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Original Scientific Paper

CORRELATION OF TPO ANTIBODY CONCENTRATION WITH THYROID HORMONES AS A PREDICTOR OF CLINICAL HYPOTHYROIDISM

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SUMMARY – This study aimed to determine the relationship between the concentration of thyroid peroxidase antibodies (TPO-Ab) and thyroid-stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) in patients with chronic lymphocytic thyroiditis. This cross-sectional study included 144 patients diagnosed with chronic lymphocytic thyroiditis whose mean age was 46±15 years at the time of diagnosis. According to their TSH concentration, the patients were either euthyroid or hypothyroid. After 59 patients were excluded due to TSH levels below the reference range, no TPO-Ab findings, substitution therapy, immune hyperthyroidism, or TPO-Ab and TSH levels outside the resolution limits of the laboratory tests, 85 patients remained in the study. A positive correlation was found between TPO-Ab and TSH concentration within the euthyroid and hypothyroid range and a negative correlation between TPO-Ab and FT4 concentration. The result suggests that the TSH and TPO-Ab concentration could be used to identify individuals at risk for the development of hypothyroidism in the general population.

Keywords: *Free thyroxine (FT4); Free triiodothyronine (FT3); Hypothyroidism; Thyroglobulin; Thyroid peroxidase antibodies (TPO-Ab); Thyroid-stimulating hormone (TSH)*

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Introduction

Hypothyroidism is defined as a decreased production of two primary thyroid hormones – thyroxine (T4) and triiodothyronine (T3). The pituitary gland regulates their production through the thyroid-stimulating hormone (TSH), also called thyrotropin (1). If there is reduced production of T4 and T3, the pituitary gland produces more TSH, which stimulates the thyroid gland to produce more T4 and T3. If the thyroid gland cannot respond with increased production of T4 and T3, TSH levels increase. Increased TSH levels are a sign of hypothyroidism. The most common cause of hypothyroidism is chronic autoimmune thyroiditis – Hashimoto's thyroiditis (2).

Chronic autoimmune thyroiditis affects 1%-2% of the total population and is one of the most common thyroid diseases (3). The disease incidence is particularly increased in people aged between 30 and 50 years but can occur in any age group, including children (4, 5). Hypothyroidism most often occurs in areas with sufficient iodine concentration. The annual incidence of chronic autoimmune thyroiditis globally is estimated to be 0.3-1.5 per 1000 individuals, and it is 10-15 times higher in women (6–8). The disease is more common today, not because of frequent examinations but because of the more potent immunogenicity of thyroid antigens caused by increased iodine intake (3, 4).

Chronic autoimmune thyroiditis is an inflammation of the thyroid gland caused by the immune system's disorder, usually with a genetic predisposition (positive family history) (4, 9). It occurs about four times more often in genetically predisposed individuals; an environmental trigger is required for the disease to develop. The autoimmune destruction mechanism includes cellular and humoral immunity and infiltration of the thyroid gland by an equal number of B lymphocytes and cytotoxic T lymphocytes (10). A decreased number or damage of suppressor T lymphocytes results in the appearance of a pathological clone of autoreactive auxiliary T lymphocytes that escape immune control and are not destroyed (11). This allows the development of B lymphocytes to convert a particular type of plasma cell with the resulting production of specific antibodies against the thyroid tissue itself (12).

Recent studies have shown that, in addition to antibody production, cellular immune disorders play an essential role in the mechanism of immune disorders (13). T lymphocytes contribute to the clinical presentation by direct cytotoxic action on thyrocytes. Cytotoxic T lymphocytes (or T–killer cells) attack antibody-coated thyrocytes through antibody-dependent cellular cytotoxicity (ADCC) (14). Large granular lymphocytes or natural killer (NK) cells and lymphotoxins, such as tumor necrosis factors (TNF) α and γ, interferons, and interleukin 2, also play an important role (15). Lymphocytes infiltrating the thyroid gland and those outside it react to thyroid antigens by producing specific antibodies, leading to the clinical presentation of the disease (12). The most common consequence of autoimmune inflammation is damage to the thyroid tissue with goiter formation and dysfunction of thyroid hormones secretion – hypothyroidism or, much less frequently, hyperthyroidism (16). Several antibodies and antigen-specific T lymphocytes have been linked to autoimmune thyroid disease (17).

