (n=194), university hospitals (n=122), and private hospitals (n=84). The results were interpreted considering the recommendations of revised ATA 2009 and AACE/AME/ETA 2010 guidelines.

The questionnaire was based on an index case: a 35-year-old man admitted to the clinic with swallowing discomfort. Thyroid US revealed a 13 mm right nodule (RN) and an 18 mm left nodule (LN). The TSH level was 0.5 mIU/L (reference range [RR]=0.5-5.5 mIU/L). The participants were asked to choose only one option for all questions.

In the first part of the survey, the questions were based on the initial diagnostic tests used for the evaluation of TNs. In the second part, the results of more detailed thyroid US and scintigraphy were provided. In the first scenario, the right solid nodule had intranodular blood flow and microcalcification on power Doppler and B-mode US, respectively. Tc-99m pertechnetate thyroid scintigraphy showed that RN was normoactive. The LN was solid and hypoechoic on US and hypoactive on scintigraphy. The choice of FNAB for each nodule or surgery was asked.

The second scenario in this section was about a RN with iso-hyperechogenicity and LN with mixed cystic-solid texture on US. The choice of FNAB for each nodule or surgery was asked again.

In the final part of the questionnaire, follow-up and treatment options were questioned. The approach to benign nodules and the estimated risk of malignancy in nodules with indeterminate cytology were asked to physicians. Informed consent was obtained from each participant.

Statistical Analysis
Statistical analyses were performed using SPSS for Windows 11.5 (IBM Corp, NY, USA). The chi-square or Fisher's exact test was performed to compare diagnostic and therapeutic approaches of physicians from different specialties and medical centers. A $p$-value of <0.05 was considered statistically significant.
Results

The case was presented as follows: a 35-year-old man admitted to the clinic with swallowing discomfort. Thyroid US revealed a 13 mm RN and 18 mm LN. The TSH level was 0.5 mIU/L (RR: 0.5-5.5 mIU/L).

Diagnostic Procedures

In the first part of the questionnaire, FNAB was used by 38.5% of responders (n=154), FNAB and scintigraphy by 25.5% (n=102), US by 24.5% (n=98), and scintigraphy by 11.5% (n=46) (Figure 1A). Among endocrinologists, US (37%) and scintigraphy (37%) were used at an equal rate, whereas general surgeons (38%) and endocrine surgeons (41%) most commonly used FNAB alone (Figure 1B). Scintigraphy followed by FNAB was more frequently recommended by general surgeons (n=79) than endocrinologists (n=14) (30% versus 14%, p=0.04), whereas no statistically significant difference was observed between disciplines regarding the selection of other initial diagnostic procedures. In all medical centers, FNAB was the most preferred initial diagnostic method and no difference regarding the selection of FNAB was observed between private and university hospitals (p=0.06) (Figure 1C). In private hospitals, FNAB was more frequently preferred as an initial approach than state hospitals (p=0.04).

The routine calcitonin measurement was not recommended by 66.5% (n=266) of physicians. The calcitonin measurement was more frequently used by endocrinologists than general surgeons (54% versus 33%, p=0.08) and endocrine surgeons (54% versus 14%, p=0.01).

In the second part of the questionnaire, the selection of invasive diagnostic methods was asked. In the first scenario, US and scintigraphy results were summarized as follows: "Right solid nodule had intranodular blood flow and microcalcification on power Doppler and B-mode US, respectively. Tc-99m pertechnetate thyroid scintigraphy showed that RN was normoactive. LN was solid and hypoechoic on US and hypoactive on scintigraphy." In the entire group, 68.5% (n=274) of responders suggested FNAB for two nodules, 15% (n=60) for RN, and 7.5% (n=30) for LN (Figure 2A). Surgery without FNAB was recommended by 9% (n=36) of clinicians. FNAB was the most preferred approach among endocrinologists (68%), general surgeons (65%), and endocrine surgeons (83%) (Figure 2B). FNAB was more commonly recommended by endocrinologists for only RN than general surgeons and endocrine surgeons (26% versus 14%, p=0.01 and 26% versus 7%, p=0.01, respectively), whereas the recommendation of FNAB for only LN was not different among the disciplines. Endocrine surgeons more commonly recommended FNAB for both nodules (83%) than general surgeons (65%) and endocrinologists (68%) (p=0.02). Surgery without FNAB was more frequently recommended by general surgeons than endocrinologists (12% versus 3%, p=0.01) and endocrine surgeons (12% versus 3%, p=0.03) (Figure 2B). Physicians from private hospitals more frequently preferred surgery (19%) than those from state hospitals (8%, p<0.01). None of the specialists from university hospitals preferred surgery without FNAB (0%).
surgery for this scenario. On the other hand, FNAB was the most preferred method for both nodules in university (86%), state (74%), and private (53%) hospitals (Figure 2C).

The second scenario of US was given as follows: "RN showed iso-hyperechogenicity and LN showed mixed cystic-solid texture". The choice of management was asked again. Approach to the nodules in the second scenario among all physicians (A), according to medical disciplines (B), and medical centers (C).

The second scenario of US was given as follows; "RN showed iso-hyperechogenicity and LN showed mixed cystic-solid texture". Choice of management was asked again. Approach to the nodules in the second scenario among all physicians (D), according to medical disciplines (E), and medical centers (F).

Figure 2: In the first scenario; the right-solid nodule had intranodular blood flow and microcalcification on the power Doppler and B-mode US, respectively. Tc-99m pertechnetate thyroid scintigraphy showed that the right nodule (RN) was normoactive. The left nodule (LN) was solid and hypoechoic on US and hypoactive on scintigraphy.

Approach to the nodules in the first scenario among all physicians (A), according to medical disciplines (B), and medical centers (C).

The second scenario of US was given as follows; "RN showed iso-hyperechogenicity and LN showed mixed cystic-solid texture". Choice of management was asked again. Approach to the nodules in the second scenario among all physicians (D), according to medical disciplines (E), and medical centers (F).

Follow-up and Treatment

The subsequent question was “which follow-up strategy you would recommend for the patient if both nodules were benign by FNAB, the thyroid volume was 30 mL and after two further years there were two further benign nodules?”. Follow-up was the most preferred strategy (46%, n=182) (Figure 4A). Surgery was more frequently recommended by endocrinologists than endocrine surgeons (43% versus 15%, p=0.01; Figure 4B). Levothyroxine or levothyroxine plus iodine treatments were more frequently preferred by general surgeons and endocrine surgeons than endocrinologists (43% versus 15%, p=0.01; Figure 4B). Surgery was recommended by 37% of physicians from private medical centers, 23% from university, and 20% from state hospitals (p<0.01; Figure 4C).
The last question was the most preferred follow-up strategy for this patient. The evaluation of US and thyroid hormones after one year and further follow-up depending on results were selected by 44% (n=176) of physicians (Figure 4D).

**Discussion**

This survey evaluated diagnostic and therapeutic approaches to TNs among thyroid specialists from different disciplines and centers in Turkey. Our results showed that controversies existed among disciplines despite the recommendations of guidelines. In addition, differences in follow-up and treatment strategies of medical centers were documented.

Since the 1980s, investigators focused on the management attitudes and practices of physicians from different centers and disciplines regarding thyroid nodularity (7,9,10,13-15). The first international survey regarding the approach to “a small TN in a 35-year-old woman” was conducted by Baldet et al. in 1989. After 10 years of this survey, Bennedbaek conducted a similar survey among 110 clinical members of the European Thyroid Association, and subsequently, the same survey was conducted among 142 North American physicians (ATA members) including endocrinologists, general surgeons, and nuclear medicine specialists (7,13). In 2007, the questionnaire was answered by 122 endocrinologists and
48 endocrine surgeons from Australia (9).

When Australian clinicians and ETA and ATA members were compared, the use of US was significantly infrequent among ATA members. Scintigraphy regardless of thyroid function was more frequently recommended in the European survey than North American and Australian surveys (9). The German Society of Endocrinology provided the same questionnaire among 50 participants including endocrinologists and nuclear medicine specialists in 2003 (10). The use of US as an initial procedure was more frequent, but FNAB was rare in Germany compared with former surveys conducted in Europe and North America (7,9,10,13). Previous data demonstrated that not only diagnostic tests but also follow-up and therapeutic strategies for benign TNs varied among physicians and medical disciplines. Thyroxine treatment was recommended by only 7.6% of Australian specialists, whereas this rate was 33% and 38% among ATA and ETA members, respectively (7,9,13). The preferred strategy in the management of benign TN was rarely surgery by ATA members (0.7%) (13). However, Australian physicians and ETA members equally (approximately 21%) and more frequently recommended surgery for benign nodules (7,9). In an Australian survey, the surgical approach was significantly and more frequently recommended by surgeons than endocrinologists (60% versus 22%) (9).

The results of these surveys pointed out the need for consensus on TN management (4). The initial “American Thyroid Association (ATA) guideline for patients with TNs and differentiated thyroid cancer” was published in 2006 (16). The revised guideline of ATA was published in 2009 (4). The major questions addressed by authors were the indications of imaging techniques (US and scintigraphy), laboratory tests, the role of FNAB, medical therapy for benign nodules, and long-term follow-up strategies. The AACE/AME/ETA guidelines for clinical practice for the diagnosis and management of TN were published in 2010 (17). ATA 2009 and AACE/AME/ETA guidelines recommend the measurement of TSH level initially and performing a radionuclide thyroid scan if the TSH level was subnormal for TN management (4). Thyroid US was recommended for all patients with known or suspected TN. If a hyperfunctioning nodule was observed on nuclear imaging, the cytologic evaluation was not recommended, as these nodules are rarely malignant (18). Although our survey was conducted after the publication of revised ATA 2009 and AACE/AME/ETA guidelines, the initial approach to TN varied broadly among clinicians from the same or different disciplines. Ultrasonography alone and FNAB without US were equally recommended by endocrinologists, whereas the initial approach to nodule was commonly FNAB followed by US among all experts. No difference was observed regarding the selection of FNAB as an initial approach between private and university hospitals. However, FNAB was more frequently preferred by physicians from private hospitals than that from state hospitals.

The use of routine calcitonin measurement in TN management is controversial. The ATA 2009 guidelines do not recommend either for or against the routine calcitonin measurement (4). On the other hand, AACE/EMA/ETA guidelines state that serum calcitonin testing may be useful in the initial evaluation of TNs with moderate-quality evidence (17). The surveys from Europe reported that 20-40% of specialists requested routine calcitonin measurement (7,15). A previous survey was conducted in Turkey between 2014 and 2015 among general surgeons (12). The survey results demonstrated that routine calcitonin measurement was recommended by only 8.4% of participants. Similar to European surveys, 33.5% of participants recommended routine calcitonin testing in our study. Endocrinologists more frequently recommended routine measurement than surgeons (7,9).

In ATA 2009 guideline, the indications of FNAB were as follows: a nodule of >5 mm in a patient with a high-risk history of thyroid malignancy, a hypoechoic nodule of >10 mm, an iso- or hyperechoic nodule of ≥10–15 mm, a spongiform nodule of ≥20 mm, a mixed cystic-solid nodule without suspicious US features of ≥20 mm, and ≥15-20 mm if any suspicious US features were present (4). The AACE/AME/ETA thyroid nodule 2010 guidelines recommend FNAB for nodules larger than 10 mm and smaller than 10 mm if had a suspicious history or US find-
ings. In our first scenario, FNAB was indicated for both nodules according to ATA 2009 and AACE/AME/ETA guidelines; RN was a normoactive, 13 mm solid nodule with suspicious US features (intranodular blood flow and microcalcification), and LN was 18 mm hypoechoic and hypooactive. The biopsy of both nodules was recommended by 65% of general surgeons, 83% of endocrine surgeons, and 68% of endocrinologists. A significant difference was observed regarding the choice of surgery without FNAB among disciplines. General surgeons more commonly requested surgery as a diagnostic procedure than other disciplines. In addition, surgery was more frequently recommended by physicians from private hospitals than those from state and university hospitals. In the second scenario, RN showed iso-hyperechogenicity and LN showed mixed cystic-solid texture. According to ATA 2009, FNAB was optional for RN but was not indicated for LN. FNAB was the most frequently chosen option in all disciplines for both nodules. Diagnostic surgery was more frequently recommended by general surgeons and physicians from private hospitals.

In the last part of the questionnaire, the risk of malignancy in nodules reported as “indeterminate” by FNAB was asked to participants. Indeterminate cytology included “follicular or Hurthle cell neoplasm” and atypia/follicular lesion of undetermined significance (AUS/FLUS) Bethesda categories (19). Follicular neoplasm and/or Hurthle cell neoplasms were reported to have a 20-30% AUS/FLUS lesions and 5-10% risk of malignancy (4,20). In our survey, 40% of physicians gave a 20% risk of malignancy for indeterminate nodules. General and endocrine surgeons provided lower risks than endocrinologists.

According to ATA 2009 and AACE/AME/ETA 2010 guidelines, the recommended strategy in the management of benign nodules was a follow-up, as false negative results were low (4,21). Serial follow-up with clinical US examination and TSH measurement in 6 to 18 months were recommended in AACE/AME/ETA 2010 guidelines. Long-term levothyroxine suppressive therapy was not recommended because of regrowth after the cessation of treatment (4,17). In our survey, follow-up was most commonly recommended for the management of benign nodules and the evaluation of US and thyroid hormones one year later was the most preferred follow-up strategy; further, follow-up depended on the results. Surgery was recommended by 32% of physicians. Endocrinologists and physicians from private hospitals advised surgery. Our results were consistent with the previous survey from Turkey that showed discrepancies in the evaluation and treatment of TNs among general surgeons (12).

In conclusion, the approach to TNs is controversial among different disciplines and institutions in Turkey despite the recommendations of guidelines. Not only invasive/noninvasive diagnostic tests but also the treatment and follow-up strategies varied among general surgeons, endocrine surgeons, and endocrinologists. In addition, the private hospital setting was associated with an increase in the frequency of surgery.

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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
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Evaluation of Hyperandrogenemia in Women with Prolactinoma

Mehmet Çağrı ÜNAL, Züleyha KARACA, Kürşad ÜNLÜHIZARCI, Fahrettin KELEŞTEMUR*
Erciyes University Faculty of Medicine, Division of Endocrinology and Metabolism, Kayseri, Turkey
*Yeditepe University Faculty of Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey

Abstract

Objective: Differential diagnosis of androgen excess disorders revealed the occurrence of hyperprolactinemia. However, an elevated level of prolactin (hyperprolactinemia) is a very infrequent cause of hyperandrogenemia in clinical practice. This study aimed to investigate the presence of hyperandrogenism/hyperandrogenemia in women with prolactinoma before and after treatment with cabergoline.

Material and Methods: Twenty women diagnosed with prolactinoma in the recent past and 15 healthy women between the ages of 18 to 50 were enrolled in the study. Patients were evaluated at the baseline and after six months of cabergoline treatment. Patients were carefully noted for any signs and symptoms of hyperandrogenemia and concentration of androgen in blood. Further, adrenocorticotropin stimulation test was performed to analyze cortisol, dehydroepiandrosterone sulfate (DHEAS), androstenedione, 11-deoxycorticisol (11-S), and 17-hydroxyprogesterone (17-OHP) responses. Results: A significantly higher level of prolactin compared to the control group was seen in prolactinoma patients, which reverted to normal levels after cabergoline treatment.

Androstenedion, DHEAS, 17-OH progesterone, 11-S, and cortisol were found to be significantly after the treatment. The levels of basal androstenedione, DHEAS, 17-OH progesterone, 11-S, and cortisol were found to be similar between the two groups. Basal and stimulated DHEAS and androstenedione levels decreased significantly after cabergoline treatment in prolactinoma patients. The presence of acne, hirsutism, and androgenic alopecia were similar in both groups. Pelvic ultrasonography revealed polycystic ovary (PCO) in nine patients with prolactinoma, which was significantly more frequent than in the control group. Among the 9 PCO patients, normal ovarian morphology was restored in three patients after the treatment.

Conclusion: From the data, it may be suggested that hyperprolactinemia may not lead to clinically significant hyperandrogenemia and hirsutism. Moreover, the treatment of hyperprolactinemia does not lead to significant improvement in hirsutism score of the patients, if exists.

Keywords: Androgen; hirsutism; hyperandrogenemia; prolactin; prolactinoma

Özet

Amaç: Hiperprolaktinimité, androjen fazlaşlığı ile seyreden hastalarının ayrıntı tanılanlara artırılmasını gerekten durumdandır. Ancak, klinik pratikte hiperprolaktinemi olan hastalarda nadiren hiperandrojenizm görülmektedir. Çalışmamızda, prolaktinomada antısm almiş kadınlarda kabergolin tedavisi ön- cesi ve sonrası hiperandrojenizmin değerlendirilmesi amaçlanmıştır. Gereç ve Yöntemler: Çalışmaya yaşı aralığı 18-50 yıl olan, prolaktinoma tanısı konulan 20 kadın hastanın ve 15 sağlıklı kontrol dâhil edildi. Hastalar bazal ve 6 aylık kabergolin tedavi- siden sonra değerlendirildi. Hiperandrojenizmin belirtileri ince- lendi ve bazal androjen düzeyleri ölçülü. Adrenokortikotropin stimülasyon testi ile kortizol, dehidroepiandrosteron sulfat (DHEAS) ve androstenedion, 11-deoksikortikol (11-S), 17-hid- roksiprogesteron (17-OHP) cevabı değerlendirildi. Bulgular: Prolaktinominin kadın hastalarda kontrol grubuna kıyasla PRL se- yileyeni yüksekti ve kabergolin tedavisi ile geriledi. E2 seyileyeni prolaktinoma hastalannında daha düştüktü ve kabergolin sonrası anlamlı bir artış görüldü. Seks hormon ba- zı dozal ve klinik sonuçlar kabergolin tedavisine yanıt verdi. Sivilce, hirsutizm ve androjenik alopesi 2 grupta benzer bulunuldu. Prolaktinoma hastalannında, bazal ve yananım DHEAS ve androstenedion seyileyeleri, kabergolin tedavisi sonrası anlamlı derece azaldı. Sivilce, hirsutizm ve androjenek alopesi 2 grupta benzer izlendi. Prolaktinominin 9 kadın hastada kontrol grubuna göre anlamlı olarak daha sik pelvik ultrasonografide polistik over (PKO) görülü. Tedaviden sonra PKO olan hastanın 3’ünde normal over morfolojisi görülü ve 6 hasta hâlâ PKO vardı. Sonuç: Çalışmamızda, hiperprolaktineminin klinik ola- rak anlamlı hiperandrojenizm ve hirsutizme yol açtığına iz- lendi. Ayrıca, hirsutizmini olan kadın hastalarda ve hirsutizm skorunda hiperprolaktinemi tedavisi sonrası anlamlı bir iyileşme gözlenmedi.

