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Original Article

TSH receptor autoantibody levels in patients with non-toxic diffuse and nodular goiter

Hatice Dörtok Demir[1], Zeynep Çetin[2], Cemil Çelik[3]

[1]Department of Medical Biochemistry, Faculty of Medicine, Amasya University, Amasya, Turkiye
[2]Deparment of Internal Medicine, Faculty of Medicine, Amasya University, Amasya, Turkiye
[3]Department of Medical Biochemistry, Faculty of Medicine, Maltepe University, İstanbul, Turkiye

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Corresponding Author: Zeynep Çetin, drzeynepcetin@gmail.com

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ABSTRACT

Aims: To investigate thyroid stimulating hormone (TSH) receptor antibody (TRAb) levels in patients with diffuse and nodular goiter and in patients which undergo thyroid operation.

Methods: 40 patients with non-toxic diffuse goiter (NTDG), 20 patients with non-toxic nodular goiter (NTNG), 20 patients undergo thyroid operation, and 34 healthy subjects were involved. Thyroid function tests, TRAb levels were measured. All the patients were done thyroid sonography. In the opereation group, preoperative, postoperative 10th and 30th day TRAbs were studied.

Results: TRAb was significantly elevated in 20% of patients with NTNG, 30% of patients with NTDG and 35% of patients in the operation group ($p>0.05$). No TRAb(+) patient was found in the control group. TRAb levels in the postoperative period in the operated group were higher than in the preoperative period.

Conclusion: TRAb was found in euthyroid patients with goiter, different from the control group. In the group operated for goiter, postoperative TRAb levels were higher than preoperative levels. These findings indicated that autoimmunity may be involved in the development of non-toxic goiter.

Keywords: TSH receptor antibody, non-toxic, diffuse goiter, nodular goiter

INTRODUCTION

Goiter is enlargement of the thyroid gland. The normal thyroid gland has a volume of 7-10 ml and weighs 10-20 g.[1] When the thyroid volume exceeds 18-19 ml in women and 25 ml in men, it is defined as goiter.[2] Goiter may be diffuse or nodular and may be accompanied by a thyroid hormone disorder. Epidemiologically, it is classified in 2 ways: goiter in more than 10% of the population or in 5% of children aged 6-12 years is called endemic goiter; if this rate is less than 5%, it is called sporadic goiter.[3] The primary cause of goiter is iodine deficiency. However, despite adequate iodine prophylaxis, goiter cannot be eradicated completely. Goiter is not observed in every individual living in the same endemic area. The incidence of goiter may also be different in regions with similar iodine intake. Based on all these, it is obvious that other factors are also involved in the development of goiter. In particular, autoimmune mechanisms have been shown to play a role in goiter development.[4]

Autoimmune diseases of the thyroid gland are the most common of all autoimmune endocrinopathies. Many factors have been shown to be indicative of autoimmunity in the thyroid. HLA-DR, a major histocompatibility complex (MHC) class II antigen, is associated with autoimmune diseases and has been shown to be present in autoimmune thyroid. Infiltration of the thyroid gland with activated T lymphocytes, decreased T suppressor/T helper lymphocyte ratio in the blood and various autoantibodies also point to this autoimmunity. These include anti-thyroid peroxidase (anti-TPO) antibody, anti-thyroglobulin (anti-TG) antibody, and thyroid stimulating hormone (TSH) receptor antibody (TRAb). While the first two cause thyroid cell damage, TRAB affects the function and growth of the gland.[5-7]

TRAbs are heterogeneous, some of them act as full agonists or full antagonists of TSH, while others act as partial agonists. Those that bind to the receptor and stimulate cell function play a role in Graves' disease, those that inhibit cell function play a role in idiopathic myxedema, those that stimulate cell growth play a role in non-toxic goiter and those that inhibit cell growth play a role in the etiology of atrophic thyroiditis.[8,9] In the literature, TRAbs have been shown to be present in the circulation of patients with non-toxic goiter at various rates. The presence of these antibodies in endemic or sporadic cases of goiter suggests that autoimmunity is involved in the pathogenesis of goiter. In these patients, it has been found that non-toxic goiter starts as a result of prolonged action of an antibody which is not as potent as in Graves' disease and the picture progresses to nodular goiter.[10-12]

The aim of this study was to determine the level of TRAB, a humoral marker of thyroid autoimmunity, in patients with non-toxic diffuse and non-toxic nodular goiter from various iodine-deficient regions in the Black Sea region of Turkiye and to determine preoperative and postoperative TRAB levels in a group of patients operated for nodular goiter.