The significant antigens are thyroglobulin (TG), thyroid peroxidase (TPO), and the thyroid-stimulating hormone receptor (TSH receptor, thyrotropin receptor). Antibodies are highly cytotoxic microsomal antibodies to thyroid peroxidase, thyrocyte membrane antigens, and/or binding complement. These antibodies cause destruction and lysis of thyrocytes (18). Thyroglobulin, synthesized in follicular cells, acts as both a precursor and a storage molecule for thyroid hormones and is one of the primary targets of autoimmunity in chronic autoimmune thyroiditis (19). Studies have shown that more than 14 polymorphisms in the thyroglobulin gene are associated with an increased incidence of chronic autoimmune thyroiditis (20). In addition, thyroid peroxidase catalyzes the iodination of tyrosine residues in thyroglobulin. Experimental autoimmune thyroiditis was induced using thyroglobulin or TPO as an antigen in mice, demonstrating the role of thyroglobulin and TPO in the pathogenesis of chronic autoimmune thyroiditis (21).

The clinical features of Hashimoto's thyroiditis include euthyroidism (when thyroid tissue compensates for destroyed thyroid cells) and hypothyroidism (when the thyroid gland does not produce enough hormones) (22). The diagnosis of autoimmune thyroiditis is based on antibodies against specific thyroid antigens, primarily thyroid peroxidase antibodies (TPO-Ab), and

ultrasound features of the thyroid parenchyma (23). Primary hypothyroidism is considered when TSH levels are increased and FT4 is reduced (22), with additionally increased TPO-Ab levels (24).

The TSH receptor antigenicity is associated with autoimmunity in Graves' disease (25). Most patients have increased serum levels of both TPO-Ab and thyroglobulin antibodies (TG-Ab). The positive TPO-Ab in serum is found in 90% of patients with chronic autoimmune thyroiditis, slightly less often in patients with Basedow's disease (75%), or patients with idiopathic myxedema (70%). TG-Ab is present in the serum at slightly lower levels than TPO-Ab (70%) and detected in one-third of patients with Basedow's disease. In addition, other antibodies may appear, such as antibodies to the colloidal antigen, thyroid hormones, and receptor antibodies, such as thyroid-stimulating antibody (TS-Ab), TSH-receptor antibody (TR-Ab), thyroid stimulating hormone subunit beta antibody (TSHB-Ab), thyroid-growth-immunoglobulins (TGI), and thyroxine-binding globulin antibody (TGB-Ab) (26–28).

This study aimed to determine if there is a correlation between TPO-Ab concentration and TSH, FT4, and FT3 concentrations in the serum of patients diagnosed with chronic lymphocytic thyroiditis.

Patients and methods

Patients

This study included 144 patients diagnosed with chronic lymphocytic thyroiditis who were referred for the examination at the Division for Thyroid Diseases of the Clinical Institute of Nuclear Medicine and Radiation Protection, University Hospital Center Osijek, Croatia, in the period from November 18, 2002, to January 28, 2013.

The main criteria for patient selection were positive TPO-Ab and/or cytological findings of chronic lymphocytic thyroiditis obtained by targeted cytological aspiration puncture guided by ultrasound.

The database used to conduct the study consisted of outpatient records and medical history of 144 patients with chronic lymphocytic thyroiditis aged 12 to 75 years, residents of Slavonia.

The study was conducted according to good clinical practice and the Declaration of Helsinki and approved by the Ethics Committee of University Hospital Center Osijek, Croatia (Ethical approval number R1/6414/2021).

