The Turkish Journal of Endocrinology and Metabolism is published 4 (March, June, September and December) times a year. Local period publication.

The Turkish Journal of Endocrinology and Metabolism is indexed in Emerging Sources of Citation Index (ESCI), British Library, CINAHL, Directory of Open Access Journals (DOAJ), EBSCO, EMBASE, SCOPUS, Tübitak / Ulakbim TR Index, TürkMedline, Türkiye Citation Index.

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Publishing House: Türkiye Klinikleri
Address: Nasuh Akar Mah. Türkocağı Cad. No:30 Balgat - Ankara Turkey
Telephone: +90 312 286 56 56
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Publication Date: 11.04.2018

ISSN: 1301-2193
E-ISSN: 1308-9846
AIMS AND SCOPE

The Turkish Journal of Endocrinology and Metabolism is the peer-reviewed periodical on clinical and experimental endocrinology and metabolism diseases and related fields. It is the official journal of the Society of Endocrinology and Metabolism of Turkey and is published quarterly (March, June, September and December) as hardcopy and an electronic journal at www.turkjem.org. The manuscripts are published in English language. The journal publishes original research papers, reviews and case reports which primarily focus on clinical endocrinology. The journal's aim is to be the essential reading for both endocrinologists and clinical practitioners.

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EDITORIAL

Dear esteemed readers of TurkJEM Family,

Industrialization and mass production globally is believed to be causing climate change with drastic temperature changes in the northern hemisphere; while very cold weather in Germany and UK but mild winter in Balkans. In this letter a focus in an area which endocrinology and metabolism discipline’s importance from a very different perspective. Study conducted in UK by Amanda Hughes and Meena Kumarand focuses on the unemployment, underweight, and obesity interaction. Statistically significant results for elevated morbidity and mortality among jobseekers may be partly explained by adiposity. Research done in 2016 asks the question whether unemployment is associated with being overweight and obesity. They used unemployment and body mass index (BMI) data for different groups to justify their hypothesis. In short they concluded that unemployment is associated with underweight and in nonsmokers, obesity. Unemployment was positively associated with underweight and negatively associated with overweight, with effects more apparent for longer-term jobseekers, men, and jobseekers from lower-income households.

In a world of want where 1.4 billion people are struggling to survive on $1.25 a day, Hedwig Lee asks the question how poverty leads to obesity and life-long problem. All these findings show that we are in vicious circle of poverty triggering obesity, and obesity and overweight positively associated by unemployment which further deteriorates the income level. Treatment of obesity and related metabolic diseases has direct affects as well as social and economic consequences where a holistic as well as differentiated approach is lacking. Global academia’s focus on humanity and its wellbeing should and will focus on these primary, secondary and tertiary relations in time.

With such focus in mind for this issue of TJEM we have the following researches as contributions: “Serum Heart Type Fatty Acid Binding Protein Levels in Primary Hyperparathyroidism”, “Clinical Value of Histogram Analysis Using Gray-Scale Ultrasound Images in Thyroiditis”, “Cytologic Comparison Between Growing and Non-growing Benign Thyroid Nodules Evaluated Using Two Different Growth Criteria”, “Ratio of Thyrotropin to Thyroglobulin as a Novel Marker for Differentiating Between Benign and Malignant Thyroid Nodules within Different Bethesda Categories”, “Implication of Findings from International Studies on Hypoglycemia for Management of Diabetes in Insulin-treated Patients in Turkey”, “A Different Cause of Malignant Hypercalcemia in a Breast Carcinoma with Bone Metastasis”, “Two Siblings with Triple-A Syndrome: Endocrinologic and Neurologic Features”, “Pregnancy-Associated Osteoporosis: Long-term Follow-up of a Patient with Two Pregnancies” and “Review of Clinical Recommendations on Prolactinoma and Pregnancy”.

Spring is not too far; let’s keep our hopes for more rigorous research for overcoming obesity, diabetes and metabolic diseases. I wish you all a happy new season and a good reading.

With my best regards,

Nilgün Başkal MD
Editor-in-Chief
Serum Heart Type Fatty Acid Binding Protein Levels in Primary Hyperparathyroidism
Primer Hiperparatiroidizmde Serum Kalp Tipi Yaş Asidi Bağlayıcı Protein Düzeyleri

Bekir Uçan*, Mustafa Şahin*, Mustafa Özbek, Mustafa Çalışkan, Muhammed Kızılöğüllü, Gülfer Öztürk**, Mehter Akif Öztürk***, Erman Çakal

Purpose: Recent studies indicate that plasma heart-type fatty acid binding-protein (H-FABP) concentration can be used as an early biochemical marker for macrovascular diseases. Patients with primary hyperparathyroidism (PHT) reportedly display an increase in cardiovascular (CV) risk factors. Our aim was to evaluate plasma H-FABP concentration in these subjects with primary hyperparathyroidism, by comparing them with healthy controls.

Material and Method: Anthropometric parameters, serum H-FABP, serum lipid, serum calcium, phosphorus, parathormone (PTH), insulin resistance (HOMA-IR), high sensitive C-reactive protein (h-CRP), 24-hour urine microalbumin excretion and carotid intima-media thickness (CIMT) were evaluated among primary hyperparathyroidism patients (8 males, 80 females) and 87 healthy subjects (12 males, 75 females).

Results: No significant difference was seen in the levels of H-FABP between patients [1086.07 (298–3744)] and controls [1113.36 (263.27–3510)]. The average values of PTH, HOMA-IR, total cholesterol, LDL cholesterol, triglycerides and calcium and mean CIMT were found to be significantly higher in patients with primary hyperparathyroidism (p<0.05). H-FABP was positively correlated with age, fasting blood glucose, BMI, and HsCRP.

Discussion: H-FABP levels correlated with some of the CV risk factors like age, fasting blood glucose, BMI, and h-CRP, moreover no difference in H-FABP levels was seen among patients of primary hyperparathyroidism having no cardiac disease.

Keywords: Hyperparathyroidism; H-FABP; cardiovascular risk

Anahtar kelimeler: Hiperparatiroidizm; H-FABP; kardiyovasküler risk

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Turkish Journal of Endocrinology and Metabolism published by Türkiye Klinikleri

DOI: 10.25179/tjem.2017-57329
Introduction

Primary hyperparathyroidism (PHT) is one of the most common endocrine disorders, resulting most commonly from parathyroid adenomas. Multiple observational studies indicate that primary hyperparathyroidism might be associated with cardiovascular diseases (1). Hypertension is commonly reported among patients with mild PHT (2–4), while other observational studies also suggest a correlation between left ventricular hypertrophy/diastolic dysfunction and PHT (5,6).

The mean carotid intima-media thickness (CIMT) is significantly higher in patients with PHPT and significantly higher levels of parathormone (PTH) are associated with the thickness of aortic and carotid artery (7,8). These results suggested that arteriosclerosis might be associated with the severity of hyperparathyroidism.

Heart-type fatty acid binding-protein (H-FABP) is a low molecular weight, cytoplasmic and non-enzymatic protein that provides the transport of long-chain fatty acids to cardiomyocytes (9). We have previously demonstrated the presence of elevated levels of H-FABP in various endocrinological and metabolic disorders including acromegaly, prediabetes and metabolic syndrome (10–12). However, these levels were not affected in hyperprolactinemia and hyperthyroidism (13,14).

The kinetics and release of this protein are similar to that of myoglobin, but unlike the latter, it is available at a higher concentration in the heart than the skeletal muscle. Thus making H-FABP more specific than myoglobin for heart tissue (15). Various studies have indicated that H-FABP is closely related to the cardiovascular risk factors, and is an independent risk factor for cardiovascular mortality (16,17). H-FABP is an excellent early marker for cardiac injury in acute coronary syndromes, providing early diagnosis of minor myocardial injury in heart failure and unstable angina (18–20).

Carotid intima-media thickness is an important marker of early changes in the atherosclerotic process and an indicator for the development of cardiovascular events. The most critical changes seen during the early, subclinical stage of atherosclerotic disease are endothelial dysfunction in the entire arterial system and increased intima-media thickness (IMC). The carotid intima-media thickness is frequently used as a strong predictor of cardiovascular events (myocardial infarction, stroke, and transient ischemic attack) (24).

The purpose of this study is to determine the level of H-FABP in primary hyperparathyroidism and also to evaluate the possible relationship of plasma H-FABP levels with CIMT, serum lipid, HOMA-IR, HsCRP, and microalbuminuria.

Material and Methods

About 88 patients with primary hyperparathyroidism and 87 control subjects in the study were enrolled in this study. Ethical committee approval and written informed consent of participants were obtained before the commencement of the study. After overnight fasting, blood samples were collected from all of the subjects to check the levels of parathormone (PTH), glucose, insulin, free T4, thyroid-stimulating hormone (TSH), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, total cholesterol, high sensitive C-reactive protein (h-CRP), and H-FABP. Excretion of 24-hour urine microalbumin was assessed in the patient and control group. Weight, height, and waist and hip circumference were measured and the BMI and homeostasis model assessment of insulin resistance (HOMA-IR) were also calculated for both the groups. Additionally, all the patients underwent high-resolution B-mode ultrasonography to measure the carotid artery intima-media thickness (CIMT), wherein the same investigator carried out all the scans and image measurements. Furthermore, all the study participants were interviewed using a standard questionnaire that included information about their demographic characteristics, concomitant disease, use of medications that could affect H-FABP levels and smoking history. Afterward, the patients underwent a physical examination, and those with a history of the acute coronary syndrome (ACS), pulmonary embolism, stroke and heart failure or with immunological or renal diseases were excluded from the study. A control group, comprising of volunteers who had no history of ACS, heart failure, cardiomyopathy, pulmonary embolism, renal diseases, immunological diseases and diabetes mellitus, was also used in this study.

Heart-type fatty acid-binding protein

The H-FABP measurements were performed with the Epoch micro-volume spectrophotometer system (BioTek, Inc., Winooski, VT, USA) using a commercially available enzyme-linked immunosorbent assay ELISA kit (Hycult Biotech,
Uden, The Netherlands). The assay range of the H-FABP ELISA kit was 102 to 25000 pg/mL and the measurements were calculated simultaneously during the same experiment. The ready-to-use solid-phase human H-FABP ELISA is based on the sandwich principle. Samples and standards were incubated together with a peroxidase-conjugated secondary antibody in microtiter wells coated with a primary antibody that recognizes human H-FABP. During incubation, the solid bound primary antibody captures the human H-FABP and the secondary antibody then binds to the captured human H-FABP, following which, the peroxidase-conjugated antibody reacts with the substrate tetramethylbenzidine (TMB; this reaction was stopped by the addition of oxalic acid). The absorbance at 450 nm was measured with a spectrophotometer.

Statistical Analysis

The statistical analysis was performed using SPSS 11.5 (SPSS, Inc) software. The presentation of the variables that were normally distributed was as mean ± standard deviation (SD) and of those that were non normally distributed was as median (min-max). Categorical variables are presented as case number and percentage (%). Student’s t-test was used to analyze normally distributed continuous variables. The significance of the difference between medians was compared by the Mann-Whitney U test and categorical variables were compared using the Pearson’s chi-square or Fisher’s exact test. Correlations were analyzed using Pearson’s and Spearman’s correlation and a p-value of <0.05 was considered to be statistically Significant for all analyses.

Results

Mean age (49.01±13.08 to 46.06±10.93, p=0.111), sex distribution and BMI were found to be similar between groups (Table 1). The prevalence of hypertension (HT) was significantly higher in the patients as compared to healthy individuals (18% to 0%, p=0.004), but the frequency of smoking was significantly lower in the patients (11% to 20%, p<0.0001). Additionally, no significant dif-

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* Values are median (minimum-maximum).
Abbreviations: BMI: Body mass index; TSH: Thyroid-stimulating hormone; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; H-FABP: Heart-type fatty acid-binding protein; CIMT: Carotid intima-media thickness, HsCRP: High sensitive C-reactive protein.
ference was observed between the patients and controls with respect to the prevalence of diabetes mellitus (DM) (4% to 0%, p=0.351). Coronary artery disease (CAD) was not seen in the patients enrolled in the study and there was no significant difference in average H-FABP value between the patients with or without HT ((1126 (819) to 1059 (956), p=0.218). The average values of PTH, HOMA-IR, total cholesterol, LDL cholesterol, triglycerides, and calcium levels and mean CIMT were significantly higher in patients with primary hyperparathyroidism (p<0.05) (Table 1). Some patients from the control group had secondary hyperparathyroidism due to vitamin D deficiency. No significant change was identified between these two groups with respect to other values (Table 1) and no significant difference was found in H-FABP levels between patients [1078.13 (298-3744)] and controls [1049.91 (263.27-4403.02)] (Figure 1).

There was a positive correlation between age, fasting blood glucose, BMI, hsCRP levels and H-FABP levels (Table 2).

Discussion

Hyperparathyroidism is known to be associated with cardiovascular mortality and morbidity and with cardiomyopathy, arrhythmia, myocardial hypertrophy, as well as atherosclerosis, and valve and cardiac calcification (8,25,26). In vivo studies have demonstrated the vascular remodeling and vascular calcification effects of the parathormone through atherogenesis, as well as its pro-sclerotic effects on the vascular smooth muscle cells (27). In addition to these direct effects, hyperparathyroidism has indirect effects on the cardiovascular disease because of its assumed positive correlation to hypertension (28-30). H-FABP is a protein present in the cytosol of cardiomyocytes that is rapidly released into circulation during the event of myocardial tissue damage. H-FABP has been indicated as a more accurate and early marker in the identification of acute myocardial damage (31,32). Several studies have indicated a positive correlation between H-FABP and cardiometabolic risk factors (2–8). Therefore, we investigated whether H-FABP level is increased in patients with PHT. We demonstrated that H-FABP levels did not differ between patients and controls. We further investigated H-FABP levels with various CV risk factors.

Age of the individual also appears to contribute to the development of CVD. In a cohort of more than 3.6 million individuals, aged 40 years or older, who underwent self-referred screening for cardiovascular disease (ankle-brachial index, carotid duplex ultrasound, and abdominal ultrasound), the prevalence of any type of vascular disease increased significantly with each decade of life (33). In addition, hs-CRP measurement is also considered to be a useful and independent marker for cardiovascular risk assessment (34). In our study, H-FABP levels showed a positive correlation with age, fasting blood glucose, BMI, and h-CRP.

American Heart Association has identified obesity as an independent risk factor (35). In a meta-analysis of studies assessing the impact of body weight on CHD, there was a 29 percent increase in CHD for every five-unit increase in body mass index (BMI) (36). Similarly, we also found a positive correlation between H-FABP levels and BMI in our study.

In one study with a large group of volunteers, serum H-FABP levels were found to be affected by age, gender, obesity, renal function, and ECG abnormality (37). Another study demonstrated that concentrations of H-FABP and h-CRP corre-
lated positively (38). Taken together these studies suggest that H-FABP levels could be influenced by some CV risk factors and/or markers, such as age, HsCRP, and BMI.

The design of our study excluded patients with overt cardiovascular or cerebrovascular disorder, although, there was an evidence of subclinical atherosclerosis in our patient group. Early atherosclerosis can be detected by CIMT and measured easily in a non-invasive manner. From the ultrasonographic point of view, CIMT measurements represent a good correlation with histology (40) where increased CIMT is associated with vascular risk factors and the presence of severe atherosclerosis (35–38), thus making CIMT level a widely accepted sign of early atherosclerosis. In our study, CIMT levels in patient group were higher than controls, suggesting that the patients with PHPT are at a higher risk for CV diseases. However, probably because of the exclusion of patients with overt CV, no correlation was found between the levels of CIMT and H-FABP.

To the best of our knowledge, this is the first study that evaluates the importance of H-FABP in patients with primary hyperparathyroidism in the literature. The findings of our study restate that cardiovascular risk factors such as hyperlipidemia and CIMT are higher in patients with primary hyperparathyroidism. In addition, we found a positive correlation between H-FABP and age, fasting blood glucose, BMI and h-CRP and similar H-FABP levels between groups, which necessitates comprehensive studies including larger populations to enlighten the relationship between H-FABP and primary hyperparathyroidism. The limitations of this study is its relatively small sample size and it being a single center study.

Conclusion

In the present study, H-FABP levels were seen to correlate with some CV risk factors such as age, fasting blood glucose, BMI and h-CRP; however, these levels did not differ in primary hyperparathyroidism patients who had no cardiac disease.

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.

Author Contributions

Idea/Concept: Bekir Uçan, Mustafa Şahin, Mustafa Özbek; Design: Bekir Uçan, Mustafa Şahin, Mustafa Özbek; Control/Supervision: Bekir Uçan, Mustafa Çalışkan, Muhammed Kızılçığlı; Data Collection and/or Processing: Bekir Uçan, Mustafa Çalışkan; Analysis and/or Interpretation: Bekir Uçan, Muhammed Kızılçığlı; Literature Review: Bekir Uçan, Muhammed Kızılçığlı; Writing The Article: Bekir Uçan, Mustafa Şahin, Güler Öztürk, Mehtem Akif Öztürk; Critical Review: Bekir Uçan, Mehtem Akif Öztürk, Erman Çakal; References and Fundings: Bekir Uçan, Muhammed Kızılçığlı, Mehtem Akif Öztürk, Erman Çakal; Materials: Bekir Uçan, Güler Öztürk, Mehtem Akif Öztürk, Erman Çakal.

References


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Clinical Value of Histogram Analysis Using Gray-Scale Ultrasound Images in Thyroiditis

Tiroiditlerde Gri Skala Ultrasound Görüntülerinin Histogram Analizinin Klinik Değeri

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Abstract

**Objective:** The main purpose of this study was to investigate the histogram analysis (HA) in terms of the differential diagnosis of thyroiditis.

**Material and Methods:** A total of 137 cases with a definitive diagnosis of thyroiditis confirmed with clinical and laboratory findings were evaluated in the study. Out of these, 23 cases were diagnosed as Graves’ disease (GD), 94 as Hashimoto’s thyroiditis (HT), and 20 as subacute thyroiditis (SAT), and 34 healthy volunteers were included in this study as a control group. The thyroid and sternocleidomastoid muscle’s (SCM) HA and thyroid-to-SCM echogenicity ratio (TRSCMR) were evaluated.

**Results:** The HA values of thyroid parenchyma and SCM (mean±SD) of patients with GD, HT, SAT, and CG were 83.49±27.91, 71.25±20.93, 70.83±13.94, 80.95±20.88, and 52.74±24.11, 58.17±18.67, 67.20±14.71, and 69.32±18.94, respectively. The HAs of thyroid parenchyma of GD, HT, and SAT were not statistically significant. The TRSCMR (mean±SD) of patients with GD, HT, SAT, and CG were 1.29±0.41, 1.09±0.26, and 1.25±0.5, respectively. Compared with control group, the GD TRSCMRs were higher than the SCM TRSCMRs (p<0.0001), compared with SAT, GD TRSCMRs were higher than SAT TRSCMRs, and these results were statistically significant (p<0.001).

