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Determination of the Frequencies of Various Thyroid Autoantibodies in Autoimmune Thyroid Diseases and Evaluation of Their Relationship with Sonographic Findings: A Single-Center Study

ABSTRACT

Objective: The frequency of thyroid autoantibodies in autoimmune thyroid disease is known to vary between countries. We aimed to determine the frequency of autoantibody positivity and the correlation between sonographic findings and autoantibodies in autoimmune thyroid disease.

Methods: Laboratory findings and ultrasound findings of 490 patients with Hashimoto's thyroiditis and 225 patients with Graves' disease were retrospectively enrolled from a tertiary center.

Results: Anti-thyroid peroxidase and anti-thyroglobulin positivity were 90.2% and 60.2% in Hashimoto's thyroiditis patients, respectively. Anti-thyroglobulin titers were significantly higher in males ($P < .01$). Mean thyroid volume of Hashimoto's thyroiditis patients was 10.5 ± 6.9 mL and 11.6% of patients had atrophic thyroiditis (<4 mL). Correlation analysis proved a positive correlation between anti-thyroid peroxidase titers, parenchymal heterogeneity (determined as mild, moderate, and severely heterogeneous) and a weak positive correlation with the thyroid volume and increasing heterogeneity ($P < .001$, $P < .001$, r=0.273,) respectively. TSH receptor antibody, anti-thyroid peroxidase, and anti-thyroglobulin positivity were found to be 84%, 75.1%, and 48.4%, respectively, in Graves' disease patients. The mean thyroid volume of the Graves' disease group was 22.2 ± 13.9 mL. When the patients were grouped according to their TSH receptor antibody levels (i.e., >30 U/L, 10-30 U/L, <10 U/L) and parenchymal heterogeneity, severe heterogeneity in the group with TSH receptor antibody >30 U/L was found to be significantly higher than the others ($P=.049$).

Conclusion: In conclusion, the most prevalent antibody detected in Hashimoto's and Graves' disease is anti-thyroid peroxidase and TSH receptor antibody, respectively, in Turkish patients. Furthermore, a significant relation was found between anti-thyroid peroxidase positivity and anti-thyroid peroxidase titer and the degree of sonographic heterogeneity.

Keywords: Autoantibody frequency, Hashimoto's thyroiditis, Graves' disease

Introduction

Autoimmune thyroid disease (AITD), including Hashimoto's thyroiditis (HT) and Graves' disease (GD), is one of the most common organ-specific autoimmune diseases, affecting approximately 5% of the general population.[1] Although the etiology of AITD has yet to be fully explained, it could be caused by a combination of genetic and environmental factors that impair immunological tolerance. Demonstrating the presence of thyroid antibodies is important in the diagnosis of AITD. Patients develop autoantibodies against common thyroid antigens such as thyroid peroxidase (TPO), anti-TPO, and thyroglobulin (Tg) anti-Tg.[2,3] Generally, anti-TPO positivity in HT is seen 80%-100%, while anti-Tg positivity is seen 60%-80% of the patients.[4,5] In GD, antibodies against TSH receptors (TRAb) are often blamed for the pathogenesis. The sensitivity and specificity of TRAb in Graves' disease were found to be 98% and 99%, respectively.[6] In addition to TRAb, 65%-80% anti-TPO positivity and 50%-60% anti-Tg positivity was reported.[4,5]

Several patients diagnosed with AITD were reported to show low anti-TPO positivity and higher anti-Tg levels.[7] In a study conducted with 70 HT, 70 GD, 50 silent thyroiditis patients, and 100 healthy controls, Nishihara et al[7] evaluated the prevalence of anti-Tg and anti-TPO positivity by using 5 different commercial kits. Anti-Tg positivity was significantly higher than anti-TPO positivity in HT patients according to 4 of those 5 kits. The frequency of anti-Tg positivity alone was found to be significantly higher than anti-TPO in both HT and silent

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thyroiditis.[7] Contrary to the classical knowledge, this study, conducted in Japan, concluded that anti-Tg should be the first choice, as a screening test, for HT.[7] Thus, population-specific autoantibody frequencies may show variations, which implied that it is important to determine the autoantibody frequencies specific to our country.