Anahtar kelimeler: Androgen; hirsutizm; hiperandrojenemi; prolaktin; prolaktinoma

Address for Correspondence: Mehmet Çağrı ÜNAL, Erciyes University Faculty of Medicine, Division of Endocrinology and Metabolism, Kayseri, Turkey
Phone: +90 352 207 66 66 E-mail: mcagrunal@erciyes.edu.tr

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Introduction

Prolactinomas are the most common form of pituitary adenomas. They account for about 40% of all pituitary adenomas (1). Prolactinoma causes amenorrhea and galactorrhea in premenopausal women, while it causes sexual dysfunction in men. Patients suffering from macroprolactinoma may show signs and symptoms like headache, visual field defects, and pituitary deficiencies. Prolactinomas are found in incidentalomas in few cases (2).

Androgens are the steroid hormones produced by the adrenal cortex, are synthesized in the adrenal glands and ovaries of women. Dehydroepiandrosterone sulfate (DHEAS) is the primary adrenal androgen and is released only in small amounts by the ovaries. The adrenal glands and the ovaries produce androstenedione and testosterone in women. Moreover, a small amount of DHEAS is converted to androstenedione and testosterone in peripheral tissues and adrenal glands (3). Although hypersecretion of DHEAS suggests adrenal hyperandrogenism, they possess little intrinsic androgenic activity. Symptoms of hyperandrogenism like hirsutism and virilization are caused primarily by the more potent androgens such as androstenedione and mainly testosterone (4). In adult women, hyperandrogenism causes hirsutism, acne, androgenetic alopecia, menstrual irregularities, and infertility. It may seldom cause virilization. The most common cause of hyperandrogenism in women is polycystic ovary syndrome (PCOS) (5,6) followed by idiopathic hyperandrogenemia, idiopathic hirsutism, and non-classic congenital adrenal hyperplasia (7,8). Although infrequent, other causes of androgen overproduction that causes hirsutism include androgen-secreting ovarian and adrenal tumors, severe insulin-resistance syndromes, Cushing’s syndrome, acromegaly, and hyperprolactinemia. But these diseases appear with more specific and frequent clinical symptoms (9-12).

Although hyperprolactinemia is commonly found in the differential diagnosis of androgen excess disorders, it appears to be a rare cause of hyperandrogenemia in clinical practice (13,14). There are very few studies that investigated the relationship between hyperandrogenemia and hyperprolactinemia. Therefore, the present study aimed to evaluate the existence of hyperandrogenism/hyperandrogenemia before and after treatment with cabergoline in women with prolactinoma.

Material and Methods

It was a prospectively designed study with 20 women with treatment naïve prolactinoma and 15 healthy women of age 18 to 50 years included as controls. Informed consent was obtained from each participant. The patients who used drugs that could increase the prolactin level and had any comorbid conditions were excluded from the study.

The patients were evaluated at the time of diagnosis and after six months of cabergoline treatment. The control group, consisting of healthy individuals, was evaluated only once. The presence of acne, hirsutism and androgenic alopecia was noted in the patients with prolactinoma and healthy controls. Modified Ferriman-Gallwey score (mFG) was exercised for evaluating hirsutism and a score of ≥8 was considered as hirsutism (15).

Basal androgens (total testosterone, androstenedione, and DHEAS), prolactin (PRL), cortisol, thyrotropin (TSH), free thyroxine (fT4), FSH, LH, estradiol (E2) and sex hormone-binding globulin (SHBG) levels were estimated. Free androgen index (FAI) was also calculated from the equation given below:

\[
\text{Free androgen index (FAI)} = \frac{\text{Total testosterone (nmol/L)} \times 100}{\text{SHBG (nmol/L)}}
\]

The adrenocorticotropic hormone (ACTH) stimulation test was performed in both groups. A single bolus of 250 µg synthetic ACTH was administered intravenously (Synacthen 0.25 mg, Novartis, Nurnberg, Germany). The test was performed in the morning, during the follicular phase of menstruation in patients with a regular cycle. Patients with oligo/amenorrhea, ovulation was excluded by low progesterone levels. Cortisol, DHEAS, androstenedione, 11-deoxycortisol (11-S), and 17-hydroxyprogesterone (17-OHP) levels were measured at 0, 30, and 60 min after the ACTH administration.
Electrochemiluminescence and chemiluminescence methods were used for estimating the hormone concentration. Serum prolactin (PRL), cortisol, total testosterone, DHEAS, SHBG, thyrotropin (TSH), free thyroxine (FT4), FSH, LH, and estradiol (E2) levels were measured by the electrochemiluminescence immunoassay (Cobas® 8000, Roche). IGF–1 and androstenedione levels were determined by chemiluminescence technique (Immulite 2000 XPi, Siemens). Serum 17-OHP level was measured by radioimmunoassay, while liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure 11-S concentration. Pelvic ultrasonography was performed to detect the polycystic ovaries (PCO) and to evaluate their morphology.

Statistical analysis was performed by using SPSS version 21.0. The data were presented as mean±standard deviation. All data were subjected to the Shapiro-Wilk test, which determines the normality of the data. A chi-square test was used for testing relationships between categorical variables. Student’s t-test or Mann-Whitney U test was used for comparing the results of both the groups, wherever appropriate. The value of p<0.05 was considered statistically significant. The area under the curve (AUC) was calculated following the trapezoid formula. Pearson’s correlation coefficient was used to calculate the association between two continuous variables.

Results

The mean age of the patients with prolactinoma and the control group showed similar results (26.9±7.4 and 26.6±4.5, respectively). Two prolactinoma patients had acne, and three of them had hirsutism that persisted after cabergoline treatment (Table 1). The patients with prolactinoma showed higher PRL levels than the control group that were restored to normal values after cabergoline treatment. The E2 levels were lower in the patients with prolactinoma, which did not increase significantly after the treatment (Table 2). SHBG levels were also found to be reduced in patients with prolactinoma, which increased significantly after the treatment. A negative correlation was detected between SHBG, and total testosterone and DHEAS levels (R=-0.58 P=0.023, R=-0.53 P=0.049, respectively). FAI was found slightly higher in patients with prolactinoma that was decreased after treatment; however, the change in data was statistically insignificant. Although the data revealed a positive correlation between PRL levels and total testosterone (R=+0.52 P=0.026), there was no correlation between PRL and FAI, androstenedione, DHEAS, 17-OH progesterone, 11-S levels.

Basal androgen and cortisol levels and responses to ACTH stimulation test are summarized in Table 3. Basal androstenedione, DHEAS, 17-OH progesterone, 11-S, and cortisol levels were found to be similar in both the groups. In patients with prolactinoma, the basal and stimulated DHEAS and androstenedione levels decreased significantly in the cabergoline treated group. Although insignificant, FAI was decreased in prolactinoma patients after cabergoline treatment.

Pelvic USG revealed PCO in nine patients with prolactinoma that was significantly more frequent than in the control group (n:0) (P=0.004). After treatment, normal ovarian morphology was restored in 3 out of 9 PCO patients.

Discussion

Women with hyperprolactinemia have been reported to have hyperandrogenemia and/or hirsutism. However, there are very few studies that investigated the relationship between hyperprolactinemia and androgen excess disorders. In clinical practice, hyperandrogenism and hirsutism are infrequently found in patients with hyperprolactinemia. In this study, prolactinoma was not found to be associated with hyperandrogenemia. Although treatment with cabergoline after six months led to a significant decrease in the basal and ACTH stimulated DHEAS levels but during the initial diagnosis, the patients showed DHEAS levels similar to healthy women.

A study by Glasow et al. showed the presence of PRL receptor using polymerase chain reaction (PCR) and immunohistochemical techniques in the human adrenal gland and adrenal primary cell cultures. PRL receptor was observed in all three zones of the adrenal cortex and marginally in the medulla. The concentrations of cortisol, aldosterone,
### Table 1. Clinical features and body composition analysis of the patients with prolactinoma and the control group.

|                      | Patients at baseline (n=20) | Control (n=15) | p₁       | Patients after treatment (n:20) | p₂       |
|----------------------|-----------------------------|----------------|----------|---------------------------------|----------|
| Age (years)          | 26.9±7.4                    | 26.6±4.5       | 0.409    |                                 |          |
| Acne                 | 2                           | 1              | 0.610    | 2                               | 1,000    |
| Androgenic alopecia  | 0                           | 0              | 1,000    | 0                               | 1,000    |
| Hirsutism (≥50)      | 3 (%15)                     | 0              | 0.224    | 3 (%15)                        | 1,000    |
| Ferriman Galwey score (median min-max) | 4.95 (0-34)                     | 3.0 (0-7)       | 0.580    | 4.10 (0-30)                     | 0.115    |
| Height (cm)          | 161.0±6.4                   | 162.2±6.0      | 0.595    |                                 |          |
| Weight (kg)          | 65.1±14.5                   | 64.6±11.8      | 0.899    | 61.9±18.1                       | 0.182    |
| BMI (kg/m²)          | 25.1±5.6                    | 4.7±5.0        | 0.800    | 25.0±5.8                        | 0.556    |
| Waist circumference (cm) | 80.7±11.6                     | 80.5±9.4       | 0.953    | 81.1±12                         | 0.575    |
| Body fat percentage (%) | 27.8±7.3                       | 29.2±7.0       | 0.583    | 28±7.8                          | 0.873    |
| Body fat mass (kg)   | 19 ±8.9                     | 19.6±8.6       | 0.857    | 18.9±8.5                        | 0.994    |

*p₁: p-value for the comparison of the control group and patients with prolactinoma at baseline, p₂: p-value for the comparison of before and after the treatment of patients.

### Table 2. Hormone levels of the patients with prolactinoma and the control group.

|                      | Patients at baseline (n=20) | Control (n=15) | p₁       | Patients after treatment (n:20) | p₂       |
|----------------------|-----------------------------|----------------|----------|---------------------------------|----------|
| IGF1 (ng/mL)         | 185.3±67.7                  | 194.4±81.8     | 0.671    | 169±45.3                        | 0.272    |
| TSH (µIU/mL)         | 2.43±1.5                    | 1.84±0.65      | 0.164    | 2.69±1.6                        | 0.330    |
| sT4 (ng/dL)          | 1.2±0.1                     | 1.2±0.1        | 0.824    | 1.18±0.1                        | 0.242    |
| PRL (ng/mL)          | 209±155                     | 21.3±8.4       | <0.001*  | 15.9±15.5                       | <0.001*  |
| FSH (mIU/mL)         | 5.95±2.3                    | 6.07±1.8       | 0.866    | 7.04±2.7                        | 0.156    |
| LH (mIU/mL)          | 8.06±4.76                   | 8.0±6.0        | 0.982    | 6.52±1.9                        | 0.244    |
| E2 (pg/mL)           | 44.9±34.4                   | 105.9±113.7    | 0.034*   | 49.4±40.6                       | 0.724    |
| TT (ng/dL)           | 37.9±23.5                   | 41.2±24        | 0.705    | 30.1±17.8                       | 0.238    |
| FAI                  | 4.1±3.7                     | 2.9±2.8        | 0.494    | 2.1±1.5                         | 0.064    |
| SHBG (nmol/L)        | 45±23                       | 72±47          | 0.059    | 59±24                           | 0.001*   |

*p₁: P-value for the comparison of the control and patients with prolactinoma at baseline, p₂: P-value for the comparison of patients with prolactinoma at baseline and after treatment. TT: Total testosterone; FAI: Free androgen index.
and DHEA were found to be enhanced after PRL stimulation in cell supernatant. Kim et al. studied basal androgen levels in 20 hyperprolactinemic women and 7 control subjects. The total testosterone and DHEA concentrations were similar in both groups. However, the free testosterone level was elevated and the E2 level was reduced in patients with hyperprolactinemia than the control group. Another study reported higher serum DHEA concentration (basal and after ACTH stimulation) in eight hyperprolactinemic women than the control group. Androstenedione levels were found similar in both groups. In the present study, the free androstenedione index and total testosterone levels were similar in the two groups, whereas prolactinoma patients had reduced E2 and SHBG concentrations. 17-OHP, 11-S, DHEA, and androstenedione responses to ACTH stimulation were also comparable in patients with prolactinoma and healthy controls. After cabergoline treatment, the patients were found to have significantly decreased DHEA response to ACTH stimulation, as previously shown. Another study by Moria et al. comprising of 122 medically and 26 surgically treated patients with prolactinoma, reported a decrease in DHEA levels after treatment. However, the study did not have a control group and the effects of hyperprolactinemia could not be evaluated at baseline. In the present study, SHBG concentration was found to be increased and the stimulated androstenedione level was found to be decreased in the treated patients. A negative correlation was found between SHBG and androgen levels and SHBG levels, but after treatment, SHBG concentration increased, which may be due to the

Table 3. Androgen and cortisol levels of the patients with prolactinoma and the control group.

| Hormone                  | Patients at baseline (n=20) | Control (n=15) | p1  | Patients after treatment (n=20) | p2 |
|--------------------------|-----------------------------|---------------|-----|-------------------------------|----|
| 17-OHP (basal) (ng/mL)   | 1.18±0.83                   | 0.93±0.65     | 0.426 | 0.93±0.47                    | 0.180 |
| 17-OHP (peak) (ng/mL)    | 2.76±1.96                   | 1.97±0.57     | 0.169 | 2.55±1.83                    | 0.192 |
| AUC (17-OHP response to ACTH) | 126.6±93.6                | 94.2±24.2     | 0.271 | 117±77.4                     | 0.280 |
| 11-S (basal) (ng/mL)     | 2.46±1.6                    | 2.19±1.17     | 0.639 | 1.99±1.1                     | 0.188 |
| 11-S (peak) (ng/mL)      | 4.28±1.4                    | 4.09±1.43     | 0.751 | 3.93±1.6                     | 0.294 |
| AUC (11-S response to ACTH) | 190.9±76.3                 | 205±69.1      | 0.574 | 188.3±85.9                   | 0.822 |
| Cortisol (basal) (µg/dL) | 12.1±4.3                    | 11.6±4.9      | 0.853 | 10.8±2.9                     | 0.128 |
| Cortisol (peak) (µg/dL)  | 25.6±4.6                    | 25.2±3.65     | 0.801 | 23.7±3.2                     | 0.033* |
| AUC (cortisol response to ACTH) | 1231±206                   | 1224±176      | 0.918 | 1133±154                     | 0.030* |
| DHEAS (basal) (µg/dL)    | 317±162                     | 293±180       | 0.886 | 208±106                      | 0.003* |
| DHEAS (peak) (µg/dL)     | 310±176                     | 310±189       | 0.899 | 201.7±111.2                  | 0.002* |
| AUC (DHEAS response to ACTH) | 18566±9830                 | 17857±10753   | 0.845 | 12320±6542                   | 0.004* |
| Androstenedione (basal) (ng/mL) | 2.7±1.3                    | 2.5±1         | 0.586 | 2.1±0.9                      | 0.048* |
| Androstenedione (peak) (ng/mL) | 4.54±1.97                   | 3.6±1.25      | 0.140 | 3.32±1.28                    | 0.005* |
| AUC (androstenedione response to ACTH) | 229±92                     | 194.1±64.2    | 0.230 | 175±73                       | 0.009* |

p1: P-value for the comparison of the control and patients with prolactinoma at baseline, p2: P-value for the comparison of the patients with prolactinoma at baseline and after treatment; AUC: Area under the curve.
A decline in androgen levels by cabergoline treatment. A positive correlation was identified between total testosterone and PRL levels as discussed in published reports (20). However, the concentration of total testosterone did not decrease after treatment in the patient group.

In the present study, the patients were evaluated for hyperandrogenism symptoms which include hirsutism, acne, and androgenic alopecia. Although hirsutism was more frequent in patients with prolactinoma, it was statistically nonsignificant and the mFG scores were found similar between the groups. Hagag et al. investigated 80 hirsute and hyperprolactinemic women with prolactinoma, neuroleptic treatment, and idiopathic hyperprolactinemia. In all women, the mFG score, Leed acne score, DHEAS, free testosterone, and androstenedione levels decreased after the treatment with a dopamine agonist drug, which was carried out for 11±1 months (20). However, the study group taken into consideration was very heterogenous and the study also included the cases of drug-induced hyperprolactinemia. It can be inferred that antipsychotic drugs may directly interfere with androgen levels (21-23). In the present study, only a homogeneous group of patients with hyperprolactinemia due to prolactinoma was taken into account. Although DHEAS and androstenedione levels decreased after the treatment as reported, the basal levels of androgens in patient and control groups were comparable in the present study. Also, no significant change was observed after the treatment in the mFG score. DHEAS is known to be a weak androgen, and testosterone is more likely to be responsible for hyperandrogenism symptoms (24). The reduction of DHEAS in patients after treatment may be due to restitution of prolactin levels or a direct effect of cabergoline or both.

For the diagnosis of PCOS, hyperprolactinemia is required to be ruled out. On the other hand, polycystic ovarian morphology may be seen in 20% of women in the reproductive age group and 5% of them have PCOS (25). In the literature, a few studies and case reports indicates a close association between PCOS and prolactinoma, but there is no prospective study evaluating ovarian morphology in patients with prolactinoma (26,27). In the present study, an increased prevalence of PCO in patients was seen with prolactinoma. After treatment, the ovarian morphology was restored to normal in three of the nine PCO patients.