**Conclusion:** This study demonstrated that HA may help in differentiating SAT from GD that show similar appearance on routine ultrasonography.

**Keywords:** Histogram analysis; subacute thyroiditis; Hashimoto’s thyroiditis; Graves’ disease; thyroid ultrasonography

Introduction

Diffuse thyroid diseases include hyperthyroidism, chronic autoimmune hyperthyroidism (Hashimoto’s thyroidism [HT]), subacute thyroiditis (SAT), and Graves’ disease (GD). The imaging features of thyroid parenchyma echogenicity are used in B-mode
imaging in the differential diagnosis of thyroiditis. However, this differentiation is subjective and depends on the examiner. The differentiation of GD and SAT in gray-scale sonograms is difficult because of their similar appearances. Although they share similar clinical, biochemical, and sonographic properties, they have different etiopathogenesis and require different treatments. Therefore, a correct diagnosis of these types of thyroiditis by ultrasound is critical. Ultrasound assessment has limited value in terms of the differential diagnosis of thyroiditis. Therefore, radiological imaging techniques may have to be developed to provide crucial information to clinicians for planning treatment algorithms.

With the developing technology, computer programs may be able to diagnose diseases similar to a radiologist in the near future. Therefore, the quantitative B-mode evaluation methods should be developed. In this study, we aimed to investigate the use of histograms in diagnosing thyroiditis.

Palpation has long been used for the examination of the thyroid. SonoeLASTography (USE), first used by Ophir et al., has replaced palpation in the examination of thyroid (1). The current literature reported the use of USE in thyroiditis by a shear wave and strain elastography (1-6). However, elastography is a new developing technology that is not currently available in several ultrasonic devices, and the contribution of the differential diagnosis of thyroiditis remains unclear.

In addition, gray-scale ultrasound imaging is a universal and easily accessible technology worldwide. Thus, new evaluation techniques should be developed for accurate diagnosis and treatment. Histogram analysis (HA) is based on the recordings of images obtained in the ultrasonographic gray-scale mode, and these images are analyzed with a special medical software program after they are transferred to a workstation.

The HA of gray-scale ultrasound images has been investigated in various organs for the differentiation of transudate from exudative ascites, breast tumor differentiation, perfusion defects of kidney disease, characterization of parathyroid gland injury after head-and-neck radiotherapy, differentiation of asymptomatic diffuse thyroid disease from normal thyroid, and investigation of the chronic thyroiditis appearance (7-15). Despite the benefits of HA in chronic thyroiditis, data regarding the use of HA in SAT, HT, and GD are limited (12-15). The capability of differential diagnosis by HA and thyroid-to-sternocleidomastoid muscle echogenicity ratio (TRSCMR) in thyroiditis is yet to be investigated. In this study, we aimed to investigate the applicability of HA and TRSCMR in different types of thyroiditis.

Graves’ Disease

GD is an autoimmune condition that presents with thyrotoxicosis induced by circulating thyroid-stimulating hormone receptor autoantibodies (TRAbs) with thyroid-stimulating activity. In addition, thyrotoxicosis is the result of different types of painless thyroiditis. To identify the exact cause of thyrotoxicosis, radioactive iodine uptake measurement is still the gold standard of clinical application. By contrast, nuclear medicine techniques are unavailable in several health facilities, though radioactive substances are contraindicated for lactating and pregnant women.

The prevalence of GD is 5 to 10 times more common in females than in males. The maximum incidences of the disease were observed during the third to sixth decades. Tremor, heat intolerance, nervousness, weight loss, goiter, fatigue, tachycardia, and exophthalmos are the most usual clinical symptoms of GD. The evaluation of TRAb levels in the blood is advantageous for differentiating GD from HT and SAT. However, TRAb analyses are not accessible in general hospitals and clinics.

Ultrasonography is a non-invasive and cost-effective imaging technique for the examination of the thyroid parenchyma. GD is identified using B-mode ultrasonography through the development of diffuse thyroid enlargement with the loss of echogenicity. In addition, it is associated with the increased intraparenchymal thyroidal flow on color flow Doppler sonography (16).

The increased thyroid vascularity is crucial evidence for GD, which is beneficial for differentiating GD from SAT and HT (17).

In theory, the differential diagnosis of GD from SAT and HT by HA is possible because of the different histopathologic characteristics and types of thyroid gland vascularities for each condition. To date, no study has compared the TRSCMRs among patients with GD and other types of thyroiditis.

Subacute Thyroiditis

SAT is an unusual, self-limiting condition that generally occurs after an upper tract viral infection, which is the result of an autoimmune response (18). Clinically, this situation is associated with severe anterior neck pain and may radiate
up to the mandible, ear, and occipital fossa. At the beginning of this condition, SAT usually causes low-grade fever with thyrotoxicosis (19). The laboratory findings of SAT have decreased thyrotropin levels, increased thyroid hormones (FT4 and FT3), and increased C-reactive protein (20). In general, the gray-scale and Doppler sonographic findings of SAT are thyroid enlargement and ill-defined focal hypoechoic areas with hypovascularity (19). After treatment, these findings usually disappear (19).

**Hashimoto’s Thyroiditis**

HT is the most usual inflammatory process of the thyroid gland, and it is the main cause of autoimmune hypothyroidism. This condition develops 15 times more in women than in men. The maximum incidences of HT were observed during the third to fifth decades (21). The prevalence of HT is approximately 5% to 15% in women. HT is commonly associated with the development of circulating anti-thyroid autoantibodies, which are the cause of cytological injury and thyroid malfunction. The detection of thyroglobulin antibody and/or thyroid peroxidase antibody in blood tests is used for the diagnosis of HT. Ultrasonography shows the loss of echogenicity, increased heterogeneity, and decreased normal vascularity with the development of septa and hypoechoic micronodules in the patients with HT (22).

The degree of thyroid fibrosis is associated with decreased echogenicity in HT. The applicability of HT for the evaluation of thyroid HA was first described by Schiemann et al. in 2003 (14).

**Material and Methods**

**Study Population**

Most of the patients were sampled from the patient population treated in the Department of Endocrinology at Trabzon Kanuni Research and Education Hospital, between November 2015 and January 2017. The patients with a suspected diagnosis of thyroiditis were identified. The patients under 18 years and above 90 years of age or without available images (B-mode ultrasonography) and laboratory tests were excluded. In addition, the patients who were diagnosed with thyroiditis were excluded.

The study was approved by the local institutional review board and all participants provided written informed consent. This prospective, single-institution study was conducted in compliance with the Helsinki Declaration and good clinical practice guidelines of the Ministry of Health of Turkey. The study was approved by the local ethics committee of Kanuni Research and Education Hospital, Turkey.

An Apio 500 ultrasound machine (Toshiba Medical Systems, Co. Ltd., Otawara, Japan) with linear 4.8 to 11 MHz transducers and elastography software was used. All the examinations were performed by one radiologist with an experience of more than 15 years in thyroid imaging. The radiologist was blinded to the clinical findings, laboratory results, early clinical suspected differential diagnosis of thyroiditis, and the final diagnosis of the patients. A total of 250 individuals examined in our radiology department from October 2015 to March 2016 were screened. Out of these individuals, 52 patients were having thyroid nodules in addition to thyroiditis, which were detected incidentally. High-quality images were not obtained from 17 patients because of retrosternal thyroid enlargement or short neck anatomical structure. A total of 69 patients were excluded from the study. Finally, 171 cases were included in the study. Out of these cases, 23 were diagnosed as GD (8 men, 15 women; 33±12 years), 94 were diagnosed as HT (8 men, 86 women; 40±13 years), and 20 were diagnosed as SAT (8 men, 12 women; 42±9 years). All cases were confirmed by clinical examination and laboratory findings. The control group (CG) consisted of 34 healthy volunteers (5 men, 29 women; 40±10 years) whose ultrasound imaging and thyroid laboratory results were normal (Table 1).

**Ultrasound Imaging**

The ultrasound examination started with gray-scale imaging. The patient was supine with a slightly hyperextended position over a special wheeled bed, which was built for thyroid imaging. The B-mode ultrasonographic evaluation of thyroid glands was performed with standard transverse and longitudinal planes. In addition, thyroid dimensions and parenchymal echogenicity were evaluated. The echogenicity of the thyroid parenchyma was defined as marked hypoechoogenicity, isoechogenicity, and hyperechogenicity. Thyroid echogenicity was evaluated by comparing neighboring neck muscles (23). During the gray-scale ultrasound, all thyroid regions suspected for thyroiditis were identified in a longitudinal scanning position.
Before saving the image in the JPEG format in ultrasound database, we checked that the selected B-mode image does not contain any thyroid nodules and lymph nodes. If necessary, patients were requested to hold their breath to prevent gray-scale artifacts.

To improve and standardize the imaging quality, we selected gray-scale time-gain compensation values between -30 and 30 dB and gain levels between 0 and 60. Finally, the focus-level interval was from 0 to 4, and we used focus level 2. We obtained three sonographic images for each of the thyroid gland and sternocleidomastoid muscle (SCM). After the examination, we selected the technically perfect image from the collected data for HA. This selected JPEG was considered valid.

**Histogram Image Analysis**

Electronically recorded sonographic data in the JPEG format were transferred to a high-resolution computer system, and ImageJ software (version 1.4.3.67, National Institutes of Health) was used for gray-scale HA. The echogenicity of the images was measured as gray-scale pixels ranging from 0 to 255 (0=black, 255=white) through HA.

A region of interest (ROI) for HA was identified. The ROI included maximum possible thyroid lobes that were affected from thyroiditis to investigate a maximal amount of the thyroid parenchyma (Figure 1). The first ROI was accepted as the target region. We then selected another ROI as a reference. The ipsilateral SCM was selected for comparing the affected thyroiditis area to depict color pixels (Figure 2). Both ROIs included maximum possible thyroid tissue and SCM. In addition, we ensured that both ROIs were placed at the same vertical axis to the transducer and in the central region of the image. The TRSCMR was obtained from the collected data.
Statistical Analysis
Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed in percentages. The normality of the continuous variables was analyzed using the Kolmogorov-Smirnov test. The categorical variables between the groups were compared using the chi-square test. The numerical variables between independent groups were compared. If the normal distribution requirement was provided, then analysis of variance was performed. If the normal distribution requirement was not provided, then Kruskal-Wallis variance analysis was used. The Mann-Whitney U test was performed to determine whether the distribution of thyroid and SCM HA and TRSCMR is different among CG, SAT, GD, and HT. In addition, the Mann-Whitney U test was used to compare the HAs and TRSCMRs of the groups.

The receiver operating characteristic curve was used to determine the cut-off point of the HA and TRSCMR of normal and experimental subjects. The p-values lower than 0.05 were considered statistically significant. In the presence of a significant threshold, sensitivity, and specificity values were calculated. To evaluate the area under the curve (AUC), the diagnostic value of the test was accepted as statistically significant when type 1 error was less than 5%. The statistical analysis was performed using SPSS 22.0 statistical software.

Results
The clinical and demographic characteristics of patients are shown in Table 1. The HA values of thyroid parenchyma (mean ± SD) of patients with GD, HT, SAT, and CG were 83.49 ± 27.91, 71.25 ± 20.93, 70.83 ± 13.94, 80.95 ± 20.88, and 52.74 ± 24.11, respectively. By contrast, the HA values of SCM (mean ± SD) of patients with GD, HT, SAT, and CG were 52.74 ± 24.11, 58.17 ± 18.67, 67.20 ± 14.71, and 69.32 ± 18.94, respectively (Table 2).

The thyroid parenchyma HAs of GD, HT, and SAT were not statistically significant, whereas SCM HAs of HT and GD were statistically significant (p = 0.009). The TRSCMR (mean ± SD) of patients with GD, HT, SAT, and CG were 1.85 ± 0.86, 1.29 ± 0.41, 1.09 ± 0.26, and 1.25 ± 0.5, respectively (Table 3 and Figures 3–5).

Compared with the CG, patients with GD had statistically significant TRSCMRs (p = 0.001). TRSCMRs presented the following ascending order: SAT < CG < HT < GD. The boxplot of the TRSCMR values to each group is shown in Figure 6. Patients with GD had statistically different TRSCMRs (p = 0.001) compared with patients with SAT. This result is noteworthy because SAT and GD share similar imaging properties in gray-scale sonography.

The cut-off points of the TRSCMR of patients with GD, HT, and SAT to the CG were 1.32 (sensitivity

Table 2. The distributions of HA results of thyroidal parenchyma (TP) and SCM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases n (%)</th>
<th>Mean±SD (TP)</th>
<th>Mean±SD (SCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>34 (19.88)</td>
<td>80.95 (20.88)</td>
<td>69.32 (18.94)</td>
</tr>
<tr>
<td>HT</td>
<td>94 (54.98)</td>
<td>71.25 (20.93)</td>
<td>58.17 (18.67)</td>
</tr>
<tr>
<td>SAT</td>
<td>20 (11.69)</td>
<td>70.83 (13.94)</td>
<td>67.20 (14.71)</td>
</tr>
<tr>
<td>GD</td>
<td>23 (13.45)</td>
<td>83.49 (27.91)</td>
<td>52.74 (24.11)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>171 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CG: Control group; HT: Hashimoto’s thyroiditis; SAT: Subacute thyroiditis; GD: Graves’ disease.

Table 3. Thyroid and sternocleidomastoideus muscle ratio (TRSCMR) ranges of different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases n (%)</th>
<th>TRSCMRs</th>
<th>Range</th>
<th>Median</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>34 (19.88)</td>
<td>0.64–2.22</td>
<td>1.17</td>
<td>1.25 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td>94 (54.98)</td>
<td>0.61–2.65</td>
<td>1.22</td>
<td>1.29 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>20 (11.69)</td>
<td>0.69–1.25</td>
<td>1.02</td>
<td>1.09 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>23 (13.45)</td>
<td>1.48–2.23</td>
<td>1.61</td>
<td>1.85 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>171 (100.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CG: Control group; HT: Hashimoto’s thyroiditis; SAT: Subacute thyroiditis; GD: Graves’ disease.
Discussion

In this study, we performed the B-mode HA of thyroid parenchyma using sonography and evaluated its applicability to differentiate thyroiditis from healthy thyroid and the subtypes of thyroiditis from one another.

We found statistically significant values in TRSCMRs in GD compared with other types of thyroiditis, indicating that TRSCMRs can be used as a supplementary sonographic parameter. Compared with the CG, the GD TRSCMR values were higher than the CG TRSCMR values and the TRSCMR values of the SAT group were lower than the TRSCMR values of the CG group, which was statistically significant (p<0.0001).

GD is an autoimmune condition that presents with thyrotoxicosis, which can also be the result of different types of thyroiditis. To identify the exact cause of thyrotoxicosis, the radioactive iodine uptake measurement is still the gold standard of clinical application. GD shows radioactive iodine uptake even when SAT does not. By contrast, nuclear medicine techniques are not easily accessible at several health centers, and radioactive materials are harmful to lactating and pregnant women. GD’s gray-scale sonographic features are thyroid enlargement with the loss of parenchymal echogenicity.

SAT is an unusual, self-limiting condition that generally develops after an upper tract infection caused by a viral infection, which is the result of an autoimmune response (18). Initially, SAT usually causes low-grade fever with thyrotoxicosis (19).

In general, the gray-scale sonographic findings of SAT are thyroid enlargement and ill-defined focal hypoechoic areas (19). The gray-scale ultrasound findings of SAT and GD are highly similar. Our study results revealed that the gray-scale sonographic imaging findings of SAT and GD showed no statistically significant differences (p=0.215).

The treatment of SAT and GD depends on their etiopathogenesis and is completely different. Non-steroidal anti-inflammatory drugs and corticosteroids are used in SAT treatment, whereas anti-thyroid-acting propylthiouracil and thiouazole are used in GD treatment. Discriminant diagnosis must be performed correctly, as they have different treatments.
In theory, the differential diagnosis of GD from SAT and HT by HA is possible because of the different histopathologic characteristics and types of thyroid gland vascularities for each condition. To date, no study has compared the TRSCMRs among patients with GD and other types of thyroiditis.

In this study, GD TRSCMR values were higher than SAT TRSCM values. TRSCMRs could statistically significantly differentiate GD from SAT; however, it could not differentiate SAT from HT and CG. This result was particularly remarkable because SAT and GD have similar imaging properties to gray-scale sonography.

The applicability of HA for the evaluation of chronic thyroiditis was first described by Schiemann et al. in 2003 (14). The current studies focused on ultrasound gray-scale HA in chronic thyroiditis (12-15,25). These studies are based on thyroid parenchymal HA rather than TRSCMR. Conversely, conventional ultrasound is a worldwide, easily accessible, inexpensive, and innocuous diagnostic method. However, ultrasound is a user-dependent imaging modality, which is the most crucial disadvantage for technique standardization. Kim et al. showed that even if experienced radiologists identify the presence of chronic thyroiditis based on the specific similar sonographic features of HT, their evaluations are inconsistent (25).

SAT is a painful and inflammatory condition (26). Pain may be bilateral or unilateral and spread to the mandible, occipital fossa, and ear. Researchers must realize that patients may choose to avoid neck movements to prevent pain, and this option may lead to an increase in muscle echogenicity. In this study, statistically significant TRSCMR differentiating SAT from GD was a result of increased muscle echogenicity in SAT, which was secondary to immobilization and decreased muscle echogenicity in GD because of the increased vascularity. This study had some limitations. First, the gender of enrolled patients was not equal because of the nature of thyroiditis. Second, cytological findings for each of the thyroiditis groups and long-term follow-up HA values after treatment were not included. Further studies in the recovery phase of thyroiditis are required to enrich the findings of this study.

**Conclusion**

The HA of sonographic imaging is applicable for differentiating patients with thyroiditis from healthy individuals; however, its differentiating value for different types of thyroiditis, such as SAT and HT, is limited. TRSCMR is valuable for differentiating patients with thyroiditis from healthy individuals; however, it has limited value in differentiating SAT-HT from GD-HT. Our study
results revealed that TRSCMR was the only useful method for differentiating GD from SAT. TRSCMR is a useful method for the differential diagnosis of GD from a healthy population or from SAT. HA would be useful in differentiating GD from other causes of hyperthyroidism to avoid overtreating SAT and provide a prognosis. Nuclear medicine studies may confirm this role; however, they involve radiation and are not always available. In addition, laboratory tests are useful but not always available. HA combined with conventional ultrasonography is an excellent tool for the discrimination of GD from SAT.

**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: Hatica Ayça Ata Korkmaz; Design: Hatica Ayça Ata Korkmaz; Control/Supervision: Mustafa Köse; Data Collection And/Or Processing: Mustafa Köse; Analysis and/or Interpreta-
References
Cytologic Comparison Between Growing and Non-growing Benign Thyroid Nodules Evaluated Using Two Different Growth Criteria

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Abstract
Objective: Thyroid nodules are frequent in the adult population. Thyroid fine-needle aspiration biopsy is performed for diagnosing cancerous nodules. It is suggested that biopsy-proven benign thyroid nodules should be followed up clinically, and if they grow, rebiopsy should be performed. However, certain growth criteria have not yet been defined.

