Original Article

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Association Between Thyroid Antibodies and Ultrasonic Imaging in Patients with Hashimoto's Thyroiditis

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Abstract

Aim: The association between high levels of anti-thyroid antibodies and the extent of destruction of thyroid tissue is well documented. The aim of the present study was to analyze the relationship between anti-thyroid antibodies, thyroid hormones, and sonographic parenchymal changes.

Methods: The study was designed as a case-control study. Four hundred and seventy-five patients with HT and 98 healthy subjects were included in the study. Serum levels of free thyroxine (fT4), free triiodothyronine (fT3), thyroid-stimulating hormone, and anti-thyroid antibodies (anti-thyroid peroxidase antibodies and anti-thyroglobulin antibodies) were measured. The ultrasonographic results of the patients were also recorded.

Results: Serum levels of anti-TPO and anti-Tg were significantly associated with hypoechogenicity, heterogeneity, and pseudonodulation ($p<0.001$). There was no significant difference between the two groups in terms of cyst and nodule formation, however, a significant difference was found in terms of thyroid volume ($p<0.001$). Thyroid volumes were higher in the HT group. As serum anti-TPO levels increased in the HT group, parenchymal hypoechogenicity increased ($p<0.001$).

Conclusion: Ultrasonography is a non-invasive method that provides information about the inflammatory activity of the thyroid gland. Significantly reduced echogenicity, heterogeneity, and multifocal pseudonodular infiltration were indicators of inflammatory activity and were associated with higher anti-TPO levels. Anti-TPO and ultrasonographical changes may be useful in the follow-up of Hashimoto's thyroiditis.

Keywords: Hashimoto thyroiditis, ultrasonic diagnosis, autoantibodies

Introduction

Hashimoto's thyroiditis (HT) is an organ- specific autoimmune disease that occurs due to immune defects caused by genetic predisposition and environmental factors. The disease is characterized by lymphocytic infiltration that causes progressive loss of thyroid function (1-3). The reported overall incidence is more than 2% in the general population and is 10 times more common in women than in men (4). The diagnosis of HT is based on clinical evaluation, laboratory results and ultrasound findings. Anti-thyroid antibodies (TPO-Ab, Tg-Ab) are widely used in the diagnosis of autoimmune thyroid diseases and are closely associated with lymphocytic infiltration and thyroiditis. Anti-thyroid antibody positivity

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is found in 83.3% of clinically hypothyroid patients (5). However, this positivity is also found in10-13% of the normal population without thyroid disease (6,7). Anti-TPO reflects current activity, stage of lymphocyte infiltration and thyroid destruction via T-cell mediated cytotoxicity (8).

Ultrasonography (US), which has been used clinically in the diagnosis and follow-up of HT since the 1990s, provides valuable information about the size and structure of the thyroid gland as well as the ability to detect possible thyroid nodules (9).

Classic US findings in HT are reduced echogenity, heterogeneous parenchyma, increased volume, hypervascularity, and micronodulations that may reflect the lymphocyte infiltration (10-12). While micronodulation was reported in 43% of patients, nodules were detected in 10% of patients with HT (13).

HT is a chronic, high-incidence autoimmune thyroid disorder that is a common cause of hypothyroidism. It is mainly manifested by positive thyroid-specific antibodies in the peripheral blood with thyroid lymphocytic infiltration and fibrosis. The best imaging modality for diagnosing thyroid diseases is US, which provides the exact dimensions of the thyroid goiters (14). Ultrasound echo intensity reflects the internal pathological basis of HT. Lymphocyte and plasma cell infiltration, for example, reduces echo intensity, whereas fibrosis causes heterogeneous echo intensity. Therefore, ultrasound has become the most important imaging method for evaluating and monitoring HT (15).

In this study, we evaluated the relationship between US findings and antibodies in patients with HT.

Materials and Methods

Compliance with Ethical Standards

Ethics committee approval was received for this study from the ethics committee of Ankara Numune Training and Research Hospital (approval number: 865, date: 21.07.2014).

Study Population

This retrospective study included 475 patients (group 1) previously diagnosed with HT (clinical signs and symptoms for thyroid disease, elevated thyroid antibodies, and US findings were used for diagnosis) and 98 control subjects (group 2) (subjects with no signs or symptoms and/or history of thyroid disease) who were admitted to the endocrinology clinic (Table 1). The demographic, laboratory, and ultrasonographic characteristics of the cases and controls who applied to the endocrinology outpatient clinic were recorded from the clinical database. HT patients were divided into two groups, hypothyroid and euthyroid, according to their thyroid functions.

