Turk J Endocrinol Metab 2019;23;141-149

Frequency Profile and Clinical Correlations of TSH Receptor Antibodies in Euthyroid and Hypothyroid Patients Having Autoimmune Thyroiditis

Otoimmün Tiroiditi Bulunan, Ötiroid ve Hipotiroid Hastalarda TSH Reseptör Antikorlarının Sıklık Profilleri ve Klinik Korelasyonları

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Abstract

Objective: The current understanding regarding the clinical significance of TSH receptor antibodies in chronic autoimmune thyroiditis is controversial. The aim of this study was to examine the frequency of elevated TSH receptor antibodies in euthyroid and hypothyroid autoimmune thyroiditis. Furthermore, their associations with the levels of thyroid hormones and autoantibodies (thyroid peroxidase and thyroglobulin antibodies [TgAb]), and the thyroid volume were also investigated.

Material and Methods: This cross-sectional study included recently diagnosed euthyroid (N=86) and hypothyroid (N=54) autoimmune thyroiditis patients along with the patients who were on levothyroxine replacement (N=66). The levels of TSH, free T4, free T3, TSH receptor antibodies, thyroid peroxidase, and thyroglobulin antibodies were measured using ECLIA (Roche Diagnostics, Switzerland). A 9 MHz transducer (Fukuda Corp., Japan) for thyroid ultrasound and a 14 MHz transducer (Ultrasonix Medical Corp., Canada) for Doppler ultrasound were employed. Statistical analyses were done with the help of IBM SPSS 19.0 software.

Results: The elevated levels of TSH receptor antibodies were observed in 6.3% of the participants (12 females and 1 male) and exclusively in hypothyroid subjects, who were either untreated (7.4%) or on levothyroxine treatment (7.6%). The highest and the lowest prevalence of positive TSH receptor antibodies were seen in patients with positive thyroglobulin antibodies & negative thyroid peroxidase (17.6%) and positive thyroid peroxidase & negative thyroglobulin antibodies (3.3%), respectively. Higher levels of TSH receptor antibodies were associated with a shorter duration of the disease (22 vs. 36 months), lower titers of thyroglobulin antibodies (281.2 vs. 400.9 UI/L), decreased thyroid volumes (9.4 vs. 14.2 cm^3) and an increased prevalence of orbitopathy (23.1 vs. 4.1%). In the whole study population, TSH receptor antibodies levels were related to fT4 (linear R2=0.271, p=0.039), titers of thyroid peroxidase (quadratic, R2=0.048, p=0.034), and thyroid volume (compound R2=0.041, p=0.011). However, in the TSH receptor antibodies positive patients, the correlation was seen only with the levels of thyroid peroxidase (compound R2=0.503, p=0.032).

Conclusion: TSH receptor antibodies positivity cannot be considered negligible in Hashimoto's thyroiditis. The elevated levels of TSH receptor antibodies might result in a predisposition to a more unstable thyroid function, lower thyroid volumes, specific profiles of thyroid peroxidase, and thyroglobulin antibodies and a higher prevalence of thyroid-associated ophthalmopathy.

Keywords: Hashimoto's thyroiditis; TSH-receptor antibodies; thyroid peroxidase antibodies; thyroglobulin antibodies; thyroid hormones; thyroid volume

Özet

Amaç: Kronik otoimmün tiroiditlerde TSH reseptör antikorlarının klinik önemi halen tartışmalıdır. Bu çalışmanın amacı, ötiroid ve hipotiroid kronik otoimmün tiroiditlerde yükselmiş TSH reseptör antikorlarının sıklığının incelenmesidir. Ayrıca, tiroid hormon-ları ile oto antikorlar (tiroid peroksidaz ve tiroglobulin antikorlar) ve tiroid hacmi ile ilişkisi de incelenmiştir.