Therefore, the current retrospective study was carried out to determine the frequency of thyroid autoantibodies (anti-Tg, anti-TPO, TRAb) in AITD patients diagnosed and followed by our tertiary endocrinology outpatient clinic in Ankara University Hospitals. Sonographic findings were also established and correlated with the autoantibody titers.

Materials and Methods

Patients over 18 years of age with AITD presenting to Ankara University, School of Medicine, Endocrinology and Metabolism outpatient clinic between January 01, 2018, and May 01, 2020, were included retrospectively in the study. Diagnosis of HT was based on the presence of thyroid autoantibodies and thyroid ultrasonography (US) findings [i.e., parenchymal heterogeneity and hypoecogenicity]. Patients' autoantibody levels at the last admission and US findings were recorded. A small number of patients (n = 9) who were negative for thyroid autoantibodies, but with parenchymal heterogeneity and hypoechogenicity, were also included as they were highly suspected of having HT. Diagnosis of GD in patients presenting with thyrotoxicosis was established based on the clinical findings (i.e., ophthalmopathy, diffuse goiter, dermopathy, acropathy), US findings [i.e., parenchymal heterogeneity, hypoechogenecity, significantly increased blood flow in Doppler ultrasonography (Vitti 2-3)]. Thyroid autoantibody and ultrasonography data of these patients at the time of diagnosis were recorded. Thyroid scintigraphy and/or radioactive iodine uptake tests were also evaluated in a small number of patients without increased blood flow (Vitti-1). Patients recently diagnosed with GD and had not received antithyroid drug therapy, as well as those with recurrent GD who did not receive antithyroid drug therapy and any ablative therapy, were included.

Patients with thyroid malignancy, a history of thyroidectomy, a history of silent thyroiditis or subacute thyroiditis, and using amiodarone and steroid therapy for Graves' orbitopathy, were excluded. Laboratory test results for anti-TPO, anti-Tg, and TRAB levels performed during the diagnosis process were retrieved from the electronic patient file system. Anti-TPO reference range was 0-9 IU/mL, anti-Tg reference range was 0-4 IU/mL, and the preferred test method was access immunoassay system (Roche Diagnostics COBAS Elecsys® Anti-TPO and Roche Diagnostics COBAS Elecsys® Anti-Tg). The reference range for TRAb was 0-9 U/L, DIAsource TRAb human RIA kit (KIP2040, DIAsource Immunoassays S.A®.) was used, and measurements were conducted with radioreceptor assay technique.

The study was approved by Ankara University, School of Medicine, Non-Invasive Clinical Research Ethics Committee (statement number: I1-71-20).

Statistical Analysis

Data were uploaded to the digital environment and analyzed on the Statistical Package for Social Sciences (SPSS) for Windows 22.0 (IBM Corp.; Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (minimum-maximum), frequency distribution, and percentage. Pearson chi-square test and Fisher's exact test were used for evaluating categorical variables. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov test/Shapiro-Wilk test). Mann-Whitney *U* test was used for assessing statistical significance between 2 independent groups for variables that were not found to be in a normal distribution, and the Kruskal-Wallis test was the preferred statistical method for 3 or more independent groups. When a significant difference was detected

Table 1. Descriptive and Clinical Characteristics of Patients with Hashimoto's Thyroiditis (HT) (n = 490) and Graves' Disease (GD) (n = 225) patients

	Hashimoto Thyroiditis	Graves' Disease
Age (years), mean ± SD (min-max)	48.2 ± 14.7 (19-84)	43.2 ± 14.0 (20-85)
Gender	n (%)	n (%)
Male	58 (11.8)	56 (24.9)
Female	432 (88.2)	169 (75.1
Disease duration (years), mean ± SD (min-max)	7.3 ± 6.1 (0-39)	*
Age at diagnosis, mean± SD (min-max)	40.9 ± 14.2 (7-79)	40.3 ± 14.4 (16-84)
Sonographic heterogeneity	n (%)	n (%)
Mild	107 21.8)	38 (16.9)
Moderate	260 (53.1)	119 (52.9)
Severe	123 (25.1)	68 (30.2)
Thyroid volume (mL), mean ± SD (min-max)	10.5 ± 6.9 (3-56.6)	22.2 ± 13.9 (10.1-83.6)
Thyroid volume grade	n (%)	n (%)
Atrophic (<4.0 mL)	57 (11.6)	None
Normal (4.0-19.9 mL)	390 (79.6)	130 (57.8)
Goitrous (≥20 mL)	43 (8.8)	95 (42.2)
Number of nodules	n (%)	n (%)
None	285 (58.2)	144 (64)
Single	107 (21.8)	36 (44.4)
≥2	98 (20.0)	45 (55.6)
Blood flow (n = 191)	n %	
Vitti-I	25 (13)	
Vitti-II	83 (43.5)	
Vitti-III	83 (43.5)	