The exclusion criteria were as follows: age <18 and >65 years; pregnancy, postpartum, or lactating periods; smoking; hormone replacement therapy, or medication that may affect thyroid hormone levels (such as amiodorane, interferone, litium, gonodotropine releasing hormone analogs, rifampicine, and anticonvulsants).

Laboratory

Thyrotropin, free T4, and free T3 levels were determined using chemiluminescence methods with the Access Hypersensitivite human TSH, Access FT3 kit, and Access FT4 kit. The normal range of TSH was 0.34-4.25 μIU/mL, FT4 level was 0.61-1.2 pg/mL, and the FT3 level was 2.5-3.9 pg/mL.

Anti-TPO and anti-TG were measured using an Elecsys Anti-TG (Roche Diagnostics, Germany) kit and an electrochemiluminescence (ECLIA) kit. The normal range of the anti-TPO level was 0-34 IU/mL, and the anti-Tg level was 0–40 IU/mL. The intra- and inter-assay coefficients of variation were all <5%.

Thyroid Ultrasonography

Thyroid US was performed in an out-patient clinic by the same experienced endocrinologist using a high-resolution ultrasound machine with a 13 megahertz high-frequency linear transducer (Hitachi EUB 7000 HV, Japan) while the patients lay supine with slightly hyperextended necks. Right and left thyroid lobes were visualized in longitudinal and transverse planes. Anteroposterior, transverse, and longitudinal measurements of both thyroid lobes were evaluated. Thyroid volume was calculated using a previously reported formula (13):

Thyroid volumes (mL) = Length (cm)x width (cm)x Depth (cm) x π /6

Besides volume, all US findings (echogenity, parenchymal structure, presence of pseudonodulation, nodules, or cysts) were recorded in detail.

Statistical Analysis

Statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The distribution of data was determined by the Shapiro-Wilk test. Continuous variables were expressed as mean±standard deviation and categorical variables as frequency and percentage. Continuous variables were compared with the Mann-Whitney U test, and categorical variables were compared using Pearson's chi-square test or the Fisher's exact test. The linear relationship between two continuous variables was evaluated by Spearman correlation analysis. A p-value less than 0.05 was considered statistically significant for all tests.

Results

Sonography revealed an increase in heterogeneity, a decrease in echogenicity, and an increase in

Table 1. Compassion of two groups for demographic, laboratory and ultrasound features			
Variable	**Group 1 (n=475)**	**Group 2 (n=98) (control)**	**p-value**
Age (year)	44.12±12.80 (26-77)	42.03±12.21 (19-67)	0.056
Gender (F/M) (%)	420 (88.4%)/55 (11.6%)	92 (93.9%)/6 (6.1%)	0.157
FT3	2.92±0.52 (0.28-4.86)	2.98±0.40 (2.26-3.84)	0.315
FT4	0.84±0.23 (0.15-1.92)	0.85±0.31 (0.29-3.37)	0892
TSH	10.45±15.46 (0.42-100)	7.31± 9.73 (0.86-100)	0.914
Anti-TPO	385.65±346.21 (45-1149)	2.73±4.67 (0.10-38.4)	**<0.001**
Anti-Tg	278.59±649.98 (0.0-4000)	1.49±1.05 (0.10-6.3)	**<0.001**
Thyroid volume (mm^3)	50.93±33.34 (20-202)	34.12±24.13 (21-159)	**<0.001**
Echogenity (n, %) Isoechoic Mild hypoechoic Overt hypoechoic	 84 (17.7%) 182 (38.3%) 209 (44%)	 56 (57.1%) 31 (31.6) 11 (11.2%)	**<0.001**
Texture (n, %) Homogen Heterogen	 94 (19.8%) 381 (80.2)	 72 (73.5%) 26 (26.5%)	**<0.001**
Pseudonodulation Absent Present	 117 (24.6%) 358 (75.4%)	 91 (92.9%) 7 (7.1%)	**<0.001**
Nodule (n, %) Absent Present	 319 (67.2%) 156 (32.8%)	 65 (66.3%) 33 (33.7)	**0.871**

FT3: Free triiodothyronine, FT4: Tetraiodothyronine, also called free thyroxine, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, Anti-Tg: Antithyroglobulin antibody, F/M: Female/male

pseudonodulation (micronodulation) in group 1 (p<0.001). Pseudonodulation was found in 75% of group 1 and in only 7% of the control group. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of echogenicity were 82.3%, 57.1%, 90.3%, and 40%, respectively, for TPO-positive HT patients. The sensitivity, specificity, PPV, and NPV of heterogenicity were 81.3%; 73.4 , 93.6%, and 43.3% for TPO-positive HT patients, respectively. The sensitivity, specificity, PPV, and NPV of pseudonodulation were 24.7%; 92.8; 94.4%; and 20.2% for TPO-positive HT patients, respectively. The sensitivity, specificity, PPV, and NPV of thyroid volume (>25 mL) were 82.9%; 34.6%; 86%; 29.5% for TPO-positive HT patients, respectively.