Gereç ve Yöntemler: Bu çapraz tasarımlı çalışmaya, yeni tanı almış ötiroid (n=86) ve hipotiroid (n=54) hastalarla birlikte, levotiroksin replasman tedavisi almakta olanlar (N=66) dâhil edilmiştir. TSH, serbest T4, serbest T3, TSH reseptör antikorları, tiroid peroksidaz ve tiroglobulin antikorlar düzeyleri, ECLIA (Roche Diagnostics, İsviçre) kullanılarak ölçülmüştür. Tiroid ultrasonografisi için bir 9 MHz'lik transduser (Fukuda Corp., Japonya) ve Doppler ultrasonografi için bir 14 MHz'lik transduser (Ultrasonix Medical Corp., Kanada) kullanılmıştır. İstatistiksel analizler, IBM SPSS 19.0 yazılım desteği ile yapılmıştır.

Bulgular: Katılımcıların %6,3 (12 kadın ve 1 erkek)'ünde, TSH reseptör antikorları düzeylerinde yükselme görülmüştür. Yükselme, tedavi altında olmayan (%7,4) veya levotiroksin tedavisi alan (%7,6) hipotiroidi hastalarında belirgindir. Pozitif TSH reseptör antikorlarının en yüksek ve en düşük prevalansı, sırası ile pozitif tiroglobulin antikorlar-negatif tiroid peroksidaz (%17,6) hastalarda ve pozitif tiroid peroksidaz-negatif tiroglobulin antikorlar (%3,3) hastalarda görülmüştür. Daha yüksek TSH reseptör antikorları düzeyleri, daha kısa hastalık süresi (22'ye karşı 36 ay), daha düşük tiroglobulin antikorlar (281,2'ye karşı 400,9 UI/L) titreleri, azalmış tiroid hacimleri (9,4'e karşı 14,2 cm^3) ve artmış orbitopati prevalansı (%23,1'e karşı 4,1) ile ilişkilidir. Tüm çalışma popülasyonunda, TSH reseptör antikorları düzeyleri fT4 (lineer R2=0,271, p=0.039) düzeyleri, tiroid peroksidaz (kuadratik, R2=0,048, p=0,034) titreleri ve tiroid hacmi ile (bileşik R2=0,041, p=0,011) ile ilişkilendirilmiştir. Bununla birlikte, TSH reseptör antikorları pozitif hastalarda, sadece tiroid peroksidaz (bileşik R2=0,503, p=0,032) ile korelasyon görülmüştür.

Sonuç: Hashimoto tiroiditinde TSH reseptör antikorları pozitifliği göz ardı edilemez. TSH reseptör antikorlarının düzeylerinin yükselmesi, daha stabil olmayan tiroid fonksiyonlarına, daha düşük tiroid hacimlerine, spesifik tiroid peroksidaz ve tiroglobulin antikorlar profillerine ve daha yüksek tiroid ilişkili oftalmopati prevalansına predispozisyon oluşturabilmektedir.

Anahtar kelimeler: Hashimoto tiroiditi; TSH-reseptör antikoru; tiroid peroksidaz antikorları; tiroglobulin antikorları; tiroid hormonları; tiroid hacmi

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Received: 18/12/2018 **Received in revised form:** 19/06/2019 **Accepted:** 19/07/2019 **Available online:** 28/08/2019

DOI: 10.25179/tjem.2018-64300

Introduction

TSH receptor antibodies (TSH-R-Ab) are one of the hallmarks of Graves' disease (GD). However, they are also found to be elevated in a small proportion of patients with chronic autoimmune thyroiditis (AIT) (1,2). The prevalence and clinical significance of these antibodies have been studied in patients with AIT and different thyroid functions (3-7). A few of the available studies focused on the hypothesis that blocking TSH-R-Ab might cause thyroid atrophy (3,8). Several studies that included hypothyroid patients showed great variability in the prevalence of elevated TSH-R-Ab, ranging from 5% to 35% (9-11). While a few surveys about euthyroid patients showed the prevalence rate between 9% and 23% (4,12). Two studies reported the prevalence of elevated antibody in 13.6% and 78.3% of hyperthyroid AIT patients, respectively (13,14). Some publications have described a negative correlation between TSH-R-Ab levels and the thyroid volume (12,15-17) or the function (12,15,18), while others did not (9). Most of the older studies used first and second generation thyroid-binding assays (3-5,19,20), which had lower sensitivity and specificity when compared to the third generation electrochemiluminescence immunoassays (e.g., ECLIA), which are widely available (10,21,22). Only very few studies have demonstrated a comprehensive and complex an7alysis of the clinical, laboratory and ultrasound correlates of TSH-R-Ab levels in AIT.