A relatively limited number of patients with prolactinoma and short follow-up time are the limitations of the present study.

The data, from the above study, suggest that hyperprolactinemia may not lead to clinically meaningful hyperandrogenemia and hirsutism. Moreover, the treatment of hyperprolactinemia does not lead to significant improvement in the hirsutism score of the patients, if exists. A well-known feature of hyperandrogenic disorders is menstrual dysfunction. Hyperprolactinemia should be considered in the differential diagnosis of menstrual disturbances whether associated with hirsutism or not. However, in accordance with the data evaluated in the present study and the published reports mentioned above, it may be suggested that hyperprolactinemia is not a cause of hyperandrogenism/hirsutism per se.

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Ethical Approval
The study was carried out according to the ethical standards of institutional research committee (Erciyes Üniversitesi Etik kurulu Karar no:2016/447 Tarih: 29/07/2016).

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Conflict of Interest
No conflicts of interest between the authors and / or family members of the scientific
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**Authorship Contributions**

Idea/Concept: Kürşad Ünlühizarci; Design: Züleyha Karaca; Control/Supervision: Züleyha Karaca; Data Collection and/or Processing: Mehmet Çağrı Ünal; Analysis and/or Interpretation: Züleyha Karaca; Literature Review: Kürşad Ünlühizarci; Writing the Article: Mehmet Çağrı Ünal; Critical Review: Züleyha Karaca; References and Fundings: Fahrettin Kelestimur; Materials: Fahrettin Kelestimur.

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Immunohistochemical Subtypes of Growth Hormone-Secrete Pituitary Adenoma and Association with the Clinical Course and Secondary Malignancy

Growth Hormon Sekrete Eden Adenomlarda Subtip Tayininin Klinik Takip ve Akromegali İlişkili Sekonder Malignansı Gelişiminde Rolü

Gamze AKKUŞ, Nuri Eralp ÇETINALP, Emine KILIÇ BAĞİR*, Mehtap EVRAN, Sinem ŞENGÖZ, Murat SERT, Suzan ZORLUDEMİR*, Tamer TETİKER

Çukurova University Faculty of Medicine, Division of Endocrinology and Metabolism, Adana, Turkey
*Çukurova University Faculty of Medicine, Department of Pathology, Adana, Turkey

Abstract

Objective: Most of the acromegaly cases are caused by growth hormone-secreting pituitary adenoma. Pituitary adenomas are classified histologically into sparsely granulated adenoma (SGA) and densely granulated adenoma (DGA). SGA has been reported to elicit a more aggressive clinical course and therapy resistance. The aim of this study was to investigate the immunohistochemical subtype of patients with pituitary adenoma and their relationship with the clinical course of the disease. Material and Methods: In the period between 2000 and 2016, about 40 (F21, M19) patients with acromegaly who were diagnosed and operated for pituitary adenoma at our university hospital were included in this study. The medical history of patients, duration of the disease, and comorbidities were assessed. Based on current guidelines for acromegaly management, we determined the serum growth hormone [with 75 g "oral glucose tolerance test" (OGTT)], insulin-like growth factor 1 (IGF-1) levels, as well as computed tomography (CT) or magnetic resonance imaging of the pituitary gland. Immunohistochemical staining of postoperative tissue materials and subtypes of pituitary adenomas were evaluated by an experienced cytopathologist. Results: Of the 40 acromegaly patients included in the study, 25 patients were evaluated as sparsely granulated and the remaining 15 patients were evaluated as densely granulated. The mean age of SG adenomas (40.6±9.7 vs. 48.6±5.7, p=0.04) was significantly lower. At the first visit, 64% of SG adenomas were macroadenoma while only 35% of DG adenomas were macroadenoma and the difference was not statistically significant (p=0.43). SG adenomas' pre-treatment GH, IGF1 values (29.2 ng/mL, 800 ng/mL versus 8.4 ng/mL, 445 ng/mL, p=0.02) and post-treatment GH, IGF1 values (4.1 ng/mL, 440 ng/mL versus 0.4 ng/mL, 152 ng/mL, p=0.03) were significantly higher. While endocrine remission is more common in DG adenomas; organomegaly, abnormal echocardiographic findings (left ventricular hypertrophy) and multinodular goiter were more common in patients with SGA. However, further studies are needed to confirm our findings.

Keywords: Acromegaly; carcinogenesis; sparsely granulated adenoma

Address for Correspondence: Gamze AKKUŞ, Çukurova University Faculty of Medicine, Division of Endocrinology and Metabolism, Adana, TURKEY
Phone: 0 506 262 92 04 E-mail: tugrulgamze@hotmail.com

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**Introduction**

Acromegaly is a rare systemic disease caused by excess growth hormone secretion (1). Increased growth hormone (GH) and insulin-like growth factor (IGF-1) levels lead to somatic overgrowth, along with multiple comorbid disorders including hypertension, diabetes mellitus, respiratory disturbances, and secondary malignancy. The estimated prevalence of acromegaly is estimated to be 40 to 480 cases per million (2).

Based on the histological, immunohistochemical and electron microscopic studies, growth hormone-secreting adenomas can be subdivided in sparsely (SGA) and densely granulated (DGA) adenomas (3,4).

In the clinical course of the disease, SGAs are more aggressive and resistant to treatment (5,6). Major differences between SGA and DGA are the intensity and size of secretory granules and the distribution of cytokeratin filament. SGAs are composed of cytokeratin filament called fibrous bodies and an eccentric nucleus. DGAs have large secretory granules and its nucleus is located centrally. Immunohistochemical stainings of the DGA show strong growth hormone secretion (7,8).

It has been suggested that immunohistochemical subtypes of these adenomas could determine the clinical outcomes of the therapy. SGAs are commonly seen in young patients and presented with large size of the pituitary mass and more invasive to adjacent tissues such as cavernous sinuses and optic chiasm (9). In this study, we evaluated the postoperative immunohistochemical pituitary adenoma subtypes and their relationship with the clinical course of the disease.

**Material and Methods**

Forty patients with acromegaly who had been referred to our endocrinology clinic between January 2000 and December 2016 were retrospectively included in the study. The diagnosis of acromegaly was assessed based on the clinical findings and hormone analysis according to the endocrine guidelines (10). We obtained data of demographical, hormonal and radiological reports from patient files. There were 40 patients with acromegaly who were eligible based on the pathological reports. In our study, the disease duration was defined as the time between the initial diagnosis of acromegaly and the current time of performing this study. The inclusion criteria were as follows: (a) confirmed acromegaly diagnosis, (b) a pituitary mass on MRI, (c) patients who had pituitary surgery and quantitatively enough specimen for histopathological diagnosis, (d) those who had colonoscopy to screen colon neoplasm at initial diagnosis of acromegaly, and (e) those who had at least one follow-up clinical evaluation after the pituitary surgery. The follow-up screening criteria and post-operative tests were as follows: (a) random GH and IGF-1 value at 12 weeks or nadir GH value after a 75 g-glucose tolerance test (GH ≤ 1 mcg/L) (b) MRI of the pituitary at least after 12 weeks (c) thyroid ultrasonography was performed if there was a palpable thyroid nodule and/or a positive family history of thyroid malignancy. (d) abdominal ultrasonography was performed on those patients given somatostatin receptor analogs for gallstone disease (10).

The endocrine remission criteria were as follows: (a) Patients who had serum IGF-1 within normal ranges considering age and gender, and GH levels <1.00 mcg/L with 75 g oral glucose load (OGTT); (b) Patients who had unsuccessful pituitary surgery and continuing active disease, and growth hormone suppressive therapy including somatostatin analogs and cabergoline have been administered (10).

Serum GH levels were measured as a chemiluminescence immunometric assay using the Immulite 2000, Siemens; ng/mL. Serum IGF-1 measurements were performed with a solid phase enzyme-labeled chemiluminescence immunometric assay using the Immulite 2000, Siemens; ng/mL. Calibration was up to 1600 ng/mL (WHO National Institute for Biological Standards and Control first International Reference Reagent [NIBSC first IRR] 87/518). The serum levels of prolactin, thyroid-stimulating hormone (TSH), free thyroxin, adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone were analyzed using an immunoassay kit (DxI 800, Beckman Coulter; ng/mL).
Radiological Evaluations

Magnetic resonance imaging of the pituitary was performed before and after intravenous contrast administration. Tumors were classified as macroadenomas (>10 mm) or microadenomas (<10 mm) as consistent with the clinical guidelines. Parasellar extensions of pituitary tumors were classified into five grades according to the Knosp classification (11). Grades 0, I and II were considered as noninvasive and grades III/IV as invasive.

We assessed the pre- and post-operative MRI/BT scans of the pituitary gland as follows: a change in the residual mass size below 10% was defined as the stabilized mass and an increase of >10% as unstabilized mass. Ultrasonographic examinations were conducted by the same experienced radiologist by using static grayscale and real-time B mode ultrasonography. Both ultrasonographic examinations (thyroid and abdomen) were performed after 8-h of fasting. Sonographic measurements for hepatomegaly were determined according to the criteria of Gosink et al (12).

Pathological Evaluation and Immunohistochemistry

Immunohistochemical stainings were performed on tissues of 5-mm sections which had been formalin-fixed and paraffin-embedded using human growth hormone antibody (GH) (1/100, Zymed Laboratories), low molecular weight keratin (LMWK) (1/70, Leica) and Ki-67 (clone MIB-1, Dako). BenchMark XT with heat-induced epitope retrieval (CC1 solution) and iView DAB detection kit (Ventana, Tucson, AZ) were used for the visualization system. Cytoplasmic staining was regarded as positive for GH. Dot-like globules of keratin staining called as a fibrous body at LMWK and fibrous bodies were defined for SGA. Granular cytoplasmic staining with LMWK was considered as DGA. Nuclear staining was accepted as positive for Ki-67 and the percentage of its staining was evaluated. Examples of different immunostaining patterns are shown in Figure 1. This study was conducted in accordance with the Declaration of Helsinki. The Local Ethics Committee of Cukurova University approved the study (No:87, 2019).

Statistical Analysis

Statistical analyses were performed using the Shapiro-Wilk, Mann-Whitney U and Chi-squared tests. For correlations between the groups, Pearson’s correlation was used for the parameters with the normal distribution, and Spearman’s tests were used for the parameters without normal distribution. The results for categorical variables were presented as n (%), while quantitative variables were expressed as mean±SE mean or median (min, max). SPSS-19 software (IBM, Armonk, NY, USA) was used for all statistical analyses.

![Figure 1: Subtypes of somatotroph adenoma. A) Hematoxylin-eosin staining in somatotroph adenoma, B) Immunohistochemical positivity of growth hormone (GH) in somatotroph adenoma, C) Distinct paranuclear fibrous bodies typical of sparsely granulated somatotroph adenoma.](image-url)
Results

There were 21 female and 19 male patients. The median age of patients was 48 years (range, 30 to 66). The mean duration of the disease was 12-years (range, 12 to 192 months). Of the 40 patients, 30 had macroadenoma (tumor size >10 mm) and 10 had microadenoma (tumor size <10 mm).

Twenty-five of the total 40 patients (62.5%) had SGA, while 15 (37.5%) had DGA. Ki-67 proliferation index was found to be 1% in the specimens of 38 patients and 2% in the specimens of two patients. The SGA was found to be higher in females than in males (15 vs. 10, \( p < 0.05 \)), respectively.

The preoperative mean serum GH value of the patients with DGA was 8.4 ng/mL. The median IGF-1 values of the patients with DGA were two times higher than the upper limit of the normal range (median 445 ng/mL). The preoperative mean GH value of the patients with SGA was 29 ng/mL and the median IGF-1 levels of the patients with SGA were four times higher than the upper limit of normal (median 800 ng/mL).

The remission rate after primary surgery in all patients with acromegaly was 9/40. Of the nine patients, two had SGA (2/25) and the remaining seven patients had a subtype of DGA (7/15) (\( p = 0.001 \), SGA vs. DGA).

The postoperative MRI scans of the pituitary revealed that 31 patients had residual lesions, 7 patients had no residual lesions, and 2 had empty sella. While 20 of 25 patients with SGA were found to have macroadenomas using MRI of the pituitary, only ten patients with DGA had macroadenomas.

Seventy percent of adenomas (28/40) were found to have cavernous sinuses invasion (CSI) at grade III (n=16) and grade IV (n=12) levels.

When compared for the cavernous sinus invasion in both adenoma types, SGA (n=18) showed a higher rate of invasion than that of the DGA, which was not significant (18 vs. 10, \( p = 0.05 \)), respectively.

Of the 40 patients, 10 (25%) had only diabetes mellitus as a comorbid disease, 5 (12.5%) only hypertension and the remaining 25 (62.5%) had no other disease.

The patients’ data including demographic, CT/MRI, serum GH/IGF-1, and histopathological characteristics are summarized in Table 1.

Complete endocrine remission (defined as the serum GH level <1 ng/mL with OGTT) was seen in 15 of 40 (37.5%) patients. Among patients with endocrine remission, nine patients had only transsphenoidal surgery (TSS) and the remaining six patients were administered somatostatin analogs (Octreotide) and gamma-knife radiotherapy in addition to TSS. The median duration to remission was 36 months considering the serum IGF-1 normalization (range, 12-192 months). When compared to the histopathological subtypes of 15 patients who achieved endocrine remission, 11 had DGA, and only four patients presented with SGA (\( p < 0.05 \)).

There were total 25 (25/40) patients showing no endocrine remission after TSS, who were followed with chemotherapy (Octreotid: 16, Lanreotid LAR:5, Cabergolin:2) and/or gamma-knife radiotherapy. Of them, 21 were SGA and 4 were DGA. The outcomes of surgical or combination therapy with respect to immunohistochemical subtypes of adenomas (SGA, DGA) are shown in Table 2.

Surveillance Findings

The results of the 31 patients who were treated with GH suppressive medical therapy and screened by abdominal ultrasonography are shown in Table 1. All patients with SGA (100%) had abnormal ultrasound findings including hepatomegaly (n=10), hepatosteatosis (n=2), splenomegaly (n=5), hepatomegaly and splenomegaly (n=4), polycystic renal diseases (n=2), and cholelithiasis (n=2).

The thyroid ultrasonography of the 40 patients indicated that 19 were with normal observations, 21 showed multinodular goiter, and of these 21 cases, most (n=14) were associated with SGA. In the diagnostic fine needle aspirations (FNA) of the patients with nodular goiter, there was only one case with thyroid follicular neoplasia. The echocardiographic findings of the 40 patients were normal in 24 cases; however, in 10 patients, it showed left ventricular hypertrophy, and in 6 patients, left ventricular diastolic dysfunction and pericardial effusion were observed. Regarding the adenoma subtypes, all patients with abnormal cardiac findings including left ventricular hypertrophy (n=10), left ventricular diastolic dys-
function (n=5) and pericardial effusion (n=1) were carrying SGA, while those with DGA had normal echocardiographic findings (SGA vs. DGA, p<0.05). The colonoscopic findings were normal in all patients. The results of thyroid ultrasonography, echocardiography, and their relevance with the immunohistochemical subtypes of the adenomas are shown in Table 1.

**Secondary malignancies associated with acromegaly**

We found four secondary malignant diseases including renal cell cancer (n=2), thyroid follicular neoplasia (n=1), and larynx cancer (n=1) in our patients with acromegaly. These four patients were operated and were clinically followed postoperatively. The different types of cancer immunostaining are shown in Figure 2. All patients with malignancy had SGA but with no statistical significance.

**Discussion**

It is well known that the primary treatment for acromegaly patients with pituitary adenoma is transphenoidal surgery to control the growth hormone excess and to prevent the acromegaly related comorbidities. An unsuccessful surgery requires the other

| Table 1. The patient data including demographics, hormonal parameters, imaging findings and comorbidities with respect to immunohistochemical subtypes of GH secreting adenomas. |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| **Duration of Disease (year)**                  | DGA (n=15)     | SGA (n=25)     | P-value         |
| Age                                             | 14             | 4              | 0.002           |
| Sex                                             |                |                | 0.04            |
| Male                                            | 9/40           | 10/40          |                 |
| Female                                          | 6/40           | 15/40          |                 |
| Cavernous Sinus invasion                       | 10 (%35.8)    | 18 (%64.2)    | 0.05            |
| Macroadenoma                                    | 10 (%33.4)    | 20 (%66.6)    | 0.43            |
| Microadenoma                                    | 5 (%70)       | 5 (%30)       |                 |
| GH*(ng/mL)                                      | 8.4            | 29.2           | 0.02            |
| IGF1* (ng/mL)                                   | 445            | 800            |                 |
| GH**(ng/mL)                                     | 0.4            | 4.1            | 0.03            |
| IGF1** (ng/mL)                                  | 152            | 440            |                 |
| Post operative residual mass                   | 9 (%31.1)     | 20 (%68.9)    | 0.01            |
| Endocrine Remission***                          | 11 (%64.7)    | 4 (%35.5)     | 0.02            |
| Hepatomegaly                                    | 2              | 10             |                 |
| Splenomegaly                                    | 2              | 5              |                 |
| Cholelithias                                    | 2              | 2              |                 |
| Abnormal echocardiography ∞                     | 2              | 14             | 0.04            |
| Nodular goiter                                  | 7              | 14             | 0.04            |
| Malignant disease δ                             | -              | 4              | 0.91            |

DG, densely granulated; SG, sparsely granulated, * Preoperative value; **Postoperative value; ***Endocrine Remission, Growth hormone (GH) <1ng/mL with 100 g OGTT; ∞, Abnormal echocardiography including left ventricular hypertrophia, left ventricular diastolic dysfunction, pericardial effusion. δ, Malignant disease including larynx, renal, thyroid neoplasia.

| Table 2. Outcomes of surgical and medical treatment in patients with DGA or SGA. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Surgical Treatment**          | **Combination Therapy**+ | **Endocrine Remission*** | **Without Endocrine Remission** |
| SGA                             | 2               | 2               | 4               | 21              |
| DGA                             | 7               | 4               | 11              | 4               |
| Total                           | 9               | 6               | 15              | 25              |

+: Surgical, Medical and/or Gamma knife RT; **: Growth hormone (GH) <1 ng/mL with 100 g OGTT.
known treatment options such as medical therapy and pituitary radiotherapy (13). Recent studies (14-17) have shown that immunohistochemical subtypes of GH-secreting adenomas (DGA, SGA) have a significant relationship with treatment response, aggressiveness, and clinical and hormonal features of acromegaly. It has been reported that the SGA subtype has a larger tumor volume, higher incidence of suprasellar extension, cavernous sinus invasion and is more common in females than the DGA subtype (5,18-20).