METHODS

This thesis study was conducted between January 1994 and December 1994 at the Ondokuz Mayıs University Faculty of Medicine Hospital. There was no ethical approval requirement at the time of this study, therefore ethical approval was not obtained. Institutional approval was obtained. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

The study consisted of 114 subjects in total. Non-toxic diffuse goiter (NTDG) was 40, non-toxic nodular goiter (NTNG) was 20, operation group was 20 and the control group consisted of 34 healthy subjects. All participants were euthyroid and were not taking any medication that would affect thyroid function. Patients with allergic, rheumatologic or other autoimmunity-related diseases and those with suspected thyroid malignancy were excluded.

The study group (NTDG+NTNG) patients came from various provinces of the Black Sea region. The patients and their numbers are given in the table below (Table 1).

Table 1. Provinces of the patients

Province	Number (%)
Samsun	31 (52)
Amasya	8 (13)
Giresun	6 (10)
Ordu	9 (15)
Çorum	2 (3.3)
Tokat	4 (6.7)
Total	60 (100)

In the first part of the study, NTDG, NTNG and healthy volunteers were compared and this part was designed cross-sectionally. In the 2nd part, preoperative and postoperative TRAb levels of 20 NTNG patients with an indication for surgery were analyzed.

Goiter was staged according to Pan American Health Office (PAHO) criteria:[13]

Grade 0a: No goiter

Grade 0b: Goiter present on palpation only. No visible hyperplasia when the neck is in extension

Grade I: Palpable goiter present. Hyperplasia visible on neck extension

Grade II: Hyperplasia visible when the neck is in neutral position

Grade III: Very large hyperplasia identifiable from a distance

Laboratory

TSH, total thyroxine (T4), free T4, total triiodothyreonine (T3) and free T3 were measured by chemiluminescence method. In the first part of the study, euthyroid individuals with goiter were identified and after the diagnosis was confirmed, blood samples were taken from the participants, centrifuged at 3500 rpm and serum was separated. Sera were stored at -20^{0}C until the time of the experiment. In the second part of the study, blood samples were taken from 20 NTNG patients with an indication for operation 1 day before the operation and on the 10th and 30th days after the operation. They were centrifuged at 3500 rpm and stored in the same way.

All serum samples of the experimental and control groups were removed from the deep freezer at the same time and TRAb levels were analyzed under the same conditions. TRAb levels were analyzed with Byk-Sangtec Diagnostica GmbH&Co.KG radioreceptor assay kit specific for TRAb. During the analysis, an ICN brand gamma counter with serial number 92122237 was used in the Central Laboratory of Ondokuz Mayıs University.

This assay method is based on the principle of competitive binding of radiolabeled TSH and TRAbs from patient serum to bind to soluble porcine TSH receptors in the experimental medium. The experimental procedure and steps are as follows:

1. 50 µl each of reference serum, positive control serum and patient serum were pipetted into test tubes. For detection of non-specific binding (NSB), the same amount of reference serum was added to the NSB tubes.
2. 100µl of porcine TSH receptor was added to each test tube and mixed with vortex. For NSB, 100 µl of distilled water was added instead of TSH receptor.
3. Allowed to stand at room temperature for 20±5 minutes.
4. 100µl of ^{125}I-labeled bovine TSH was added to all tubes and mixed with vortex.
5. Again incubated for 2 hours at room temperature.
6. 1 ml polyethylene glycol (PEG) was added to all tubes and mixed with vortex.
7. Centrifuged in a refrigerated centrifuge at 3000 rpm for 30 min.
8. After centrifugation, the supernatant was immediately aspirated with an aspirator and pasteur pipette and removed from the experimental medium.
9. The radioactivity of the pellet remaining at the bottom of the tubes was counted in a gamma counter in the ^{125}I channel.