Purpose

The purpose of this study was to determine the clinical value of TSH-R-Ab levels in euthyroid and hypothyroid patients with AIT. This was achieved by examining the frequency profile of elevated TSH-R-Ab titers and their associations with the thyroid hormones, antibody levels, the thyroid volume, and other clinical parameters.

Material and Methods

Materials (Subjects/Design)

This was a cross-sectional study that included newly diagnosed euthyroid and hypothyroid patients with AIT, as well as the patients who were on previously initiated Levothyroxine replacement. The participants were from a tertiary hospital-based specialized endocrine unit (60% inpatients and 40% outpatients). This study was approved by the Ethical Committee of the Scientific Council at the Medical University (Research Project 4-D/2016) and was conducted in compliance with ethical standards and the Declaration of Helsinki. All the patients read and signed informed consent prior to any procedure and all procedures were part of the routine clinical workup in AIT. The following were the inclusion criteria: age above 18 years, newly diagnosed euthyroid or hypothyroid AIT or undergoing current Levothyroxine replacement therapy, and willingness to participate. Current or recent treatment with glucocorticoids or interferon or any other type of immune-modulating agent for the concomitant disease was the exclusion criterion.

The diagnosis of AIT was based on the following criteria (23): Presence of at least 2-fold increase in the levels of thyroid peroxidase antibodies (TPOAb) and/or thyroglobulin antibodies (TgAb); Diffused changes in the thyroid parenchyma during ultrasound (hypoechogeneity and heterogeneity, normal or reduced blood flow); Clinical findings and status appropriate with AIT (such as hard thyroid gland on palpation, low radionuclide uptake on thyroid scintigraphy and compliant cytology results if available).

Methods

Factors like past and present medical history, smoking habits, presence of other autoimmune diseases and family history for thyroid disease were registered for each participant. Physical examination included detection of eye symptoms, cardiovascular signs, and palpation of the thyroid.

The measurement of serum levels of thyroid hormones (TSH, free T_4, and free T_3) and antibodies (TSH-R-Ab, TPOAb, TgAb) in the fasting morning blood sample was performed with the help of third-generation ECLIA assays (Roche Diagnostics, Switzerland) on an Elecsys 2010 analyzer. TSH-R-Abs were therefore assessed in a competitive binding assay as TSH-R-binding inhibitory immunoglobulins. The reference ranges for TSH, fT_4, fT_3, TPOAb, TgAb, and TSH-R-Ab were 0.27-4.2 mIU/L,

12-22 pmol/L, 3.54-6.47 pmol/L, 0-34 IU/mL, 0-115 IU/mL, and <1.75 IU/L, respectively. Gray-scale thyroid ultrasound was performed at 9 MHz, on a Fukuda-Denshi 550 device (Fukuda Corp., Japan) followed by Doppler ultrasound at 10-14 MHz, on an Ultrasonix device (Ultrasonix Medical Corp., Burnaby, Canada). The thyroid volume was calculated in mL (cm^3) according to the formula by Brunn et al. (24):
Thyroid Volume of the studied lobe (cm^3) = anteroposterior size × transverse size × length × 0.479 (all in centimeters).
The presence of nodules was noted and the vascularity and blood flow were assessed for differentiation with GD (25). Available technetium scans were reviewed and normal or decreased patchy radionuclide uptake by the thyroid was used as a confirmation criterion (26). Study subjects were evaluated by clinical endocrinologists and experienced ophthalmologists for the presence of thyroid-associated ophthalmopathy (TAO). If found to be present, the TAO severity was recorded according to the NOSPECS classification and its activity according to the clinical activity score (CAS) (27).