Similarly, we observed that the clinical course and outcomes of acromegaly treatment were related to the immunohistochemical subtypes of the GH-secreting adenoma. Among the 40 patients with acromegaly, 25 had SGA while 15 had DGA. Of the total 40 patients who had acromegaly treatment, 15 were in endocrine remission, and 11 of them (11/15) were due to DGA. Although it did not reach the statistical significance, pituitary macroadenomas were found to be higher in patients with SGA than in patients with DGA (20 vs. 10; p=0.435), respectively. In addition, the cases with SGA showed more frequent invasion of the cavernous sinuses than those with DGA (18 vs. 10; p=0.05, respectively). As reported in the other studies, SGA was found to be more common in females.

Although in a few studies (20-22), the baseline serum GH and IGF-1 levels were high in patients with DGA, in most of the other studies (5,16,21-23), the baseline serum
GH and IGF-1 levels were reported to be higher in patients with SGA than the patients with DGA subtypes. Consistent with the later reports, we found higher baseline (pretreatment) serum GH and IGF-1 levels in patients with SGA than the patients with DGA (29 ng/mL and 800 ng/mL vs. 8.4 ng/mL and 445 ng/mL; \( p = 0.03 \)), respectively.

Several studies (21,23,24) reported that the surgical response rate of patients with DGA was higher than patients with SGA. Kiseljak et al. (20) reported that acromegaly caused by DGA showed a higher remission rate than acromegaly by SGA (65.7\% vs. 14.3\%; \( p < 0.001 \)). Bakhtiar et al. (16) reported that patients with SGA (n=30) had a higher tumor volume and a lower surgical cure rate (42.3\%) compared to patients with DGA (n=111, 60.4\%). Similarly, we found a significant correlation between the postoperative remission rate (47\%) and DGA (\( p = 0.001 \)).

Another aspect of the study is to investigate acromegaly related comorbidities such as morbidity and mortality (25-27). Hypertension, impaired glucose metabolism, sleep apnea, osteoarthritis, visceromegaly, multinodular goiter, and malignancy elsewhere in the body are the most common concomitant diseases (28-30). The most frequent disorders in the cardiovascular system are left ventricular hypertrophy, decreased ventricular diastolic filling, and reduced left ventricular ejection fraction (31). Acromegaly related cardiomyopathy is found in most of the patients at initial diagnosis. Although hypertension is the best-known causative factor in cardiac hypertrophy, several studies have suggested that cardiac hypertrophy was an initial finding in the heart, even in patients without hypertension (32). In this study, we found left ventricular hypertrophy in ten patients (25\%), left ventricular diastolic dysfunction in five patients and pericardial effusion in one patient using echocardiography. When compared to the patients with DGA, all cases with abnormal cardiac findings belonged to SGA (SGA vs. DGA, \( p < 0.005 \)).

Previous studies, with respect to the intra-abdominal morbidities of acromegaly, reported that the higher serum GH/IGF1 levels and the higher visceromegaly incidence including of hepatomegaly, splenomegaly, cystic renal disease in patients were due to SGA (12,33-36). Consistent with these studies, we found that all our patients with SGA (25/40; 100\%) had hepatomegaly (n=10), splenomegaly (n=5), hepato-splenomegaly (n=4), cholelithiasis (n=2) and cystic renal disease (n=2). However, there were only six patients (n=15, 40\%) with abnormal abdominal findings due to DGA (Table 1).

Several preliminary studies have reported that patients with acromegaly have three times increased risk of the corresponding type of tumor. However, it is still under debate whether the cancer risk increases or not. In some retrospective studies, nearly 15\% to 24\% of deaths of acromegaly were attributed to various types of cancer including colon, thyroid, breast, hematopoietic and renal cell (37,38). In order to understand the underlying mechanisms of pathogenesis, GH/IGF-1 pathways are considered as the key factor in the induction of mitosis and predisposition to malignancy (39-41).

In line with the reported observation of increased secondary malignancy in patients with acromegaly, we found four (4/40) patients with renal cell carcinoma, laryngeal carcinoma, and thyroid neoplasia, which were due to SGA. Although we could not find a statistically significant difference due to the small sample size, our results should not be neglected in the management of acromegalic patients for secondary malignancies.

In conclusion, an immunohistochemical subtype of pituitary adenoma whether SGA or DGA seems to affect the clinical course, comorbidities, and therapy responses. SGA was revealed as more prone to cavernous sinus invasion, comorbidity and resistance to therapy. Moreover, all patients with malignancy had SGA. Hence, to perform immunohistochemical staining of the pituitary adenoma, it is important to predict the clinical course of the patients with acromegaly.

**Study Limitations**

This is a retrospective study based on collected data. The sample size is very small since some of the patients underwent surgery in other tertiary centers and we could not collect pathologic specimens from these centers.
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: Gamze Akkuş; Design: Gamze Akkuş, Sinem Şengöz; Control/Supervision: Murat Sert; Data Collection and/or Processing: Nuri Eralp Çetinalp, Gamze Akkuş, Emine Kılıç Bağır; Analysis and/or Interpretation: Mehtap Evran; Literature Review: Murat Sert, Gamze Akkuş; Writing the Article: Gamze Akkuş; Critical Review: Murat Sert, Tamer Tetiker, Suzan Zorludemir; Materials: Gamze Akkuş, Nuri Eralp Çetinalp.

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Endocrine Effects of Coffee Consumption

Kahvenin Endokrin Etkileri

Ceyda DINÇER, Tuğçe APAYDİN, Dilek GOGAS YAVUZ

Marmara University Pendik Training and Research Hospital, Department of Endocrinology and Metabolism, Istanbul, Turkey

Abstract

Caffeine has been found to exert various biological effects including, antiangiogenic, antiproliferative, antimetastatic activity, increased fat oxidation and mobilization of glycogen in muscle, increased lipolysis, and reduction of body fat. The aim of this review is to analyze the endocrine effects of coffee consumption. A systematic literature search was conducted on PubMed and Web of Science databases seeking articles published until May 2019, dealing with coffee consumption and diabetes, osteoporosis, thyroid gland, adrenal, and gonad. The results of the most epidemiologic studies reported that coffee consumption has positive effects on combating type 2 diabetes risk, has no significant effects on bone mineral density levels but fracture risk was shown to be higher in the high coffee consumer group. Coffee intake has no significant effect on thyroid cancer, increased sex hormone binding globulin levels, has no effect on fertility but higher consumption was related to spontaneous abortion. Studies pertaining to coffee consumption and diabetes, osteoporosis, thyroid gland, adrenal and gonad hormonal; osteoporosis; thyroid diseases

Keywords: Coffee; diabetes mellitus; fertility; gonadal hormones; osteoporosis; thyroid diseases

Introduction

Coffee is one of the most popular beverages consumed worldwide, thereby enhancing its market demand (1). Statistics reveal that the average consumption of coffee ranges from 2-4 cups per day in western societies. Western countries prefer to consume brewed coffee while instant coffee is popular among the North Europeans. Boiled coffee is frequently consumed by the inhabitants of the Balkan area, Ireland, North Africa, and Turkey. Scientists have extracted numerous bioactive compounds, such as chlorogenic acids (CGA), polyphenols, diterpenes, caffeine, and caffeine metabolites (1,2) from this complex beverage.

Özet

Kafeinin; antiproliferatif, antiangiyojenik, antimetastatik etkileri, artmış yağ oksidasyonu, glikojenin kas içinde mobilizasyonu, artmış lipoliz gibi çeşitli biyolojik etkileri göstermiştir. Bu çalışmadaamaxımız, kahve tüketiminin endokrin sistem üzerine olan etkilerini incelemektir. Mayıs 2019’a kadar kahve tüketimi ve diyabet, osteoporoz, tiroid hastalıkları, adrenal ve gonad fonksiyonları hakkında yarımilyan makaleler PubMed ve Web of Science veri tabanlarında sistematik olarak tanıland. Epidemiyolojik çalışmalar, kahve tüketiminin Tip 2 diyabet riskini azaltmada olumlu etkisi olduğunu, kemik mineral yoğunluğu üzerinde anlamlı bir etkisinin olmadığını, ancak yüksek kahve tüketen grupa kırık riskinin daha yüksek olduğunu, tiroid kanserleri üzerinde anlamlı etkisinin olmadığını, seks hormon bağlayıcı globulin seviyelerini artırdığını, doğuranınkses hormon bağlayıcı globulin seviyelerini artırdığını, ancak spontan abortus riskini artırdığını göstermiştir. Kahve tüketimi ve endokrin etkiler üzerine yapılan çalışmaların çeşitli sonuçları olup net etkilerini belirleyebilmek için uzun takip süreli klinik çalışmalar gereklidir.

Anahtar kelimeler: Kahve; diabetes mellitus; fertilit; gonadal hormonlar; osteoporoz; tiroid hastalıkları

Address for Correspondence: Tuğçe APAYDİN, Marmara University Pendik Training and Research Hospital, Department of Endocrinology and Metabolism, Istanbul, Turkey

Phone: +90 554 4036978 E-mail: tugceapaydin88@hotmail.com

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Owing to the presence of these bioactive components, there is an increased scientific interest in the potential health benefits of regular coffee consumption. Recent studies have reported that coffee exerts functional effects on human health. The effect of coffee intake on malignancy has already been reported in various studies. Presently, research is mostly focused on exploring the scientific aspect of the effect of coffee on type 2 diabetes, cardiovascular, and cerebrovascular diseases. All these medicinal effects of coffee are attributed to the presence of various bioactive compounds like caffeine, chlorogenic acid, and cafestol. Caffeine has been known to exhibit several biological effects, such as antiproliferative, antiangiogenic, antimetastatic activities, increased fat oxidation, and mobilization of glycogen in muscles, increased lipolysis, and decreased body fat (3).

Recent clinical and laboratory data implicate that endocrine tissues could also be the targets of coffee compounds. The present review aims to analyze the clinical trials that evaluated the consequences of coffee consumption on endocrine disorders.

**Methods**

A systematic literature search was conducted on PubMed and Web of Science databases seeking articles published until May 2019 using a combination of the following Medical Subject Headings terms and keywords: coffee 'AND' (diabetes 'OR' type 2 diabetes 'OR' thyroid 'OR' goiter 'OR' hypothyroid 'OR' hyperthyroid 'OR' thyroid cancer 'OR' infertility 'OR' osteoporosis 'OR' vitamin D 'OR' adrenal).

Constraints were used for advanced search: adults, human, clinical trial, and search fields: title/abstract. Additionally, we scrutinized references within identified papers as well as articles that had come to our attention through other means.

**Coffee and Type 2 Diabetes**

**Q1: Does coffee consumption reduce type 2 diabetes risk?**

Although the correlation of coffee with the pathogenesis of diabetes is still controversial, special attention has been given to estimate the possible beneficial effects of coffee on developing type 2 diabetes.

Epidemiological studies conducted among different populations throughout the world highlighted the positive effects of coffee in combating the risk of type 2 diabetes, in a dose-dependent manner. Studies suggested that 3-4 cups of coffee per day were capable of reducing the risk of development of T2DM (Type 2 Diabetes Mellitus) by approximately 25%, as compared to those consuming less than 2 cups per day. The relative risk of T2DM for the highest level of coffee intake (>6 cups/day) was estimated to be 0.71 (0.67-0.76) for caffeinated coffee and 0.79 (0.69-0.91) in case of decaffeinated coffee (1,4). In addition, increasing coffee consumption +1 cup/day incurred a decrease of 11% on T2DM risk in the following four years. On the other hand, reducing coffee consumption by more than 1 cup/day increased the risk of development of T2DM in four years by 17% (1,5). Both caffeinated and decaffeinated coffee, filtered and instant coffee were associated with reduced risk of T2DM (6-8). Studies also suggested that this inverse association was applicable to very high levels of coffee consumption. Dose-response analysis suggested that 12% (0.88 (0.86-0.90)) reduced risk of T2DM was attributed to the intake of every 2 cups/day of caffeinated coffee. On the other hand, intake of every 2 cups/day of decaffeinated coffee was associated with an 11% (0.89 (0.82-0.98)) reduced risk (9). A recent study from a low coffee consumer country, Iran also concluded a lower risk of diabetes or pre-diabetes among the coffee consumer group (10).

However, few epidemiologic studies failed to report any correlation between coffee consumption and type 2 diabetes risk (11).

**Q 2: Is it the caffeine in coffee that causes diabetes?**

Published reports opined that both caffeinated and decaffeinated coffee consumption lowered the risk of type 2 diabetes (7,10,12,13).

There are controversial studies about the effect of decaffeinated coffee on type 2 diabetes risk. A prospective cohort study reported a negative relationship between caffeinated coffee and type 2 diabetes risk. However, this study failed to establish any effect of decaffeinated coffee on the development of T2DM (14).
Q3: Does coffee consumption affect glycemic control?

There are a few studies reflecting the outcome of coffee consumption on glucose levels in type 2 diabetic patients. Studies suggested that exaggerated postprandial glucose and altered insulin responses owing to the presence of caffeine might lead to impaired glycemic control (15). This contradictory effect might result from the acute effect of coffee. However, habitual consumption might indulge in the positive effects of this beverage. Therefore, it is difficult to compare the studies investigating acute metabolic effects.

Q4: Are there any effects of coffee consumption on glucose metabolism and insulin sensitivity in healthy individuals?

Oral glucose tolerance test (OGTT) was conducted among euglycemic individuals where subjects were provided with a 75-gram glucose load and the blood glucose level was estimated, henceforth. Such cross-sectional studies among healthy populations revealed that second-hour glucose levels, following the glucose load, were lower in the coffee consumer group (16). Another study also reported second-hour glucose levels in a coffee consumer group to be lower among 1328 healthy individuals (17). Randomized controlled studies in healthy individuals observed reduced insulin sensitivity attributed to acute caffeine load. However, the plasma glucose levels remained unaffected. Contrary to these findings, one study indicated a disruption in the second-hour glucose levels in healthy volunteers with a single dose of coffee (18). Contradictory reports are available regarding the effect of coffee consumption on postprandial glucose levels. Some data indicated that tolerance to the adverse consequences of coffee on insulin sensitivity may occur as a long term effect.

In a randomized controlled clinical trial from Japan, overweight men were randomly classified into three groups: one group receiving five cups of caffeinated coffee, the second group consumed five cups of decaffeinated coffee, and the third group did not receive any coffee for 16 weeks. Second-hour glucose levels were found to be lower for the caffeinated group. When adjusted according to waist circumference, caffeinated and decaffeinated groups were associated with a modest reduction in glucose levels post 2-hour glucose load (19). Randomized controlled trials were also conducted estimating the short-moderate term effects of coffee on glucose metabolism and insulin sensitivity. In a trial, the glucose and insulin levels were recorded during the second week of the regular coffee intake period and two weeks of abstinence period in 26 healthy volunteers. Fasting plasma glucose levels were similar but fasting insulin levels were higher after the coffee intake period (20).

Another study among euglycemic individuals compared the insulin sensitivity among 5 cups/day caffeinated, 5 cups/day decaffeinated, and no coffee consumers for a period of eight weeks. Though insulin sensitivity was reduced due to short term effects of coffee, positive effects on adipocyte and liver functions were witnessed (21).

Q5: What is the main mechanism of the beneficial effects of coffee?

The main mechanism behind the beneficial effects of coffee on glucose metabolism has not been elucidated so far. Some in vivo and in vitro studies have been conducted to screen the pharmacological activity of the biological compounds present in coffee. In a cross-over trial, 12 gram decaffeinated coffee, 1 gram chlorogenic acid, 500 mg trigonelline, and 1 gram mannitol used as placebo were compared. Chlorogenic acid and trigonelline ingestion were associated with significantly lower glucose and insulin concentrations, 15 min after an oral glucose load was given. However, OGTT insulin and glucose under the curve areas were not reduced when compared with the placebo. So it was claimed that the positive effects on glucose metabolism may be due to chlorogenic acid and trigonelline, present in coffee (22). Studies involving animal models postulated that the reduction of blood glucose concentration by chlorogenic acid was mediated by the activation of adenosine monophosphate-activated protein kinase (AMPK), affecting fat and glucose metabolism at the cellular level (1,23). Evaluation of the effect of chronic consumption of caf-
The effect of Glut 4 transporters present in the skeletal muscles and AMPK activity along with the anti-oxidant and anti-inflammatory effects of coffee were hypothesized to contribute to glucose metabolism (1). The results of observational and clinical studies about the effects of coffee on glucose metabolism are contrary. Clinical trials mostly focus on the short term effects of coffee on glucose and insulin parameters, long term randomized controlled studies are essential to establish the preventive effect of coffee from type 2 diabetes.

Conclusion
The results of the most epidemiologic studies establish the dose-dependent beneficial effect of coffee consumption on reducing type 2 diabetes risk. Although the number of patients was high and the follow-up period was long in epidemiological studies, the coffee drinking and dietary habits of the participants were evaluated with a validated self-reported questionnaire. However, lengthy follow up period of these cohort studies, often result in miss assessments. Differences in cup sizes and brewing types also need to be taken into consideration.

In randomized-controlled studies, there may be bias as most of the studies in the literature are not double-blinded. Coffee consumption failed to portray significant beneficial effects on glucose control in diabetic patients.

Coffee and Osteoporosis

Q1: Does coffee consumption has an association with increased osteoporosis risk?
As coffee is one of the most consumed beverages, its outcomes on bone health were also studied extensively. In experimental studies, high doses of caffeine were shown to suppress osteogenesis (24) and increase osteoclastic formation (25). However, these unfavorable consequences on bone health were in contradiction with the results obtained from clinical and observational studies.