Method

Serum concentrations of TPO-Ab, TSH, FT3, and FT4 were determined by immunoenzymatic assays (Beckman Coulter, Brea, California, USA) including the Access TPO Antibody assay, HYPERsensitive TSH assay, free T3, and free T4 assays.

TPO-Ab was considered positive if serum concentration was >100 U/mL. The reference range for TSH was 0.46–4.68 mIU/L, for FT3 5.10–9.65 pmol/L, and for FT4 10.0–28.2 pmol/L. The study included euthyroid and hypothyroid patients with TSH concentration estimated to be within the laboratory reference values. To investigate the likelihood of clinical hypothyroidism development in patients with positive

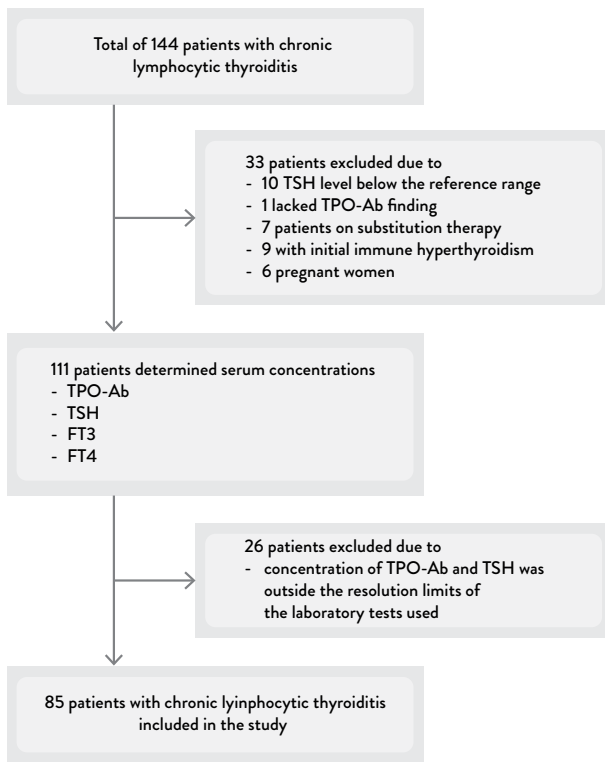

Fig. 1. Flowchart of patient selection. TSH— thyroid-stimulating hormone, TPO-Ab— thyroid peroxidase antibody

TPO-Ab concentration, we excluded 33 patients from the analysis including 10 patients who had a TSH level below the reference range at the time of diagnosis, one who lacked a TPO-Ab finding, and seven who had already started taking substitution therapy. Also, we excluded patients who developed initial immune hyperthyroidism (n=9) and pregnant women (n=6). Moreover, 26 patients were excluded due to the concentration of TPO-Ab and TSH outside the resolution limits of the laboratory tests (for TPO-Ab >3000 U/mL and for TSH >100 mIU/L). Thus, 85 patients were included in the analysis (Fig. 1).

The analyzed data included medical history, outpatient records, thyroid ultrasound findings, cytological findings of thyroid punctures, and other diagnostic tests performed during treatment and follow-up, patient data (sex, age), disease course (time to clinical hypothyroidism), and laboratory findings (TPO-Ab, TSH, FT4, FT3).

Statistical analysis

Statistical analysis was performed with the statistical program SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Differences in the TPO-Ab, TSH, FT3 and FT4 concentrations between euthyroid and hypothyroid patients were determined using Mann-Whitney U test. In addition, the correlation between the concentrations of TPO-Ab, TSH, FT3, and FT4 was determined using the Spearman correlation coefficient. The level of statistical significance was set at $p<0.05$.