Statistical analysis

The sample size was calculated based on the population size of AIT patients in the local area of 10 000, with a 90% confidence interval and a 5% margin of error. About 264 patients were required for the statistical power but only 206 agreed to participate in the research. Therefore, the power of the study was 86%. The participants were recruited over a period of 18 months (between Jan 2016 and June 2018).
Statistical analyses were done with the help of IBM SPSS 19.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics and variation analysis were performed initially and the normal distribution was examined by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Descriptive statistics were compared between the groups via Kruskal-Wallis and Mann-Whitney tests, which are non-parametric tests for continuous variables. ANOVA, correlation and regression analyses were performed and missing values were excluded from the analysis. Statistical significance was set as $p<0.05$ and all tests were two-tailed.

Results

Around 206 subjects (191 females and 25 males), aged between 18-74 years, having AIT agreed to participate in the study. The participants included 86 newly diagnosed euthyroid patients, 54 newly diagnosed hypothyroid patients and 66 subjects who were under Levothyroxine treatment (mean dose 59±38 µg; range 7.5-150 µg). The ranges of TSH, fT_4 and fT_3 of the participants were 0.34 to 95.0 mIU/L, 3.4 to 22.0 pmol/L and 2.59 to 6.00 pmol/L, respectively. The titer ranges of the thyroid antibodies TSH-R-Ab, TPOAb and TgAb were 0.3 to 6.58 IU/L, 4.38 to 6000.0 IU/L, and 2.64 to 4000.0 IU/L, respectively. Almost half of the participants, i.e., 46%, were both TPOAb and TgAb positive, whereas, only positive TPOAbs were present in the other 42%, and the remaining 12% had only positive TgAbs. The thyroid volume during ultrasound ranged from 1.0 to 60.0 mL. Single nodules and multiple nodules were found, during an ultrasound, in 18.7% and 2.7% of the participants, respectively.
Table 1 gives a summary of the thyroid hormones, antibodies and the thyroid volumes of the participants. The newly diagnosed hypothyroid subjects were observed to be a bit younger than the remaining participants. There was no difference in the fT_4 levels between euthyroid and hypothyroid subjects on Levothyroxine treatment; the same was true for fT_3. No significant inter-group differences were seen in the levels of TSH-R-Ab. The titers of TPOAb and TgAb were found to be highest in the newly discovered hypothyroid subjects, followed by the newly diagnosed euthyroid cases. The TPOAb titers were highest in the presence of positive TgAb, while TgAb levels were highest in the presence of positive TPOAb. The thyroid volume was highest in the newly diagnosed euthyroid group and lowest in the subgroup with positive TgAb and negative TPOAb. Atrophic thyroiditis was found in 14.0% of the total participants. Of them, it was observed in 2.5% of the newly discovered euthyroid participants, in 23.1% of the newly discovered hypothyroid patients, and in 25.0% of the patients on replacement therapy ($p<0.001$ for the difference between the euthyroid subgroup and the other participants). As indicated by the antibody status,

Table 1. The thyroid parameters (hormonal and antibody levels, thyroid volume) of the participants shown as medians and min-max range (in brackets).