Material and Methods: We retrospectively reviewed thyroid fine-needle aspiration records of all patients at Dokuz Eylül University Hospital between January 2006 and June 2009. The nodules that underwent second biopsies were evaluated using two different growth criteria: at least 50% increase in the nodule maximal diameter and 20% increase in at least two nodule dimensions with a minimal increase of 2 mm.

Results: From a total of 4217 thyroid nodules, we evaluated the cytological results of 117 benign thyroid nodules, which underwent follow-up biopsies. No significant difference was observed in the cytological results of the growing group (n:21), which had at least 50% increase in the maximal nodule diameter, and the non-growing group (n:96) (p=0.999). In addition, using the growth criteria of 20% increase in at least two nodule dimensions with a minimal increase of 2 mm, no significant difference was observed in the cytological results of the growing (n:47) and non-growing (n:70) benign thyroid nodules (p=0.700).

Conclusion: According to two different growth criteria, the growth of a benign nodule is not an additional risk factor for cancer.

Keywords: Thyroid nodule; nodule growth; thyroid fine-needle aspiration biopsy

Özet

Gereç ve Yöntemler: Dokuz Eylül Üniversitesi Hastanesi’nde Ocak 2006-Haziran 2009 tarihleri arasında uygulanan TIİAB kayıtları geriye dönük olarak incelendi. İkinci bir TIİAB yapılan benign nodüllerin izlemeksi Steele’leri iki farklı büyüme kriteri kullanılarak (en büyük nodül çapında %50 ve ya üzerinde artış, nodülün en az iki çapında 2 mm’den az olmamak üzere %20 veya üzerinde artış) değerlendirildi.

Bulgular: Ince iğne aspirasyon biyopsisi yapılan 4217 tıroid nodülden izlem biyopsileri olan benign nodüller incelemeye alındı. İzlemde maximum çapta %50 ve üzerinde artışa göre büyüyenler (n: 21), bu kritere göre büyümeyenler (n:96) benign nodüller incelendiğinde aralardan anlamlı sitolojik fark saptanmadı (p=0.999). Benzer şekilde nodülün en az iki çapında ~2 mm’den az olmamak üzere %20 veya üzerinde boyut artışa sahip anlamlı sitolojik fark saptanmadı (p=0.700).

Sonuç: Çalışmada kullanılan ikinci büyüme kriterine göre benign nodüllerin izlemde büyümeleri kanser varlığı için ek risk oluşturmadı.

Anahtar kelimeler: Tiroid nodülü; nodül büyümesi; tiroid ince iğne aspirasyon biyopsisi
Introduction

A thyroid nodule is a frequently observed health problem. Thyroid nodules can be detected in 4% of the population by palpation and in up to 67% by ultrasonography (1). Thyroid nodules are clinically crucial, as they have 5% to 10% probability of thyroid cancer (2). A clinical approach to thyroid nodules aims at detecting cancerous nodules. The simplest and safest method to screen thyroid cancer is thyroid fine-needle aspiration biopsy (TFNAB; 3).

It is recommended that benign nodules diagnosed by TFNAB should be followed up with physical examination and thyroid ultrasonography (3). One of the most crucial criteria to be assessed during follow-up is the nodule growth. Rebiopsy is recommended for a growing nodule, which was initially benign (3). However, studies have shown that benign nodules can also grow in their natural course (4–6). There is no consensus about the “growth” criteria of thyroid nodules. Several criteria were used to establish the nodule “growth” (>50% increase in the maximal dimension of the nodule, >15% increase in the nodule volume, >30% increase in the nodule volume, etc. (7). American Thyroid Association (ATA) has defined a 20% increase in at least two nodule dimensions with a minimal increase of 2 mm as an acceptable growth criterion (3). However, till date, no evidence exists that cancer risk increases in benign nodules growing at follow-up.

This study aimed to compare the TFNAB results of the growing nodules, which had initially benign cytology, with non-growing nodules. We used two different growth criteria (i.e., at least 50% increase in the nodule maximal diameter and 20% increase in at least two nodule dimensions with a minimal increase of 2 mm) to detect whether the cancer risk of nodules growing at follow-up increases compared with the ones that do not grow.

Material and Methods

We retrospectively reviewed all results of ultrasonography-guided fine-needle aspiration biopsies performed at Dokuz Eylül University Hospital between January 2006 and June 2009. We recorded nodules that had repeated biopsies. Age, sex, radiation history to the neck, additional malignancy history, thyroid surgery history, familial history, and thyrotrophin stimulating hormone (TSH) level of the patient and the dimensions of the nodules were recorded. The nodules which had repeated biopsies were classified as growing and non-growing. Growth classification and evaluation have been performed separately according to two different criteria (at least 50% increase in the nodule maximal diameter and 20% increase in at least two nodule dimensions with a minimal increase of 2 mm), as no consensus exists about the exact growth criteria of thyroid nodules.

The biopsy results were classified as benign, intermediate, or malign. Nodules that are Thy 2-3-4 according to Bethesda classification were classified as intermediate. The existence of surgical intervention toward the nodule after the second fine-needle aspiration biopsy was ascertained. If there was any surgical intervention, we recorded the pathological diagnosis of the nodule.

The study protocol was approved by the local ethics committee.

Statistical analysis was performed using SPSS V15.0, SPSS Inc., Chicago. Nominal data were evaluated using chi-square test; continuous variables were assessed using independent sample’s t-test or Mann-Whitney U test. Logistic regression analysis was performed for assessing the independent impact of a specific variable on other parameters.

Results

We retrospectively analyzed 4217 thyroid nodule biopsies from 3202 patients at Dokuz Eylül University Hospital between January 2006 and June 2009 (Figure 1). The general characteristics of the nodules are summarized in Table 1.

Out of the initial benign nodules (3509), 117 nodules that had repeated biopsy were included in the statistical analysis. There were 47 growing and 70 non-growing nodules using the growth criteria of 20% increase in at least two nodule dimensions with a minimal increase of 2 mm (Group 1); and there were 21 growing and 96 non-growing nodules using the growth criteria of at least 50% increase in the nodule maximal diameter (Group 2).

Group 1

No difference was observed in terms of age, TSH, and the initial maximal diameter of the nodule between growing and non-growing groups (Table 2). Follow-up time was longer in the growing group than the non-growing group (2.76 ±1.30 years vs. 2.13 ±1.33 years, p = 0.050). In addition, no difference was observed in the cytological results of rebiopsies between the two
Two nodules with non-benign cytology in the growing group were operated; both nodules were defined as papillary thyroid cancer (PTC). A total of 5 nodules out of 45 nodules with benign cytology in the growing group were operated; 3 of these nodules were defined as PTC. There were five nodules with intermediate cytology in the non-growing group; three of these nodules were operated, and two PTC and one benign nodule were established (Table 3). All of the five nodules with benign cytology in the non-growing group had benign pathology after surgery.

In the linear regression model including age, sex, TSH level, and follow-up period, the follow-up period was 1.5 times high in the growing nodules (Table 4).

**Group 2**

No difference in terms of age and TSH levels was observed between the growing and non-growing groups. The non-growing group had higher initial diameter than the growing group (Table 2). In addition, no difference was observed in terms of cytological results between the growing and non-growing groups (p=0.999). In both groups, malignant cytology result was not found. After the surgical excision of the nodule with intermediate cytology in the growing group, PTC was found. Out of the 20 growing nodules, which had benign cytology, three were operated and all of them had benign pathology. Furthermore, four nodules with intermediate cytology were excised surgically in the non-growing group, although PTC was detected in three of them (Table 3). In addition, seven of the non-growing nodules with benign cytology were operated, although three of them had PTC.

When the data were evaluated with linear regression analysis, age, sex, initial TSH level, and follow-up period were not associated with nodules, which grow according to Group 2 criteria (Table 5).

A total of seven malignant and eight benign pathology results were found. The growth rate of benign nodules was 1.81 [-2, 35-12, 42] mm/year, and the growth rate of malignant nodules was 1.21 [-1, 12-4, 44] mm/year.

**Discussion**

In this study, we retrospectively examined the biopsies of thyroid nodules. Considering the two different growth criteria, we did not find an increased rate of malignancy in growing, initially benign nodules.

The natural course of benign thyroid nodules is not completely understood. Although the growth of the benign nodule can be attributed to its natural course, most guidelines recommended rebiopsy to exclude malignancy in growing, initially benign nodules.

The growth rate of the benign nodule can be attributed to its natural course, most guidelines recommended rebiopsy to exclude malignancy in growing nodules (3). There were different growth criteria used for defining the nodule “growth.” The rate of nodule growth differed when different growth criteria were used. Erdogan et al. reported the rates of nodule growth for different criteria (32% for ≥15% change in the nodule volume, 24.1% for ≥30% change in the nodule volume, and 4.1%...
for ≥50% change in the maximal diameter of the nodule; 7). Lim et al. reported that 11.8% and 9.4% of benign nodules grew using ≥50% increase in volume and ≥20% increase in at least two nodule dimensions with a minimal increase of 2 mm as a cut-off value, respectively (8).

ATA recommended the rebiopsy of initially benign nodules if ≥20% increase was observed in at least two nodule dimensions with a minimal increase of 2 mm (3). Durante et al. reported that 15.4% (n=153) of the benign nodules grew using the ATA criteria (9). Only two malignancies were detected from these nodules after second TFNAB. Rosario et al. reported the rebiopsy results of the initial benign nodules (10). Out of 86 growing nodules (using ATA criteria), only three malignancies (3.5%) were detected after the second biopsy. In addition, Kim et al. reported a growth rate of 21.1% considering the criteria of 50% change in the nodule volume (11). Moreover, they found only one malignancy among 172 growing nodules. The results of all these studies suggested that a proportion of benign nodules grow over time and the malignancy rate of these growing nodules in follow-up is very low.

In a recent study, malign nodules were found more likely to grow more than 2 mm/year compared with benign nodules (12). As a limited number of patients had final pathology in our study, no difference was observed between benign and malign nodules in terms of nodule growth speed. In addition, the follow-up time for growing nodules was longer than that for non-growing nodules in Group 1. No data exist in the literature comparing the follow-up time and growth; therefore, additional studies are required to interpret this finding.

We have found intermediate cytological results in growing and non-growing nodules during follow-up. The reason for changing the cytological characteristics of the nodules may be the false negative rate of the initial TFNAB rather than that the growth in follow-up increases the malignancy risk. As in a study in which growth has not been used as a criterion and the rebiopsies of benign nodules during follow-up have been examined, rebiopsies in 13.2% of benign nodules showed intermediate or malign cytology (13). Similarly, in another study including the rebiopsies of benign nodules with suspicious ultrasonographic features, in 17.4% of them, suspicious cytology was found (14). Therefore, in some studies, TFNAB is recommended for benign thyroid nodules in follow-up (15).

We faced various limitations during our study. First, a retrospective approach weakens the strength of the study. In this study, we used the biopsies from non-growing nodules as a control group. However, data were not available indicating the reason for taking a biopsy from the non-growing nodules during follow-up. Repeated biopsy may have been taken from a group with

<table>
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<tr>
<th>Group 1 Growing (N=47)</th>
<th>Cytology (n)</th>
<th>Pathology (n)</th>
<th>Group 1 Nongrowing (N=70)</th>
<th>Cytology (n)</th>
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<tr>
<td>Benign: 45</td>
<td>Benign: 2</td>
<td>PTC: 3</td>
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<td>Intermediate: 2</td>
<td>Benign: 0</td>
<td>PTC: 2</td>
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<th>Group 2 Growing (N=21)</th>
<th>Cytology (n)</th>
<th>Pathology (n)</th>
<th>Group 2 Nongrowing (N=96)</th>
<th>Cytology (n)</th>
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<tr>
<td>Benign: 20</td>
<td>Benign: 3</td>
<td>PTC: 1</td>
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<tr>
<td>Intermediate: 1</td>
<td>Benign: 0</td>
<td>PTC: 1</td>
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| Group 1: 20% increase in at least two nodule dimensions with a minimal increase of 2 mm. Group 2: At least 50% increase in nodule maximal diameter. PTC: Papillary thyroid cancer. |

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<th>Cytology (n)</th>
<th>Pathology (n)</th>
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<tbody>
<tr>
<td>Benign: 90</td>
<td>Benign: 4</td>
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<tr>
<td>Intermediate: 6</td>
<td>Benign: 1</td>
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<th>Table 4. Factors predicting “20% increase in at least two nodule dimensions with a minimal increase of 2 mm”.</th>
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<td><strong>ODDS ratio</strong></td>
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<td>Follow-up period</td>
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<td>TSH</td>
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*p<0.05.

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<th>Table 5. Factors predicting at least 50% increase in nodule maximal diameter.</th>
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<td><strong>ODDS ratio</strong></td>
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high clinical cancer suspicion even there was no dimensional growth. Therefore, the control group may be a group with high risk. Intermediate risk category is a very heterogeneous group, and possible discordance between the cytologists is another limitation.

Conclusions and Recommendations

The most outstanding clinical importance of thyroid nodule is a cancer risk. In this study, two different growth criteria were used, and it was observed that the cancer risk of growing benign nodules was not higher than the non-growing ones. It is necessary that these findings are supported by broad and prospective studies.

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

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

Authorship Contributions

Idea/Concept: Mehmet Muhittin Yalcın, Sena Yeşi; Design: Mehmet Muhittin Yalcın, Barış Akıcı, Abdurrahman Çömlekci, Fırat Bayraktar; Control/Supervision: Abdurrahman Çömlekci, Fırat Bayraktar, Sevinç Eraslan, Aytaç Gülçü, Tülay Canda; Data Collection and/or Processing: Mehmet Muhittin Yalcın, Sinan Unal; Analysis and/or Interpretation: Mehmet Muhittin Yalcın, Barış Akıcı, Sena Yeşi; Literature Review: Mehmet Muhittin Yalcın, Barış Akıcı, Sena Yeşi; Writing the Article: Mehmet Muhittin Yalcın, Barış Akıcı; Critical Review: Abdurrahman Çömlekci, Fırat Bayraktar, Sevinç Eraslan, Aytaç Gülçü, Tülay Canda; References and Fundings: Abdurrahman Çömlekci, Fırat Bayraktar, Sevinç Eraslan.

References

Ratio of Thyrotropin to Thyroglobulin as a Novel Marker for Differentiating Between Benign and Malignant Thyroid Nodules within Different Bethesda Categories

Benign and Malign Tiroid Nodüllerinin Ayrımında ve Farklı Bethesda Kategorilerinde Yeni bir Belirteç Olarak Tirotropin Tiroglobulin Oranı

Abstract

Objective: We aimed to determine whether the ratio of thyrotropin (TSH) to thyroglobulin (Tg) (TSH/Tg) would be able to assist in predicting malignancy in thyroid nodules.

Material and Methods: Euthyroid patients operated between the year 2007 and 2014 were retrospectively reviewed. Patients who previously had thyroid disease or surgery and those with increased levels of anti-thyroglobulin antibodies were excluded from this study. Clinicopathological features, as well as serum TSH, Tg, and TSH/Tg were compared between histopathologically benign and malignant groups.

Results: Data related to 370 (60.3%) benign and 244 (39.7%) malignant patients were analyzed. The malignant patients exhibited significantly higher TSH, TSH/Tg, and total thyroid volume, and a lower Tg compared to the benign patients (p<0.001 for each). There were 924 (74.2%) benign and 321 (25.8%) malignant nodules. Cytological distribution of the nodules was observed to be as follows: 343 (27.6%) nondiagnostic, 637 (51.2%) benign, 121 (9.7%) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), 39 (3.1%) follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), 64 (5.1%) suspicious for malignancy (SM), and 41 (3.3%) malignant. TSH, Tg, and TSH/Tg were significantly different in different Bethesda categories (p<0.001 for each). Median TSH/Tg was the lowest in benign (0.013), and highest in SM (0.054) and malignant (0.086) cytopathologies. TSH/Tg was significantly higher in the malignant nodules compared to benign nodules, in AUS/FLUS, FN/SFN, and SM categories (p=0.001, p<0.001, and p=0.003, respectively). In the regression analysis, TSH/Tg demonstrated higher diagnostic performance compared to TSH and Tg (p<0.001).

Discussion: Preoperative TSH/Tg could be used as a novel marker for differentiating between benign and malignant thyroid nodules. It could also assist in the prediction of risk of malignancy and management decisions when the cytology is indeterminate.

Keywords: Thyrotropin; thyroglobulin; thyroid malignancy; TSH/Tg; Bethesda

Özet

Amaç: Malign vebenign tiroid nodüllerinin aynında yeni bir prediktif markırolaraktirotropin (TSH) tiroglobulin (Tg) oranını araştırmaya amaçladık.

Gereç ve Yöntemler: 2007 ve 2014 arasında oper edilen ötiroid hastalar retrospektif olarak değerlendirildi. Tiroid hastalığı veya cerrahi öyküsü olanlar ve artmış anti-tiroglobulin antikorları olanlar dışlandı. Histo patolojik olarak benign ve mal ign gruplar klinikopatolojik özellikler ve serum TSH, Tg, TSH/Tg oranını açıdan karşılaştırıldı.

Bulgular: 370 (%60.3) benign ve 244 (%39.7) malign hastada benign hastalara göre malign hastalarda ani lar olarak yüksek TSH, TSH/Tg ve total tiroid hacmi ve düşük Tg vardı (her biri için, p<0.001). (%74.2) benign ve 321 (%25.8) malign nodul vardı. Sito patolojik dağılım şöyledi: 343 (27.6%) nondiagnostic, 637 (51.2%) benign, 121 (9.7%) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), 39 (3.1%) follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), 64 (5.1%) suspicious for malignancy (SM), ve 41 (3.3%) malign. TSH, Tg, ve TSH/Tg farklı Bethesda kategorilerinde anlamlı olarak farklıydı (her biri için p<0.001). Median TSH/Tg belirlenemeyen benign (0.013) ve malign (0.086) ortalamaları arasında farklıydı. TSH/Tg benignde (0.013) en düşük ve MŞ (0.054) ve malign sitolojilerde (0.086) en yüksekti. ÖBA/OBFL, FN/SFN ve MŞ kategorilerinde, TSH/Tg oranını malign nodüllerde benign nodüllere göre büyük bulundu (srasıyla, p=0.001, p<0.001 ve p=0.003). Regresyon analizinde TSH/Tg; TSH ve Tg’ye göre daha yüksek tansal performansa sahipti (p<0.001).

Tartışma: TSH/Tg ameliyat öncesinde benign ve malign tiroid nodüllerinin aynında yeni bir marker olarak kullanılabilir. Ayrca indeterminate sitolojisi olan nodüllerde malignite riskinin belirlenmesine ve yönetimine yardımcı olabilir.