Q2: How coffee consumption affects BMD (Bone Mineral Density) levels?
Studies pertaining to the effect of coffee on BMD have contrary results. Studies reported positive, negative or neutral effects of coffee consumption on BMD, as shown in Table 1. Most of the studies measured BMD with QUS (quantitative ultrasound) or with DXA (Dual-energy x-ray absorptiometry) in premenopausal, postmenopausal women or male population. Coffee consumption was negatively correlated with the prevalence of osteoporosis in postmenopausal and premenopausal women (26). Yu et al. evaluated the prevalence of osteoporosis among 992 Chinese men, aged between 30-90 years. Osteoporosis was recorded according to T scores and the prevalence of osteoporosis was found to be less frequent in Chinese men with moderate coffee intake (27). Yang et al. (2015) evaluated the association between osteoporosis and coffee consumption in postmenopausal women. Osteoporosis in 1,817 participants was investigated by calcaneus quantitative ultrasound and T scores were recorded (26).

A study from the Korean premenopausal population of 1,761 participants failed to ascertain the significant correlation between BMD and coffee consumption (28). Choi et al. (2016) explored 4,066 postmenopausal women according to BMD levels measured by DXA and coffee consumption. The participants in the highest quartile for coffee consumption had 36% lower odds for osteoporosis (29). França et al. from Brazil, found adverse effects of coffee on BMD, in a study population of 156 postmenopausal women (30). A study from Turkey, in 2005, analyzed a study population of 200 postmenopausal women and found no association between BMD levels and coffee consumption (31). A recent study from the Taiwanese population examined the association between coffee consumption and T scores. The study revealed higher T scores in the coffee consumer group but half of the women in the study were premenopausal, so the evaluation of this study should be done carefully (32).

Q3: Does coffee consumption have an association with increased fracture risk?
Conflicting results of clinical studies are available in the literature regarding the ef-
| Study               | Country | Sample size | Sample type                        | Design           | Osteoporosis diagnosis                     | Coffee dose | Result                                                                 |
|---------------------|---------|-------------|------------------------------------|------------------|--------------------------------------------|-------------|-------------------------------------------------------------------------|
| Yu et al., 2016     | China   | 992 men     | 30-90 years                        | Cross-sectional  | BMD measured at calcaneus by quantitative US | Seldom or moderate | Prevalence of osteoporosis less frequent in Chinese men with moderate coffee intake |
| Yang et al., 2015   | China   | 1,817 women | Postmenopausal                     | Cross-sectional  | BMD measured at calcaneus by quantitative US | Seldom, sometimes, always | Coffee consumption was negatively correlated with the prevalence of osteoporosis |
| Choi et al., 2016   | Korea   | 4,006 women | Postmenopausal                     | Cross-sectional  | L1-4 and femoral DXA                       | 9 categories almost no- 3/day | Coffee consumption may have protective effects on bone                  |
| França et al., 2015 | Brazil  | 156 women   | Postmenopausal and osteoporotic    | Cross-sectional  | L1-4 and femoral DXA                       | 3 day food diary | Caffeinated beverages exert a negative effect on BMD                    |
| Demirbag et al., 2005 | Turkey | 200 women   | Postmenopausal                     | Cross-sectional  | L1-4 and femoral DXA                       | Cups/day     | No relation between coffee consumption and BMD                           |
| Chang et al., 2017  | Taiwan  | 2,929 women | 1,366 premenopausal, 1,593 postmenopausal | Cross-sectional  | Quantitative US                             | Cup/day      | Positive effect in premenopausal and neutral in postmenopausal           |
| Choi et al., 2014   | Korea   | 1,761 women | Postmenopausal                     | Cross-sectional  | L1-4 and femoral DXA                       | <1/day, 1/day, 2/day, 3/day | No significant association between BMD and coffee consumption          |
Effect of coffee consumption on the risk of fracture. There are large cohort studies that claim increased fracture risk in the high coffee consumer group (>4 cups/day). The studies pertaining to coffee consumption and fracture risk are enlisted in Table 2. A cohort study in the United States (US) for a period of six years among middle-aged women consuming more than 4 cups of coffee per day was found to be associated with a three-fold increase in the risk of low impact trauma hip fractures (33). Another cohort study with 31,527 Swedish middle-aged women found a modestly increased fracture risk in the 4 cups/day or more coffee consumer group (34). Jokinen correlated the risk factors for cervical and trochanteric fractures with coffee consumption of over 5 cups per day and he found increased trochanteric fractures among the coffee consumers (35). A recent study from China also documented that the coffee intake of four or more cups was associated with increased fracture risk in both men and women (36).

Literature also contains few studies that negate the effect of coffee on fracture risk. Halstrom et al. (2012) explored the effects of coffee in a cohort of 61,433 women and high coffee consumption was associated with a small reduction in bone density. However, no significant association between high coffee intake and an increase in fracture risk was witnessed (37). Albrand et al. also failed to correlate caffeine consumption with fracture risk (38).

Most of the studies focus on the risk of fracture in postmenopausal women. Few studies also investigated the risk in men. Halstrom et al., in 2014, examined and found no increase in fracture risk in the coffee consumer group comprising of a cohort of 42,978 middle-aged men (39). Another cohort study among the geriatric population failed to establish any association between hip fractures and caffeine intake (40).

Another interesting observation was obtained from a clinical study that scrutinized the risk of fracture in postmenopausal women based on the fracture site. Coffee consumption was found to have a positive association with a wrist fracture, whereas there was no significant association with hip or vertebral fracture (41).

A meta-analysis report documented no significant increase in fracture risk with coffee consumption; however, the relative risk increased from the highest to the lowest category of coffee intake, when compared with those who never drank coffee. The pooled RR of hip fracture was found to be 1.13 (95%CI: 0.86 to 1.48) for individuals with the highest level of coffee consumption (42).

Q4: What are the possible mechanisms of action of coffee on the bone?

As the studies in the literature have contradictory results, possible mechanisms for the negative effects of coffee were predicted to be due to increased urinary calcium excretion and decreased intestinal calcium absorption (41). The estrogenic effects, antioxidant activity or anti-inflammatory property of coffee might contribute to its positive effects on bone. Allred et al. demonstrated that trigonelline, a compound present in coffee, can function as an estrogen receptor agonist in estrogen receptor-positive breast cancer (29,43). Studies have also highlighted prominent antioxidant property of coffee as compared to grape juice, orange, raspberry (44). Chlorogenic acid, an ingredient in coffee, was shown to exhibit an inhibitory effect on osteoclastogenesis (29,45). The anti-inflammatory activity of coffee was attributed to nitric oxide synthase and COX–2 inhibition (46).

Earlier studies have mostly been based on food questionnaires such as the number of cups per day, while randomized controlled trials are lacking. Therefore, further studies are needed with detailed information on confounding factors, coffee types, and longer follow-up periods.

Conclusion

Cohort studies indicated increased fracture risk in high coffee consumer group >4 cups/day coffee consumption. The results of the studies should be interpreted carefully as some of the studies employed QUS, which is not the gold standard for the assessment of BMD. Also, the coffee intake was assessed with a self-reported food questionnaire in most of the studies. Thus standardization of the cup sizes and brewing types was not considered in most of the studies.
| Study                  | Country        | Sample size   | Sample type         | Design | Follow up time | Coffee dose | Result                                                                 |
|-----------------------|----------------|---------------|---------------------|--------|----------------|-------------|-------------------------------------------------------------------------|
| Hernandez et al., 1991| USA            | 84,484 women  | Middle aged women   | Cohort | 6 years        | Cup/day     | >4 cups/day increase osteoporotic fracture risk                           |
| Hallstrom et al., 2006| Sweeden        | 31,527 women  | 40-76 aged          | Cohort | 10.3 years     | Never to 4 times or more       | Daily intake of 330 mg of caffeine or more (>4 cups/day) is associated with modest increased risk of fracture |
| Jokinen et al., 2010  | Finland        | 1,681 women   | Age 70-73 years     | Cohort | 10 years       | Cups/day    | >5 cups/day coffee consumption is associated with trochanteric fractures |
| Hallstrom et al. 2012 | Sweeden        | 61,433 women  | Middle aged and older | Cohort | 19.4 years     | Cups/day    | Reduction in bone density but no increased fracture risk                 |
| Hansen et al.         | USA            | 34,703 women  | 55-69 years, postmenopausal | Cohort | 6.5 years     | Cups/day    | Modest association between coffee and fracture risk varying by site       |
| Hallstrom et al., 2014| Sweeden        | 42,978 men    | 45-79 years men     | Cohort | 11.2 years     | Cups/day    | High coffee consumption was not associated with increased fracture risk  |
| Albrand et al., 2003  | Europe         | 1,039 women   | 31-89 years         | Cohort | 5.3±1.1 years  | Cups/day    | No association between caffeine consumption and fracture risk            |
| Zhaoli et al.         | China          | 27,959 men, 35,298 women | 45-74 years       | Cohort | 5 years        | Cups/day    | >4 cups/day is associated with increased fracture risk                   |
Randomized controlled studies are required.

**Coffee Consumption and Adrenal Hormones**

Limited resources are available regarding the effects of coffee consumption on adrenal hormone metabolism. A double-blind, randomized, cross-sectional study investigated the effect of caffeine ingestion on plasma renin activity and catecholamines. The results suggested that caffeine ameliorated the plasma renin activity by 57%, plasma norepinephrine by 75%, and plasma epinephrine by 207% (47). Caffeine’s effect on the hypophysis adrenal axis was investigated previously. Caffeine was found to enhance the levels of ACTH (adrenocorticotropic hormone) and cortisol and remained for 3 h after caffeine ingestion. This suggested that caffeine led to a cortisol rise with the action of ACTH. The blockage of adenosine receptors and interference with cyclic adenosine monophosphate were the possible mechanisms behind this action of caffeine (48).

A placebo-controlled study explored the effects of caffeine on heart rate, blood pressure, and urinary excretion of catecholamines at work and home. The results portrayed increased levels of urinary epinephrine in the caffeine group. Blood pressure and heart rate were also elevated in this caffeine group (49).

A more recent study that investigated the acute effects of caffeine on appetite, inflammation, and energy intake, reported no effect on these parameters. However, this study reported high levels of cortisol in the morning in the caffeinated coffee group (50). In another randomized, double-blind, placebo-controlled trial, caffeine was found to have an insignificant effect on DHEA-S, androstenedione levels. Contrary to the previous research, this study failed to display the ameliorating effect of caffeine on cortisol levels (51).

**Conclusion**

Though coffee consumption failed to show promising effects on steroid metabolism, it was associated with elevated urine catecholamine levels.

**Coffee Consumption and Thyroid**

**Q1: Does coffee consumption affect thyroid function in healthy people?**

Limited data indicated that coffee has no effect on thyroid function tests in healthy individuals.

In a prospective study from the Netherlands, 2 gr/day Arabica and Robusta oils were given to 11 healthy volunteers for three weeks and serum total and free thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) remained unaffected in all the study subjects (52).

To date, no study was found in the literature that correlated the effect of coffee consumption on thyrotoxicosis, goiter or hypothyroidism.

**Q2: Does coffee consumption affect levothyroxine treatment?**

Clinical and experimental data indicated that coffee may negatively affect levothyroxine absorption in hypothyroid patients.

In a prospective study in 2008 from Italy, 8 hypothyroid patients and 9 volunteers were given LT4 with only water or coffee, and water one hour after coffee. Italian-style coffee physically interacted with LT4 and reduced the intestinal absorption of LT4 (53).

**Q3: Is there a relation between coffee consumption and thyroid cancer?**

Clinical and epidemiological data provided controversial results regarding the effect of coffee consumption and differentiated thyroid cancer.

A total of 24 studies about thyroid cancer and coffee were found, out of which a total of 10 studies were included after the exclusion of duplications (n=8) and ‘not suitable’ (n=14) articles.

As documented in Table 3, no association between coffee consumption and thyroid cancer could be extracted from the population-based cohort studies (54-56) and meta-analyses of cohort and case-control studies (57,58).

While a few case-control studies reported contradictory results. Two case-control studies detected coffee consumption reduced the risk of thyroid cancer (59,60) and two studies [a case-control (61), a cohort study...
showed drinking only caffeinated coffee had a preventive effect on thyroid cancer. In all these studies coffee intake was assessed by a food-frequency questionnaire. The mechanism behind this effect was postulated to be increasing intracellular adenosine monophosphate levels. The antioxidant nature of this adenosine monophosphate yielded an inhibitory effect on tumor growth.

Only two studies have explored the effect of caffeinated or decaffeinated coffee consumption on thyroid cancer (TC) separately. The first one was a case-control study from Germany and detected that drinking decaffeinated coffee was associated with an increased risk of TC, whereas drinking caffeinated coffee had a preventive effect on thyroid cancer (57,61). The second one was a prospective cohort study from the USA with 167,720 non-white and white participants with 15.3 years follow-up period. This study also observed an inverse association of coffee consumption with thyroid cancer and TC was prevalent among participants who consumed caffeinated coffee, but not in those who consumed decaffeinated coffee. The associations with TC were mostly manifested in whites and it was a dose-dependent relationship (62).

A meta-analysis of 7 seven studies (2 prospective cohorts, 5 case-control studies) also failed to detect any significant association of coffee consumption with thyroid cancer (57). Similar findings were observed in another pooled meta-analysis of 14 case-control studies where no consistent association between coffee intake and thyroid cancer could be derived (58).

### Conclusion

- Results from most of the cohort studies and meta-analyses reported that coffee consumption does not affect the progression of thyroid cancer.
- Only two studies discriminated between caffeinated and decaffeinated coffee and detected that caffeinated coffee had a preventive effect on thyroid cancer (57).

### Coffee Consumption and Gonads

We retrieved 159 studies from a literature search in PubMed and Web of Science. After removing duplicates (n=18) and not suitable (n=109), a total of 32 studies were considered to identify the relationship of coffee consumption with the fertility-fecundability.
Q 1: Does coffee consumption affect female fertility?

Controversial results were reported. Numerous studies recognized a nonsignificant association between coffee or total caffeine consumption and female fertility, as represented in Table 4. Several studies showed coffee or total caffeine consumption led to reduced fertility (63-72). Most of this effect was found to be dose-dependent (63,64,66-68,70-72). Hakim et al. (72) evaluated coffee consumption with a dietary diary, whereas, other studies used a questionnaire. Higher fecundability was reported with moderate caffeine (400-700 mg/day) intake. On the other hand, heavy caffeine intake (>700 mg/day) was inversely proportional to fecundability (73).

Q 2: Does coffee consumption affect male fertility?

A literature survey portrayed a limited number of studies with men. All of these studies documented coffee consumption by interviews. Coffee intake was positively correlated with SHBG (sex hormone-binding globulin) levels in men (74,75). Cross-sectional studies showed caffeine intake was associated with reduced semen volume (76), sperm concentration, total sperm count, and higher (%14) testosterone levels (75,77). A pregnancy follow-up cohort showed higher maternal coffee consumption at the time of pregnancy was associated with diminished semen volume and testosterone levels (77). Inversely, current male caffeine intake was associated with increased (%14) testosterone levels, but with normal semen quality (77).

Two cross-sectional studies found no association between male coffee consumption and semen quality (78,79). Contrary to this, Adelusi et al. (80), Marshburn et al. (81), and Sobreiro et al. (82) found a positive association between coffee consumption and sperm motility. Nonetheless, Parazzini et al. (83) reported coffee intake increased the propensity of poor semen quality.

Few prospective studies also reported that male caffeine consumption was associated with reduced fecundability (70,73). Coffee intake might indulge in DNA breaks and aneuploidy (84). Curtis et al. (65), Wesselink et al. (85) also found a slight decrease in fecundability with coffee consumption. Male caffeine intake had no significant influence on fertilization, pregnancy or live birth delivery (86,87).

Q 3: Does coffee consumption affect gonadal hormone metabolism?

Coffee consumption positively affected SHBG concentrations in women (88,89). Numerous studies indicated conflicting results between free estradiol level and coffee/caffeine intake. Significant associations between caffeine consumption and sex hormone levels in women were detected in some studies (88). Higher caffeine intake (≥200 mg/d), which was evaluated by dietary recalls, was inversely associated with free estradiol concentrations among white women and positively associated among Asian women (90). Other studies estimated higher estrogen levels (88,91) and total testosterone (75) resulting from coffee consumption. Kotsopoulos et al. (89) determined the inverse correlation between increased caffeine intake and luteal free E2 levels. Reduced duration of the menstrual cycle was observed in subjects with a daily caffeine consumption >300 mg (92). On the other hand, androgen or estrogen concentrations remain unaffected as a result of caffeinated beverage consumption (74,93).

Choi et al. (87) determined that higher caffeine intake inversely affected peak E2 in women, undergoing infertility treatment. Elevated or insufficient E2 concentrations can inhibit ovulation. However, altered E2 levels associated with moderate coffee intake failed to modulate ovulatory function (90). Besides, the caffeine levels in serum and follicular fluid were correlated (94). Caffeine levels were inversely related to the number of eggs. However, the association was not established between the success rate of pregnancy and caffeine consumption (94).

Q 4: Does coffee consumption affect menopause time?

A cross-sectional study from Norway with 2123 women found that coffee consumption, as estimated by a questionnaire, remained unassociated with early menopause (95). Kinney et al. (96), Cramer et al. (97), and Nilsson et al. (98) also indicated no association between caffeine and age of menopause.
Contrary to this, another cross-sectional study found that a higher intake of coffee was inversely associated with later menopause after controlling for age, total energy, parity, menarche age, and relative weight (99). A case-control study with postmenopausal women found a positive association between SHBG and caffeinated coffee. However, no relation was observed with caffeinated coffee and sex hormones (93). Similar results were also reported in a cross-sectional study with 2377 women (100).

Nonselective blocking of adenosine receptors by caffeine, chlorogenic acid, and other phytochemicals, resulted in enhanced intracellular concentration of cyclic AMP. As a result of improving liver function by blocking liver adenosine receptors, serum SHBG concentrations may increase.