All (Men/Women)	Whole group (N=206; 25 M/191 F)	On Levothyroxine (N=66; 4 M/62 F)	Newly diagnosed Hypothyroid (N=54; 8 M/46 F)	Newly diagnosed Euthyroid (N=86; 12 M/74 F)	Only TPOAb + (N=90; 14 M/76 F)	Only TgAb + (N=17; only Females)	Both Ab + (N=99; 11 M/88 F)
Age, years	42.5 (18-74)	47.0 (22-73)	40.0 (18-72)[a]	43.0 (18-74)	46.0 (18-74)	46.0 (18-70)	38.5 (18-72)[b]
AIT duration, months	N/A	33.0 (6-300)	N/A	N/A	6.0 (0-300)	5.0 (0-192)	15.0 (0-240)[b]
TSH, mIU/L	3.3 (0.4-99.8)	3.1 (0.4-73.6)	6.6 (4.2-50.9)	1.9 (0.4-4.2)[a]	2.6 (0.4-95.0)	3.6 (0.4-10.5)	4.1 (0.4-73.6)[b]
Free T_4, pmol/L	14.6 (3.4-22.0)	15.8 (8.3-22.0)	13.5 (4.8-16.8)	15.4 (3.7-20.7)	14.7 (3.4-22.0)	14.3 (9.3-19.9)	14.7 (4.8-20.8)
Free T_3, pmol/L	4.7 (2.6-6.0)	4.8 (3.1-5.6)	3.6 (2.6-6.0)	4.7 (2.8-6.0)	4.8 (2.9-5.8)	4.6 (3.0-6.0)	4.5 (2.6-6.0)
TSH-R-Ab, IU/L	0.4 (0.3-6.6)	0.4 (0.3-6.6)	0.3 (0.3-2.5)	0.4 (0.3-3.0)	0.4 (0.3-2.9)	0.3 (0.3-6.6)[b]	0.4 (0.3-4.3)
TPOAb, IU/L	214.4 (4.4-1000.0)	207.7 (4.4-1000.0)	315.9 (9.6-1000) [a]	188.9 (5-1000)	199.5 (34.5-1000)	12.0 (4.4-27.0)	261.0 (40.7-1000)[b]
TgAb, IU/L	198.2 (2.6-4000)	236.6 (10.0-4000)	205.0 (15-4000)[a]	183.7 (2.6-3671)	24.0 (2.6-110.0)	418.7 (131.7-1960)	347.0 (115-4000)
Thyroid volume, ml	13.0 (1.0-60.0)	12.0 (3.0-60.0)	12.0 (6.0-31.0)	13.0 (6.0-45.0)	13.0 (1.0 -45.0)	11.5 (6.0-15.0)[b]	12.0 (3.0-60.0)

[a] The differences with the other two subgroups (columns 3-5) are significant. The Mann-Whitney test was performed.
[b] The differences with the other two subgroups (columns 6-8) are significant. The Mann-Whitney test was performed.

thyroid atrophy was found in 10.3% of patients were positive for TPOAb and negative for TgAb, 26.7% of patients were positive for TgAb and negative for TPOAb, and 15.4% of patients were positive for both antibodies, underlining a significant association of low thyroid volumes with isolated TgAb positivity ($p<0.001$ for the difference in isolated TgAb positivity compared to isolated TPOAb positivity; and $p=0.02$ compared to combined positivity of both antibodies).

The levels of TSH-R-Ab were positive (≥1.75 IU/L) in 13 participants (6.3%); 12 females and 1 male. Negative titers of TSH-R-Ab were seen in 169 females and 24 males (Female: male ratio of 7). TSH-R-Ab levels were seen to be elevated among 7.6% of patients on Levothyroxine treatment, 4.7% of the newly diagnosed euthyroid subjects and 7.4% of the newly diagnosed hypothyroid subjects. TSH-R-Ab positivity was found in 7.1% of patients positive with both TPO- and Tg-antibodies, 3.3% of cases with positive TPOAb only, and 17.6% of the subjects with positive TgAb only.

Table 2 gives a summary of the differences between the subjects with positive and negative titers of TSH-R-Ab. When compared to the participants with negative TSH-R-Ab, the TSH-R-Ab positive ones underwent a shorter duration of Levothyroxine treatment; their TSH and thyroid volumes were higher. Among the TSH-R-Ab negative patients, 52.0% had positive titers of both TPOAb and TgAb; 39.9% had positive levels of TPOAb only and 8.1% had TgAb positivity only. About 50.0% of the participants with positive TSH-R-Ab showed positivity of the other two thyroid antibodies, whereas 25.0% had elevated TPOAb only and another 25.0% had elevated TgAb only. Therefore, isolated TgAb positivity was seen more frequently in the participants who had positive TSH-R-Ab. GO was more frequent in TSH-R-Ab positive patients, but the low number of patients did not allow further analysis. Positivity of TSH-R-Ab was associated with a higher prevalence of smoking and a higher number of cigarettes per day when compared to negative TSH-R-Ab patients. During US, no thyroid nodules were found in the patients with positive TSH-R-Ab, while in 22.5% of negative TSH-R-Ab cases, nodular lesions consisting of mostly single nodules were present. The prevalence