Anahtar kelimeler: Tirotropin; tiroglobulin; tiroid malignitesi; TSH/Tg; Bethesda

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Received: 09/11/2017 Accepted: 21/02/2018

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Turkish Journal of Endocrinology and Metabolism published by Türkiye Klinikleri

DOI: 10.25179/tjem.2017-58803
Introduction

Nodular thyroid disease is the most common thyroid pathology observed in clinical practice. More than 50% of the population has at least one thyroid nodule under ultrasonography examination (1). Although nodule prevalence is very high, only 5-10% of the nodules are malignant and require surgical and/or medical management (2). The major concern is to discriminate the malignant lesions from the benign ones, preoperatively. Fine-needle aspiration biopsy (FNAB) for thyroid is the gold standard test used for this purpose. Although it is a rapid, cost-effective, safe, and reliable procedure, the nondiagnostic results requiring re-biopsy and the indeterminate cytology defined as the “gray zone” are the limitations of this method (3, 4). Atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm or suspicious for follicular neoplasm (FN/SFN), and suspicious of malignancy (SM) categories in the Bethesda classification may be considered indeterminate cytologies. These groups are representative of morphologically abnormal findings that are related to an increased risk of malignancy, though not enough to confirm a malignant lesion (5). Majority of the nodules with indeterminate cytology are surgically excised due to their malignancy potential, which confronts the patients with risks of morbidity and complications related to unnecessary surgeries (6). Therefore, it is comprehensible that in addition to cytology, certain other parameters are required to predict malignancy preoperatively. Gender, age, nodule diameter, exposure to radiation, and certain ultrasonography (US) features have been demonstrated to be associated with the risk of malignancy in previous reports (3, 7). Preoperative high serum thyrotropin (TSH) has also been demonstrated to increase the risk of malignancy in various studies. Boelaert et al. (8) evaluated serum TSH in patients with nodular or diffuse goiter, and observed that even though within normal ranges, high serum TSH was associated with thyroid malignancy.

Thyroglobulin (Tg) is a glycoprotein produced specifically in the thyroid follicular cells, regardless of whether they are of malignant or benign nature (9). It is a well-known marker for persistent or recurrent differentiated thyroid cancer (10). Although the role of Tg in the postoperative period has been clearly defined, its use as a predictive marker in the preoperative period is debatable (9, 11, 12). Routine Tg measurement is not recommended as an initial laboratory investigation for thyroid nodules. It is not sensitive or specific for thyroid cancer as it exhibits increased levels in several other thyroid diseases (13).

In the present study, our aim was to evaluate the role of the ratio of TSH to Tg (TSH/Tg) as a novel marker for the prediction of malignant nodules in patients with the euthyroid nodular disease. We also attempted to investigate whether this ratio could be used to predict malignancy in different Bethesda categories and thus, assist in determining the optimal management in case of indeterminate cytology.

Material and Methods

A retrospective analysis of 2,900 patients operated in our center between January 2007 and December 2014 was performed. Since the high anti-thyroglobulin (anti-Tg) levels could cause a confounding effect on absolute Tg, patients with high anti-Tg and the patients without simultaneous measurement of serum anti-Tg were excluded from this analysis. Patients with undetectable preoperative Tg levels (<1 ng/mL) were also excluded from the analysis because this was suggestive of occult antibody interference. Clinical or subclinical hypothyroidism or hyperthyroidism, radiation to head and neck, history of thyroid surgery, and previous or current use of antithyroid or thyroid hormone replacement therapy were the other exclusion criteria.

Age, sex, preoperative thyroid functions, antithyroid autoantibodies, US features (thyroid volume, the presence of nodule/nodules, and nodule size and number), and FNAB results were evaluated.

Serum TSH, free triiodothyronine (FT3), free thyroxine (FT4), anti-thyroid peroxidase (anti-TPO), and anti-Tg levels were measured using chemiluminescence methods (Immuno 2000, Diagnostic Products Corp., Los Angeles, CA, USA; UniCel Dxi 800, Beckman Coulter, Brea, CA). The normal levels for TSH, FT3, and FT4 were 0.4-4 µIU/mL, 1.57-4.71 pg/mL, and 0.85-1.78 ng/dL, respectively. Anti-TPO levels higher than 35 IU/mL, and anti-Tg levels higher than 40 IU/mL were considered positive. The normal range for Tg was 0-78 ng/mL.

The three diameters obtained from thyroid ultrasonography (maximal length × width × depth) were multiplied with π/6 to calculate the volume of each lobe, and the sum of volumes of the two lobes was defined as the thyroid volume. US (GE
Logiq 200 Pro with a 7.5 MHz probe, Kyunggi-do, Korea) guided FNAB was performed in nodules >1 cm in size. Also, cytological evaluation was performed when suspicious US features (hypoechogenicity, taller-than-wide shape, microcalcification, infiltrative margins, increased nodular vascularization, and absence of peripheral halo) were observed in a subcentimeter nodule. Cytological diagnosis was classified according to Bethesda system, which has the following categories: nondiagnostic (ND), benign, AUS/FLUS, FN/SFN, SM, and malignant. Patients and nodules were grouped as benign and malignant based on their histopathological results. Demographical and clinicopathological features, serum TSH, Tg, TSH/Tg, and Tg/TSH were compared between these two groups. Hashimoto thyroiditis was determined histopathologically. TSH and Tg levels in the patient were considered as the values of the nodule. In case of more than one nodule in a patient, values for each nodule were recorded. The local ethical committee approved the study protocol.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, USA) and MedCalc 11.4.2 (MedCalc Software, Mariakerke, Belgium). Shapiro-Wilk test was used to determine whether the variables were normally distributed. Normally distributed variables were expressed as a mean ± standard deviation, and the non-normally distributed variables were expressed as median (min-max). Numbers and percentages of categorical variables were calculated. Student’s T-test and Mann-Whitney U test were used to compare the numerical values in malignant and benign patients. Categorical variables were compared using Chi-square and Fisher’s exact tests. Numerical values in the Bethesda groups were compared using ANOVA (post-hoc: Bonferroni) and Kruskal-Wallis H test (post-hoc: Dunn’s test). Significant prognostic factors for malignancy determined in the univariate analysis were included in the stepwise backward elimination multivariate logistic regression model, and independent predictors were identified. ROC analysis was performed and area under the curve was used to identify the diagnostic discrimination among independent predictors. Youden’s index method was used in order to determine the prediction point of TSH/Tg ratio and Tg/TSH ratio for malignancy. In the statistical analysis, p<0.05 was considered to be significant and the ORs were presented with their respective 95% confidence interval.

Results

Data related to 614 euthyroid patients with nodular thyroid disease were analyzed. Histopathological diagnosis was benign in 370 (60.3%) and malignant in 244 (39.7%) patients. Malignancy was an incidental finding in 67 (27.5%) of the malignant patients. Age, sex, mean age, nodule number, mean fT3, mean fT4, and anti-TPO positivity were similar in benign and malignant patients (p>0.05 for each) (Table 1). Malignant patients exhibited a higher rate of histopathologically confirmed Hashimoto thyroiditis compared to benign patients (24.6% vs. 15.9%; p=0.008). Median total thyroid volume obtained was 31.4 mL in malignant, and 20.9 mL in benign patients (p<0.001). Malignant patients exhibited significantly higher median TSH (1.4 µIU/mL vs. 1.1 µIU/mL; p<0.001), and lower median Tg (34.1 ng/mL vs. 59 ng/mL; p<0.001) compared to benign patients. Median TSH/Tg was higher (0.04 µIU/ng vs. 0.02 µIU/ng; p<0.001), and median Tg/TSH was lower (23.8 ng/µIU vs. 53.1 ng/µIU; p<0.001) in malignant patients, compared to benign patients (Table 1). Preoperative cytological evaluation was performed in 1245 nodules of 614 patients; the cytological diagnosis was benign in 637 (51.2%), nondiagnostic in 343 (27.6%), AUS/FLUS in 121 (9.7%), FN/SFN in 39 (3.1%), SM in 64 (5.1%), and malignant in 41 (3.3%) nodules. Histopathologically, 924 nodules (72.4%) were benign and 321 (25.8%) were malignant. Mean fT4 was similar in all categories of Bethesda; whereas, mean fT3 was higher in FN/SFN category compared to the other Bethesda categories (p=0.033) (Table 2). Median TSH was similar in nondiagnostic and benign nodules, and lower than that in the other categories. AUS/FLUS and FN/SFN categories presented similar median TSH, which were lower than those observed in SM and malignant categories (p<0.001). There was no significant difference in median Tg between nondiagnostic, benign, AUS/FLUS, and FN/SFN cytology; however, these categories exhibited significantly higher Tg compared to the SM and malignant categories (p<0.001). Median TSH/Tg was lower in nondiagnostic, benign, and AUS/FLUS categories compared to the FN/SFN category; it was higher in the SM category compared to the FN/SFN category, and in the malignant category compared
to the SM category (p<0.001). Median Tg/TSH was highest in nondiagnostic, benign, and AUS/FLUS categories; whereas, it was significantly lower in the FN/SFN category compared to these categories. SM category exhibited significantly lower Tg/TSH compared to FN/SFN category, and malignant category exhibited significantly lower Tg/TSH compared to SM category (p<0.001 for each) (Table 2).

Bethesda categories were analyzed separately, and histopathologically confirmed malignancy was observed in 80 (23.3%) of the nondiagnostic, 99 (15.5%) of the benign, 37 (30.5%) of the AUS/FLUS, 11 (28.2%) of the FN/SFN, 53 (82.8%) of the SM, and 41 (100%) of the malignant cytology (Table 3). In all Bethesda categories, mean fT3 was similar in histopathologically benign and malignant nodules. In the nodules with preoperative nondiagnostic and AUS/FLUS cytology, mean fT4 was higher in malignant nodules compared to benign nodules (p=0.017 and p=0.019, respectively). Benign and malignant nodules presented similar median TSH when the Bethesda categories were considered separately, except in the nondiagnostic category where malignant nodules demonstrated higher median TSH compared to the benign ones (1.2 µIU/mL vs. 1.0 µIU/mL; p=0.012). Median Tg was significantly higher in histopathologically benign nodules compared to malignant nodules in AUS/FLUS, FN/SFN, and SM cytology (p=0.001, p<0.001, and p=0.002, respectively). Median TSH was significantly lower in benign nodules compared to malignant nodules in AUS/FLUS, FN/SFN, and SM categories (p=0.001, p<0.001, and p=0.003, respectively). Benign and malignant nodules demonstrated similar TSH/Tg in the nondiagnostic and benign Bethesda categories (p=0.347 and p=0.580, respectively). These significant and nonsignificant results for each Bethesda category were also valid for Tg/TSH, however, with inverse relations (Table 3).

Variables associated with malignancy were examined by multiple logistic regression analysis model (Table 4). In Model I, the presence of Hashimoto thyroiditis, total thyroid volume, TSH, and Tg were included as variables. In addition to the variables of Model I, TSH/Tg and Tg/TSH were included in Model II and III, respectively. Total thyroid volume (OR=1.045; p<0.001) and serum TSH (OR=1.260; p=0.043) were identified as independent predictors for malignancy in Model I. Every 1 mL increase in the volume and every 1 µIU/mL increase in TSH was associated with 1.045 and 1.260 times increased risk of malignancy, respectively. In Model II, total thyroid volume (OR=1.035; p<0.001) and TSH/Tg (x100) (OR=1.162; p<0.001) were identified as independent predictors for malignancy. Malignancy risk increased by 1.162 times for every 1% increase in TSH/Tg. In Model III, total thyroid volume (OR=1.035; p<0.001) and Tg/TSH (x100) (OR=0.993; p<0.001) were identified as independent predictors for malignancy, and a 1% decrease in Tg/TSH was associated with 1.007 (1/0.993) times increased risk of malignancy. Model II and III demonstrated higher model per-

### Table 1a. Clinicopathological features of patients with benign and malignant histopathology (categorical variables).

<table>
<thead>
<tr>
<th></th>
<th>Benign (n=370)</th>
<th>Malignant (n=244)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>84 (22.7%)</td>
<td>60 (24.6%)</td>
<td>0.589</td>
</tr>
<tr>
<td>Female</td>
<td>286 (77.3%)</td>
<td>184 (75.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Hashimoto thyroiditis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>311 (84.1%)</td>
<td>184 (75.4%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Present</td>
<td>59 (15.9%)</td>
<td>60 (24.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Nodule number</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>52 (14.1%)</td>
<td>41 (16.8%)</td>
<td>0.352</td>
</tr>
<tr>
<td>Multinodular</td>
<td>318 (85.9%)</td>
<td>20 (83.2%)</td>
<td></td>
</tr>
</tbody>
</table>

Categorical variables are shown as numbers (%).

### Table 1b. Clinicopathological features of patients with benign and malignant histopathology (numerical variables).

<table>
<thead>
<tr>
<th></th>
<th>Benign (n=370)</th>
<th>Malignant (n=244)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>47.9±10.8</td>
<td>48.6±2.5</td>
<td>0.429</td>
</tr>
<tr>
<td><strong>Total thyroid volume (mL)</strong></td>
<td>(5.6-150.1)</td>
<td>(5.9-256.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Anti TPO positivity</strong></td>
<td>36 (9.7%)</td>
<td>21 (8.6%)</td>
<td>0.639</td>
</tr>
<tr>
<td>fT3 (pg/mL)</td>
<td>3.26 ±0.5</td>
<td>3.24 ±0.5</td>
<td>0.700</td>
</tr>
<tr>
<td>fT4 (ng/dL)</td>
<td>1.20 ±0.18</td>
<td>1.19 ±0.18</td>
<td>0.898</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>1.1 (0.4-3.8)</td>
<td>1.4 (0.4-4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tg (ng/mL)</td>
<td>59.0</td>
<td>34.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH/Tg (µIU/ng)</td>
<td>0.02</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tg/TSH (ng/µIU)</td>
<td>53.1</td>
<td>23.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Numerical variables are shown as mean ±standard deviation or median (min-max).

Anti-TPO: Anti-thyroid peroxidase; fT4: Free thyroxine; fT3: Free triiodothyronine; TSH: Thyrotropin; Tg: Thyroglobulin.
The analysis was made with 1245 nodules. Numerical variables are shown as mean ± standard deviation or median (min-max).

| Bethesda categories | n   | ftT4   | p    | ftT3   | p    | TSH   | p    | Tg     | p    | TSH/Tg | p    | Tg/TSH | p    |
|---------------------|-----|--------|------|--------|------|-------|------|--------|------|--------|------|--------|------|-------|
| ND                  | 343 | 1.19±0.17 | 0.990 | 3.25±0.46 | 0.013 | 1.0   | 0.016 | 66.8   | 0.011 | <0.001 | (0.94-2137.5) | <0.001 |
| Benign              | 637 | 1.20±0.17 | 0.9    | 3.37±0.36 | 0.017 | 1.2   | 0.033 | 77.8   | 0.001 | <0.001 | (0.92-2137.5) | <0.001 |
| AUS/FLUS           | 121 | 1.20±0.16 | 0.9    | 3.29±0.99 | 0.033 | 1.1   | 0.016 | 1.0    | 0.001 | <0.001 | (2.32-531.9) | <0.001 |
| FN/SFN            | 39  | 1.19±0.21 | 0.978 | 3.44±0.53 | 0.019 | 1.2   | 0.037 | 58.0   | 0.001 | <0.001 | (3.43-802.86) | <0.001 |
| SM                 | 64  | 1.19±0.19 | 0.978 | 3.16±0.50 | 0.037 | 1.6   | 0.054 | 18.5   | 0.001 | <0.001 | (0.45-719.42) | <0.001 |
| Malignant         | 41  | 1.21±0.20 | 0.978 | 3.14±0.41 | 0.037 | 1.6   | 0.086 | 11.6   | 0.001 | <0.001 | (2.45-300.68) | <0.001 |

The TSH/Tg ratio and Thyroid Nodules

Table 2. Comparison of ftT3, ftT4, TSH, Tg, TSH/Tg and Tg/TSH levels in different Bethesda categories.

The diagnostic performance of total thyroid volume, TSH, Tg, TSH/Tg, and Tg/TSH for the prediction of malignancy was evaluated using ROC curve analysis. TSH and Tg/TSH demonstrated similar diagnostic performance compared to Model I (Nagelkerke R² = 0.310, Model II: 0.203; p < 0.001). In addition, the diagnostic performance of total thyroid volume, TSH, and Tg/TSH for the prediction of malignancy was evaluated using ROC curve analysis.

Discussion

Clinical follow-up is sufficient for most of the thyroid nodules after the clinical or cytologic exclusion of malignancy. The major challenge for thyroid cancer patients is detecting those who might have malignant thyroid nodules within normal limits, with a higher risk of malignancy. Various risk factors for the clinician (14). Varying levels of TSH-receptor mRNA are expressed in nearly all papillary thyroid cancer cells (15). Varying levels of TSH-receptor mRNA in thyroid carcinoma cells have been well described in previous studies (16, 17). Varying levels of TSH-receptor mRNA are expressed in nearly all papillary thyroid cancer cells (18). Increasing serum TSH levels, although within normal limits, have been reported to be associated with a higher risk of malignancy (19). Shi et al. (14) demonstrated that the prevalence of differentiated thyroid carcinoma (DTC) increased significantly as the serum TSH level increased. A serum TSH level of 15-30 mIU/L and 50-150 mIU/L were observed to be associated with a 5.5-fold and 16.5-fold increased risk of DTC, respectively, compared to a serum TSH level of 0.5-5 mIU/L.

Increasing serum TSH levels, although within normal limits, have been reported to be associated with a higher risk of malignancy (19). Shi et al. (14) demonstrated that the prevalence of differentiated thyroid carcinoma (DTC) increased significantly as the serum TSH level increased. A serum TSH level of 15-30 mIU/L and 50-150 mIU/L were observed to be associated with a 5.5-fold and 16.5-fold increased risk of DTC, respectively, compared to a serum TSH level of 0.5-5 mIU/L.
The analysis was made with 1245 nodules. Numerical variables are shown as mean ± standard deviation or median (min-max).