Experimental results were evaluated with the following formula:

$$F\%= 100 X [1 - cpm_{sample} - cpm_{NSB} / cpm_{reference\ serum} - cpm_{NSB}]$$

F%: Level of immunoglobulin that inhibits the binding of labeled TSH

cpm_{sample}: cpm (count per minute) of patient serum

$cpm_{reference\ serum}$: cpm of reference serum

1: The number at which binding of specific ^{125}I-TSH is considered 100%.

Normal population studies have shown that TSH binding inhibition (F%) is not greater than 15% in normal subjects. TSH binding inhibition greater than 15% indicates the presence of TRAb activity.[14]

Values with F%<15% are considered negative for antibody. TRAbs measured by this method are called immnunglubulins that inhibit TSH binding.

Test characteristics were studied for quality control of the experiment;

a. According to the assay procedure, the binding of the reference serum had to be 20-30%.

It was evaluated by the formula BN= ($cpm_{reference\ serum}$/total activity) x 100 and found to be 28%.

b. Nonspecific binding had to be below 9%.

BNSB= ($cpm_{non\text{-}specific\ binding}$/total activity) x 100 and was found to be 7.4%.

c. Assay precision was assessed by repeated measurements of the positive control and intra-assay variation was found to be 8.3%.

Measurement of Thyroid Gland Volume

To examine the correlation of TRAbs with thyroid volume, thyroid ultrasonography (USG) was performed by the same radiologist. The volume of each thyroid lobe was calculated by the formula (height x width x thickness) x (π/6) and then the volume of each lobe was summed to determine the total thyroid volume. Total thyroid volume was divided by body weight to obtain the thyroid volume/body weight ratio.[15]

Statistical Analysis

Data were expressed as mean ± standard error. For statistical analysis, Student's t test was used to compare the mean age of the patient group with NTDG, the patient group with NTG (diffuse+nodular) and the control group. Mann-Whitney U test was used to compare the mean age of the patients with NTNG, patients operated for nodular goiter and the control group. Mann-Whitney U test was used to compare the mean age between genders. Chi-square test was used to compare the gender distribution between groups and chi-square test was used to compare the distribution of patients with TRAb (+). Chi-square test in dependent groups was used to compare the distribution of patients with TRAb (+) in the preoperative and postoperative (10^{th} and 30^{th} day) period in the operation group. Friedman test was used to compare the F% values of the operation group and Wilcoxon paired two-sample test was used for pairwise comparisons. Correlation analysis was used to evaluate the relationship between thyroid volume and F% values reflecting TRAb levels in patients with TRAB (+) in the whole patient group (NTDG+NTNG).

Table 2. Mean age and gender distribution in the groups

Groups		Patient number	Age (mean±SD)
1	Non-toxic diffuse goiter Male Female	40 5 35	31.38±1.52 35.2±4.43 30.83±1.62
2	Non-toxic nodular goiter Male Female	20 2 18	34.83±2.12 22±2 36.28±2.09
3 (1+2)	Non-toxic goiter (diffuse+nodular) Male Female	60 7 53	32.53±1.24 33.43±3.93 32.68±1.32
4	Control group Male Female	34 5 29	32.41±1.03 35±3.84 32.1±1.03
5	Operated for NTNG Male Female	20 3 17	38.3±1.9 36.33±3.18 38.65±1.73

SD: Standard deviation, NTNG: Non-toxic nodular goiter

Table 3. Comparison of groups according to TSH level

Groups		TSH (μIU/ml)	p
1	Non-toxic diffuse goiter	0.98±0.106	NA
2	Non-toxic nodular goiter	0.817±0.7	
3 (1+2)	Non-toxic goiter (diffuse+nodular)	0.927±0.08	
4	Operated for NTNG	0.681±0.108	
5	Control	0.964±0.08	

TSH: Thyroid stimulating hormone, NTNG: Non-toxic nodular goiter

Table 4. F% levels and TRAb(+) patient distribution of the groups

Groups		Patient number	F%	Number of TRAb (+) patients (%)
1	Non-toxic diffuse goiter	40	11.18±1.76	12 (30)
2	Non-toxic nodular goiter	20	6.27±1.67	4 (20)
3 (1+2)	Non-toxic goiter (diffuse+nodular)	60	9.54±1.49	16 (26.6)
4	Operated for NTNG Pre-operatif Post-operatif 10^{th} day Post-operatif 30^{th} day	20	 7.91±2.1 23.84±1.83 21.98±2.01	 7 (35) 17 (85) 15 (75)
5	Control group	34	4.21±0.79	0 (0)

TRAb: TSH receptor antibody, TSH: Thyroid stimulating hormone, NTNG: Non-toxic nodular goiter

RESULTS

A total of 114 people participated in the study. The NTDG group consisted of 40, the NTNG group of 20, and the control group of 34 healthy volunteers. Other 20 people underwent total thyroidectomy due to nodular goiter. There was no difference in gender distribution between the groups. The operated group had a higher mean age than the other groups (p<0.05) (Table 2).