Table 2. Thyroid parameters were stratified according to levels of TSH-R-Ab (positive versus negative subjects). The medians, as well as the ranges (min.-max.), are shown.

Thyroid variable	Positive TSH-R-Ab (N=13)	Negative TSH-R-Ab (N=193)
Age, years	46.0 (19-68)	42.0 (18-74)
AIT duration, months	8.0 (0-120)	2.0 (0-300)[a]
TSH, mIU/L	2.2 (0.4-73.6)	3.3 (0.3-95.0)[a]
Free T_4, pmol/L	13.5 (8.3-20.8)	14.7 (3.40-22.00)
Free T_3, pmol/L	5.0 (2.8-5.2)	4.6 (2.59-6.00)
TSH-R-Ab, IU/L	2.5 (1.9-6.6)	0.3 (0.30-1.71)[a]
TPOAb, IU/L	197.0 (4.4-600.0)	218.9 (5.0-1000.0)
TgAb, IU/L	211.2 (10.0-636.0)	197.0 (2.6-4000.0)
Thyroid volume, mL	8.6 (3.0-21.0)	13.0 (1.0-60.0)[a]
Levothyroxine, µg daily (if applicable)	50.0 (10.0-100.0)	50.0 (0-150.0)
Presence of thyroid-associated orbitopathy,%	3 subjects, 23.1%	8 subjects, 4.1%[a]
The activity of thyroid-associated orbitopathy-CAS (if applicable)	0	1.6 (0-2)
Severity thyroid-associated orbitopathy-NOSPECS (if applicable)	2.3 (2-3)	3.6 (3-4)
Current smokers,%	6 subjects, 46.2%	67 subjects, 34.7%
Cigarettes daily (if applicable)	20 (0-40)	14 (1-30)

[a] $p<0.05$ for the difference between participants with positive and negative titers of TSH-R-Ab. The Mann-Whitney test was performed.

of thyroid atrophy in patients with negative and positive TSH-R-Ab was 12.5% and 36.4%, respectively ($p<0.001$).

The bivariate correlation analyses of the whole study group indicated significant correlations between the levels of TSH-R-Ab and TPOAb (Spearman's Rho=0.131, p=0.049) and between TSH-R-Ab levels and the thyroid volume (Spearman's Rho=0.174, p=0.028). No other significant correlations were present between TSH-R-Ab and the other parameters in the TSH-R-Ab positive subgroup.

The results of the regression analysis of the TSH-R-Ab titers (as the independent variable) with different clinical and laboratory parameters (as dependent variables) are shown in Table 3. The TSH-R-Ab titers did not relate with other thyroid parameters in the study group as a whole (combining both TSH-R-Ab positive and negative participants), except fT_4, TPOAb titers, and thyroid volumes. All of the three regression coefficients were found to be significant, but for their very small size (describing a very weak association). The linear relationship between the levels of TSH-R-Ab and fT_4 displayed the strongest association. In the multiple regression model, fT_4 remained as the single variable that very weakly related to the levels of TSH-R-Ab ($R^2=0.09$, $p=0.002$). In subjects with positive TSH-R-Abs, only a single regression model with the levels of the TPOAb was found and no other relationships could be established.