<table>
<thead>
<tr>
<th>Bethesda categories</th>
<th>n</th>
<th>fT4 p</th>
<th>fT3 p</th>
<th>TSH p</th>
<th>Tg p</th>
<th>TSH/Tg p</th>
<th>Tg/TSH p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic</td>
<td>B 263</td>
<td>1.18±0.17</td>
<td>0.017</td>
<td>3.23±0.47</td>
<td>0.147</td>
<td>1.0</td>
<td>0.012</td>
<td>67.4</td>
</tr>
<tr>
<td></td>
<td>M 80</td>
<td>1.23±0.16</td>
<td>3.31±0.41</td>
<td>0.012</td>
<td>57.6</td>
<td>0.017</td>
<td>0.825</td>
<td>0.347</td>
</tr>
<tr>
<td>Benign</td>
<td>B 538</td>
<td>1.20±0.17</td>
<td>0.406</td>
<td>3.26±0.45</td>
<td>0.192</td>
<td>0.9</td>
<td>0.330</td>
<td>80.1</td>
</tr>
<tr>
<td></td>
<td>M 99</td>
<td>1.18±0.18</td>
<td>3.33±0.51</td>
<td>0.011</td>
<td>90.4</td>
<td>0.016</td>
<td>0.013</td>
<td>0.580</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>B 84</td>
<td>1.17±0.16</td>
<td>0.019</td>
<td>3.24±0.46</td>
<td>0.060</td>
<td>1.3</td>
<td>0.922</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>M 37</td>
<td>1.25±0.16</td>
<td>3.42±0.53</td>
<td>0.014</td>
<td>41.9</td>
<td>0.043</td>
<td>0.014</td>
<td>0.001</td>
</tr>
<tr>
<td>FN/SFN</td>
<td>B 28</td>
<td>1.20±0.21</td>
<td>0.638</td>
<td>3.37±0.50</td>
<td>0.255</td>
<td>1.1</td>
<td>0.233</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td>M 11</td>
<td>1.16±0.22</td>
<td>3.59±0.60</td>
<td>0.14</td>
<td>22.0</td>
<td>0.055</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SM</td>
<td>B 11</td>
<td>1.16±0.18</td>
<td>0.594</td>
<td>3.09±0.46</td>
<td>0.584</td>
<td>1.4</td>
<td>0.715</td>
<td>86.6</td>
</tr>
<tr>
<td></td>
<td>M 53</td>
<td>1.20±0.19</td>
<td>3.18±0.51</td>
<td>1.5</td>
<td>17.5</td>
<td>0.077</td>
<td>0.027</td>
<td>0.003</td>
</tr>
<tr>
<td>Malignant</td>
<td>M 41</td>
<td>1.21±0.20</td>
<td>3.14±0.41</td>
<td>1.6</td>
<td>23.9</td>
<td>0.086</td>
<td>0.027</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 3. Comparison of fT3, fT4, TSH, Tg, TSH/Tg and Tg/TSH levels in histopathologically benign and malignant nodules with different Bethesda categories.

fT4: Free thyroxine; fT3: Free triiodothyronine; TSH: Thyrotopin; Tg: Thyroglobulin; ND: Nondiagnostic; AUS/FLUS: Atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN: Follicular neoplasm/suspicious for follicular neoplasm; SM: Suspicious for malignancy.
TSH: Thyrotropin; Tg: Thyroglobulin.
Model I: Hashimoto thyroiditis, total thyroid volume, TSH, and Tg were included.
Model II: Hashimoto thyroiditis, total thyroid volume, TSH, Tg and TSH/Tg were included.
Model III: Hashimoto thyroiditis, total thyroid volume, TSH, Tg and Tg/TSH were included.
OR: Odds ratio, CI: Confidence interval.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β±SE</th>
<th>OR</th>
<th>lower</th>
<th>upper</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I</td>
<td></td>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total thyroid volume</td>
<td>0.044±0.006</td>
<td>1.045</td>
<td>1.014</td>
<td>1.033</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH</td>
<td>0.231±0.114</td>
<td>1.260</td>
<td>1.007</td>
<td>1.576</td>
<td>0.043</td>
</tr>
<tr>
<td>Nagelkerke R² = 0.203; p &lt; 0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Model II          |        | 95% CI |       |       |    |
| Total thyroid volume | 0.034±0.003 | 1.035 | 1.012 | 1.029 | <0.001 |
| TSH/Tg            | 0.150±0.020 | 1.162 | 1.117 | 1.209 | <0.001 |
| Nagelkerke R² = 0.310; p < 0.001* |

| Model III         |        | 95% CI |       |       |    |
| Total thyroid volume | 0.034±0.003 | 1.035 | 1.012 | 1.029 | <0.001 |
| Tg/TSH            | -0.008±0.001 | 0.993 | 0.990 | 0.996 | <0.001 |
| Nagelkerke R² = 0.308; p < 0.001* |

Table 4. The predictive variables of malignancy.

TSH/Tg Ratio and Thyroid Nodules

of 1.0–1.9 mIU/L. Higher TSH has also been observed to be associated with an increased risk of lymph node metastasis and an advanced disease stage (14). Another study suggested an association between higher serum TSH and significantly increased risk of DTC. The risk of malignancy increased by 25% when the TSH levels were in the range of 0.40–1.39 mIU/L, while the
increase was 35% when the TSH levels were in the range of 1.40-4.99 mIU/L (p=0.002) (20). Similar to these findings, we also observed higher serum TSH (within normal ranges) in histopathologically malignant patients compared to benign patients. In contrast to these findings, Kim et al. (21) reported similar serum TSH levels in both papillary thyroid cancer (PTC) and benign nodules. There are also additional studies that reported lack of association between TSH and malignancy (6, 22).

Tg is closely associated with the synthesis and deposition of thyroid hormones, and small amounts of physiological Tg are released into peripheral circulation in healthy people. Detection of Tg in serum after total thyroidectomy in patients with DTC is suggestive of recurrent or persistent disease (23). Unlike the setting of postoperative cancer follow-up, the use of Tg in the preoperative assessment of thyroid nodules is debatable (12). Guarino et al. (24) did not find any diagnostic or prognostic value of preoperative Tg measurement in thyroid nodules. Another study examining the role of preoperative Tg, in addition to the US features, in predicting malignancy in thyroid nodules demonstrated that it could not be used for differentiating malignant nodules from the benign ones. The authors concluded that the only predictive factors for malignancy were suspicious US features (25). On the contrary, there exist studies which suggest a possible role of Tg in the preoperative diagnosis of malignancy (26, 27). However, Tg measurement is not yet a recommended laboratory examination during the preoperative assessment of thyroid nodules (13).

In the present study, we observed that both TSH and Tg were risk factors for malignancy in euthyroid patients with thyroid nodules. The other risk factors determined were as follows: the presence of Hashimoto thyroiditis, increased total thyroid volume, increased TSH/Tg and decreased Tg/TSH. However, the diagnostic value of total thyroid volume was higher than that of serum TSH and Tg. In the regression analysis, total thyroid volume, TSH, TSH/Tg, and Tg/TSH were identified as independent predictors of malignancy. ROC curve analysis demonstrated that both TSH/Tg and Tg/TSH were better predictors compared to the other risk factors. As confirmed in the present study, TSH/Tg appears to be one of the promising thyroid malignancy markers under investigation. Wang et al. (28) evaluated TSH/Tg in 158 benign and 242 malignant nodules, and suggested that a high preoperative serum TSH/Tg was a risk factor for thyroid cancer and that TSH/Tg correlated with malignancy better than serum TSH. Sensitivity and specificity of TSH/Tg for prediction of malignancy were 74.3% and 61.5%, respectively, in patients with normal anti-Tg levels. In the mentioned study, however, patients with positive anti-Tg and patients with Tg non-producing and TSH unresponsive tumors, such as medullary and anaplastic cancer, were not excluded from the analysis. In another study including 134 benign and 68 malignant patients, preoperative TSH and Tg were not useful in detecting thyroid cancer, and although TSH/Tg was obtained as a predictor for malignancy in the univariate analysis, it did not remain statistically significant in the multivariate analysis. Patients on antithyroid or thyroid hormone replacement therapy, along with one patient with thyroid metastasis from renal cell carcinoma, were also included in the mentioned study (29). In comparison with the previous two studies, the sample size was higher and the study population was more uniform in our study. Similar to the report by Wang et al. (28), both TSH and TSH/Tg were obtained as predictors for malignancy in the present study, and TSH/Tg was observed to be superior to TSH.

The risk of malignancy and recommended clinical management have been described for each category in The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) (30). A benign cytology accurately predicts a benign nodule that can be followed-up, while a malignant cytology carries a 97-99% risk of cancer, requiring thyroidectomy. AUS/FLUS, FN/SFN, and SM categories are cytologically indeterminate groups that present a 5-15%, 15-30%, and 60-75% risk of malignancy, respectively. In these categories, the samples are adequate for cytological evaluation and abnormal morphological findings are also observed, which are, however, not enough to confirm malignancy (31). Clinical risk factors, US features, and molecular testing results ought to be considered during the management of nodules with SM and FN/SFN cytologies. In the nodules with AUS/FLUS cytology, repeat FNAB or molecular tests may be performed, according to their clinical and US features (13). Thyroidectomy is suggested in this category in case of nondiagnostic or persistent AUS/FLUS cytology, or in case of a cytological result associated with greater
malignancy potential obtained in repeated FNAB. If repeated FNAB is benign, clinical follow-up is adequate (32). When cytology is indeterminate, both the patient and the physician stand at a crossroad and require reliable markers to choose the right path. We tried to evaluate whether we could use TSH/Tg to decide the management of nodules with different Bethesda categories, particularly the indeterminate ones. We demonstrated that TSH/Tg was markedly higher in malignant nodules compared to the benign ones in the AUS/FLUS, FN/SFN, and SM categories. In addition, it was increasing in the malignant nodules as the cytology moved from the Bethesda category with lower to higher risk of malignancy. A similar situation was noted for Tg/TSH. In the AUS/FLUS, FN/SFN, and SM categories, Tg/TSH was lower in histopathologically malignant nodules compared to benign nodules, and it decreased as the cytology moved from the Bethesda category with lower to higher risk of malignancy.

There were certain limitations in our study. We attempted to conduct a uniform group study, however, while doing that, a selection bias might have occurred inherently. The ratio of histopathologically confirmed malignant nodules was higher than expected in all the Bethesda categories. Our center is one of the biggest tertiary referral centers in our country, and a considerable number of patients with high suspicion of malignancy are referred from other centers. Another limitation was that the number of nodules was low in certain Bethesda categories. We are aware that patients with multiple nodules might have dominated the findings, particularly TSH/Tg and Tg/TSH, in the module-based analysis. In such patients, the nodule exhibiting highest malignancy potential in the cytological examination might have been chosen instead of including each nodule separately in the analysis. However, this approach would lead to consideration of only the nodule with the highest risk of malignancy and the other nodules would not be represented. In general practice, when cytology is benign, thyroidectomy is suggested as an option only in case of large lesions or indeterminate results such as follicular neoplasm. This might partly explain the higher Tg levels in the histopathologically benign group. Lastly, we included only euthyroid patients in our study. This may also be considered a strong aspect of our study. However, from another perspective, patients with normal TSH, fT3, and fT4 displaying hot nodules in scintigraphy might exhibit lower TSH than the patients displaying warm or cold nodules. We could not exclude this possibility as we did not perform thyroid scintigraphy.

Conclusion

High serum TSH, even within normal ranges, was observed to be associated with an increased risk of thyroid malignancy. However, TSH/Tg proved to be a better predictor of malignancy compared to TSH or the other risk factors identified in this study. TSH/Tg could serve as a novel, cost-effective, practical, and applicable index for risk stratification of thyroid nodules, and when combined with other factors, it could assist clinicians in forming important decisions regarding the management of nodules. Further prospective and large-scale research are required to support these findings.

Ethics: Ethics Committee Approval and Informed Consent: Ethical review board of Yıldırım Beyazıt University Atatürk 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

References


Implication of Findings from International Studies on Hypoglycemia for Management of Diabetes in Insulin-treated Patients in Turkey

Hipoglisemi ile İlgili Uluslararası Çalışmalardaki Bulguların Türkiye'de Insülin Tedavisi Gören Hastalarda Diyabet Yönetimine Etkileri

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Received: 24/07/2017
Accepted: 18/08/2017

Abstract

Recent estimates indicate that diabetes occurs in 16.5% of the Turkish population, which is in keeping with the increasing global prevalence of diabetes. Following rapid economic growth, increase in life expectancy and changes in lifestyle over the past decade have resulted in the placement of a tremendous cost burden on the Turkish economy by diabetes and associated co-morbidities, representing 20% of overall spending on healthcare.

Maintaining good glycemic control is vital for effective diabetes management. Long-term studies have demonstrated the ability of intensive glucose-lowering strategies to prevent or delay co-morbidities associated with diabetes. However, insulin intensification is commonly associated with hypoglycemia, which is regarded as the most significant barrier to attaining and maintaining good glycemic control. Patients are often unaware of the potential negative impact of hypoglycemia on their long-term health, as well as on daily functions. In addition, hypoglycemia and its associated symptoms or co-morbidities greatly influence adherence and dosing behavior, patients’ quality of life (QoL), and significantly affect economic productivity. The aim of this study is to review the impact of hypoglycemia in patients with diabetes, focusing on the implications of findings from international and Turkish studies for the management of hypoglycemia in Turkish patients.

Keywords: Hypoglycemia; nocturnal hypoglycemia; Turkish diabetic patients; insulin analog; pre-mixed insulin; co-morbidity; quality of life; glucose monitoring

Özet

Son yapılan çalışmalar, Türkiye nüfusunun %16,5’inde diyabet olduğunu göstermektedir ki bu diyabetin artan kuresel prevalansını ile uyumluştur. Hızlı ekonomik büyümenin ardından, geçtiğimiz on yılda yaşam beklentisinin artması ve yaşam tarzındaki değişiklikler, diyabet ve buna eşlik eden komorbiditelerin Türkiye ekonomisine muazzam bir maliyet yükü getirmesine ve toplam sağlık harcamalarının %20’sini oluşturmasıyla sonuçlanmıştır.


Anahtar kelimeler: Hipoglisemi; gece hipoglisemi; Türk diyabetik hastalar; insülin analogu; premiks insülin; komorbidite; yaşam kalitesi; glukoz takibi

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Turkish Journal of Endocrinology and Metabolism published by Türkiye Klinikleri

DOI: 10.25179/tjem.2017-57369
Introduction

The prevalence of diabetes is increasing worldwide and according to recent global estimates, there will be approximately 642 million adults with diabetes by 2040 (1). The age-standardized prevalence of diabetes in Turkey ranges from 13.7% to 17.0%, with diabetes being more common among women than men (17.2% vs. 16.0%, p=0.008) (2). Recent figures indicate that over 6.5 million adults in Turkey have been diagnosed with diabetes, which represents an increase of 90% over the past 12 years (2). The International Diabetes Federation predicts that the prevalence will rise to nearly 12 million by 2035 (3).

Following rapid economic growth in the past decade, the increase in the life expectancy and changes in lifestyle (2) has resulted in diabetes placing an enormous cost burden on the Turkish economy, accounting for ~1% of the national gross domestic product and approximately 20% of the overall spending on healthcare (4). Therefore, maintenance of good glycemic control by keeping glycosylated hemoglobin levels and pre- and post-prandial blood glucose levels within recommended limits are vital in the management of diabetes (5, 6). However, the management of diabetes cannot follow a ‘one size fits all’ approach. Rather, treatment customization by balancing the benefits of glycemic control with its potential risks such as the adverse effects of glucose-lowering medications (particularly hypoglycemia), as well as the patient’s age and health status among other concerns, is a more viable option (6).

Long-term studies have demonstrated that intensive glucose-lowering strategies (e.g., insulin-based treatment regimens) can prevent or delay co-morbidities such as long-term vascular complications which are commonly associated with diabetes (7-10). However, the most common adverse condition associated with insulin therapy for the management of type 1 and type 2 diabetes mellitus (T1DM or T2DM) is hypoglycemia (11), which is regarded as the most significant barrier preventing patients from attaining and maintaining good glycemic control (12).

Hypoglycemic episodes are usually defined as either asymptomatic, wherein patients are unaware of a current episode, or symptomatic, wherein the hypoglycemia ranges from mild to moderate (non-severe) which patients can self-treat, to severe episodes requiring third-party assistance and which can potentially be life-threatening (13). The aim of this article is to review the impact of hypoglycemia in patients with diabetes, focusing on the implications of data from international and Turkish studies for the management of hypoglycemia in Turkish patients.

Incidence of Hypoglycemia

The development of hypoglycemia, although sometimes asymptomatic, is often initiated by a combination of neurogenic or autonomous (including palpitations, sweating, shaking, hunger) and neurological symptoms (behavioral changes, inability to concentrate, confusion, seizures) (13). The patterns of symptoms vary with individuals and patients can learn to recognize their own pattern of hypoglycemic symptoms (13). Discussing these patterns with a healthcare professional can help in prediction or early recognition of hypoglycemic episodes before they become severe. However, patients often do not report non-severe hypoglycemia, which leads to lack of practical data on the incidence of hypoglycemia and its impact on the management of diabetes in Europe in general, and specifically in Turkey, the Middle East, and North African regions.

The difficulty in estimating the incidence of hypoglycemia is complicated by a high proportion of patients with unrecognized mild to moderate hypoglycemic episodes. Several studies have assessed the incidence of hypoglycemia, including the PREDICTIVE study (14), two European online surveys (15, 16), and two Danish surveys (17, 18). The baseline data from the PREDICTIVE study, which was a large, prospective, international observational study of >19,000 adults with uncontrolled T1DM or T2DM on current treatment and initiating basal insulin, showed an overall incidence of 47.5 episodes and 9.2 episodes of hypoglycemia per patient-year among patients with T1DM and T2DM, respectively. The incidence rates for severe hypoglycemia and nocturnal hypoglycemia were reported as 3.0 and 13.8 episodes, respectively, in patients with T1DM, and 0.8 and 3.4, respectively, in patients with T2DM (14). The baseline frequency of hypoglycemia in insulin-treated patients in the PREDICTIVE study increased with the duration of diabetes, a number of daily injections, and variation in fasting glucose. A similar trend was observed in the Turkish cohort of the study, which included 613 patients with T1DM and 2092 patients with T2DM (14). Another survey recently assessed the self-reported, non-severe hypo-
glycemic episodes in Europe, by recruiting patients via consumer panels, nurses, telephone recruitment, and family referrals to complete four online questionnaires. This survey reported an annual hypoglycemic incidence of 94 episodes per year in T1DM and 21 to 36 episodes per year (depending on regimen) in insulin-treated patients with T2DM (15). A single-center, cross-sectional survey in Denmark, which had recruited successive patients with T2DM with a previously arranged outpatient appointment, assessed patients based on a questionnaire seeking information on the number of hypoglycemic episodes experienced in the past, status of awareness regarding hypoglycemia, and socio-demographic information. This study reported an incidence of <0.5 episodes per patient-year (16). Another survey conducted in Germany, France, and the UK assessed patients via 11 key questions, including their understanding, perceptions, and daily experiences of hypoglycemia, during a 10-min online questionnaire. Also, a cross-sectional questionnaire survey including patients with T1DM from six Danish healthcare institutions evaluated severe hypoglycemic episodes reported during the preceding year, and mild hypoglycemia during the preceding week. Both these studies reported an incidence of severe hypoglycemic episodes of ~1.0-2.4 episodes per patient-year among patients with T1DM (17, 18). Patients with diabetes regularly experience non-severe hypoglycemic episodes (19), with daytime episodes being more common than nocturnal episodes. Patients are often unaware of non-severe hypoglycemic episodes, and often do not report their self-managed mild to moderate hypoglycemic episodes to their primary care team (15). This makes it difficult to estimate the true incidence of non-severe hypoglycemic episodes. In addition, surveys have shown that nocturnal episodes cause anxiety or worry and concern about the potential negative impact on long-term health (19-21). Furthermore, nocturnal episodes appear to have a greater impact on patients’ daily functioning, and the discomfort experienced during a nocturnal episode can carry over to the next day, causing tiredness, irritability, lack of concentration, and fluctuating blood sugar levels (21).