Caffeine and other bioactive substances in coffee are known to possess a high affinity for the estrogen receptor (101). Besides, both caffeine and estradiol are metabolized by the hepatic enzyme CYP1A2, thus a common metabolism pathway may be responsible for the effect of caffeine on estradiol levels (101).

Q5: What are the effects of coffee consumption on pregnancy?

Caffeine can traverse the placental barrier and therefore, the fetus gets exposed to the same level as that of the mother. This results in an increased level of catecholamines and cyclic adenosine monophosphate that can impact placental blood flow. As a consequence, high dose coffee consumption (≥300 mg/d) is associated with an increased number of spontaneous abortions (94,101,102). Studies have shown that those who consumed more than 300 mg/day caffeine were associated with fetal growth restriction compared to those consuming below 100 mg/day (103). The European Food Safety Authority (EFSA, 2015) and the WHO recommend a daily caffeine intake below 200 mg and 300 mg, respectively.
Conclusion

- Relation of coffee consumption with increased SHBG levels, testosterone levels, estradiol level results were conflicting.
- Fecundability studies were also contradictory. However, higher consumption was related to spontaneous abortus.

Summary

Coffee consumption may exert positive effects on reducing type 2 diabetes risk, may have negative effects on the bone with increased risk of fracture over 4 cups/day of coffee consumption, may enhance abortion risk, and have no effect on thyroid cancer development. There are limited data about the effects of coffee consumption on thyroid and adrenal hormones.

Long term randomized controlled studies are essential to elucidate any possible biological interactions of coffee on the endocrine system.

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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: Dilek Gogas Yavuz; Design: Dilek Gogas Yavuz; Control/Supervision: Dilek Gogas Yavuz; Data Collection and/or Processing: Dilek Gogas Yavuz, Ceyda Dinçer, Tuğçe Apaydin; Analysis and/or Interpretation: Dilek Gogas Yavuz, Ceyda Dinçer, Tuğçe Apaydìn; Literature Review: Dilek Gogas Yavuz, Ceyda Dinçer, Tuğçe Apaydìn; Writing the Article: Ceyda Dinçer, Tuğçe Apaydìn; Critical Review: Ceyda Dinçer, Tuğçe Apaydìn.

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A Rare Combination: Multiple Endocrine Neoplasia Type 1 and Follicular Thyroid Carcinoma

Nadir Bir Kombinasyon: Multipl Endokrin Neoplazi Tip 1 ve Foliküler Tiroid Karsinomu

Ahmet GÖRGEL, Sacit Nuri GÖRGEL*, Mustafa DEMİRPEÇE, Mitat BAHÇECİ

Atatürk Training and Research Hospital, Department of Endocrinology and Metabolism, İzmir, Turkey
*Atatürk Training and Research Hospital, Department of Urology, İzmir, Turkey

Abstract

Multiple endocrine neoplasia Type 1 (MEN-1) is an inherited syndrome characterized by the development of endocrine tumors of the pancreas, parathyroid, and pituitary glands. Mesenchymal tumors and adrenal neoplasms might also accompany this syndrome. However, the syndrome is rarely associated with thyroid tumors in contrary to the multiple endocrine neoplasia Type 2 that includes medullary thyroid carcinoma. This case study presents a 44-year-old woman who was diagnosed with MEN-1 on the basis of her clinical characteristics, laboratory data, and the presence of endocrine tumors. Follicular thyroid carcinoma was detected in the patient when she was being operated for nodular goiter, 12 years ago. We report this rare case which is likely the third case in the available scientific literature.

Keywords: Multiple endocrine neoplasia Type 1; follicular thyroid carcinoma, nephrolithiasis; hypoglycemia; acromegaly

Introduction

Multiple endocrine neoplasia type 1 (MEN-1) is an autosomal-dominant hereditary syndrome associated with pituitary, parathyroid, and enteropancreatic endocrine tumors. Its estimated prevalence ranges from 1 in 10,000 to 100,000 (1). The syndrome arises from mutations of a putative tumor suppressor gene at chromosome 11q13 which encodes a 610-amino acid protein, menin. Although the cellular and biochemical functions of menin are not well-known, loss of heterozygosity of the MEN1 locus appears in MEN1-related tumors (2). Patients with MEN-1 inherit an inactivated copy of MEN-1 in all cells; a second inactivation occurs postnatally in certain cells and neoplasia results from clonal expansion of the cells with dual inactivation (3).

Keywords: Multiple endocrine neoplasia Type 1; follicular thyroid carcinoma, nephrolithiasis; hypoglycemia; acromegaly
It has been reported that MEN-1 is associated with several mesenchymal neoplasms such as facial angiofibroma, skin collagenoma, cutaneous lipoma, and leiomyoma. Patients with MEN-1 less commonly may also present with tumors of other endocrine organs, including thyroid and adrenal glands. Notably, the coincidence of follicular thyroid carcinoma and MEN-1 is very rare. We found only two cases in the literature, the first being the Hurthle-cell thyroid carcinoma and the second being micro-invasive follicular thyroid carcinoma (4,5). Herein, we report a case with the coincidence of MEN-1 and follicular thyroid carcinoma and review the related literature.

Case Report
A 44-year-old female patient with acromegaly and follicular thyroid cancer was referred to our clinic for recurrent hypoglycemia. Hypoglycemic episodes were first detected in the urology clinic. Two months ago, this patient was hospitalized for percutaneous nephrolithotomy operation because of renal stone. The patient was 32 years old when she was diagnosed with acromegaly, and she had been operated for visual complaints due to pituitary macroadenoma. In the following four years, the patient is undergoing conventional radiotherapy and being treated with octreotide until now. She underwent thyroidectomy because of nodular goiter when diagnosed with acromegaly. The pathology of thyroidectomy was reported as follicular thyroid carcinoma. The patient received radioiodine therapy after the surgery, and she is taking levothyroxine, 125 mcg per day. Furthermore, she has a history of recurrent upper gastrointestinal bleeding.

On physical examination, she has an acromegalic face, enlarged hands and feet, a transverse incision scar on the neck, and two brownish skin lesions that were consistent with facial angiofibroma were detected in the nose (Figure 1). Laboratory tests revealed hyperinsulinemic hypoglycemia, elevated levels of gastrin and calcitonin, normocalcemic hyperparathyroidism, and hypogonadotrophic hypogonadism. Additionally, the results of the oral glucose tolerance test revealed that the serum level of growth hormone (GH) was not suppressed (Table 1). On conducting the abdominal computed tomography scan, we detected a 4 cm sized

Table 1. The laboratory findings of the patient.

| Test                | Result  | Reference Range |
|---------------------|---------|-----------------|
| Fasting Glucose     | 37 mg/dL| 70-100          |
| Fasting Insulin     | 42 µIU/mL| 2.4-29.1       |
| Fasting Gastrin     | 677 pg/mL| 25-125          |
| Calcium             | 9.6 mg/dL| 8.4-10.2       |
| Parathyroid Hormone| 247 pg/mL| 12-65           |
| FSH                 | 1.5 mIU/mL| 23-116         |
| LH                  | 0.5 mIU/mL| 16-54          |
| Estradiol           | <11 pg/mL| 0-40            |
| Prolactin           | 26.4 ng/mL| 2.8-29.2       |
| GH*                 | 3.84 ng/mL| 0-2             |
| IGF-1               | 252 ng/mL| 101-267        |
| TSH                 | 0.01 µIU/mL| 0.4-4.5       |
| Free T3             | 2.45 pg/mL| 2.3-4.2        |
| Free T4             | 1.08 ng/dL| 0.89-1.72      |
| Basal Cortisol      | 16.1 µg/dL| 4.6-22.8       |
| ACTH                | 16.2 pg/mL| 7.2-63.3       |
| Calcitonin          | 52.1 pg/mL| <0.5 **        |

ACTH: Adrenocorticotropic Hormone; FSH: Follicle Stimulating Hormone; GH*: Minimum Value of Growth Hormone after Orally Glucose Tolerance Test; IGF-1: Insulin-like Growth Factor-1; LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; ** in athyreoidal individuals.
calcified mass and a 6.5 cm sized solid mass on the body-tail junction of the pancreas and the left adrenal gland, respectively (Figure 2). The overnight dexamethasone suppression test and the measurement of 24-hour urinary fractionated metanephrines and normetanephrines excluded subclinical Cushing’s syndrome and pheochromocytoma, respectively. The patient had neither hypertension nor hypokalemia, which is why plasma renin activity and serum aldosterone concentration were not measured. Thereupon, the adrenal mass was considered non-functional. On conducting neck ultrasonography, a 1 cm sized hypoechoic nodular area consistent with parathyroid adenoma was found on the right inferior lobe of the thyroid. Moreover, the presence of parathyroid adenoma was confirmed by Technetium-99m-MIBI scintigraphy. On the dual-energy x-ray absorptiometry was detected osteoporosis. There was no residual tissue or recurrent mass of adenohypophysis on sellar magnetic resonance imaging. Based on these results, we considered that she had acromegaly, insulinoma, and primary hyperparathyroidism. As a result, the operation was recommended for the pancreatic and parathyroid tumors. Unfortunately, the patient insistently refused both operations despite our strong recommendations. On the other hand, the genetic analysis, which may contribute to the definitive diagnosis of MEN-1, could not be performed for technical reasons.

Discussion
Acromegaly is the pituitary component of MEN-1 in our patient. It is well-known that thyroid tumors can develop by the hyperstimulation of GH in patients with acromegaly. Therefore, the combination of MEN-1 and follicular thyroid carcinoma may not be surprising. Even though a few cases with the coexistence of MEN-1 and papillary thyroid carcinoma have been reported, the occurrence of follicular thyroid carcinoma with MEN-1 is rare (4,5). On the other hand, some of the recent classification systems suggest that follicular thyroid carcinoma should be assumed as a variant of papillary thyroid carcinoma rather than assigning it a distinct clinical entity. Nevertheless, all types of thyroid tumors are not included in the diagnostic criteria of MEN-1, unlike tumors of the pituitary, parathyroid, and pancreas. We aimed to emphasize that the tumor of the thyroid and/or adrenal glands are unusual in the patients with MEN-1. Primary hyperparathyroidism (PHPT) is the most frequent and earliest manifestation of MEN-1; however, in our case, it was demonstrated 12 years after the diagnosis of acromegaly. The presence of nephrolithiasis and osteoporosis was also consistent with PHPT. Moreover, hypogonadism might have also contributed to the development of osteoporosis in the patient. Enteropancreatic tumor component of the syndrome was probably insulinoma in our case even though gastrinomas account for the largest percentage of these tumors in MEN-1. Hyperinsulinemic hypoglycemia, together with a calcified solid mass on the pancreas, was documented. Also, fasting hypergastrinemia was detected in the patient. Hypercalcemia is a potent stimulus for gastrin secretion. Therefore, this can be a probable explanation for the occurrence of hypergastrinemia in a patient with PHPT; however, our patient was normocalcemic. Therewithal, the history of recurrent upper gastrointestinal bleeding led to the speculation of Zollinger-Ellison Syndrome, the eponym for the clinical syndrome, which is characterized by autonomous and excess gastrin production by a gastrinoma. Because of these conditions, we believe that the pancreatic mass secreted not only insulin but also gastrin. This could not be
proven as the patient refused the excision of pancreatic mass despite the fact that the clinical picture has suspected gastrinoma. Although some immunohistochemical studies have shown that the pancreatic endocrine tumors have the potential for multiple hormone production, the clinical manifestations are often related to hypersecretion of only one type of hormone. Several instances of sequential transition from one type of syndrome to another have been reported (6,7). Only one case in the literature has clearly demonstrated two clinically recognizable syndromes (Zollinger-Ellison Syndrome and insulinoma, concurrently), arising from a single pancreatic lesion, but this patient did not meet the criteria for MEN-1 (8).

Pituitary adenomas in MEN-1 may lead to headache and visual-field defects owing to tumor growth. Also, the production of excess hormones can cause various other symptoms. The majority of these lesions are reported to be prolactinomas. The first manifestation of the syndrome in our case was acromegaly; however, we did not find any residual or recurrent mass on sellar magnetic resonance imaging, although non-suppressible GH levels were detected. It might be explained by GH hypersecretion in the patients with MEN-1, which may occasionally be secondary to the ectopic secretion of growth hormone-releasing hormone. The other peptides that may rarely be over-secreted by pancreatico-duodenal neuroendocrine tumors in MEN1 include parathyroid hormone-related peptide, adrenocorticotropic hormone, somatostatin, and calcitonin. The serum levels of calcitonin which are expected to be too low to measure in an athyroidal individual were observed to be high in our case, even though she underwent total thyroidectomy. We assumed that calcitonin was hypersecreted from the pancreatic mass presumably because the patient had no residual thyroid tissue on neck ultrasonography. Cutaneous abnormalities such as angiofibromas, collagenomas, and lipomas are also common in MEN-1 patients. It has been shown that these lesions are also associated with the allelic loss of the MEN-1 gene, suggesting that they are benign neoplasms arising from clonal expansion. Facial angiofibromas were detected in our patient with MEN-1. Over and above, we found an adrenal mass which may be considered as an uncommon component of MEN-1.

MEN-1 may also include uncommon neoplastic components such as adrenal and thyroid tumors. Since the role of menin as a tumor regulator in many organs remains to be established, it is difficult to distinguish between random observations and etiological relationships between MEN-1 cases and atypical tumors. Loss of heterozygosity of the MEN1 locus was examined in previous case series of MEN-1 patients with thyroid carcinoma (9,10); however, their results did not show any loss of heterozygosity and indicated no etiological relationship between the presence of MEN1 mutation and thyroid carcinoma. Therefore, these tumors appear to develop along pathogenetic pathways that are different from classical MEN-1-associated tumors. Nevertheless, further studies and additional case reports are required to clarify the possible mechanisms underlying the development of atypical tumors in MEN-1 patients.

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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: Ahmet Görgel; Design: Mustafa Demirpençe; Control/Supervision: Mitat Bahçeci; Data Collection and/or Processing: Sacit Nuri Görgel; Analysis and/or Interpretation: Sacit Nuri Görgel; Literature Review: Mustafa Demirpençe; Writing
the Article: Ahmet Görgel; Critical Review: Sacit Nuri Görgel; References and Fundings: Mitat Bahçeci; Materials: Ahmet Görgel.

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Coexistence of Primary Mucosa-Associated Lymphoid Tissue Lymphoma of Thyroid and Papillary Thyroid Microcarcinoma in a Background of Hashimoto’s Thyroiditis

Hashimoto Thyroiditis Zemininde Tiroidin Primer Mukoza İlişkili Lenfoması ve Papiller Mikrokarsinom Birlikteliği

Hakan Düğer, Murat Çalışkulu, Bekir Ucan, Erman Çakal, Mustafa Özbek, Demet Yılmazer, Kutsal Doğan, Muhammed Erkam Sencar

University of Health Sciences Düşkapı Yıldırım Beyazıt Training and Research Hospital, Department of Endocrinology and Metabolism, Ankara, Turkey
*University of Health Sciences Düşkapı Yıldırım Beyazıt Training and Research Hospital, Department of Pathology, Ankara, Turkey

Abstract

Papillary thyroid carcinoma (PTC) is the most common endocrine cancer; however, extranodal marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT) of the thyroid gland is quite rare. The comorbid condition of both cancers is an infrequent event. In the present study, we report a 65-year-old woman, who underwent a thyroid ultrasound examination due to palpable thyroid nodules. The reports of the thyroid ultrasound revealed thyroiditis and the presence of multiple nodules on both lobes. Although the results revealed that biopsies were benign, total thyroidectomy was performed due to the presence of multiple nodules that may become difficult for the subsequent follow-up. Histopathological analysis exhibited the presence of papillary thyroid microcarcinomas in two foci, Hashimoto’s thyroiditis accompanied with MALT lymphoma. No metastasis was found in postoperative computed tomography scans. However, bone marrow biopsy indicated uniform marginal zone lymphoma. From the result, it can be concluded that PTC and primary thyroid lymphoma may be associated with Hashimoto’s thyroiditis, and for both the diseases multimodal approach is required.

Keywords: Papillary thyroid carcinoma; lymphoma; Hashimoto’s thyroiditis

Özet

Papiller tiroid karsinomu [papillary thyroid carcinoma (PTC)] en yaygın endokrin kanserdir; bununa birlikte tiroid bezinin mukoza ile ilişkili lenfoid dokusunun [mucosa-associated lymphoid tissue (MALT)] ekstranodal marginal zon lenfoması olduğu nadirdir. Her 2 kanserin eşik eden durumu nadir görülür bir durumdur. Bu çalışmada, ele gelen tiroid nodülleri nedeni ile tiroid ultrasonu yapılan 65 yaşındaki bir kadın oltuyu sundu. Tiroid ultrasonunda tiroidit ve her 2 lobden fazla nodül saptandi. Biyopsi sonuçları benign olmasına rağmen, nodül sayısının çok olması bağlı takip zorluğunu nedeni ile total tiroidektomi yapıldı. Histopatolojik analiz, Hashimoto tiroiditi zemininde MALT lenfomaya eşik eden 2 odakta papiller tiroid mikrokarsinomu varlığı göstergi. Postoperatif bilgisayarlı tomografi taramalarında metastaz saptanmadı. Bununla birlikte, kemik iliği biyopsisinde marjinal zon lenfoma saptandi. Sonuç olarak, PTC ve primer tiroid lenfomasinin Hashimoto tiroiditi ile ilişkili olabileceğinin ve her 2 hastalık için de multimodal yaklaşımın gerekli olduğu sonucuna varilabilir.

Anahtar kelimeler: Papiller tiroid karsinomu; lenfoma; Hashimoto tiroiditi
Introduction
Thyroid cancer is the most common endocrine neoplasia. Between 2004 and 2013, the estimated incidence of thyroid carcinoma increased by an average of 5% annually. However, the incidence rate has been recently stabilized (1). Among all thyroid cancers, papillary thyroid cancer (PTC) accounted for 75-85% of all cases and occurs predominately in women with excellent prognosis (2). On the other hand, primary thyroid lymphoma is a rare condition, where only 2% of extranodal lymphomas develop in the thyroid gland. These lymphomas account for less than 2% of thyroid cancers (3). The co-occurrence of PTC and PTL is a rare condition. The purpose of this case report is to emphasize the incidence of primary thyroid lymphoma in adjunct with thyroid papillary carcinoma, which may have some association in people with a background of Hashimoto’s thyroiditis.