Discussion

In this study, we examined the frequency profile and clinical correlates of elevated TSH-R-Ab levels in newly diagnosed euthyroid and hypothyroid patients with autoimmune thyroiditis, and in patients who were on Levothyroxine replacement therapy. TSH-R-Ab levels were elevated in 6.3% of the participants (almost all females). The prevalence of positive TSH-R-Abs was highest in patients with positive TgAb and TPOAb, whereas it was lowest in those with positive TPOAb and negative TgAb. TSH-R-Ab positivity was found to be associated with a shorter duration of the disease (if previously known), lower titers of TgAb, reduced thyroid volumes and a higher prevalence of associated orbitopathy. The levels of TSH-R-Ab correlated with fT_4, the titers of TPOAb, and thyroid volumes in the whole study population. Further, in patients with positive TSH-R-Abs, a significant correlation was seen with the levels of TPOAb.

Table 3. The best models from the regression analyses are listed. Eight regression curves were tested: linear, quadratic, cubic, inverse, compound, power, logarithmic, exponential ones. The titers of TSH-R-Ab were the independent variable and the related thyroid parameters were the dependent variables.

	Best fitting regression model (Confidence interval 95%)	
Dependent variables	**Whole study population (N=206)**	**Only TSH-R-Ab positive subjects (N=13)**
Age, years	No regression model available	No regression model available
AIT duration, months	No regression model available	No regression model available
TSH, mIU/L	No regression model available	No regression model available
Free T_4, pmol/L	Linear, R2=0.271, p=0.039[a] Beta=-0.203[b]	No regression model available
Free T_3, pmol/L	No regression model available	No regression model available
TPOAb, IU/L	Quadratic, R2=0.048, p=0.034[a] Beta=-0.099/-0.119[b]	Compound, R2=0.503, p=0.032, Beta=-0.597[b]
TgAb, IU/L	No regression model available	No regression model available
Thyroid volume, mL	Compound, R2=0.041, p=0.011[a], Beta=0.818[b]	No regression model available
Levothyroxine, µg daily (if applicable)	No regression model available	No regression model available

[a] Other models also available (although weaker ones with lower R2); [b] Standardized beta-coefficients are shown.

One of the first papers that reported the elevation of TSH-R-Abs in euthyroid or hypothyroid patients with AIT was published in 1990 (3). The authors described the prevalence as 3.7% in euthyroid, 9.4% in subclinical hypothyroid participants, and in 46% of atrophic thyroiditis patients. Their conclusion suggested that TSH-R-Ab was possibly linked to the development of hypothyroidism and thyroid atrophy (3). During the same year, another group reported positive TBII and TSH stimulated cAMP response inhibitory antibodies (blocking TSH-R-Ab) in 5 out of 28 pregnant women with AIT (4). A prevalence of 9.2% was reported in 54 Singaporean patients with AIT (euthyroid and hypothyroid) which was linked to TSH-R-Ab positivity (5). Positive TSH-R-Ab levels in AIT were also observed when different assays for TSH-R-Ab measurement were validated (10,19,20,28). A recent study reported a 5.5% prevalence of positive thyroid-stimulating antibodies in 700 consecutive and unselected patients with Hashimoto's thyroiditis, while their prevalence was 68.2% in the presence of Hashimoto's thyroiditis and thyroid-associated orbitopathy (29). The authors concluded that TSH-R-Ab stimulation may contribute to the pathophysiology of TAO. Another recent study found the prevalence of TSH-receptor blocking antibodies in Hashimoto's thyroiditis to be 9.3% (6). This study revealed that the presence of those specific TSH-R-Abs was a relevant and important factor in the identification of potentially reversible hypothyroidism (6). A commentary on that data stated that the occurrence of thyroid-stimulating or blocking antibodies might be seen in 4-9% of patients with AIT (7). Almost all these investigations focused on studying the prevalence of TSH-R-Abs and did not extensively explore its correlations with ultrasound and laboratory parameters. Nonetheless, the prevalence of positive TSH-R-Abs among the patient populations in these studies is quite similar to what we found in our study. Some studies have not reported positive TSH-R-Abs in AIT, yet others have published surprisingly high percentages of TSH-R-Ab positivity (2,14).