Risk Factors for Hypoglycemia
Risk factors for hypoglycemia are individual-specific and include endogenous insulin deficiency, duration of diabetes, history of hypoglycemia, misinformation or complete lack of awareness on symptoms of hypoglycemia, intensive insulin regimens or stringent glycemic targets, recent moderate or intensive exercise, disrupted sleep patterns, and renal failure (13). Among the risk factors, a high proportion of patients with diabetes reports unawareness or altered awareness about symptoms of hypoglycemia (15) as one factor affecting detection of mild hypoglycemic episodes. In patients with the lack of awareness about hypoglycemia, the risk of severe hypoglycemia increases threefold (16), as mild hypoglycemia, which often precedes severe episodes, is not recognized (13).

Insulin regimens, particularly those with a high number of injections, have also been associated with an increased risk of hypoglycemia (14). In the multicenter, international prospective observational TREAT study containing a Turkish cohort of patients, the incidence of hypoglycemia was higher in patients treated with a basal-bolus regimen compared with a basal-only regimen at six months of treatment (22). The trend remained unchanged over the 2-year study period, with 75% of patients on basal-bolus regimens being injected four times per day (22). Similar long-term findings in the UK cohort were reported by the GAPP2 survey (20). One or more hypoglycemic episodes were reported during the previous month by 29% of patients on basal insulin and 46% on basal-bolus insulin, while 11% and 14% of patients, respectively, reported hypoglycemia during the past year (20).

Impact of Hypoglycemia
Hypoglycemia and associated symptoms or co-morbidities greatly impact adherence and dosing behaviors (23), the patients’ quality of life (QoL) (19, 21), and significantly affect economic productivity (24-26).

Dosing irreularities
A high proportion of patients with diabetes report being worried about hypoglycemia. Nocturnal episodes cause patients more concern than daytime episodes (19), as they can remain undetected during the night. Many physicians have indicated that they would prescribe intensive insulin therapy more frequently if it were not for concerns over hypoglycemia (23). In addition, there is a high level of fear of hypoglycemia among patients who have previously experienced a severe hypoglycemic episode (27). This fear often results in missed injections and non-
adherence to treatment, as patients attempt to take corrective action to avoid recurrent hypoglycemic episodes (23, 28). A recent single-center survey (n=345) in Turkey showed that patients with T1DM, who had experienced severe hypoglycemia, not only had greater levels of fear and anxiety over recurrent hypoglycemia but also displayed higher treatment adherence behavior than patients with T2DM (29). This behavior may be partially attributable to the fact that patients with T1DM acknowledge that insulin treatment is indispensable for their well-being, resulting in higher levels of treatment control and self-efficacy to avoid potentially life-threatening hypoglycemic episodes than patients with T2DM.

Another study assessing dosing irregularities in response to self-treated hypoglycemic episodes was the GAPP2 - a multinational, cross-sectional survey (19). In the UK cohort of the GAPP2 survey, 15-25% of patients either reduced, missed, or mistimed at least one dose of insulin during the month prior to assessment. In the majority of cases, this action was intentional, due to concerns over hypoglycemia (20). Similar results were observed in the Canadian cohort of GAPP2, wherein 23%, 26%, and 13% of patients reported missed, mistimed, or reduced doses of insulin during the month before assessment (28). These patients also cited concern over the risk of hypoglycemia as the most common reason for intentional dose irregularity (28). Many patients have also been reported to intentionally maintain a state of hyperglycemia to give themselves a ‘safety margin’ to avoid hypoglycemia (17), and to adopt other behaviors associated with avoidance of hypoglycemia.

Health-related quality of life

It has been demonstrated that self-reported hypoglycemic episodes have a strongly negative impact on the QoL of patients with T2DM (30). Patients with symptoms of hypoglycemia have reported significantly higher rates of shakiness, sweating, excessive fatigue, drowsiness, impaired concentration, dizziness, hunger, asthenia, and headache, compared with patients without hypoglycemia (31). The increase in frequency and severity of hypoglycemic symptoms not only had a detrimental effect on patients’ rating of their QoL (30-32), but the mere experience of hypoglycemia was also associated with a higher likelihood of developing depression (30).

Economic impact of hypoglycemia

In addition to the impact on patients’ QoL and treatment efficacy, the economic impact of hypoglycemia can be categorized into direct costs to treat severe hypoglycemic episodes, and indirect costs that arise due to lost work productivity following severe or non-severe episodes (26). Almost half of the Turkish patients with diabetes are aged between 40 and 64 years (2), and good glycemic control to reduce the risk of hypoglycemia is essential for the economic well-being of patients and their families.

In 2010, it was estimated that the direct cost of managing major hypoglycemia in Turkey was 87 Turkish lira per acute episode (33). Patients have been demonstrated to increase blood glucose monitoring in response to hypoglycemic episodes (19), which may also increase costs associated with the management of hypoglycemia (26). However, the wider economic impact of severe and non-severe episodes (e.g., absence from work, impacts on QoL and on careers) is largely unknown, as few studies based in Turkey have analyzed this parameter till date. Data from other studies in Europe or the US provide a clear indication of the impact. A recent report of two surveys assessing daytime and nocturnal hypoglycemia in 300 patients with T1DM and T2DM highlighted that daytime and nocturnal hypoglycemic episodes negatively affected patients’ sleep and work productivity (26). Nocturnal episodes appeared to have the highest impact on patients, with 29% of respondents going to work late, 16% leaving work early, and 12% missing at least one day of work due to a nocturnal episode (26). Nocturnal episodes, in particular, have been shown to not only affect the individual experiencing hypoglycemia, but also their bed partner (26).

A large UK survey on 861 patients with T1DM and T2DM reported that health-related QoL and work-related productivity decreased with increase in the frequency and severity of hypoglycemia (25). Each episode of nocturnal non-severe hypoglycemia was reported to be responsible for the loss of productivity corresponding to 3.3-7.5 h (24). A European study reported that 10% of patients surveyed had taken time off work due to severe hypoglycemia in the past 12 months (17), while severe hypoglycemia resulted in 34 emergency room visits per 1000 patients with diabetes in the US (34).
Reducing the Risk of Hypoglycemia

More than half of all hypoglycemic episodes can be predicted by regular self-monitoring of blood glucose levels (35). Hence, self-monitoring of blood glucose levels by patients, supported by appropriate training on the signs and symptoms of hypoglycemia, is an important strategy in raising awareness on hypoglycemia and reducing the risk of future severe hypoglycemic episodes. However, a recent single-center, questionnaire-based Turkish study (n=380) showed that patients with T1DM, particularly those with chronic disease, often did not achieve optimal glycemic outcomes and adopted fewer self-management behaviors (36), emphasizing the need for an integrated approach toward monitoring diabetes and patient support. Another study suggested that patients’ levels of anxiety and fear of hypoglycemia should be assessed regularly by the primary care team and that patients should be encouraged to record their concerns or hypoglycemic experiences in a diary (29).

Modern long-acting basal insulin analogs such as insulin glargine (IGlar), insulin detemir (IDet), and the ultra-long-acting insulin degludec (IDeg), may reduce the risk of hypoglycemia associated with diabetes. Published clinical evidence has reported that IGlar and IDet have similar, low-risk rates of hypoglycemia. Differences in efficacy among modern basal insulin analogs were demonstrated in 2886 patients of the Turkish cohort in the multinational observational SOLVE study, wherein IDet was associated with a lower risk of minor hypoglycemia than IGlar (37). The findings highlighted the need to customize treatment of individual patients to match their clinical requirements in an environment, which is slow to intensify diabetes treatment to include insulin therapy (13). However, a pre-planned meta-analysis of seven phase III clinical trials comparing IDeg with IGlar on patients with T1DM or T2DM, reported a significantly lower risk of hypoglycemia associated with IDeg compared to IGlar, in the overall pooled population. The reduction in risk of hypoglycemia associated with IDeg was more evident after stabilization of the dose of insulin (>16 weeks) (38), indicating that intensive blood glucose monitoring may be required shortly after initiation of insulin therapy, and also that treatment with insulin analogs was generally a viable option for treatment intensification.

Premixed insulins containing bolus injections of short-acting insulin (basal-bolus) are an easier alternative to the intensification of basal insulin, for patients unable to achieve glycemic targets on basal insulin alone. However, premixed insulin formulations have been associated with a slightly higher degree of hypoglycemia and weight gain than basal regimens (5). This view was recently challenged, when it was demonstrated that switching from a biphasic human insulin premix to a premixed insulin analog could reduce the incidence of hypoglycemia (39). The results from a subgroup analysis showed that such a switch decreased the rates of severe as well as overall hypoglycemia, and improved glycemic control (40). Several patients preferred premixed insulin analogs over premixed human insulin, owing to their more favorable pharmacokinetic profiles, which allowed dosing immediately before or after a meal and helped avoid late postprandial hypoglycemia (41). However, despite premixed insulins offering better glycemic control and improved convenience than basal-bolus regimens, studies still reported varied effects on the incidence of hypoglycemia. PREFER, a study comparing premixed insulin and basal-bolus regimens, demonstrated a lower incidence of severe hypoglycemia for premixed therapy, compared with the basal-bolus regimen, while the rates of non-severe hypoglycemia, weight gain, and nocturnal hypoglycemia were similar (42). In contrast, comparison of premixed insulins and basal insulin therapy demonstrated a higher overall risk of hypoglycemia with premixed insulin analogs (4, 43).

Guidelines on the Management of Hypoglycemia

The treatment and management of T1DM, T2DM, and diabetes-related complications are governed by international guidelines, which are regularly updated (5, 6). In addition, several countries have developed local guidelines to allow for a more tailored and relevant treatment approach. Turkish guidelines for the management of diabetes and its complications are published and regularly updated by the Society for Endocrinology and Metabolism of Turkey (44). These guidelines include specific recommendations for the prevention and management of hypoglycemia (Table 1). One key element highlighted in the Turkish guidelines was the recommendation for increased awareness of, and adherence to, international and local guidelines on the prevention and management of hypoglycemia, in order to minimize the risk of severe and nocturnal hypoglycemia. However, despite the existence of national guidelines (5, 45) and high
SEMT Approaches and Recommendations

Hypoglycemia prevention

Education
- After treatment for any hypoglycemic episode, the causes must be reviewed and training should be repeated where necessary
- Training must be provided to individuals with diabetes and family members in a timely fashion to increase knowledge and skills in diabetes self-management
- Individuals with T2DM who use insulin or insulin secretagogues must be assessed for the risk of hypoglycemia

Monitoring
- All individuals with diabetes and family members must be trained in PG measurement to enable them to adjust therapy on the basis of the results
- Individuals with T2DM, especially elderly patients identified with hypoglycemia associated with sulfonylurea use must be monitored for 24-48 h
- In T1DM SMPG must be an integral part of treatment

Treatments and glycemic targets
- Since the risk of symptomatic hypoglycemia and nocturnal hypoglycemia is lower in individuals receiving basal insulin, long-term acting insulin analogs are preferable to NPH in patients at high hypoglycemic risk
- Glycemic targets must be established for pre-pubertal children to minimize hypoglycemia (especially nocturnal):
  - Age < 6 years: FPG, 100-180 mg/dL; NPG, 110-200 mg/dL; HbA1c, 7.5-8.5%
  - Age 6-12 years: FPG fast, 100-180 mg/dL; NPG, 100-180 mg/dL; HbA1c < 8.0%
  - Age 13-18 near-adult glycemic targets must be achieved: FPG: 80-120 mg/dL; NPG, 90-130 mg/dL; HbA1c, 6.5-7.0%, 48-53 mmol/mol

Nutrition and rescue treatment
- In the individuals with T2DM, the absorption of proteins may increase insulin response without increasing blood glucose concentration. Consequently, proteins must not be used in acute hypoglycemia or nocturnal hypoglycemia
- To avoid recurrent hypoglycemia, after the hypoglycemia has resolved, main meals and snacks must be given at planned intervals. If there is a period of more than one hour between one meal and the next, a snack consisting of 15 g carbohydrate and protein must be given.
- It is preferable that individuals with diabetes do not take alcohol. Consumption of alcohol may cause various health problems in individuals with diabetes with impaired glycemic control, individuals at high risk of hypoglycemia or with uncontrolled hyperlipidemia
- Individuals with T1DM must be warned of increased risk of late hypoglycemia if they take alcohol. To reduce hypoglycemic risk, reduction of alcohol must be exercised and measures such additional carbohydrate consumption, reduction of insulin dose and more frequent SMBG may be applied

Hypoglycemia management

Treatment
- Any carbohydrate source containing glucose may be used in case of hypoglycemia, through ingestion of 15-20 g glucose is preferred
- Fat-containing products (e.g., chocolate or wafer) should not be used
- Response to treatment of hypoglycemia must be obtained within 10-20 min
  - Mild hypoglycemia must be treated with 15 g oral carbohydrates (4 sugar cubes, or 150 mL fruit juice or lemonade). PG must be measured after 15 min, if < 80 mg/dL an additional 15 g of carbohydrates must be given
  - Moderate hypoglycemia must be treated with 20 g carbohydrates (5 sugar cubes, or 200 mL fruit juice or lemonade). PG must be measured after 15 min, if < 80 mg/dL an additional 15 g of carbohydrates must be given
  - Severe hypoglycemia must be treated with s.c. or i.m. glucagon injection and emergency medical assistance must be summoned
- The relatives of patients with high risk of hypoglycemia should be taught how to administer a glucagon injection
- An unconscious patient with severe hypoglycemia, which does not resolve with glucagon should receive 10-25 g i.v. glucose (20-50 mL 50% dextrose within 1-3 min or 50-150 mL 20% dextrose within 5-10 min)

Monitoring
- PG levels must be measured one hour after the hypoglycemic event and if necessary additional treatment must be given.

Table 1. Turkish (SEMT) recommendations for hypoglycemia management (44).

FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; i.m.: Intramuscular; i.v.: Intravenous; NPG: Nocturnal plasma glucose; OAD: Oral antidiabetic drug; PG: Plasma glucose; s.c.: Subcutaneous; SEMT: Society of Endocrinology and Metabolism of Turkey; SMPG: Self-monitored plasma glucose; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.
levels of input from primary care and specialist teams, optimal outcomes are often not seen in clinical practice (36). To allow patients and physicians to make informed treatment decisions, and to encourage improved monitoring and prevention of hypoglycemia, country-specific data on rates of hypoglycemia and QoL/health-economics impacts are required. Therefore, patients identified to be at risk of hypoglycemia or those having high levels of fear or anxiety of hypoglycemia should be monitored closely and offered specialist counseling support.

Conclusions and Strategies for the Future
As the prevalence of diabetes increases in Turkey, the Middle East, and North African regions, treatment of diabetes and ultimately treatment intensification with insulin is inevitable. Intensive blood glucose-lowering strategies to attain glycemic control are closely associated with the risk of hypoglycemia, which has a strong negative impact on patient QoL, as well as important health-economic consequences. Altered awareness of hypoglycemia, coupled with a lack of communication or under-reporting of hypoglycemia between patients and physicians has created lack of real-world data on the actual incidence of non-severe and severe hypoglycemic episodes in insulin-treated patients with diabetes and the scale of the burden of hypoglycemia, in clinical practice in Turkey.

A multinational study monitoring the symptoms, management, and prevention of hypoglycemia is required. One such study, the IO-HAT (International Operations Hypoglycemia Assessment Tool) (46), which enhanced the hitherto limited information on the prevalence of hypoglycemia in countries such as Turkey, has recently been completed. The IO-HAT study consisted of two parts that retrospectively and prospectively assessed severe and non-severe hypoglycemia using patient-led self-assessment questionnaires, and explored associations between hypoglycemia, patient co-morbidities, treatment regimen, and QoL. In addition to the IO-HAT study results, which included a large Turkish cohort (>2000 patients), regular patient self-monitoring of blood glucose levels, improved patient awareness regarding symptoms of hypoglycemia, and integrated communication between patients and primary care teams will help in the improvement of hypoglycemia awareness, and reduction in the burden of overall and particularly severe hypoglycemia in insulin-treated patients with diabetes.

Acknowledgements: Medical writing support was provided by ApotheCom and funded by Novo Nordisk Region, International Operations AG.

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.

Funding source: Funding for the development of this review was provided by Novo Nordisk A/S.

Authorship Contributions
Rifat Emral, Ramazan Sari and Serdar Güler have critically reviewed and contributed to every stage of the manuscript development.

References


**Case Report**

**A Different Cause of Malignant Hypercalcemia in a Breast Carcinoma with Bone Metastasis**

Kemik Metastazı Olan Meme Kanserli Bir Hastada Malign Hiperkalseminin Farklı Bir Nedeni


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**Abstract**

Hypercalcemia refers to a condition of calcium levels in blood above the normal range. Most common causes of hypercalcemia include overactivity of parathyroid glands, also known as primary hyperparathyroidism (PHPT), and malignancies. These two disorders contribute to 90% of etiologies leading to hypercalcemia. Various types of cancer manifest hypercalcemia, including breast carcinoma, lung carcinoma, and multiple myeloma. For instance, hypercalcemia is observed in 30% to 40% of patients with breast cancer. The occurrence of hypercalcemia in cancers is attributed to bone metastasis and paraneoplastic syndromes. Malignancies may also be accompanied by PHPT. Therefore, in cancer patients with hypercalcemia, serum parathyroid hormone (PTH) level should be assessed. In the present study, we present a case of breast cancer with hypercalcemia to emphasize the role of PHPT in malignancies.

**Keywords:** Breast cancer; hypercalcemia; positron emission tomography; primary hyperparathyroidism

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**Introduction**

The most common causes of hypercalcemia are Primary Hyperparathyroidism (PHPT) and malignancies. These two disorders cover 90% of etiologies leading to hypercalcemia (1). The cancers where hypercalcemia is mostly seen are breast carcinoma, lung carcinoma, and multiple myeloma. Hypercalcemia is observed in 30-40% of breast cancer patients (2). Breast carcinoma is most frequent cancer in women and the second cause of death after lung cancer. The most frequent causes of hypercalcemia in cancer patients are bone metastasis and paraneoplastic syndromes (1-4). Malignancies might also be found with PHPT. Therefore, in cancer patients with hypercalcemia, serum parathyroid hormone (PTH) level should be assessed (5). We present this case in order to further emphasize the fact that PHPT might accompany malignancies.