Increased antibody levels were negatively correlated with the echogenicity of the thyroid gland, but there was no difference between the two groups according to the presence of nodules. Nodule frequency was found in 32.8% of HT patients and 33.7% of controls (p=0.871). In patients with positive antibodies, anti-TPO was positively correlated with both TSH and thyroid volumes (p<0.001). There was a positive correlation between anti-Tg and TSH levels (p<0.001), but not with thyroid volume (p=0.341) (Table 2).

Group 1 was divided into two categories according to thyroid status: euthyroid and hypothyroid. The hypothyroid group had higher thyroid antibodies and more heterogeneous and hypoechoic thyroid parenchyma compared to the euthyroid group. However, no significant difference was found between the two groups in terms of thyroid volume or the presence of pseudonodulation (Figure 1).

Discussion

In this study, we documented a higher incidence of decreased echogenicity, increased heterogeneity, increased thyroid volume, and pseudodulation in the HT group compared to healthy individuals (p<0.001), and there was no difference in terms of the presence of nodules and cysts between the two groups. Both anti-tpo and anti-Tg levels were found to be positively correlated with TSH; however, only the anti-level was correlated with thyroid volume.

The most accurate method for the diagnosis of HT is histological diagnosis, but this method is rarely used because of its invasive nature. Physical examination, laboratory testing, and US examination are used to

Table 2. Relationship between TSH levels and thyroid antibodies of thyroid volumes				
	TSH		**Thyroid volume**	
	r	p	r	p
Anti-TPO	0.235	<0.001	0.272	<0.001
Anti-Tg	0.231	<0.001	0.047	0.341

TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, Anti-Tg: Antithyroglobulin antibody

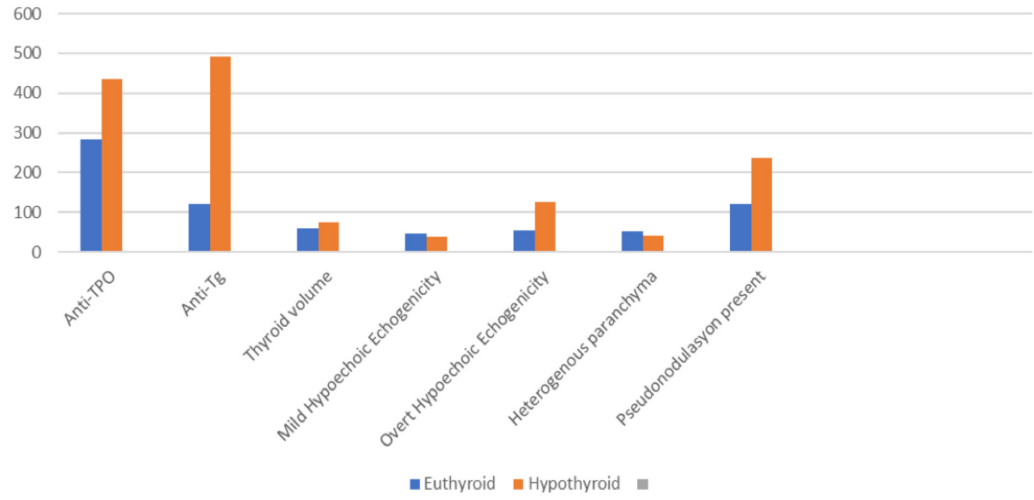

Figure 1. Comparison of thyroid antibodies and USG feature between patients with euthyroid and hypothyroid patients

Anti-TPO: Anti-thyroid peroxidase antibody, Anti-Tg: Antithyroglobulin antibody, USG: Ultrasonography

diagnose and monitor HT patients. The ease of use and non-invasiveness make the US a key procedure in the diagnosis and follow-up of HT (10).