SD, standard deviation.
*Data of the patients with GD at the time of diagnosis were included.

MAIN POINTS

- Anti-TPO and anti-Tg positivity were 90.2 and 60.2% in patients with Hashimoto's thyroiditis, respectively.
- TRAb, anti-TPO, and anti-Tg positivity were found to be 84%, 75.1%, and 48.4%, in patients with Graves' disease, respectively.
- Anti-TPO titer was found to be significantly related with decreasing thyroid volume and increasing parenchymal heterogeneity in Hashimoto's thyroiditis. No significant relation was found between anti-Tg titer and sonographic features.
- Hashimoto's thyroiditis patients with both anti-TPO and anti-Tg positivity, had higher sonographic parenchymal heterogeneity.
- TRAb titers was significantly higher in Graves' disease with severe sonographic parenchymal heterogeneity.

Table 2. Distribution of Descriptive and Clinical Characteristics in HT Patients According to Study Groups

	Group-I (n = 256) Anti-TPO (+) Anti-Tg (+)	Group-II (n = 186) Anti-TPO(+) Anti-Tg (−)	Group-III (n = 39) Anti-TPO (−) Anti-Tg (+)	Group-IV (n = 9) Anti-TPO (−) Anti-Tg (+)	*P*
Age (years)	47.7 ± 15.4 (19-84)	47.8 ± 13.4 (19-80)	53.8 ± 15.1 (20-82)	48.8 ± 14.7 (29-69)	.129
Gender					
Male	44 (17.2)	10 (5.4)	3 (7.7)	1 (11.1)	**.002***
Female	212 (82.8)	176 (94.6)	36 (92.3)	8 (88.9)	
Disease duration (years)	7.4 ± 5.8 (0-39)	7.2 ± 6.2 (1-38)	7.3 ± 7.8 (1-32)	5.4 ± 5.1 (1-17)	.554
Age at diagnosis	40.3 ± 14.5 (7-79)	40.5 ± 13.6 (14-76)	46.4 ± 15.4 (19-74)	43.3 ± 12.2 (28-63)	.135
Sonographic heterogeneity					
Mild	43 (16.8)	40 (21.5)	18 (46.2)	0	
Moderate	142 (55.5)	98 (52.7)	17 (43.6)	3 (33.3)	**<.001****
Severe	71 (27.7)	48 (25.8)	4 (10.3)	6 (66.7)	**<.001****
Thyroid volume (mL)	11.0 ± 7.2 (3-43.3)	10.3 ± 6.8 (3-56.6)	9.2 ± 5.9 (0.7-25.0)	7.8 ± 7.1 (1.2-25.4)	.130
Thyroid volume grade					
Atrophic (<4.0 mL)	31 (12.1)	19 (10.2)	5 (12.8)	2 (22.2)	
Normal (4.0-19.9 mL)	196 (76.6)	157 (84.4)	31 (79.5)	6 (66.7)	.343
Goitrous (≥20 mL)	29 (11.3)	10 (5.4)	3 (7.7)	1 (11.1)	
Number of nodules					
None	148 (57.8)	108 (58.1)	24 (61.5)	5 (55.6)	
Single	57 (22.3)	43 (23.1)	4 (10.3)	3 (33.3)	.545
≥2	51 (19.9)	35 (18.8)	11 (28.2)	1 (11.1)	
Nodule presence					
No	148 (57.8)	108 (58.1)	24 (61.5)	5 (55.6)	.974
Yes	108 (42.2)	78 (41.9)	15 (38.5)	4 (44.4)	

