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

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

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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) cytologies. 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 ve benign tiroid nodüllerinin ayırımında yeni bir prediktif markır olarak tirotropin (TSH) tiroglobulin (Tg) oranını araştırmayı amaçladık.

Gereç ve Yöntemler: 2007 ve 2014 arasında opere edilen ötiroid hastalar retrospektif olarak değerlendirildi. Tiroid hastalığı veya cerrahi öyküsü olanlar ve artmış anti-tiroglobulin antikorları olanlar dışlandı.Histopatolojik olarak benign ve malign gruplar klinikopatolojik özellikler ve serum TSH, Tg, TSH/Tg oranı açısından karşılaştırıldı.

Bulgular: 370 (%60.3) benign ve 244 (%39.7) malign hasta vardı.Benign hastalara göre malign hastalarda anlamlı 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 nodül vardı. Sitopatalojik dağılım şöyleydi; 343 (%27.6) nondiagnostik, 637 (%51.2) benign, 121 (%9.7) önemi belirlenemeyen atipi/önemi belirlenemeyen folliküler lezyon (ÖBA/ÖBFL), 39 (%3.1) folliküler neoplazi/folliküler neoplazi şüphesi (FN/FNŞ), 64 (%5.1) malignite şüphesi (MŞ) ve 41 (%3.3) malign. TSH, Tg ve TSH/Tg Bethesda kategorilerinde anlamlı olarak farklıydı (her biri için p<0.001). Median TSH/Tg benignde (0.013) en düşük ve MŞ (0.054) ve malign sitolojilerde (0.086) en yüksekti. ÖBA/ÖBFL, FN/FNŞ ve MŞ kategorilerinde, TSH/Tg oranı malign nodüllerde benign nodüllere göre yüksek bulundu (sırasıyla, p=0.001, p<0.001 ve p=0.003). Regresyon analizinde TSH/Tg; TSH ve Tg'ye göre daha yüksek tanısal performansa sahipti (p<0.001).

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

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

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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 (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA; UniCel Dxl 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 Bethesta 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

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

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

Categorical variables are shown as numbers (%).

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

	Benign (n=370)	Malignant (n=244)	p
Age (years)	47.9±10.8	48.6 ±2.5	0.429
Total thyroid volume (mL)	20.9 (5.6-150.1)	31.4 (5.9-256.8)	<0.001
Anti TPO positivity	36 (9.7%)	21 (8.6%)	0.639
fT3 (pg/mL)	3.26 ±0.5	3.24 ±0.5	0.700
fT4 (ng/dL)	1.20 ±1.18	1.19 ±0.18	0.898
TSH (µIU/mL)	1.1 (0.4-3.8)	1.4 (0.4-4.0)	<0.001
Tg (ng/mL)	59.0 (1.8-2175.0)	34.1 (1.3-1000)	<0.001
TSH/Tg (µIU/ng)	0.02 (0.001-1.09)	0.04 (0.001-2.24)	<0.001
Tg/TSH (ng/µIU)	53.1 (0.92-1587.3)	23.8 (0.45-2137.5)	<0.001

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.

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/Tg 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 2. Comparison of fT3, fT4, TSH, Tg, TSH/Tg and Tg/TSH levels in different Bethesda categories.

Bethesda categories	n	fT4	p	fT3	p	TSH	p	Tg	p	TSH/Tg	p	Tg/TSH	p
ND	343	1.19±0.17	0.990	3.25±0.46^{d,e,f}	0.033	1.0 (0.4-3.4)^{c,d,e,f}	<0.001	66.8 (1.8-1000)^{e,f}	<0.001	0.016 (0.001-1.065)^{d,e,f}	<0.001	64 (0.94-2137.5) ^{d,e,f}	<0.001
Benign	637	1.20±0.17		3.27±0.46^{d,e,f}		0.9 (0.4-3.7)^{c,d,e,f}		80.3 (1.8-2175)^{e,f}		0.013 (0.001-1.088)^{d,e,f}		77.86 (0.92-2137.5)^{d,e,f}	
AUS/FLUS	121	1.20±0.16		3.29±0.49^{d,e,f}		1.3 (0.4-4.0)^{a,b,e,f}		79.6 (4-1000)^{e,f}		0.017 (0.002-0.431)^{d,e,f}		58.80 (2.32-531.9)^{d,e,f}	
FN/SFN	39	1.19±0.21		3.44±0.53^{a,b,c,e,f}		1.2 (0.5-3.7)^{a,b,e,f}		59.0 (8.8-894)^{e,f}		0.037 (0.001-0.291)^{a,b,c,e,f}		27.00 (3.43-802.86)^{a,b,c,e,f}	
SM	64	1.19±0.19		3.16±0.50^{a,b,c,d}		1.6 (0.4-3.3)^{a,b,c,d}		22.3 (1.3-1000)^{a,b,c,d}		0.054 (0.001-2.240)^{a,b,c,d,f}		18.59 (0.45-719.42)^{a,b,c,d,f}	
Malignant	41	1.21±0.20		3.14±0.41^{a,b,c,d}		1.6 (0.5-3.8)^{a,b,c,d}		23.9 (2.1-439)^{a,b,c,d}		0.086 (0.003-0.408)^{a,b,c,d,e}		11.6 (2.45-300.68)^{a,b,c,d,e}	