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Turkish Journal of Endocrinology and Metabolism published by Türkiye Klinikleri

DOI: 10.25179/tjem.2017-56502
Case Report

A 37-year-old female patient was admitted to our medical oncology center nearly three months ago with a mass in the left breast and left axilla. Ultrasonographic examination of the breast revealed a 2.5-cm mass lesion with irregular contoured microcalcifications. Pathological examination of tru-cut biopsy revealed invasive ductal carcinoma. Positron emission tomography-computerized tomography (PET-CT) was conducted that revealed a multi-centric tumor in the left breast, metastatic lymph nodes in left axilla, and a lytic metastatic lesion in the eighth thoracic vertebra (T8) (Figure 1A). The patient underwent modified radical mastectomy combined with axillary lymph node dissection at another center. The postoperative pathologic examination of the patient showed estrogen receptor (ER), negative; progesterone receptor (PR), 35% moderately positive; and c-erb-2, 3 positive (+) invasive papillary carcinoma. Thus, the patient was diagnosed with metastatic left breast carcinoma (T3N3M1, bone metastasis). At admission, the patient presented with weakness, loss of appetite, and weight loss. Physical examination revealed a blood pressure of 110/70 mmHg, heart rate of 88/min, respiratory rate of 18/min, and body temperature of 36.8 °C. Operation scars were present in left breast and left axilla. Initial examinations revealed a hemoglobin (Hb) level of 11.1 g/dL (13.6–17.2); white cells, 7,000 mm$^3$ (5,200–12,400 mm$^3$); creatinine, 0.9 mg/dL (0.6–1.3 mg/dL); calcium, 12.4 mg/dL (8.8–10.6 mg/dL); and phosphate, 2 mg/dL (2.5–4.5 mg/dL). Magnetic resonance imaging (MRI) verified bone lesion seen in PET-CT. The lesion at T8 found in the thoracic vertebrate MRI with contrast was considered metastasis, and hypercalcemia was predicted to be related to bone metastasis. The treatment consisted of chemotherapy with docetaxel (80 mg/m$^2$/d) + trastuzumab (8 mg/kg loading dose on day 1) followed by 6 mg/kg on day 1 every 21st day. For bone metastasis, treatment comprised zoledronic acid (4 mg/every 21st day). During chemotherapy, follow-up calcium values were between 12.0 and 12.5 mg/dL; no symptom due to hypercalcemia was observed. PET-CT was used to evaluate the response to therapy after six cure chemotherapy; it revealed metastasis in the eighth thoracic vertebra. Further, diffusely increased F-18 Florodeoksiglukoz (FDG) uptake by the skeletal system was noted, particularly by all costochondral junctions, sternum, and iliac bones as signs of a metabolic bone disorder (Figure 1B). Due to a pre-diagnosis of PHPT, PTH and 25-OH vitamin D3 levels were measured: PTH was 1,256 pg/mL (15–65 pg/mL), and 25-OH vitamin D3 was 28.6 ng/mL (20-100 ng/mL). Parathyroid ultrasound (USG) examination revealed a 25 × 16-mm hypoechoic parathyroid lesion in the inferior right thyroid lobe. The Tc-99m methoxyisobutyl isonitrile (MIBI) scintigraphy further reported it to be parathyroid adenoma (Figure 2). Thus, hypercalcemia in the patient was attributed to parathyroid adenoma, for which ultrasonographic laser ablation and percutaneous ablation alternatives were suggested. Surgery was performed after knowing patient’s preference. Postoperative pathological diagnosis was parathyroid adenoma. After parathyroid surgery, PTH and calcium levels returned to normal values.

Literature Review and Discussion

Hypercalcemia is a life-threatening electrolyte disorder. Its detection in a patient with breast cancer suffers from poor prognosis, primarily because the presence of hypercalcemia usually indicates skeletal metastasis (4).

The occurrence of malignancy-associated hypercalcemia depends on three mechanisms, namely the production of local cytokines due to osteolytic metastasis (osteoclast activating factors), the secretion of parathyroid hormone-related protein (PTHrP) by tumor cells, and the synthesis of 1,25-dihydroxyvitamin D (calcitriol) by tumor cells (1). In patients with non-metastatic solid tumors, PTHrP is the most common cause of hypercalcemia (3,5,6). However, since in most patients with hypercalcemic malignancy, cancer is clinically proven, the measurement of PTHrP levels is not required. Binding of PTHrP to PTH receptors results in various biological properties of PTH. Breast cancer cells upon metastasis cause the bones to synthesize PTHrP more frequently than soft tissue tumors or primary tissue tumors. The resulting osteolysis can be cured by administration of anti-PTHrP antibodies, leading to a rise in serum calcium levels, which, in turn, is associated with morbidities related to hypercalcemia (3).

Primary hyperparathyroidism is an autonomous disorder that occurs due to excessive secretion of PTH from parathyroid glands. Of primary hyperparathyroidism (PHPT), 80 to 85% depends on a single parathyroid adenoma (7). PHPT is common in cancer patients. Also, the incidence
of cancer is high in patients with primary hyperparathyroidism. The mechanisms underlying the co-existence of PHPT with certain malignancies, including breast cancer, are still unknown (8). Since PHPT may accompany cancer cases, PTH levels should be measured in cancer patients with hypercalcemia. A high serum level of both PTHrP and PTH indicates PHPT besides malignancy. In mild hypercalcemia, PHPT should be first taken into account; if acute and severe hypercalcemia is present, hypercalcemia related to malignancy should be considered. In cases where serum PTH is high and PTHrP is low, hypercalcemia is attributed to PHPT (3). In our patient, PTH was high but PTHrP was not assessed. The 18 F-FDG PET-CT scanning is an effective and highly specific method in tumor staging, re-staging in recurrent metastatic disease, and follow-up of response to therapy in breast cancer (9). It

Figure 1: Maximum intensity projection (MIP) images of two different 18 F-FDG PET/CT scanning that was performed for staging and evaluation of response to the therapy in a patient with breast cancer. The first MIP image demonstrates increased F–18 FDG uptake in tumor tissue, axillary metastatic lymph nodes, and bone metastasis in the eighth thoracic vertebrae (black arrow) (A). Second 18 F-FDG PET/CT MIP image reveals metastasis in eighth thoracic vertebrae (black arrow) beside diffusely increased F–18 FDG uptake at skeletal system, particularly at all costochondral junctions, sternum and iliac bones as signs of a metabolic bone disorder (B).

Figure 2: Tc-99m MIBI parathyroid scintigraphy demonstrating parathyroid adenoma in the inferior of right thyroid lobe.
also contributes to the evaluation of non-oncological pathologies as in the case presented. However, 18 F-FDG PET-CT scans must be evaluated with caution so that benign cases are not mistakenly reported as metastasis. In our case, the cause of hypercalcemia was first considered as bone metastasis related to breast cancer. However, since signs of a metabolic bone disorder were observed in second 18 F-FDG PET-CT, biochemical analysis and imaging were performed, and a parathyroid adenoma was found as the cause of hypercalcemia. Clinical presentation of hypercalcemia include gastrointestinal (nausea, vomiting, abdominal cramp, pancreatitis, peptic ulcer), neuromuscular-psychiatric (attention and memory deficits, lethargy, confusion, coma), renal (nephrolithiasis, diabetes incipitus, nephrocalcinosis, dehydration, renal insufficiency), cardiovascular (hypertension, arrhythmia, short QT), and bone symptoms (pain, cystitis fibrosis). Additionally, weight loss might be observed in accordance with the etiology (10). None of these were present in our patient. The laboratory results play a key role in determining the severity and etiology of hypercalcemia. Hypercalcemia is considered mild if calcium value is 10 to 12 mg/dL; moderate if 12 to 14 mg/dL, and hypercalcemic crisis if 14 to 16 mg/dL. Intact PTH levels have a critical role in the investigation of etiology of hypercalcemia. Low PTH levels are indicative of malignancy. In the absence of malignancy, other endocrinopathies (hyperthyroidism, acromegaly, surrenal insufficiency) should be questioned. If PTH value is normal or high, 24-hour urine analysis should be performed. A low value of 24-hour urine calcium reflects familial hypocalciuric hypercalcemia; if the value is high, primary or tertiary hyperparathyroidism should be investigated (10). The treatment of primary hyperparathyroidism is surgical. Alternatives to surgery include ultrasonographic laser ablation and percutaneous ablation. Both methods can be done when surgery is contraindicated. Ablation is performed by USG-guided percutaneous interstitial laser photoagulation (ILP) that can detect the diseased parathyroid gland. Additionally, ethanol might be injected into parathyroid adenoma percutaneously or angiographically for ablation (10). As our patient had stage 4 breast carcinoma (bone metastasis) with a life expectancy of nearly two years, ethanol injection was planned. But since the patient refused, surgery was performed. In conclusion, the involvement of PHPT in malignancy cases should be considered. Therefore, patients with malignancy and hypercalcemia, even if there is a bone metastasis, should be evaluated for PTH to exclude PHPT.

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.

ConflictofInterest: 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.

Author Contributions

Concept: Bünyamin Aydın Design: Sevim Süreyya Çerçi Data Collection or Processing: Murat Koçer, Banu Kale Analysis or Interpretation: Pınar Talip Yörükoglu, Fathı Çolak Literature Search: Mustafa Yıldız, Celal Çerçi Writing: Bünyamin Aydın Çıkar

References

Two Siblings with Triple-A Syndrome: Endocrinologic and Neurologic Features

Triple A Sendromlu İki Kardeş: Endokrinolojik ve Nörolojik Özellikler

Abstract

Triple-A syndrome is a rare, multi-systemic disease and is characterized by adrenal insufficiency, achalasia, or alacrimal. The symptoms may also involve neurologic manifestations, including pyramidal findings and peripheral motor neuropathy. Other findings include autonomic dysfunction and cerebellar ataxia. In the present study, we present the case of two siblings with triple-A syndrome with neurologic manifestations. The neurologic abnormalities can lead to misdiagnosis owing to resemblance to the manifestations of other neurologic disorders. Therefore, the cases with triple-A syndrome should be carefully evaluated and examined for clinical and neurophysiologic signs.

Keywords: Triple-A syndrome; adrenal insufficiency; peripheral neuropathy

Özet


Anahtar kelimeler: Triple-A sendromu; adrenal yetmezlik; periferal nöropati

Introduction

Triple-A syndrome is an autosomal recessive disorder that was first described in 1978 by J. Allgrove (1). Most prominent characteristics of triple-A syndrome include esophageal achalasia, alacrimal, and adrenal insufficiency. It is a rare and multi-systemic syndrome, characterized by progressive nerve degeneration; autonomic nerve system findings could also be present (2). It is a genetic disorder with an autosomal recessive pattern involving a mutation in the AAAS (Achalasia-Addisonism-Alacrimia Syndrome) gene coding for the protein called ALADIN (Alacrima Achalasia aDrenal Insufficiency Neu-

rologic disorder) (3,4). The neurologic manifestations of the syndrome have largely been ignored and not extensively studied for a long time. Gazzarian et al. defined this syndrome as 4A instead of 3A owing to the association with autonomic nervous system findings (5). In the present study, we present two cases with the diagnosis of triple-A syndrome where we studied the association of this syndrome with neurologic manifestations.

Case 1

A 28-year-old female patient presented with hyperpotassemia, hyperpigmentation, and hypocortisolism and was initially diagnosed with adrenal...
insufficiency. She was referred to our hospital with complaints of weakness and fatigue at the age of 11 years. She was later referred to the gastroenterology department with complaints of dysphagia when she was 18 years old and on hydrocortisone treatment. Esophagography revealed achalasia and balloon dilatation was performed (Figure 1a, 1b). After the detection of xerophthalmia, she was diagnosed with triple-A syndrome due to the presence of alacrima with adrenal insufficiency and achalasia. As her weakness and fatigue persisted, she was examined for neurologic signs. Deep tendon reflexes were increased with the presence of proximal muscle weakness. The findings of electromyography (EMG) were compatible with axonal sensory-motor polyneuropathy (Table 1). Autonomic neuropathy tests were normal.

Case 2
A 21-year-old male patient (brother of Case 1) was examined at the age of three years owing to family history and diagnosed with adrenal insufficiency and achalasia. He was referred to our clinic with the development of xerophthalmia and was diagnosed with triple-A syndrome. We conducted balloon dilatation to treat achalasia in the gastroenterology department. His blood pressure was 100/60 mmHg, and he complained of weakness and fatigue. His 25-hydroxyxylase vitamin D level was 35 nmol/L and was administered the initial loading dose of vitamin D replacement. Later, the maintenance dose was preferred. Neurologic examination revealed increased deep tendon reflexes and dysarthria. Muscles were atrophic in all extremities (Figure 2a, 2b). EMG findings suggested polyneuropathy. However, motor neuropathy was the dominant manifestation in EMG (Table 2). Autonomic neuropathy tests were normal.

Discussion
The patients with triple-A syndrome usually present endocrinologic and gastroenterologic symptoms at the time of diagnosis. Neurologic manifestations could be seen later, leading to these getting missed out if the clinician is unaware of the presence of neurologic manifestations. Here, we present two cases with triple-A syndrome with neurologic manifestations. Triple-A syndrome is primarily characterized by adrenal insufficiency, achalasia, and alacrima. Adrenal insufficiency and achalasia are usually manifested during the first decade of life. Achalasia is present in about 75% of cases and usually manifests as dysphagia, especially for liquids, whereas adrenal insufficiency manifests as hypoglycemia and hypotension. Main features of ad-

<table>
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<tr>
<th>Amplitude (CMAP: mV, SNAP: µV)</th>
<th>Distal latency (msec)</th>
<th>Conduction Velocity (m/sec)</th>
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<tr>
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<tr>
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<tr>
<td>Peroneal CMAP</td>
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<td>3.8 (&gt;4.7)</td>
</tr>
<tr>
<td>Posterior tibial CMAP</td>
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<tr>
<td>Sural SNAP</td>
<td>NR (&gt;8)</td>
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renal insufficiency include fatigue, loss of appetite, weight loss, low blood pressure, and darkening of the skin. It occurs due to primary adrenal insufficiency. Mineralocorticoid production is preserved in most patients with triple-A syndrome but may be impaired in 15% of patients. The third major feature of triple-A syndrome is a reduced or absent ability to secrete tears (alacrima). Most people with triple-A syndrome have all three features, although some present only two (6).

The retrospective study conducted by Vallet et al. (7) presented eight cases of triple-A syndrome with neurologic manifestations. Age at the onset ranged from one month to 20 years. Two cases manifested symptoms during early childhood and included adrenal insufficiency and achalasia. Neurologic symptoms in these cases were noticed during teenage. The other six cases presented neurologic symptoms as initial symptoms that occurred from early childhood to early adulthood. There was a delay of at least 17 years before the diagnosis of triple-A syndrome was made. When adrenal insufficiency and achalasia are present at the time of diagnosis of triple-A syndrome, neurologic dysfunction could be coexisting. In pediatric cases of triple-A syndrome, adrenal insufficiency, achalasia, and alacrima are usually present at the time of diagnosis; however, these could be missing in adult- or late-onset triple-A syndrome. Thus, in patients presenting neurologic dysfunction as the predominant clinical manifestation, it is useful to search for the history of achalasia and alacrima and perform hormone assay for adrenal insufficiency. The main neurologic pathology, in eight cases reported by Vallet et al. (7), included pyramidal syndrome and peripheral neuropathy. Peripheral neuropathy resulted in distal wasting and orthopedic deformity such as pes cavus (7). Further, motor fibers were more affected, and few patients had a sensory deficit. In the present study, the cases manifested sensory–motor neuropathy. The EMG findings of the two cases are presented in Table 1 and 2. A decrease in amplitude values was compatible with axonal neuropathy. EMG values revealed motor neuropathy to be more prominent. Needle EMG also ruled out myopathy. The EMG recordings pointed to sensory-motor neuropathy in our cases that resulted in wasting of hand muscles and pes cavus in case 2. The differential diagnosis of axonal sensorimotor peripheral polyneuropathy revealed a dominant hereditary polyneuropathy due to distinct muscular atrophy in distal muscles. However, this diagnosis was eliminated owing to the absence of similar symptoms in upper zones and the absence of an increase of deep tendon reflexes in the neurologic examination. The laboratory test, done for acquired polyneuropathy, revealed nor-

Table 2. Neurophysiologic examination of case 2.

<table>
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<th></th>
<th>Amplitude (CMAP:mV, SNAP: µV)</th>
<th>Distal latency (msec)</th>
<th>Proximal latency (msec)</th>
<th>Conduction Velocity (m/sec)</th>
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<tr>
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<td>4.5 (&gt;3.1)</td>
<td>10.2</td>
<td>34 (&gt;50)</td>
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<tr>
<td>Peroneal CMAP</td>
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<td>6.1 (&gt;4.7)</td>
<td>16.1</td>
<td>31 (&gt;40)</td>
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<tr>
<td>Posterior tibial CMAP</td>
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<td>34 (&gt;40)</td>
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<tr>
<td>Sural SNAP</td>
<td>10 (&gt;8)</td>
<td>3.7 (&gt;3.8)</td>
<td></td>
<td>38 (&gt;45)</td>
</tr>
</tbody>
</table>

mal values, and no medication was reported in patient’s history that could lead to polyneuropathy. In the light of all these, neuropathy was considered to be associated with this syndrome along with the presence of usual symptoms of the syndrome. Autonomic neuropathy and ataxic gait originating from cerebellum were present in the cases studied by Vallet et al. (7). These were not observed in our cases. Autonomic dysfunction, such as orthostatic hypotension, bladder dysfunction, diarrhea or constipation, sexual dysfunction, and dyshidrosis, was present in six cases reported by Vallet et al. (7). Bulbar and facial deficiencies were observed in all eight patients; velar insufficiency, tongue atrophy, and orbicularis oris dysfunction were observed to various degrees (7). In our study, case 2 had dysarthria as a bulbar dysfunction. Bulbar symptoms can be confusing and difficult to differentiate from achalasia. Both bulbar dysfunction and achalasia can cause swallowing difficulties. In bulbar dysfunction, dysphagia is associated with dysfunctioning of IX, X, and XI cranial nerves. On the other hand, achalasia only causes lower dysphagia of esophagus. In our cases, no autonomic dysfunction existed.

The cognitive deficit can be present as neurologic manifestation. For instance, cases studied by Vallet et al. (7) presented with frequent and mild cognitive dysfunction; however, this finding was not present. The neurologic findings misdiagnosed in all patients at the beginning were juvenile amyotrophic lateral sclerosis (ALS), spinocerebellar ataxia, spinal muscular atrophy, mitochondrialopathy, adrenoleukodystrophy, multiple sclerosis, and Charcot-Marie-Tooth neuropathy (7). The final diagnosis of the triple-A syndrome with neurologic manifestations in the study by Vallet et al. (7) was made by a team of neurologist, gastroenterologist, and endocrinologist. In these eight cases, genetic analysis was also performed, which, unfortunately, could not be performed in our cases.

Apart from the study of Vallet et al., other reports from the literature describe peripheral neuropathy and pyramidal syndrome as other neurologic manifestations (2, 8–10). The most common neurologic manifestation was lower limb weakness in the study of Nakamura et al. (11) who described six cases. All patients presented upper and lower motor neuron signs. Sensory disturbance and autonomic dysfunction were observed in 29% and 57%, respectively. This study group indicated hyperreflexia as a remarkable finding of triple-A syndrome that distinguished this disease from other causes of peripheral neuropathy. In the present study, increased deep tendon reflexes reflected pyramidal syndrome.