There was no significant difference between the groups in terms of TSH level (Table 3).

Table 4 shows the TRAb distribution and F% values of the experimental and control groups. As can be seen, 30% of the NTDG group, 20% of the NTNG group, 26.6% of the diffuse+nodular NTG group and 35% of the operation group (in the preop period) had TRAb (+) patients. While there was no significant difference between the experimental groups in this respect, there was a significant difference between the experimental groups and the control group because of the absence of TRAb (+) patients (p<0.001). When the distribution of TRAb (+) patients in the group operated for NTNG in the preoperative and postoperative 10th and 30th days, the distribution of TRAb (+) patients was higher in the postop 10th day compared to the preop (p<0.001). There were more TRAb (+) patients on postop 30^{th} day compared to preop (p<0.05). There was no significant difference between postop days 10 and 30 (Table 4).

The distribution of TRAb (+) patients and F% values were compared by gender in the experimental and control groups. Women in the NTDG group had more TRAb (+) patients than women in the control group ($p<0.001$). There were also significantly more TRAb (+) women in the NTNG group ($p<0.05$). The distribution of TRAb (+) patients in men ($p<0.05$) and women ($p<0.001$) in the operation group was higher than in the control group (Table 5).

Table 5. F% levels and TRAb(+) patient distribution of the groups according to gender

Groups		Gender	Number	F%	Number of TRAb (+) patients (%)
1	Non-toxic diffuse goiter	Male	5	6.04±2.69	0 (0)
		Female	35	11.97±2.28	12 (34.2)
2	Non-toxic nodular goiter	Male	2	17.4±4.9	1 (50)
		Female	18	5.03±1.6	3 (16.6)
3 (1+2)	Non-toxic goiter (diffuse + nodular)	Male	7	9.28±2.82	1 (15)
		Female	53	9.57±1.65	15 (37.5)
4	Operated for NTNG	Male	3	13±6.6	2 (66.6)
		Female	17	7.01±2.22	5 (29.4)
5	Control group	Male	5	5.72±2.23	0 (0)
		Female	29	3.51±0.82	0 (0)

TRAb: TSH receptor antibody, NTNG: Non-toxic nodular goiter

Thyroid volume was measured by USG in patients with non-toxic goiter. The correlation between thyroid volume and F% levels in TRAb (+) patients was investigated. As shown in Table 6, no significant correlation was found (Table 6).

Table 6. Correlation between thyroid volume and F%

Patient group	Patient number (%)	F%	Tiroid volume (ml/kg)	r	p
TRAb (+) patients in the NTNG+NTDG group	16 (26.6)	26.02±2.25	0.47±0.45	0.26	>0.05

TRAb: TSH receptor antibody, NTNG: Non-toxic nodular goiter, NTDG: Non-toxic diffuse goiter

DISCUSSION

In the first part of this study, TRAb levels were studied to investigate the role of autoimmune mechanism in the development of goiter in patients with non-toxic diffuse goiter and nodular goiter admitted to our hospital from various provinces of the Black Sea region. In the second part of the study, the changes in TRAb levels before and after the operation in a group of patients with an indication for operation due to NTNG were analyzed.