If the diagnosis of HT was based only on physical examination and laboratory results, at least half of the patients with HT would be overlooked. The clinical and laboratory features of HT may vary. Sometimes HT can be totally asymptomatic (16). The US may be a very practical diagnostic tool for thyroid disease (17). Previous studies have shown that 13% of HT may have normal thyroid antibodies. Moreover, thyroid antibodies may be found in 10% of people who have no thyroid disease (6,7,9). US can provide additional information in the gray zone of HT. The use of US with clinical and laboratory findings significantly increases the sensitivity and specificity of an accurate diagnosis because of its easy accessibility and non-invasive nature (7,9,16).

High-resolution US is a quick, reliable, inexpensive, and highly sensitive tool for both diagnosis and guidance in the therapeutic intervention of thyroid disease and nodules (18,19). During the course of HT, US findings vary significantly, which defines the stage of histopathological progress. Moreover, Marcocci et al. (20) reported that the US is more predictive in detecting patients who are prone to hypothyroidism and reflects different stages of HT.

The normal thyroid parenchyma has a unique hyperechogenic and homogenous structure due to its follicular structure and colloid. However; in HT patients, thyroid parenchyma echogenicity is reduced because of the disruption of normal tissue and lymphocyte infiltration (21).

The PPV of reduced echogenicity for HT was 88.3%, and the NPV was found to be 93% (2,5). In accordance with previous studies, we found PPV as 30.3%. However, NPV was found to be lower than literature with 40%. Willms et al. (10) reported isoechogenicity in 21.5%, mild hypoechogenicity in 32.7% and overt hypoechogenicity in 45.7% of patients with HT. In accordance with their results, isoechogenity, mild hypoechogenity, and overt hypoechogenicity were found in 17.7, 38.3%, and 44% in our series, respectively. Autoimmune diseases exclude 95% with an isoechoic pattern in the US (22).

In a recent study, thyroid volume was higher in patients with thyroid antibodies than in patients without thyroid antibodies. But thyroid volume did not differ between the hypothyroid group and controls (3). Similarly, we found that thyroid volume was higher in HT than controls. However, there was no difference in the thyroid volume between the euthyroid and hypothyroid groups. We also found that thyroid volume was correlated with anti-TPO. Similar to our results, Anderson et al. reported that elevation of TSH and thyroid volume was correlated with thyroid antibodies (23). In contrast to our results, Willms et al. (10) did not find any correlation between thyroid volumes and thyroid antibodies.

The presence of multiple discrete hypoechoic micronodules (1-6 mm in size) is strongly suggestive of chronic thyroiditis. Thin echogenic fibrous septae may produce a pseudolobulated appearance in the parenchyma. Micronodulation was more common in the group with thyroid antibodies than in the controls (10,11). Similar to previous studies, we found pseudonodulation was more common in HT patients than in controls. The micronodular pattern has been found to have a 95% predictive value for the diagnosis of HT (23,24). Alidrisi et al. (25) found that hypoechogenicity and pseudonodulation were significantly

associated with high anti-TPO for diagnostic. Another study suggested that pseudonodulation and hypoechogenity reflect high inflammatory activity (26). Acar et al. (11) reported that micronodulation was not observed in control; 43% in euthyroid HT and 26% in hypothyroid HT. Like their results; in our study, micronodulation was found to be higher in HT patients than in controls. We did not observe any differences between euthyroid and hypothyroid patients.

This study demonstrated that thyroid antibodies in the parenchymal heterogeneous group were higher than those in the homogeneous group ($p<0.001$). These findings were consistent with the literature (10,27,28). Willms et al. (10) reported that nodules were found 21.9% in HT patients. In our study, nodules were found in 32.8% of HT patients; there were no significant differences between the two groups in terms of the presence of nodules. Conversely, Anderson reported that nodules were found in 4% of HT (23).

Study Limitations

An important limitation of our study is that no fine needle aspiration biopsy was performed, which is the gold standard examination for diagnosing HT. However, ultrasound evaluation using the same endocrinology specialist and the inclusion of 475 patients in the study are the study strengths.

Conclusion

US is a simple, non-invasive, and useful method. The USG provides helpful information about the HT stage. Heterogeneous thyroid parenchyme, hypoechogenicity, and pseudonodulation predict the diagnosis and follow-up of HT and are risk factors for elevated concentrations of thyroid antibodies. Also, it is an ideal tool to detect complications such as malignant nodules.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ankara Numune Training and Research Hospital (approval number: 865, date: 21.07.2014).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: I.D.D., Design: M.U., Data Collection or Processing: E.K., Analysis or Interpretation: M.N.B., Literature Search: S.G., Writing: M.U.

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

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