HT, Hashimoto's thyroiditis; Tg, thyroglobulin; TPO, thyroid peroxidase.
Continuous variables were presented as "mean ± standard deviation (minimum-maximum)," and categorical variables were presented as "number (column percentage)" Pearson chi-square test was used to evaluate categorical variables, and Kruskal-Wallis test for the comparison between 3 or more independent groups (*corrected $P < .05$ ** $P < .01$).

between 3 or more independent groups, Bonferroni correction was applied in post hoc pairwise comparisons for the source of the difference. The relationship between the variables was evaluated with the Spearman correlation test. The correlation coefficient was interpreted as "weak" if between 0 and 0.25, "moderate" between 0.26 and 0.50, "strong" between 0.51 and 0.75, and "very strong" between 0.76 and 1.00. The statistical significance level was accepted as $P < .05$.

Results

We examined data of 715 patients, 490 of whom were diagnosed with HT and 225 with GD. Descriptive and clinical characteristics of patients with HT and GD are given in Table 1.

Patients diagnosed with HT were divided into 4 study groups according to their antibody positivity: anti-TPO(+)-Anti-Tg(+) as Group-I, anti-TPO(+)-Anti-Tg(−) as Group-II, anti-TPO(−)-Anti-Tg(+)as Group-III, and anti-TPO(−)-Anti-Tg(−) values as Group-IV (suspected HT). Accordingly, 256 (52.2%) of 490 patients diagnosed with HT constituted Group-I, 186 (38%) Group-II, 39 (8%) Group-III, and the remaining 9 (1.8%) Group-IV. A statistically significant difference was found between the study groups in terms of gender and degree of sonographic heterogeneity in patients with HT ($P = .002$; $P < .001$, respectively) (Table 2). Among patients with HT, in Group-I, the percentage of males was significantly higher than the other groups. In addition, the percentage of patients with severe heterogeneity among patients in Group-IV was significantly higher than the other study groups (Table 2).

A statistically significant difference was found between the degrees of heterogeneity and thyroid volume (TV) grades of patients in terms of disease duration ($P = .019$; $P < .001$, respectively). Post hoc pairwise comparisons revealed that the significant difference in TV grades was caused by those with small TV, and the significant difference in the heterogeneity was caused by the difference observed in those with mild heterogeneity and those with severe heterogeneity. The duration of disease in HT patients with a small TV was significantly higher than that of patients with medium and large TV. In addition, the disease duration in patients with HT with a mild heterogeneity was significantly lower than that of patients with a severe heterogeneity (Table 3).

A statistically significant difference was found in terms of anti-TPO titers between the thyroiditis grades and TV classes of HT patients ($P < .001$ for both). Anti-TPO titer of HT patients with mild degree of heterogeneity was significantly lower than those of moderate and severe ones. Post hoc pairwise comparisons showed that the significant difference in the degree of heterogeneity was caused by those with mild heterogeneity, while the significant difference in the TV group was caused by more than one group. In addition, the anti-TPO titer of patients with a large TV was significantly higher than those with normal and atrophic patients. Accordingly, the anti-TPO titer in patients with normal TV was significantly higher than those with atrophic ones (Table 4). In addition, anti-Tg titers of male patients were significantly higher than those of females ($P < .001$) (Table 4).

Table 3. Distribution of Disease Duration by Gender, Degree of Heterogeneity, Thyroid Volume, and Presence of Nodules in Hashimoto Thyroiditis Patients

		n	Disease Duration (Years) Mean ± SD (Min-Max)	p
Gender	Male	58	6.1 ± 4.8 (1-23)	.236[a]
	Female	432	7.4 ± 6.3 (0-39)	
Sonographic heterogeneity	Mild	107	6.3 ± 5.7 (0-32)[3]	**.019[b]****
	Moderate	260	7.2 ± 6.3 (1-39)	
	Severe	123	8.2 ± 6.0 (1-38)	
Thyroid volume	Atrophic	57	11.9 ± 7.8 (1-38)[2,3]	**<.001[b]****
	Normal	390	6.6 ± 5.4 (1-39)	
	Large	43	7.1 ± 7.3 (0-32)	
Nodule presence	No	285	7.1 ± 5.7 (0-38)	.787[a]
	Yes	205	7.5 ± 6.6 (1-39)	

n, number of patients; SD, standard deviation; [a]Mann-Whitney *U* test; [b]Kruskal Wallis test; [2]Post hoc paired comparison revealed a significant difference from "moderate"; [3]Post hoc pairwise comparison showed a significant difference from "severe/large" (*corrected $P < .05$,**$P < .01$).