Autoimmune diseases are caused by disturbances in immunoregulation. These disorders trigger autoimmune diseases by leading to the activation of T lymphocytes that cannot tolerate the body's autoantigens. In this process of abnormal immune reaction to the body's own antigens, environmental agents may play a role in the onset of the disease. In autoimmune diseases, target cell integrity and functions are altered in affected tissues and organs. Clinical symptoms occur as a result of tissue abnormalities due to humoral or cellular immunity or both. Autoimmune thyroid diseases occur with similar mechanisms, have a wide clinical spectrum and may lead to hypothyroidism or hyperthyroidism or the individual may become euthyroid despite the disease.[16]

The stimulation of thyroid growth by increased TSH due to iodine deficiency is accepted as the main factor in the development of goiter. However, various clinical situations have been observed which show that this mechanism alone is not sufficient to explain goiter formation. These are; thyroid growth despite TSH suppression with thyroxine, recurrence in operated patients despite thyroxine treatment, goiter in hyperthyroid patients with low TSH, similar thyroid function tests in patients with and without goiter living in the same region, failure to eradicate goiter despite adequate iodine prophylaxis. Today, many factors such as genetics, natural goitrogens, radiation, metabolic syndrome and obesity have been shown to cause goiter.[17-20] When Graves' disease is considered from this point of view, it has been found that antibodies similar to TRAbs observed in this disease are also present at various rates in patients with toxic and non-toxic goiter. The TSH receptor is encoded by a gene localized on chromosome 14q31 and functions through 4 different G proteins. These are Gs, phospholipase C, G13 and Gi. The TSH receptor is most highly expressed on the basolateral membrane of thyrocytes and regulates the functioning of the thyroid gland.[21] TRAbs' affinity for the TSH receptor is higher than TSH. Among these antibodies, those that stimulate adenylate cyclase lead to Graves' disease, those that inhibit adenylate cyclase lead to hypothyroidism, those that stimulate phosphotidyl inositol pathway lead to goiter formation and those that inhibit this pathway lead to atrophic thyroiditis, whichever type is predominant leads to clinical development.[22-24]

Non-toxic goiter is TSH-independent enlargement of the thyroid gland and patients can be euthyroid or hypothyroid. Patients are mostly euthyroid. It may be diffuse or nodular.[25,26] In our study, all participants were euthyroid. The distribution of patients was as follows: 40 non-toxic diffuse goiter, 20 non-toxic nodular goiter and 20 operated non-toxic nodular goiter (Tables 2, 3).

The incidence of goiter increases with age and the incidence of multinodular goiter is higher in the elderly.[4,20] In our study, it is not a coincidence that the operation group was statistically older.

In many studies conducted since 1986, TRAb has been detected in the serum of patients with non-toxic goiter.[27] In our study, TRAb was present in 26% of 60 patients with non-toxic goiter, while TRAb was negative in the control group. In addition, while 30% of patients with NTDG had TRAb (+), this rate was 20% in patients with NTNG, which is statistically similar (Table 4). These results are consistent with the above literature suggesting that autoimmune mechanisms may play a role in the development of goiter.

In studies, antibody positivity or increase in the amount of antibodies is observed after thyroid surgery and radioactive iodine treatment. The reason for this has been shown to be the development of antibody response against thyroid antigens released into the circulation or the passage of intrathyroidal accumulated antibodies from the thyroid to the circulation.[28,29] In our study, the number of TRAb(+) patients and TRAb levels on the 10th and 30th postoperative days were found to be higher compared to the preoperative period. This was again considered as a sign of the presence of autoimmunity.

Table 5 shows that the majority of the participants in our study were women. In addition, the number of TRAb(+) women is more dominant in the experimental groups than in the control group. Female gender is a risk factor for goiter. The female dominance in our study is consistent with the literature.[4]

In a study by Lee et al.[30] no correlation was found between TRAb level and thyroid volume in patients who underwent surgery for Graves' disease. In another study, thyroid volume was measured by thyroid tomography in hyperthyroid patients and no correlation was found with TRAb.[31] In our study, the relationship between TRAb level and thyroid volume was also examined in patients with TRAb(+) and no correlation was found as above (Table 6).

Limitations

This study has some limitations. The most important one is that this is an old study conducted 30 years ago. Recent studies are needed to support our findings.

CONCLUSION

In this study, TRAb was found in euthyroid patients with goiter, different from the control group. In the group operated for goiter, postoperative TRAb levels were higher than preoperative levels. These findings indicated that autoimmunity may be involved in the development of non-toxic goiter.

ETHICAL DECLARATIONS

Ethics Committee Approval

This specialty thesis was conducted in 1994, ethical approval was not obtained for the study as there was no requirement for ethical approval at that time, it was conducted after institutional approval was obtained.

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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