Case Report
In September 2018, a 65-year-old woman underwent a thyroid ultrasound examination due to the presence of palpable nodules in the thyroid gland. Thyroid ultrasonography (USG) of the nodules exhibited being them solid, hypoechoic, having a well-defined margin. The largest of which was 2.5 cm in the right lobe and 2 cm in the left lobe. Fine needle aspiration biopsies revealed benign cytopathology for two nodules. However, the patient was advised to undergo surgery for having a considerable number of nodules, which may cause difficulty in subsequent follow-ups.

The outcomes of the routine laboratory tests were found to be well within the normal range. Estimation of serum hormone revealed the concentration of thyroid-stimulating hormone was 1.80 mU/L (reference range 0.27-4.2 mU/L), free thyroxine (T₄) was 0.85 ng/dL (reference range 0.58-1.6 ng/dL), thyroglobulin antibodies was 133 IU/mL (reference range 0-4 IU/mL) and peroxidase autoantibody was 1.3 IU/mL (reference range 0-9 IU/mL). In November 2018, total thyroidectomy was performed on the patient, keeping the lymph nodes intact. Histopathological analysis revealed the presence of papillary thyroid microcarcinomas in the right lobe was 0.45 cm and in the left lobe was 0.6 cm in diameter. A predominant follicular pattern was observed in the left lobe (Figure 1A) accompanied with extranodal marginal zone lymphoma. The alterations were observed in diffuse large B-cell lymphoma (DLBCL) along with lymphoid cell infiltration, necrosis, and necrobiosis impairing the thyroid parenchyma. Most of the cells are of medium size and large notched cells were detected in layers at several foci. It was observed that the identified cells developed to form follicular epithelium in some foci and further formed lymphoepithelial a lesion or sometimes aggregates in the follicular. The identified cells were mostly CD20 positive with a small number of CD3+ T lymphocytes. Infiltration of CD5, CD23, cyclinD1, and CD43 was absent, whereas the presence of diffuse positive CD10 and bcl-6 was noted. Bcl2 staining in medium-sized cells showed 10% prevalence (Figure 1B), while Ki67 proliferative activity was above 90% (Figure 1C). Lymphoepithelial lesions and lymphoid balls were made observable after staining it with CK7, whereas C-myc was found negative.

No B symptoms related to lymphomas such as fever, weight loss or nocturnal sweating were absent in the patient. The staging procedures that included contrast-enhanced computed tomography of the neck, thorax/mediastinum, and abdomen revealed no evidence of metastasis, but bone marrow biopsy showed consistent marginal zone lymphoma metastasis. No thyroid tissue or pathological lymph node was detected after post-operative third-month thyroid and neck ultrasound.

Discussion
Primary thyroid lymphoma mostly originates from B-cell lineage. Of thyroid lymphomas, 60-80% are Diffuse Large B-Cell Lymphoma (DLBCL) followed by MALT lymphoma which represents nearly 10% to 23% of cases (4). MALT lymphoma is the third most common form of Non-Hodgkin Lymphoma (NHL) and accounts for 5-8% cases after DLBCL and follicular lymphomas (FL) (5). The incidence of the lymphoma is found almost equal in men and women and the average age recorded at the time of diagnosis is generally 60 years. Usually, two-third of the pa-
Patients are diagnosed with stage I-II disease, while only a few have more advanced disease. B symptoms are generally absent and involvement of bone marrow is rare. MALT lymphomas rarely transform into a more aggressive lymphoma and usually turn out to form DLBCL (6). The lack of reliable biomarkers makes it difficult to diagnose histological transformation of the tissue. Transformed lymphomas are more difficult to manage than de novo aggressive lymphomas (7). Extranodal marginal zone lymphoma is commonly found in the gastrointestinal tract but may arise in tissues such as lung, thyroid, ocular adnexa, and breast. In most cases, the disease is localized to a primary site and regional lymph nodes (8).

MALT lymphoma is frequently coupled with autoimmune conditions (e.g., Sjögren syndrome, Hashimoto thyroiditis) or infections (Helicobacter pylori associated gastritis or hepatitis C). The presence of accompanying autoimmune disease causes lymphocytes to infiltrate the thyroid tissue (9,10).

A study published by Ling Chen et al. stated that Hashimoto’s thyroiditis was present in 81.25% of patients, which included all patients with mucosa-associated lymphoid tissue (MALT) lymphoma. The study also stated that the incidence of Hashimoto’s thyroiditis differed significantly among the four lymphoma groups: MALT, DLBL, MALT + DLBL, and small lymphocytic lymphoma (P=0.014) (11).

Although most cases have Hashimoto’s thyroiditis in the background, it has been reported that MALT lymphoma can also develop as de novo without the presence of the former (12).

The first and important step in the diagnosis of thyroid lymphoma depends on the experience of the clinician and his ability to suspect the disease. Most patients with thyroid lymphoma exhibit symptoms such as goiter due to rapid growth of the thyroid gland and causes hoarseness due to compression. However, extranodal marginal zone lymphoma of MALT takes an indolent course. The diagnosis of extranodal marginal zone lymphoma may be delayed due to poor systemic symptoms. The occurrence of B symptoms and cytopenia is very rare in this disease (11,12).

Hematological tests along with few additional tests are required for patients with MALT lymphoma that are standard for patients with NHL required at the time of diagnosis. HCV and HIV testing are recommended for the patients, considering its association with MALT lymphoma. Other recommended tests include assessment of β2-microglobulin, serum protein electrophoresis, immunofixation, and serum-free light chain assay. For detection and staging of the disease, neck, thorax, abdomen, and pelvic CT scans and bone marrow biopsy should be taken into consideration. Since gastric involvement may be present in non-gastric MALT lymphomas, it is recommended to evaluate this aspect as well (13).

Herein, we presented a case with concomitance of thyroid papillary microcarcinoma and MALT in a background of Hashimoto’s thyroiditis. In contrast to published reports, despite transformation to DLBCL, the patient in the present study was asymptomatic and further evaluated only for the presence of a considerable number of thyroid nodules.
After staging workup, bone marrow involvement with marginal zone lymphoma (not DLBCL) was revealed. The prognosis of the extranodal marginal zone lymphoma of MALT localized to the thyroid is excellent and the 5-year disease-specific survival rates for it are more than 95%; however, it is known that patients with extrathyroidal invasion or transformation to high-grade lymphoma have a poor prognosis. In addition, current guidelines do not have the optimal treatment and standardized follow-up protocol (12). As a result, extranodal marginal zone lymphoma of the thyroid gland is rare and its pathogenesis is not fully understood. Despite the rarity of PTL, it can simultaneously exist with PTC, especially in patients with Hashimoto thyroiditis. The treatment for such cases has to be standardized and priority should be given to the stage and condition of the tumor.

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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: Hakan Düğer, Muhammed Erkam Sencar; References and Fundings: Hakan Düğer.

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Granulomatosis Polyangiitis Presented with Diabetes Insipidus
Granülomatöz Polianjitisin Nadir Bir Tutulumu: Diabetes İnsipidus Olgusu

Emine KOCA, Emine Figen TARHAN*, Funda DİNÇ ELİBOL**, Neşe ÇINAR***

Muğla Sıtkı Koçman University Faculty of Medicine, Department of Internal Medicine, Muğla, Turkey
*Muğla Sıtkı Koçman University Faculty of Medicine, Department of Rheumatology, Muğla, Turkey
**Muğla Sıtkı Koçman University Faculty of Medicine, Department of Radiology, Muğla, Turkey
***Muğla Sıtkı Koçman University Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Muğla, Turkey

Abstract
The pathogenesis of granulomatosis polyangiitis (GPA), systemic vasculitis of small and medium-size vessels, is not completely understood. GPA mainly affects the upper and lower respiratory tract. However, it may involve the central nervous system (CNS) as well. The most common manifestation of CNS involvement is necrotizing vasculitis, leading to peripheral neuropathies or cranial nerve palsies. CNS disorder is less common. CNS involvement in GPA can manifest itself in three ways: Vasculitic involvement, granuloma spread from adjacent anatomical areas, and new granuloma formation in brain tissue. We present a case of GPA presented with diabetes insipidus.

Keywords: Diabetes insipidus; pituitary insufficiency; granulomatosis polyangiitis; vasculitis

Our case was presented as a poster at the 19th National Rheumatology Congress, 26-30 September 2019, Bodrum, Muğla, Turkey.

Introduction
Granulomatosis polyangiitis (GPA) is a systemic disease characterized by small vascular vasculitis of unknown etiology. GPA is involved in the class of vasculitis associated with antineutrophil cytoplasmic antibodies (ANCAs). It generally involves the upper and lower respiratory tract, kidneys, ear, nose, and throat. However, GPA may affect any organ or tissue (1).

Diabetes insipidus (DI) is a rare, water homeostasis disorder characterized by abnormal hypotonic urinary excretion. Polyuric symptoms start developing if >90% of the

Address for Correspondence: Emine KOCA, Muğla Sıtkı Koçman University Faculty of Medicine, Department of Internal Medicine, Muğla, Turkey
Phone: +90 252 214 13 23 E-mail: emine_koca@hotmail.com.tr

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vasopressinergic neurons in the supraoptic and paraventricular nuclei of the posterior pituitary get damaged (2).

Pituitary dysfunction is one of the rare findings of GPA. DI is the most common presentation of pituitary involvement in GPA (3). Ahlstrom et al. reported the first case in 1953 (4). Since then, only case reports or small case series have been published (5-10). In this case report, we review pituitary involvement, which is an extremely rare presentation of GPA.

Case Report
In March 2017, a 24-year-old woman was admitted to the Mugla Sitki Kocman University Department of Endocrinology and Metabolism Disorders with complaints of headache, polydipsia, and polyuria for about 3 years. Her vital signs were as follows: temperature 36.7°C, heart rate 90/min, and arterial blood pressure 100/80 mmHg. Her physical examination was normal. Her serum sodium level was 141.9 mmol/L and urine density was 1003 g/L. A water deprivation test was performed and the results were consistent with central DI. Table 1 shows the patient’s levels of the anterior pituitary hormone. Her adrenocorticotropic hormone (ACTH) level was 24.4 pg/mL (7.2-63.3 pg/mL) and cortisol level was 4.49 µg/dL (6.2-19.4 µg/dL). The results of the Synacthen stimulation test showed that cortisol values at 30 and 60 min were 17.27 and 11.94 µg/dL, respectively (<18-20 µg/dL is significant for diagnosis). Sella magnetic resonance imaging (MRI) showed a complex cystic lesion, 13×15×10 mm, with a 7x5 mm cystic part, extending to the suprasellar region. There was no compression of the optic chiasm and a bright spot of the neurohypophysis was not observed (Figure 1, Figure 2). Trans-sphenoidal pituitary surgery was performed and histopathological examination revealed granulomatous necrotizing vasculitis. For the differential diagnosis of granulomatous necrotizing vasculitis; the following tests were performed: C-reactive protein 39.6 mg/L (normal; <5 mg/L) and first hour sedimentation value 26 mm/h (<17 mm/h). Antinuclear antibody, anti dsDNA, anti-centromere antibody, antiJo-1, anti-Scl-70, anti-Sm, anti-SSA and -SSB, rheumatoid factor, antiphospholipid antibodies, anticardiolipin antibodies, and lupus anticoagulant were all negative. Moreover, her angiotensin-converting enzyme level for sarcoidosis was 25.40 U/L, which was in the normal range of 8-52 U/L, and purified protein derivative (PPD) test for tuberculosis was also negative. Her cytoplasmic antineutrophilic cytoplasmic antibodies (c-ANCA) were positive (70.4 and 98.4 U/mL, normal <20 U/mL) and perinuclear antineutrophilic cytoplasmic antibodies (p-ANCA) were neg-

| Blood Test  | Result  | Normal Range   |
|-------------|---------|----------------|
| ACTH (pg/mL)| 24.4    | 7.2-63.3       |
| CORTISOL (µg/dL) | 4.49 | 6.2-19.4       |
| TSH (µIU/mL)    | 1.61    | 0.27-4.2       |
| FT4 (pmol/L)    | 20.87   | 12-22          |
| FT3 (pmol/L)    | 5.44    | 3.1-6.8        |
| FSH (mIU/mL)    | 5.43    | 3.5-12.5       |
| LH (mIU/mL)     | 13.15   | 2.4-12.6       |
| ESTRADIOL (pg/mL)| 125.5 | 12.4-233       |
| PROLACTIN (ng/mL)| 55.63 | 4.79-23.3      |
| GH (ng/mL)      | <0.03   | 0.126-9.88     |

ACTH: Adrenocorticotropic hormone, TSH: Thyroid-stimulating hormone, FT4: Free T4, FT3: Free T3, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, GH: Growth hormone.

Figure 1: T2-weighted coronal image shows a complex cystic mass with a 1.5 cm diameter and a hypointense solid component with a diameter of 7 mm in the sellar cavity. The area marked with an arrow indicates the lesion and the red star shows solid component.
In the paranasal sinus tomography, mucosal thickening in the ethmoid cells in the right maxillary sinus, erosions in the inferior part and the sellar region of the sphenoid sinus, soft tissue formation filling the sphenoid sinus, and focal defect in the central section of the nasal septum were observed (Figure 3, Figure 4). Thoracic tomography showed an asymmetric ice-glass density of 8 mm in diameter in the right upper lobe apical, focal thickening of the left upper lobe posterior fissure, and subpleural nodular density of 2 mm in the posterior lobe of the left lung. The patient was started on 1 mg/kg/day prednisolone and 15 mg/week methotrexate. One month after the beginning of the treatment, there was a considerable improvement in the symptoms of polyuria and polydipsia. Sedimentation value (5 mm/h) and C-reactive protein level (0.4 mg/L) were in the normal range after the treatment.

Discussion
GPA is a rare autoimmune disease characterized by necrotizing granulomatous vasculitis. Although it may frequently involve the upper and lower respiratory tract and kidneys, GPA may affect any other organ or tissue (1). Neurological involvement is seen in one-third of patients with GPA, mainly as a peripheral neuropathy due to small vessel vasculitis, and specifically, CNS involvement is seen in 7-18% of all GPA cases (2). Pituitary involvement in GPA, identified in 1953, is a rare condition and can be seen in about 1% of all GPA cases (3,4). There are some other diseases in the differential diagnosis of the granulomatous pituitary lesions such as idiopathic giant cell granulomatosis, sarcoidosis, Crohn’s disease, tuberculosis, and Takayasu vasculitis (11). The pathophysiology of pituitary involvement in GPA and all related mechanisms have not been accurately described, but three primary reasons seem responsible. The most common reason is the direct intracranial extension of granulomatous process from the nose or paranasal granuloma to the pituitary gland. The second prevailing reason is the vasculitis of pituitary vessels and the third is granuloma development in the pituitary gland (5,12). Symptoms of pituitary insufficiency uncommonly co-occur with GPA but these symptoms are
generally combined with the clinical symptoms, months or years after diagnosis. These symptoms may be nonspecific, such as fatigue, weakness, or headache, and this may lead to a delay in diagnosis (1). Our case, unlike the literature, was diagnosed with GPA with the observation of pituitary insufficiency symptoms. Out of the 23 cases of pituitary involvement in GPA that were reviewed, in 8 cases, clinical features related to pituitary involvement were present at onset and preceded other organ involvement in 3 patients (13). Of these 8 cases, only one had isolated pituitary involvement at presentation, while in the other 7 cases, at least one other organ was involved (13). In one of the studies reviewed, the mean age at the time of diagnosis of pituitary insufficiency was 38 years and 74% of the patients were women (13). In the French Vasculitis Study Group cohort, nine patients with GPA had a mean age of 51 years (24-77 years) at the time of diagnosis, and 5 of these patients were women (1). Kapoor et al. found that the male to female ratio was equal and the average age at the time of diagnosis was 48 years among 8 cases (3). A clinical manifestation usually occurs in the form of DI or isolated anterior pituitary insufficiency (6-10). De Parisot et al. reported that the most common endocrinopathy in GPA is secondary hypogonadism (78%) followed by DI (71%) (1). A decrease in gonadotropin-releasing hormone secretion can be related to other pathophysiological mechanisms such as malnutrition, hyperprolactinemia, acute illness, or use of drugs (1). The mechanisms behind hypogonadism are still unclear. Treatment with glucocorticoids and cyclophosphamide suppresses the hypothalamic-pituitary-gonadal axis (14). Other hormonal pathologies in GPA-associated pituitary dysfunction are not as common as hypogonadism and DI. Some other hormone diseases may be reported as follows: central hypothyroidism in 54% of patients, secondary adrenal insufficiency in 39%, hyperprolactinemia in 37%, and growth hormone deficiency in 20% (1). The most common finding in MRI is pituitary gland enlargement. Other findings seen in MRI include cystic changes, infundibular thickening, and increased contrast enhancement. Another significant finding is the lack of characteristic hyperintense signal in T1 imaging (13). In our case, there was a 13×15×10 mm noncontrast lesion in the sellar cavity with a 7×5 mm complicated cystic nodule in the cranial part of the lesion. The remission induction therapy in GPA involves high-dose glucocorticoids combined with oral or intravenous cyclophosphamide (3). In the literature review of De Parisot et al., 69% of the patients were treated with conventional treatment and after five years of follow-up and 11% had a recurrent systemic disease (1). Induction therapy without using cyclophosphamide is related to recurrence in 50% of patients (3). Patients who are resistant to conventional treatment can be effectively treated with rituximab (15). GPA patients treated with rituximab achieve complete remission more frequently than those treated with cyclophosphamide but the experience of treatment with rituximab in GPA is limited (16). The follow-up of patients should include the evaluation of pituitary imaging and pituitary deficiency. In spite of the remission of systemic disease and the regression of radiological findings, the regeneration capacity of the pituitary function is limited because of the irreversible damage caused by necrotizing granulomatous lesions (1,3,13). GPA is a vasculitic disease that may cause multiple organ dysfunction. Pituitary involvement is rarely seen in GPA. In the case of anterior or posterior pituitary insufficiency in a patient with a histopathological diagnosis of granulomatous necrotizing vasculitis of the pituitary masses, pituitary GPA should be considered in the differential diagnosis of the disorder.
entific 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: Emine Koca, Emine Figen Tarhan, Neşe Çınar; Design: Emine Koca, Emine Figen Tarhan, Neşe Çınar; Control/Supervision: Emine Koca, Emine Figen Tarhan, Neşe Çınar; Data Collection and/or Processing: Emine Koca, Emine Figen Tarhan, Neşe Çınar, Funda Dinç Elibol; Analysis and/or Interpretation: Emine Koca, Emine Figen Tarhan, Neşe Çınar; Literature Review: Emine Koca, Emine Figen Tarhan, Neşe Çınar; Critical Review: Emine Koca, Neşe Çınar; References and Fundings: Emine Koca, Emine Figen Tarhan, Neşe Çınar.