Peripheral nerve pathology is not a well-known finding of triple-A syndrome with an unclear etiology. The presence of sural nerve biopsy has been described by a few reports. The two cases in the present study presented axonal degeneration and a loss of myelinated and unmyelinated nerve fibers (8, 10). Previous reports have attributed the manifestation of neuropathy to a defect in ACTH receptors present on neurons/glia with secondary demyelination; however, further studies did not support this theory (12).

The study of Nakamura et al. (11) involved adult- or late-onset triple-A syndrome with achalasia where the diagnosis was made after neurologic symptoms appeared. Achalasia is an important manifestation of triple-A syndrome diagnosis. All patients in the study conducted by Nakamura et al. had alacrima. On the contrary, adrenal insufficiency was absent in late-onset triple-A cases in the study. Regular follow-ups of these patients are required to keep a check on the levels of cortisol hormone, the non-compliance to which may lead to the missing out of the crisis.

**Conclusion**

Peripheral neuropathy is a common neurologic finding in patients with triple-A syndrome. The chances of neurologic manifestations getting misdiagnosed as some other neurologic pathologies remain high. Therefore, an association of neurologic manifestations with triple-A syndrome should be considered during its diagnosis. Achalasia and alacrima serve as important clues for the diagnosis. An awareness of the association of neurologic manifestations with triple-A syndrome allows us to follow incomplete neurologic findings with appropriate tests, leading to a better diagnosis.

**Author Contributions**

Conflict of Interest: No conflict of interest is declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References


Pregnancy-Associated Osteoporosis: Long-term Follow-up of a Patient with Two Pregnancies

Gebelekli İlişkiyi Osteoporoz: İki Gebeliği Boyunca Hastanın Uzun Dönem Takibi

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DOI: 10.25179/tjem.2017-56508

Abstract

Pregnancy and lactation-associated osteoporosis (PLO) is a rare condition. Its pathogenesis and etiology are unknown. It is frequently observed in the last trimester of pregnancy and during lactation period in primigravida. The symptoms may begin with back and hip pain. In addition, it could be complicated by osteoporotic fractures and disability. Here, we report a long-term (7 years) follow-up of a 29-year-old patient who underwent two pregnancies and was diagnosed with PLO both the times. The first time this patient was admitted to our outpatient clinic with a complaint of severe back pain, she was in the lactation period of her first pregnancy. Laboratory findings, X-ray imaging and densitometry revealed osteomalacia (25-OH Vit D = 6.8 ng/dL), multiple vertebral fractures (T6-T9-T11-T12-L1), and osteoporosis (L1–L4 T-score: −4.6). After the treatment for vitamin D deficiency, she was treated with risedronate and Ca-vitamin D supplementation. At the end of the 2-year follow-up, she terminated the use of risedronate [by her own decision] and continued with the Ca–vitamin D supplementation. Six years after the first pregnancy, she became pregnant again. In the postpartum period, she complained about gait difficulty, and severe hip and back pain. With the help of laboratory results, imaging, and densitometry (L1–L4 T-score: −3.9), she was diagnosed with PLO second time, and was treated with zoledronic acid, in addition to Ca–vitamin D supplementation. Once back pain occurs in the postpartum period, PLO should be considered in differential diagnosis even if the patient is taking Ca–vitamin D supplementation.

Keywords: Pregnancy; osteoporosis; lactation; vitamin D; bisphosphonates

Özet


Anahtar kelimeler: Gebelik; osteoporoz; laktasyon; D vitamini; bisfasonatlar
Introduction
Osteoporosis (OP) is the deficiency of bone mineral density (BMD) and microarchitectural deterioration of bone tissue (1). Although OP is a common condition, pregnancy and lactation-associated osteoporosis (PLO) is a rare condition, characterized by the occurrence of fractures during late pregnancy or postpartum period without any underlying disorders (2). Approximately 100 cases have been described in the literature since the first definition of PLO (3). Here, we report a 7-year follow-up of a PLO patient, with lower back pain due to several vertebral fractures during her first pregnancy, and hip pain during the second pregnancy. This case differs remarkably from the other PLO cases in the literature due to the development of OP after both the pregnancies.

Case
A 22-year-old primigravid woman was admitted to the outpatient clinic with a complaint of lower back pain that began in the last trimester of her pregnancy. Her pain worsened during the lactation period.

The patient had no history of any chronic disease, including endocrine or metabolic disorders, smoking, and drug or alcohol use, which could affect bone metabolism. Upon physical examination, palpation of the processus spinosi of thoracolumbar vertebrae and paravertebral muscles were painful, and the range of motion in the thoracolumbar region was restricted. Anteroposterior and lateral roentgenograms of thoracolumbar vertebrae revealed multiple vertebral fractures (T6-T9-T11-T12-L1). Dual-energy X-ray absorptiometry (DXA) analysis revealed decreased BMD in the lumbar spine (L1-L4 T-score: −4.6) and proximal femur (femur neck T-score: −2.4). The laboratory analysis revealed decreased levels of 25-OH vit D (6.8 ng/dL), increased levels of alkaline phosphatase (ALP) (141 IU/L), and parathyroid hormone (PTH) (98 ng/L) in blood, along with uncountable levels of Ca in urine. Total blood count, sedimentation rate, C-reactive protein levels, levels of Ca and P in blood, and the thyroid and liver function tests were normal.

On the basis of the clinical and laboratory findings, the patient was diagnosed with osteomalacia and PLO. After normal vitamin D levels were achieved with oral supplementation of elementary Ca (1200 mg/day), vitamin D (800 IU/day) and risedronate (35 mg/week) treatment was initiated. She was asked to cease breastfeeding. The patient was recommended the use thoracolumbosacral orthosis. A home-based rehabilitation program, including muscle strengthening, and range of motion and relaxation exercises, was prescribed.

In the third month of the follow-up, her back pain decreased, with normal levels of vitamin D, PTH, and ALP. At the end of the first year of follow-up, she had no complaint of back pain. Her medical treatment (Ca, vitamin D, and risedronate) was continued for two years. She complained about the weekly regime of risedronate and discontinued the bisphosphonate treatment. However, she adhered to the daily Ca and vitamin D supplementation. The vitamin D levels and the DXA results of the patient are listed in Table 1.

After six years, she became pregnant with her second child. Pregnancy was uneventful. The patient’s back pain and severe hip pain appeared at two months postpartum. She experienced difficulty in walking. Upon physical examination, the range of motion in the thoracolumbar region and hips were painful and restricted, with greater pain in the hips. Laboratory evaluations (including vitamin D, PTH, Ca, and ALP) were normal.

The results of spinal radiography and magnetic resonance imaging (MRI) of the hips were examined. DXA revealed osteoporosis, with L1-L4 T-score = −3.9 and femur neck T-score = −1.5. No new fractures were detected on the thoracolumbar roentgenogram, and the MRI revealed bilateral bone marrow edema of femoral head and neck (Figure 1).

The clinical picture was identified as PLO. The patient was advised to cease breastfeeding. Zoledronic acid (5 mg/year) treatment was added to the daily Ca and vitamin D supplementation after the cessation of breastfeeding. Rest, activity limitation, and non-steroidal anti-inflammatory drugs were recommended for pain control. Her pain and difficulty in gait diminished within three months.

Discussion
PLO was first described by Nordin & Roper. The exact prevalence of PLO is unknown to date because the literature about this condition is limited to a small number of case reports (3). It is defined as a rare condition that develops in the last trimester of pregnancy or postpartum in lactation period, primarily in the first pregnancy. It mostly becomes complicated with fractures in the vertebrae, hip, and sacrum (1). Its etiology is not certain and a precise mechanism has not been
substantiated to date. On the other hand, there are particular risk factors, which include poor nutrition, family history of osteoporotic fractures, genetic factors, physical inactivity, low body weight, vitamin D deficiency, smoking, drug usage (corticosteroids, etc.), and the secondary causes of OP (4). When our patient was first diagnosed with PLO, the risk factors identified were low body weight and vitamin D deficiency, and that she was a primigravida. She had no chronic disorder or drug usage history, and no other factor leading to secondary OP. During pregnancy, calcium absorption from the intestine increases to ensure fetal calcium needs. If this adaptation is not enough to fulfill the calcium demand, maternal bones face resorption during the third trimester. Trabecular bone mineral density may decrease up to 5%–10% in the subsequent lactation period, due to various hormonal changes that occur in order to supply calcium to milk (5). However, maternal skeleton recovers approximately 6–12 months after weaning, by means of uncertain mechanisms (6). This physiological resorption and recovery process is not related to the number of gravidities, breastfeeding length, or to further diagnosis of osteoporosis or fragility fractures (7–9).

The role of vitamin D in the modulation of increased intestinal transport of calcium needs to be further clarified. Despite the fact that our patient’s vitamin D levels were normal, both before and during the second pregnancy, OP was observed again. It is difficult to provide a clear comment due to the varied results of different studies examining the relationship between BMD and vitamin D. Although certain studies have reported an association between low vitamin D levels and low BMD (10), this relationship has not been demonstrated in other studies (11).

The most common symptom of PLO is back pain, especially in the thoracolumbar region. Intense pain is observed when PLO is complicated with fractures. This intense and sustained pain limits...
the patient’s daily life activities and even leads to difficulty in gait (2). While our patient was suffering from severe back pain during her first pregnancy, difficulty in gait was the predominant symptom during her second pregnancy.

Thoracolumbar pain during pregnancy should be examined carefully, and imaging may be performed when required. The patients with ongoing symptoms after delivery or during the lactation period should be investigated through radiological examination, including thoracolumbar vertebrae/hip radiography, MRI, and DXA. Our patient had multiple vertebral fractures, with low BMD in the first pregnancy. Then, in the second pregnancy, patient’s DXA scores and bilateral hip MRI were compatible with OP, without a new fracture. Consistent with the literature, our patient’s BMD in the lumbar spine was observed to be lower than that in the femoral region in the repeated DXA measurements (12).

The limited literature available regarding the treatment of PLO concerns the use of bisphosphonates, strontium ranelate, and teriparatide, together with supplementation of Ca and vitamin D (1). The literature lacks a treatment algorithm or guidelines concerning the efficacy of different treatments. Therefore, the medical treatment should be customized for each individual patient through a detailed assessment, together with the appropriate rehabilitation program.

In our case, we first initiated risedronate treatment together with Ca and vitamin D supplementation. In the second pregnancy of the patient, another bisphosphonate—zoledronic acid—was preferred due to the single-dose regimen, which was more convenient for our patient. In conclusion, PLO may reoccur with each pregnancy and this should be kept in mind. Therefore, it is important to continue the follow-up with the patient. Further, bisphosphonates are safe agents that could be used in the treatment of PLO.

Author Contributions


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

Review of Clinical Recommendations on Prolactinoma and Pregnancy

Prolaktinoma ve Gebeliğe Dair Klinik Önerilerin Gözden Geçirilmesi

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Abstract
Prolactinomas are the most common hormone-secreting pituitary adenomas. Prolactinomas account for nearly 30–40 percent of all the pituitary adenomas. Although it affects individuals over a wide age range, it is more common in 20–40-year-old female patients, who are in their reproductive age. Prolactinomas may cause hypogonadism, menstrual cycle dysfunction (oligomenorrhea or amenorrhea) and infertility (luteal phase abnormalities or anovulation) in premenopausal women. When pregnancy is excluded, hyperprolactinemia in approximately 10 to 20 percent of the patients results in amenorrhea. Women with untreated prolactinomas are generally unable to achieve pregnancy, as the hyperprolactinemia affects the pulsatility of gonadotropin-releasing hormone (GnRH) and diminishes follicle-stimulating hormone (FSH) as well as luteinizing hormone (LH) secretion. The sum of these effects induces amenorrhea, infertility, and hypogonadism, thereby posing difficulties in fertility. Therefore, ovulation and fertility usually improve after proper diagnosis and treatment of prolactinoma. Therefore, during the surveillance of these patients, the onset of pregnancy is a common phenomenon. Management of these pregnancies may sometimes be challenging and require a multidisciplinary approach involving an endocrinologist, a gynecologist, a radiologist and an experienced neurosurgeon in order to achieve the best outcomes both for the patient as well the infant. In this report, the authors aim to summarize the consensus statements and the current guidelines for clinical practice.

Keywords: Prolactinoma; pregnancy; dopamine agonists

Özet

Anahtar kelimeler: Prolaktinoma; gebelik; dopamin agonistleri

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Prolactinomas are the most common out of all the hormone-secreting pituitary adenomas. Women with untreated prolactinomas are not able to achieve pregnancy, as the hyperprolactinemia affects the pulsatility of GnRH, diminishes FSH and LH secretion and induces amenorrhea, infertility, and hypogonadism, thereby posing difficulties in fertility (1). For this reason, in most cases, prolactinoma is diagnosed prior to conception. Nevertheless, ovulation and fertility are normally improved by proper diagnosis and treatment. As a result, prolactinomas in a pregnant woman are certainly challenging and require a multidisciplinary approach involving an endocrinologist, a gynecologist, a radiologist and an experienced neurosurgeon to achieve the best outcome. In this report, the authors aim to summarize the consensus statements and the current guidelines for clinical practice.

**Guideline Recommendations Regarding Preconception Period in Patients with Prolactinomas**

The risk of enlargement of a microprolactinoma during pregnancy is nearly 1.5–4.5% with symptomatic growth occurring in about 2% of the cases. However, the risk of symptomatic enlargement of a macroadenoma is greater than 15% (2, 3). The currently available dopamine agonists (DAs) include bromocriptine, cabergoline, and quinagolide (the latter is not approved for use in the United States). It should be noted that the restoration of ovulation, once DA therapy has been started, occurs even before normoprolactinemia is achieved and the patient should be informed about this outcome (4). In women who are treated before conception, DA may also induce shrinkage of the pituitary tumor (a reduction of greater than 25% is expected in the tumor size in around 70% of the patients) (5).

- Achieving a normalization of PRL levels and a tumor size <10 mm before conception is recommended for macroadenomas (6, 7).
- In women of childbearing age, the use of mechanical contraception should be advised once drug treatment has been initiated for macroadenomas. This is because ovulation and fertility may rapidly be recovered after the normalization of PRL levels (6, 8).
- Transsphenoidal adenomectomy may be an option for women with microadenoma or macroadenoma that is either intolerant or refractory to DAs, or prepregnancy tumor debulking by surgery (thereby decreasing the risk of clinically significant enlargement during pregnancy) would be an option in cases of macroadenomas that do not decrease in size with DA treatment or in those who cannot tolerate bromocriptine or cabergoline (6, 8). Transsphenoidal surgery may also cause hypopituitarism, thus requiring the subsequent use of assisted reproduction techniques such as induction of ovulation with gonadotropins and lifelong hormone replacement therapy (6-9). It is for this reason that resumption of the DA is probably less harmful to the mother and the fetus as compared to surgery (6). Therefore, the teamwork of multidisciplinary specialists is required for the careful planning of pregnancy in women with prolactinoma. Ideally, this should arise before conception, so that a full assessment of the risks and benefits of DA therapy can be assessed during pregnancy (10).
- Whenever pregnancy is planned or detected in macroadenomas, cabergoline should be discontinued and bromocriptine should be introduced although this drug also crosses the placental barrier (6). Bromocriptine is the “oldest” of all the dopamine agonists and has been tested more extensively than the other compounds, but there is far less published experience with cabergoline (8). Therefore, bromocriptine has been shown to be safe for use during early gestation (up to the first four weeks after conception, a critical period for early organogenesis).
- In women with microprolactinomas who want to become pregnant, the use of clomiphene citrate or gonadotropin therapy is suggested when ovulation cannot be restored by DAs alone (6).

**Guideline Recommendations Related to pregnancy in Patients with Prolactinoma**

- Women with prolactinomas must be instructed to discontinue DA therapy as soon as they discover that they are pregnant (6-8, 10). In selected patients with macroadenomas who are on DA therapy and become pregnant and who have not had prior tumor debulking by surgery, it may be sensible to continue DA throughout the pregnancy, especially if the tumor is invasive or is abutting the optic chiasm (6, 8, 10).
- When symptomatic tumor growth occurs (presence of a headache or visual deterioration), treatment with bromocriptine should be restarted, if previously discontinued (6, 8). If the enlarged tumor does not respond to reinstitution with DA therapy within 2–3 weeks, transsphenoidal surgery (in the second trimester) or deliv-
ery (if the pregnancy is far enough) must be considered (6-8, 10).

■ There is no evidence of increased teratogenicity associated with the use of bromocriptine or cabergoline during pregnancy (6, 8, 10). Quinagolide, on the other hand, has shown a poor safety profile in the relatively small number of pregnancies that have been reported, and it should not be prescribed to women who wish to become pregnant (8).

■ In pregnant patients with prolactinomas, performing serum prolactin measurements during pregnancy is not recommended. This is for the reason that in normal pregnancy, serum prolactin levels increase 10-fold, reaching levels of 150 to 300 g/Liter by term (6-8).

■ In general, microprolactinomas and macroprolactinomas that are localized to the sella do not undergo symptomatic growth during pregnancy, therefore the use of routine pituitary MRI during pregnancy is not recommended in these patients. Because the risk of symptomatic tumor growth is low, these patients may be followed up by clinical examination during each trimester, unless there is clinical evidence of tumor growth by symptoms such as headaches or visual deterioration (6-10). However, formal assessment of the visual fields in macroprolactinoma should be performed every three months or even more frequently if the adenoma prior to conception is close to the optic chiasm (8). When such clinical manifestations appear, imaging must be performed with unenhanced MRI. If the growth of the pituitary mass is identified, re-institution of DA (preferably bromocriptine) for the remainder of the pregnancy may provide a control over the tumor and in addition, monthly clinical assessment is required (including visual fields). Therefore, the onset of a new or a worsening headache, or a change in vision or both, mandates the urgent performance of formal visual field testing and a pituitary MRI without the use of gadolinium (6-8).

**Guideline Recommendations Related to the Postpartum Period in Patients with Prolactinoma**

■ Women wishing to breastfeed their infants should not be given DA because the resulting decrease in serum PRL levels will impair lactation. There are no data available suggesting that breastfeeding leads to an increase in tumor size (6, 7).

■ The spontaneous remission of hyperprolactinemia has only been reported in women with microprolactinomas. In these cases, long-term discontinuation of treatment with DAs after birth, along with regular monitoring for at least five years may be considered (6).

**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:** Sema Yarman, Gülşah Yalın; Design: Gülşah Yalın, Sema Yarman; Control/Supervision: Sema Yarman; Data Collection and/or Processing: Gülşah Yalın, Sema Doğançan; Analysis and/or Interpretation: Sema Yarman, Gülşah Yalın; Literature Review: Gülşah Yalın, Sema Doğançan; Writing the Article: Sema Yarman, Gülşah Yalın; Critical Review: Sema Yarman; References and Fundings: Sema Yarman; Materials: Gülşah Yalın, Sema Yarman.

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