Our study raised the question as to how useful the information from measurements of TSH-R-Abs might be in Hashimoto's thyroiditis. Firstly, we found several studies describing that the role of the TSH-R-Abs swings from hypothyroidism to hyperthyroidism and vice versa (30-35). It appears that Graves' disease with hyperthyroidism might change to a hypothyroid phase that is very similar to AIT (34,35). The opposite of this, i.e., hypothyroidism in Hashimoto's thyroiditis may spontaneously recover or

switch to hyperthyroidism just like in Graves' disease (30-33,36). Therefore, positive TSH-R-Abs in AIT might implement a more unstable thyroid function. The optimal approach to investigate this would be to measure the functionality and biological function of the TSH-R-Abs by using a cell-based assay that could differentiate between stimulating and neutral or inactive antibodies (31,37-39). In some cases, the change in the thyroid function can be explained by detecting a change in the predominant class of TSH-R-Ab (31).

Unfortunately, we were not able to differentiate between subclasses of TSH-R-Abs, which limited the scope of our data. However, we could detect the association of TSH-R-Ab positivity with slightly lower levels of thyroid volumes, a finding that was attributed to the effect of blocking or neutralizing TSH-R-Abs, as discussed above. The possible link between blocking TSH-R-Abs and thyroid atrophy had been mentioned in the initial studies which explored that subject (3). Neutral TSH-R-Abs were supposed to induce apoptosis via activation of mitochondrial ROS (mROS) (8,38,40). Moreover, in our study, the patients with positive TSH-R-Abs showed far less nodular changes on US, a novelty finding that needs further attention.

Since the role of TSH-R-Abs in TAO pathogenesis is well established (41-43), so it is not surprising that these antibodies are highly prevalent in Hashimoto's thyroiditis with associated orbitopathy (29). We also found a high prevalence of associated orbitopathy in TSH-R-Ab positive patients (23.1%), compared to TSH-R-Ab negative ones (4.1%). J. Orgiazzi reported the prevalence of 6% of TAO in patients with Hashimoto's thyroiditis (44).

Finally, the question of positive TSH-R-Abs in pregnant women with Hashimoto's thyroiditis is still under investigation. Hence, it is important to reveal a possible induction of neonatal thyroid dysfunction, mainly hypothyroidism, using these antibodies (4,41).

Strengths and limitations

The major strength of our study is the use of a third-generation TSH-R-Ab assay (ECLIA) for analysis. Some previous studies, including the porcine ones, have employed first- and second-generation assays (19,20). However, the third generation assays are much efficient as they have excellent reproducibility and higher analytical and clinical sensitivity and specificity (10,21,22). Our study also has a number of limitations. Firstly, the biological activity of the detected TSH-R-Abs was not assessed. This was because the cell-based bioassays are not available in everyday practice. Sometimes in one patient's serum, there could be more than one type of TSH-R-Abs such that their concentration and affinities could impair the proper determination (31,45). Secondly, the number of participants is modest which prevents us from finding the true prevalence as well as additional significant associations. Finally, only euthyroid and hypothyroid patients were selected, as the differentiation between thyrotoxicosis in Graves' disease or Hashimoto's thyroiditis with positive TSH-R-Abs is an ambitious task, even in the presence of cytological data (13,14).

Conclusion

It was concluded that measuring TSH-R-Abs in the setting of Hashimoto's thyroiditis is plausible from the clinician's point of view. The TSH-R-Ab positivity is not negligible. Moreover, elevated TSH-R-Abs might result in a predisposition to a more unstable thyroid function, lower thyroid volumes, specific combinations of TPOAbs and TgAbs, and a higher prevalence of TAO. Further investigations are needed for determining the biological role of TSH-R-Abs in patients with Hashimoto's thyroiditis.

Author Contributions

Ralitsa Mekova conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, reviewed drafts of the paper.

Mihail Boyanov conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Grants

This study was partly supported by the Ph.D. program 2016 at the Medical University Sofia.

Declaration of Conflict of Interest

The authors have no conflict of interest to declare.

Ethical Considerations

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers): Ethical clearance for this study was obtained from the Medical Ethics Committee of the Medical University of Sofia.

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