The analysis was made with 1245 nodules.
Numerical variables are shown as mean ± standard deviation or median (min-max).
fT4: Free thyroxine; fT3: Free triiodothyronine; TSH: Thyrotropin; 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.
^{a}p<0.05 vs. ND, ^{b}p<0.05 vs. Benign, ^{c}p<0.05 vs AUS/FLUS, ^{d}p<0.05 vs. FN/SFN, ^{e}p<0.05 vs SM, ^{f}p<0.05 vs. Malignant.

formance compared to Model I. (Nagelkerke's R^2 for Model III: 0.308, Model II: 0.310, Model I: 0.203; p<0.001).

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/Tg and Tg/TSH demonstrated similar diagnostic performance, which was higher than that of total thyroid volume, TSH, and Tg (p<0.001). In addition, the diagnostic performance of total thyroid volume was higher than that of TSH and Tg (p<0.001) (Figure 1).

A cut-off value of >0.03 for TSH/Tg could predict malignancy with 61.9% sensitivity and 74.1% specificity. A Tg/TSH value of ≤25.85 also demonstrated 61.1% sensitivity and 76.8% specificity in predicting malignancy.

Discussion

Clinical follow-up is sufficient for most of the thyroid nodules after the clinical or cytological exclusion of malignancy. The major concern is to identify malignant lesions preoperatively. Detecting the minority of thyroid cancer patients is a great challenge for the clinician (14). Various risk factors that might assist in predicting malignancy in thyroid nodules have been investigated to date. High serum TSH is one of those risk factors. TSH plays an important role in the regulation of thyroid differentiation genes, growth factors, and receptors (15). Mitogenic effect of TSH on thyroid carcinoma cells has been well described previously (16, 17). Varying levels of TSH-receptor mRNA are expressed in nearly all papillary and follicular carcinomas (18).

Increasing serum TSH levels, although within normal limits, have been reported to be associated with a higher risk of malignancy in patients with thyroid nodules (8, 19). Shi et al. (14) demonstrated that the prevalence of differentiated thyroid cancer (DTC) increased significantly as the serum TSH levels increased. A serum TSH level of 1.9-4.8 mIU/L and a serum TSH level >4.8 mIU/L were observed to be associated with 1.57 (95% CI: 1.03-2.40; p=0.038) times and 5.71 (95% CI: 2.31-14.14; p=0.0002) times of the likelihood of malignancy, respectively, compared to a serum TSH level