Anti-TPO, Anti-Tg, and TRAb positivity was 75.1%, 48.4%, and 84% of the patients with GD, respectively. In total, 225 GD patients included in the study were divided into 3 study groups, regarding their TRAb titers. Those with TRAb serum levels <9 IU/mL (Group-I), those between 9 and 29.9 IU/mL (Group-II), and those ≥ 30 IU/mL were named as Group-III. Table 5 summarizes demographic and sonographical findings with regard to study groups. Group-III had a significantly higher sonographic heterogeneity than the other study groups. There were no significant differences for other variables between groups.

Discussion

Hashimoto thyroiditis, also known as chronic lymphocytic thyroiditis, has an incidence rate of 3.5-5/1000 in women and 0.6-1/1000 in men.[8,9] The exact causes have yet to be explained. Hashimoto thyroiditis occurs 8 times more frequently in women.[10,11] Mean age of our patients was 48.2 (18-84), the mean age at diagnosis was 40.9 years, and the F/M ratio was found to be 7.47 : 1.

Prevalence of autoantibodies in HT patients had been reported at around 90% for anti-TPO and 25% to 50% for anti-Tg.[12] Similarly, the prevalence of anti-TPO positivity was 90.2%, but that of anti-Tg was slightly higher 60.2% in our patients. In a study conducted to investigate the possible difference between autoantibody positivity in HT patients with regard to gender, anti-TPO positivity was found to be 50.7% in women and 28.2% in men with typical sonographical findings.[13] A study from Turkey, which evaluated 769 HT patients retrospectively, showed that thyroid autoantibodies were positive in more than 90% of patients.[14] Anti-Tg and anti-TPO positivity was reported to be 92.8% and 98.4%, respectively, for Turkish female patients and 93.2% and 100% among Turkish male patients, no significant difference was reported between genders.[14] Similarly while there was no significant difference between genders in terms of anti-TPO, anti-Tg positivity, the anti-Tg titer was found to be significantly higher in males. Reported prevalence of HT with negative thyroid autoantibodies is generally around 5%, while we found 1.8% of the HT patients had negative autoantibodies with typical sonographic features.[15] Severe heterogeneity was significantly higher in this group. Since we had no histopathological diagnoses in these cases, other rare causes such as silent thyroiditis could still be kept responsible for primary hypothyroidism in a few of these cases.

Ultrasonographic findings in HT tend to vary dramatically in different studies, probably due to different interpretation of sonographers, especially for nodule/pseudonodule formations. Yarman et al[16] reported that 19 (39.6%) of 48 patients with HT had diffuse enlargement, 20 (41.7%) had nodular goiter, and 9 (18.7%) had solitary nodules. In the large study mentioned earlier, 12.9% of female patients had normal findings on US, 23.6% had diffuse enlargement, 52.2% and 11.3% had single and multiple nodules, respectively.[14] Same study reported 12% and 36% of male patients had normal findings and diffuse enlargement, respectively. Out of nodular glands, 32% had single and 20% had multiple nodules. No significant difference between genders was reported.[14] In the current study, nodules were found in 58.2% of patients (i.e., single 21.8% and more than one 20%). Mean TV was 10.5 ± 6.9 (min-max: 0.3-56.6) mL, and 11.6% of them had atrophic (<4 ml) thyroid glands. Although the 2019 guideline of the Society of Endocrinology and Metabolism of Turkey states the upper limit of TV as 10 and 15 mL for male and female individuals,

Table 4. Distribution of Antibody Titers by Gender, Degree of Heterogeneity, Thyroid Volume, and Nodule Status in Hashimoto Thyroiditis Patients (n = 490)