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Steroid Secreting Dedifferentiated Liposarcoma: A Unique Presentation

Steroid Salgılayan Dediferansiye Liposarkom: Özgün bir Sunum

Lucas Ribeiro dos Santos, Márcio Luis Duarte*, José Viana Lima Júnior**

Clinic of Endocrinology and Metabolism at Medical Sciences Faculty of Santos, São Paulo, Brazil
*Radiologist of Webimagem. Avenida Marquês de São Vicente, 446, São Paulo, Brazil
**Clinic of Endocrinology and Metabolism at Medical Sciences Faculty of Santa Casa de Misericórdia de São Paulo, São Paulo, Brazil

Abstract

Adrenal tumors are cancerous or non-cancerous growths on the adrenal glands. It can originate from the cortex or the medulla, and may or may not have the capacity to secrete hormones. We report a case of a 76-year-old man who was diagnosed with an adrenal mass during abdominal pain investigation. His biochemical profile and magnetic resonance images are compatible with adrenocortical carcinoma, while the pathology reports are compatible with liposarcoma. So far, to the best of our knowledge, there is no report on steroid-secreting tumors.

Keywords: Adrenal cancer; dedifferentiated liposarcoma; Cushing syndrome

Özet

Adrenal tümörler, adrenal bezlerdeki kanseröz veya non-kanseröz büyümelerdir. Korteks veya medulla kaynaklıları ve hormon salgılama kapasitesine sahip olabilir veya olmayabilirler. Burada, kann ağrısı muayenesi sırasında adrenal kitle tanısı konan 76 yaşındaki bir erkek olguyu sunduk. Olgunun biyokimyasal profili ve manyetik rezonans görüntüleri adrenokortikal karsinom ile patoloji raporları liposarkom ile uyumlu idi. Bildiğimiz kadarla, şimdiye kadar steroid salgılayan tümörler hakkında bir olgu sunumu bulunmamaktadır.

Anahtar kelimeler: Adrenal kanser; dediferansiye liposarkom; Cushing sendromu

Introduction

Although adrenal tumors are a common condition, affecting up to 10% of the general population, most are benign adenomas, discovered incidentally on abdominal imaging (1). The malignant form, called adrenocortical carcinoma (ACC) is a rare condition with an incidence of 0.72 cases per million people according to the Surveillance, Epidemiology, and End Results (SEER) database (2). ACC seems to affect persons between the fourth and sixth decades of life (3,4). The clinical presentation differs according to tumor size and the ability to overproduce steroids, which occurs in about 50% to 80% of the patients (3,5); one-third of the patients may present with local symptoms of mass growth, such as flank pain (3), and in 30% of the cases, the diagnosis occurs through an incidental finding of imaging diagnosis techniques (6).

After evaluation of the hormonal secretion profile, the confirmation of the diagnosis is made through histopathology based on the Weiss score (7).

Herein, we report a case that presented itself as an ACC but had unique histopathology of a liposarcoma, which is an extremely rare form of an adrenal tumor and was never described as a steroid secreting tumor. Informed consent was obtained from the patient.
**Case Report**

A 76-year-old male patient with a history of arterial hypertension and diabetes mellitus diagnosed about six months ago was referred to the endocrinology service center of Santa Casa de Misericórdia de São Paulo after a left adrenal mass was encountered on abdominal ultrasound, performed during the investigation of left flank pain. The patient had no typical features on physical examination.

Abdomen magnetic resonance imaging was performed with adrenal MRI protocol that showed a mass of 10.2×8.0×7.9 cm, irregular, with foci of necrosis, compromising pancreas, left adrenal and left kidney, and also a nodule on the lung base (Figure 1), which was better evaluated by CT scanning that showed sparse nodules bilaterally, with nonspecific characteristics.

Evaluation of hormonal production (Table 1) led us to the diagnosis of cortisol and androgens oversecretion.

The patient underwent left adrenalectomy plus distal pancreatectomy and left nephrectomy, and was later taken to standard postoperative care of Cushing’s syndrome. After the surgery, the patient had normal blood pressure and glucose levels even after the withdrawal of antihypertensive and oral antidiabetic drugs.

The postoperative hormone profile showed lower levels of cortisol and androgens (Table 1), reinforcing our diagnosis of steroid overproduction. It was not possible to wean from prednisone at a dose of 5 mg per day due to postural hypotension.

The anatomopathological analysis of the specimen showed fusocellular neoplasia, infiltrating the kidney, body, and tail of the pancreas and left adrenal gland, but an adrenal of normal characteristics; immunohistochemistry was positive for vimentin in diffuse pattern, cd34, s100 and cd68 protein in focal pattern, but negative for smooth muscle actin, myogenin, AE1/AE3, desmin, melan A, inhibin and chromogranin A, with Ki67 30%, resulting in analysis compatible with dedifferentiated liposarcoma. This anatomopathological analysis was re-evaluated by another pathologist, who maintained the diagnosis.

During follow-up visits, thorax tomography showed an increase in the number and volume of nodules, with metastatic character.

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**Table 1. Hormonal secretion profile.**

| Hormone | Pre operative | Post operative | Normal Range |
|---------|--------------|----------------|--------------|
| Basal Cortisol | 23.7 ug/dL | *3 | 3–18 ug/dL |
| Cortisol-Liddle 1 test | 2.4 ug/dL | *3 | < 1.8 ug/dL |
| ACTH1 | 8.6 pg/mL | - | 5–65 pg/mL |
| DHEA-S | 994 ng/dL | 243 ng/dL | 120–870 ng/dL |
| 17-OH-Progesterone | 3.4 ng/mL | 2.5 ng/mL | 0.5–2 ng/mL |
| Androstenedione | 6.10 ng/mL | 0.60 ng/mL | 0.6–3.1 ng/mL |
| Total Testosterone | 599 ng/dL | 329 ng/dL | 250–1000 ng/dL |
| Plasma metanephrines | 12 pg/mL | - | < 65 pg/mL |
| Plasma Normetanephrines | 85 pg/mL | - | < 196 pg/mL |

1. Adrenocorticotropic hormone; 2. Dehydroepiandrosterone-sulfate; 3. Not performed due to inability to wean from prednisone; 4. Aldosterone levels were not measured for technical reasons.

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istics. The patient was then referred to the oncology department where he is currently under radiotherapy.

Discussion
We presented a clinical case of a patient with imaging and hormonal secretion pattern compatible with adrenocortical carcinoma, but with discordant anatomopathological analysis. What seems to be the most interesting about this patient was his hormonal profile, indicating primary hypercortisolism and adrenal androgen excess; unfortunately, we were unable to evaluate the co-secretion of aldosterone due to logistic issues. The clinical evolution in the postoperative period, with hormonal re-evaluation demonstrating lower levels of testosterone and DHEA-S, correction of systemic arterial hypertension and the impossibility to wean from prednisone led us to believe that the sarcoma was indeed producing these steroids.

After performing a literature review in the Lilacs and Medline databases, we found no reports on steroid-secreting sarcomas. There are some reports of sarcomas with peptide hormone secretion, such as ACTH and chorionic gonadotropin (8-10). There are some reports on myxoid ACCs, which are a rare subtype, presenting degeneration of the tumor or myxoid material produced by stromal fibroblasts (11); within this subtype of ACC, there are reports on lipomatous metaplasia. The lipomatous metaplasia of the ACC is still a matter of debate but could correspond to a reactive process in response to degeneration or a metaplastic process of the neoplastic cells (12).

Despite this different histological features, lipomatous metaplasia in myxoid ACCs still holds an identical immunohistochemical phenotype to conventional ACC-positive for inhibin, melan A, synaptophysin, and vimentin, with the variable reaction for cytokeratin (11), distinguishing it from our report, that was positive only for vimentin (which can occur in sarcomas) (13).

Primary liposarcomas of the adrenal gland are extremely rare, with only two reports in the literature, and it is extremely hard to differentiate it from retroperitoneal liposarcomas (14), which do not secrete steroids. Radiological differentiation between liposarcomas and ACC can be challenging. On computed tomography, it usually presents negative Hounsfield unit (HU) values due to the high presence of fat (varying from -60 HU to +5 HU), contrasting with a usually high HU values obtained from ACC (above 20 HU); however, on MRI images, both can present high signal on T2-weighted sequences (15). Based on imaging techniques, the presumptive diagnosis of ACC will prevail, and differentiation will appear upon histopathological and immunohistochemistry analysis.

To the best of our knowledge, this is the first report on the clinical presentation of liposarcoma.

Ethics
In compliance with ethics guidelines, informed consent was obtained from the patient included in the study.

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: José Viana Lima Júnior; Design: José Viana Lima Júnior; Control/Supervision: José Viana Lima Júnior; Data Collection and/or Processing: Lucas Ribeiro dos Santos; Analysis and/or Interpretation: Lucas Ribeiro dos Santos; Literature Review: Lucas Ribeiro dos Santos; Writing the Article: Márcio Luis Duarte; Critical Review: Márcio Luis Duarte; References and Fundings: José Viana Lima Júnior.
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Introduction

Due to their inhibitory effect on proliferation, motility, apoptosis, and metastasis, the epidermal growth factor receptor (EGFR) inhibitor erlotinib and vascular endothelial growth factor receptor (VEGF) and EGFR inhibitor vandetanib are viable therapeutic options for the treatment of advanced non-small cell lung carcinoma (NSCLC) and thyroid cancer, respectively (1). NSCLCs and medullary or differentiated thyroid carcinomas (MTC and DTC) may show bone metastasis along with bone pain, elevated serum calcium, or elevated alkaline phosphatase levels. We present the interesting cases of two patients who were diagnosed with NSCLC and MTC with bone metastasis. Both were treated with EGFR inhibitors and presented at our outpatient clinic with hypocalcemia as a result of hypoparathyroidism.

Case Reports

Case 1

A 48-year-old Caucasian woman was admitted to our outpatient clinic with asymptomatic hypocalcemia. She described her history of total thyroidectomy due to micro-papillary thyroid carcinoma (PTC) five years ago, and was not treated with radioactive iodine ablation. She was indicated to be in remission for PTC and was receiving thyroid hormone suppression therapy.
Two years ago, she was diagnosed with metastatic (bone) lung adenocarcinoma and was receiving erlotinib treatment for the past six months. She was referred from the oncology department due to hypocalcemia. She had a body mass index (BMI) of 33.6 kg/m². Physical examination on admission was unremarkable, except for her obesity and a positive Chvostek sign. Biochemical analysis showed severe hypocalcemia with a corrected calcium level of 7 mg/dL (normal range 8.2-10.2). Other laboratory findings associated with hypocalcemia, such as the levels of phosphorus [4 mg/dL (2.5-4.5)], 25 (OH)-vitamin D [28 ng/mL (20-50)], and magnesium [2 mg/dL (1.8-2.6)], were within the normal reference ranges, and although the parathormone levels (PTH) were within the normal reference range despite hypocalcemia [intact PTH 35 ng/mL (12-65)], they were considered to be inappropriately low for the observed calcium level.

Hypocalcemia was initially thought to be associated with her neck surgery, but after evaluation of her calcium levels after the surgery, it was observed that the calcium levels declined after initiation of erlotinib therapy for NSCLC with osteolytic bone metastasis. Her historical calcium levels ranged in the upper normal reference range due to bone metastasis (8.9-9.8 mg/dL) before initiation of erlotinib therapy, and remained so until the completion of four months of erlotinib therapy. For the last two months, her calcium levels were observed to be within the medium and lower normal of the reference range. There was no information about her PTH levels before admission to the endocrinology department. The patient probably did not receive bisphosphonate therapy for bone metastasis, which could also result in hypocalcemia. Denosumab was given for bone metastasis instead of bisphosphonates. Calcium and calcitriol replacement therapy was initiated for hypocalcemia and normal calcium levels were achieved. With the continuation of erlotinib therapy, bone metastasis neither progressed nor resolved, and these replacement therapies were synchronously given because of existing hypoparathyroidism and hypocalcemia. She is still on 150 mg/day of erlotinib therapy.

**Case 2**

A 56-year-old man was diagnosed with MTC ten years ago and had neck surgery including total thyroidectomy and dissection of the central lymph node. Four years ago, the disease progressed and extensive metastases occurred in the lung, liver, and bone. After a couple of months of therapy with vandetanib, a multi-kinase inhibitor targeting EGFR, RET, and VEGFR, the biochemical analysis revealed extremely low calcium levels [corrected calcium 4.9 mg/dL (8.2-10.2)]. The patient showed severe muscle cramps in his hands, with slight numbness and tingling in the perioral area.

The levels of magnesium (2.2 mg/dL) and phosphorus (4.2 mg/dL) were within the normal reference range, while level of 25(OH) vitamin D [5 ng/mL (20-50)] was low, and that of PTH [34 ng/mL (12-65)] was within the normal range despite severe hypocalcemia. Like the first case, we observed hypoparathyroidism after the initiation of vandetanib (300 mg once daily) treatment. His calcium levels were within the normal reference range (9-9.5 mg/dL), and his previous PTH levels were unknown. Calcium supplements and calcitriol treatment were initiated to maintain a normal calcium level. In addition to vandetanib therapy, he also received denosumab therapy (120 mg subcutaneously once every four weeks) for bone metastasis. He died after eight months of therapy, because of septicemia due to pneumonia.

**Discussion**

Targeted therapies with EGFR have become a cornerstone in the treatment of several metastatic cancers. A majority of patients show general side effects of EGFR, although endocrine-related adverse effects are comparatively rare. Endocrine-related adverse effects of these agents include thyroid dysfunction, gonadal dysfunction, adrenal dysfunction, or disorders of glucose metabolism. However, scarce information is available on parathyroid dysfunction. Although recent reports have described secondary hyperparathyroidism in patients taking kinase inhibitors, side effects such as hypoparathyroidism and hypocalcemia were also reported.
In fact, PTH receptor 1 and the calcium-sensing receptors are the members of the G protein-coupled receptor (GPCR) superfamily and do have a direct relation with tyrosine kinases. It is hypothesized that there may be a cross-talk between certain receptor tyrosine kinases and GPCRs (2). Parathyroid cells express EGFR, which then mediates the stimulation of parathyroid cellular proliferation. Erlotinib was found to inhibit parathyroid growth and decrease the levels of proliferating cell nuclear antigen (PCNA) and TGF-α (3). Apart from changes in cellular proliferation, apoptosis may also play a role in parathyroid-related side effects.

It was shown that the activation of EGFR promotes parathyroid hyperplasia and enhances the parathyroid levels in rats with kidney disease. Therefore, the inhibition of EGFR with erlotinib was observed to prevent the growth of parathyroid cells, self-promotion of the Transforming Growth Factor-α (TGFα), and reduction of the 25 (OH) vitamin D receptor (4,5). EGFR inhibitors can cause hypomagnesemia accompanied by wasting of renal magnesium (Mg), due to the inhibition of transient receptor potential melastatin 6 protein channel (TRPM6), which is responsible for Mg handling (6). However, we did not observe any episodes of hypomagnesemia in our patients.

An association was observed between parathyroid hormone-related protein (PTHrP) gene and EGFR signaling (1). The treatment with EGFR inhibitors has been demonstrated to decrease the production and gene expression of PTHrP (40-80%), as well as the total plasma calcium concentrations in hypercalcemic mice (7).

A sudden decline in calcium levels after initiating EGFR inhibitors, in contrast to the evidence of bone metastasis in these cases, indicated that these agents may have prevented parathyroid cell growth, and the resulting decreased PTH levels led to hypocalcemia.

In order to the best of our knowledge, although several reports have demonstrated the hypocalcemic effects of erlotinib monotherapy when compared to erlotinib and emibetuzumab combination therapy (8), and vandetanib therapy when compared to placebo (9), none have described the probable and exact etiology of hypocalcemia. The cases described in our study are the first in the literature to demonstrate the occurrence of hypocalcemia due to hypoparathyroidism from the use of erlotinib and vandetanib, despite both the patients having widespread bone metastasis. Although a history of neck surgery may be a reason for hypoparathyroidism in these cases, the possible effects of these agents on parathyroid glands should not be underestimated. A prospective controlled study may prove our hypothesis. Until then, hypocalcemia should be considered as a possible side effect of EGFR inhibitors, especially in patients with a history of neck surgeries.

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Conflict of Interest
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Authorship Contributions
Idea/Concept: Asena Gökçay Canpolat; Design: Asena Gökçay Canpolat; Control/Supervision: Sevim Güllü, Murat Faik Erdoğan; Data Collection and/or Processing: Asena Gökçay Canpolat, Şule Canlar, Berna İmge Aydoğan; Analysis and/or Interpretation: Asena Gökçay Canpolat; Literature Review: Asena Gökçay Canpolat; Writing the Article: Asena Gökçay Canpolat; Critical Review: Murat Faik Erdoğan; References and Fundings: Asena Gökçay Canpolat.
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