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

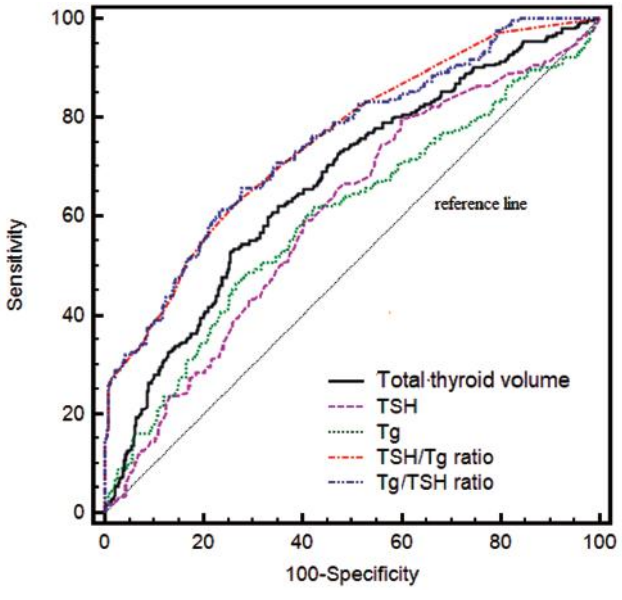
Bethesda categories		n	fT4	p	fT3	p	TSH	p	Tg	p	TSH/Tg	p	Tg/TSH	p
Nondiagnostic	B	263	1.18±0.17	0.017	3.23±0.47	0.147	1.0 (0.4-3.4)	0.012	67.4 (1.8-1000)	0.825	0.016 (0.001-0.414)	0.347	64.0 (2.41-1587.3)	0.347
	M	80	1.23±0.16		3.31±0.41		1.2 (0.4-3.1)		57.6 (2.2-1000)		0.017 (0.001-1.065)		60.54 (0.94-2137.5)	
Benign	B	538	1.20±0.17	0.406	3.26±0.45	0.192	0.9 (0.4-3.7)	0.330	80.1 (1.8-2175)	0.643	0.013 (0.001-1.088)	0.580	78.13 (0.92-1587.3)	0.580
	M	99	1.18±0.18		3.33±0.51		1.1 (0.4-3.3)		90.4 (5.4-1000)		0.016 (0.001-0.380)		61.43 (2.63-2137.5)	
AUS/FLUS	B	84	1.17±0.16	0.019	3.24±0.46	0.060	1.3 (0.4-4.0)	0.922	104.2 (4.4-1000)	0.001	0.014 (0.002-0.230)	0.001	70.6 (4.3-531.9)	0.001
	M	37	1.25±0.16		3.42±0.53		1.4 (0.4-3.8)		41.9 (3.9-591)		0.043 (0.003-0.431)		23.23 (2.32-291.25)	
FN/SFN	B	28	1.20±0.21	0.638	3.37±0.50	0.255	1.1 (0.5-3.7)	0.233	81.4 (11.1-894)	<0.001	0.017 (0.001-0.091)	<0.001	59.70 (10.99-802.86)	<0.001
	M	11	1.16±0.22		3.59±0.60		1.4 (0.6-2.7)		22.0 (8.8-56.9)		0.055 (0.037-0.291)		18.33 (3.44-27.0)	
SM	B	11	1.16±0.18	0.594	3.09±0.46	0.584	1.4 (0.6-3.3)	0.715	86.6 (13.3-1000)	0.002	0.027 (0.001-0.086)	0.003	37.73 (11.63-719.42)	0.003
	M	53	1.20±0.19		3.18±0.51		1.5 (0.4-3.5)		17.5 (1.3-500)		0.077 (0.002-2.240)		13.00 (0.45-476.19)	
Malignant	M	41	1.21±0.20	-	3.14±0.41	-	1.6 (0.5-3.8)	-	23.9 (2.1-439)	-	0.086 (0.003-0.408)	-	11.6 (2.45-300.68)	-

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

Table 4. The predictive variables of malignancy.

Variables	β±SE	OR	95% CI lower	95% CI upper	p
Model I					
Total thyroid volume	0.044±0.006	1.045	1.014	1.033	<0.001
TSH	0.231±0.114	1.260	1.007	1.576	0.043
		Nagelkerke R^2=0.203; p<0.001*			
Model II					
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 R^2=0.310; p<0.001*			
Model III					
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 R^2=0.308; p<0.001*			

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.



	AUC	SE	95% CI
Total thyroid volume	0.668	0.022	0.629 to 0.705
TSH	0.603	0.023	0.563 to 0.642
Tg	0.597	0.024	0.557 to 0.636
TSH/Tg ratio	0.751	0.019	0.715 to 0.785
Tg/TSH ratio	0.748	0.020	0.712 to 0.782

TSH/Tg ratio ~ Tg/TSH ratio	
Difference between areas	0.003
Standard Error	0.003
Significance level	P = 0.327
TSH/Tg ratio ~ Total thyroid volume	
Difference between areas	0.083
Standard Error	0.025
Significance level	P < 0.001
TSH/Tg ratio ~ TSH	
Difference between areas	0.148
Standard Error	0.026
Significance level	P < 0.001
TSH/Tg ratio ~ Tg	
Difference between areas	0.155
Standard Error	0.020
Significance level	P < 0.001
Total thyroid volume ~ TSH	
Difference between areas	0.065
Standard Error	0.027
Significance level	P = 0.018
Total thyroid volume ~ Tg	
Difference between areas	0.0708
Standard Error	0.0255
Significance level	P = 0.0054
TSH ~ Tg	
Difference between areas	0.006
Standard Error	0.032
Significance level	P = 0.845

Figure 1: Diagnostic performance of risk factors for the prediction of malignancy.

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 Ataturk Training and Research Hospital approved the study protocol.

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Authorship Contributions

Surgical and Medical Practices: Abbas Ali Tam, Nuran Sungu, Mustafa Ömer Yazıcıoğlu; Concept: Abbas Ali Tam, Didem Özdemir, Bekir Çakır; Design: Abbas Ali Tam, Didem Özdemir, Cevdet Aydın, Muhammet Cüneyt Bilginer; Data collection and Processing: Abbas Ali Tam, Didem Özdemir; Analysis or Interpretetion: Abbas Ali Tam, Didem Özdemir, Reyhan Ersoy, Bekir Çakır, Cevdet Aydın, Muhammet Cüneyt Bilginer; Literature Search: Abbas Ali Tam, Didem Özdemir, Nuran Sungu, Mustafa Ömer Yazıcıoğlu Writing: Abbas Ali Tam, Didem Özdemir.

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