			Anti-TPO IU/mL			Anti-TG IU/mL	
		n	Mean ± SD (Min-Max)	P[a]	n	Mean ± SD (Min-Max)	P[a]
Gender	Male	58	352.7 ± 366 (0.2-1155)	.433	58	311.0 ± 580 (0.9-2503)	**<.001***
	Female	432	323.2 ± 342 (0.2-1219)		428	171.1 ± 461 (0.9-2503)	
Heterogeneity	Mild	107	233.3 ± 300 (0.2-1155)[2,3]	**<.001****	107	164.0 ± 454 (0.9-2503)	
	Moderate	260	335.6 ± 354 (0.2-1219)		258	191.5 ± 500 (0.9-2503)	.704
	Severe	123	389.7 ± 347.1 (1.1-1155)		121	200.9 ± 456 (0.9-253)	
Thyroid volume	Atrophic	57	184.0 ± 242 (0.4-988)[2,3]	**<.001****	57	254.5 ± 552 (0.9-2503)	
	Normal	390	325.8 ± 339 (0.2-1218)[3]		386	164.8 ± 439 (0.9-2503)	.109
	Large	43	524.2 ± 414 (0.2-1155)		43	305.2 ± 671.1 (0.9-2503)	
Nodule presence	No	285	309.7 ± 337 (0.2-1219)	.373	281	213.4 ± 523.4 (0.9-2503)	.811
	Yes	205	350.2 ± 354 (0.2-1155		205	152.6 ± 407.7 (0.9-2503)	

n, number of patients; SD, standard deviation; [a]Mann-Whitney *U* test; [b]Kruskal Wallis test; **$P < .01$; [2]Post hoc paired comparison revealed a significant difference from "moderate"; [3]Post hoc pairwise comparison showed a significant difference from "severe/large" (corrected *$P < .05$,** $P < .01$).

Table 5. Distribution of Age, Gender, Disease Age, Thyroiditis Grade, and Thyroid Volume by TSH Receptor Antibody (TRAb) Titer Group in GD Patients

	Group-I (n = 36) (TRAb <9 U/L)	Group-II (n = 73) (TRAb 9-29.9 U/L)	Group-III (n = 116) (TRAb ≥ 30 U/L)	*P*
Age, mean ± SD (min-max)	44.2 ± 14.8 (20-69)	41.3 ± 13.9 (20-85)	44.1 ± 13.8 (20-76)	.313[a]
Gender				
Male	9 (25.0)	17 (23.3)	30 (25.9)	.924[b]
Female	27 (75.0)	56 (76.7)	86 (74.1)	
Age at diagnosis, mean ± SD (min-max)	42.7 ± 15.2 (19-68)	38.3 ± 14.4 (16-84)	40.9 ± 14.2 (16-73)	.230[a]
Heterogeneity, n (%)				
Mild	8 (22.2)	17 (23.3)	13 (11.2)	
Moderate	20 (55.6)	40 (54.8)	59 (50.9)	**.049*[b]**
Severe	8 (22.2)	16 (21.9)	44 (37.9)	
Thyroid volume (mL), mean ± SD (min-max)	23.5 ± 13.7 (6.2-68.6)	19.8 ± 13.2 (1.5-81.0)	23.2 ± 14.4 (5.3-83.6)	.133[a]
Thyroid volume grade, n (%)				
Normal (<20.0 mL)	17 (47.2)	49 (67.1)	64 (55.2)	.101[b]
Goitrous (≥20 mL)	19 (52.8)	24 (32.9)	52 (44.8)	
Nodule presence, n (%)				
No	20 (55.6)	49 (67.1)	75 (64.7)	.486[b]
Yes	16 (44.4)	24 (32.9)	41 (35.3)	
Number of nodules (n = 81), n (%)				
Single	5 (31.3)	11 (45.8)	20 (48.8)	.482[b]
Multiple	11 (68.8)	13 (54.2)	21 (51.2)	

n, number of patients; %, column percentage; SD, standard deviation; [a]Kruskal Wallis test; [b]Pearson's chi square test; [2]Post hoc paired comparison revealed a significant difference from "Group-II; [3]Post hoc paired comparison showed a significant difference from "Group-III" (*corrected $P < .05$).

respectively, considering that most of the patients participating in this study grew up during the iodine-deficient years, the arbitrary previous upper normal limit of 20 mL was chosen. Another interesting finding was that the age of disease onset in patients with nodules was significantly higher than those without. Thus, HT patients with nodules were significantly older. This finding propose that nodule formation increases if the gland does not atrophy during the years pass by with the disease. Willms et al[17] correlated autoantibody activity with US findings and found that the mean TV of 223 patients with HT was 15.8 mL, while in our study, the mean TV was 10.5 mL. In the same study, cystic and nodular structures were detected in 6.7% and 21.9% of patients, respectively. Thyroid parenchyma was reported as homogeneous in 32.3% and heterogeneous in 67.7%. Echogenicity was recorded as isoechoic, mildly hypoechoic, and hypoechoic in 21.5%, 32.7%, and 45.7% of the patients, respectively. Heterogeneous echo structure was significantly higher than homogeneous echo structure and was found to be positively correlated with anti-TPO concentrations.[17] There was no significant relationship between echogenicity and anti-Tg levels.[17] Similarly, a significant difference was observed between anti-TPO positivity and anti-TPO titer and the degree of sonographic heterogeneity, and there was no difference between anti-Tg and the degree of sonographic heterogeneity in the current study. In the aforementioned study, no significant correlation was found between the presence of nodules or cysts on sonography, TV, and autoantibody activity. We also found no relationship between the presence of nodules and the positivity of anti-TPO and anti-Tg.

Grave's disease usually peaks between the ages of 30 and 50, and F/M has been reported to range between 4/1 and 10/1.[18,19] This was found to be 3/1 in our group, probably due to the higher referral of male patients, who are usually more difficult to treat, to our tertiary center. Generally speaking, TRAb is found to be positive in 90% of GD patients (i.e., TSH receptor stimulating antibodies in 73%-100% and TSH receptor blocking antibodies in 25%-75%) and 0%-20% of HT patients.[20] Anti-TPO autoantibody positivity had been reported in 80% and anti-Tg antibodies in 50%-60% of GD patients.[20-22] While anti-TPO antibodies had been reported to have a cytotoxic effect on thyrocytes in HT, this effect may not be observed in GD.[23] Current study revealed 84%, 75.1%, 48.4%, TRAb, anti-TPO, and anti-Tg positivity, respectively, in GD patients, which is consistent with the published data.[24]

A comprehensive review examining the results of studies conducted between 1986 and 2006 for TRAb sensitivity and specificity revealed that TRAb test performed with the TBII test (porcine thyrocytes membrane TRAK test, BRAHMS® (formerly Henning, Germany) had approximately 80% sensitivity and 100% specificity. Recombinant TRAb test (Dyno human, BRAHMS®, Germany) has been reported to have 98% sensitivity and 100% specificity.[25] TRAb diagnostic kit used in our center (i.e., DIAsource® TRAb human RIA kit) has a sensitivity of 95% and specificity of 100% as reported by the manufacturer.

Mean TV in our GD patients was 22.2 ± 13.9 mL which is concordant with an Italian study that investigated 42 GD patients before treatment (i.e., mean 23.9 ± 13.9 mL).[26] Contrary to the study by Sawicka et al, we found no relationship between TRAb levels and TV before treatment.[27]

A detailed review of current literature for the presence of nodules accompanying GD detected one or more accompanying nodules in 44% of patients undergoing surgery for GD.[28] Similarly, Cantalamessa et al[29] examined the thyroid US of 315 patients with GD at the time of diagnosis and during follow-up and found that 106 (34%) patients had thyroid nodules of 8 mm or larger. Our findings are concordant

with these (i.e., detected nodules in 36% of patients). Schiemann et al[30] compared autoimmune activity and gray-scale US findings in GD patients. They observed that parenchymal echogenicity was significant in patients with high TRAb levels when compared to patients with normalized TRAb levels after treatment.[30] We also found that the severe heterogeneity was significantly higher in patients with higher TRAb levels (i.e., >30 U/L). In this context, we can suggest that heterogeneity and hypoechogenecity increase with increased blood flow and lymphocytic infiltration in more active disease. Actually, Vita et al[26] published sonographic findings in GD supporting this opinion.

In conclusion, the most prevalent antibody detected in HD and GD is Anti-TPO and TRAb, respectively, in Turkish patients. Furthermore, a significant relation was found between anti-TPO positivity and anti-TPO titer and the degree of sonographic heterogeneity.

Ethics Committee Approval: The study was approved by Ankara University, School of Medicine, Non-Invasive Clinical Research Ethics Committee (statement number: I1–71–20).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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