Table 1. Demographic and clinical characteristics of patients (N=85)

Variable	Mean ± SD / N (%)
Age (years)	46 ± 15
Sex (men)	7 (8.2%)
Euthyroid	38 (44.7%)
Hypothyroid	47 (55.3%)
Cytological puncture performed	68 (80%)
Time (years)*	3.8 ± 3.9
10-year follow up	
Patients with no hypothyroidism	38 (44.7%)
Patients on substitution therapy	47 (55.29%)

SD – standard deviation. *The time passed from the diagnosis to treatment. Numerical variables are presented as mean ± SD and categorical variables as number (percentage)

Results

The results obtained in the present cross-sectional study included 85 patients with a confirmed diagnosis of chronic lymphocytic thyroiditis, of whom 78 (91.8%) were women and 7 (8.2%) were men (Table 1). Chronic lymphocytic thyroiditis predominantly occurred in women. The mean age of the patients at the time of diagnosis was 46±15 years. The youngest patient was 12 and the oldest 75 years old. Descriptive analyses of the TPO-Ab, TSH, FT3 and FT4 concentrations are shown in Table 2.

Within a 10-year period (from 2002 to 2013), 38 patients (44.7%) did not develop either clinical or laboratory hypothyroidism, while 47 patients (55.3%) were taking substitution therapy due to the development of clinical hypothyroidism. Of these 47 patients under treatment, 32 (68.1%) started taking substitution therapy after the first examination by a nuclear medicine specialist, and the remaining 15 (31.9%) patients developed clinical hypothyroidism over an average of 9.7 months. The differences in TPO-Ab, TSH, FT3, and FT4 concentrations between euthyroid and hypothyroid patients are shown in Table 3.

Spearman correlation coefficients between TPO-Ab, TSH, TF3, and TF4 concentrations are presented in Table 4.

Discussion

The leading biomarker for detecting autoimmune thyroid disease is the determination of TSH concentration (29). Our study underscores the importance of TPO-Ab as a biomarker in detection of thyroid disease in general population. Some earlier prospective studies investigated TSH and TPO-Ab concentrations in patients with TSH concentrations within reference values. Thyroid disease can be caused by too little or excessive iodine intake (30). Insufficient iodine intake is managed by iodizing table salt. A higher proportion of individuals who ingested smaller amounts of iodized

Table 2. Descriptive analyses of the TPO-Ab, TSH, FT3, and FT4 concentrations (N=85)

Variable	Mean ± SD	Min	Max	Median (IQR)
TPO-Ab (IU/mL)	758.12 ± 710.59	3.00	2941.00	600 (1091-173.5)
TSH (mIU/L)	11.58 ± 19.56	0.66	121.00	4.36 (10.85-2.56)
FT3 (pmol/L)	5.43 ± 1.39	1.31	8.36	5.74 (6.32-4.67)
FT4 (pmol/L)	12.95 ± 14.57	1.64	142.00	11.70 (13.65-9.72)

SD – standard deviation; IQR – interquartile range

Table 3. Medians and interquartile ranges of the TPO-Ab, TSH, FT3, FT4 concentrations and time between euthyroid and drug therapy diagnosis and the differences between euthyroid and hypothyroid patients (N = 85)

Variable	**Euthyroid** (n=38) median (IQR)	**Hypothyroid** (n=47) median (IQR)	p-value**
TPO-Ab (IU/mL)	309.5 (803-126.7)	773 (1322-373)	0.02
TSH (mIU/L)	2.7 (3.7-1.7)	8.7 (16.3-4.4)	<0.001
FT3 (pmol/L)	6.1 (6.5-5.4)	5.4 (6.2-4.4)	0.04
FT4 (pmol/L)	12.4 (14.6-11.1)	10.5 (12.9-8.2)	0.003
Time (years)*	8 (5-9)	0 (0-1)	<0.001

IQR – interquartile range; *The time passed from the diagnosis to treatment. **Mann-Whitney U test

Table 4. Correlations (Spearman's rho) of TPO-Ab, TSH, FT4, and FT3 (N=85)

Variable	TPO-Ab	TSH	FT4	FT3
TSH	0.422**			
FT4	-0.356*	-0.505**		
FT3	-0.179	-0.289**	0.318*	
Time (years)†	-0.283**	-0.213	0.153	0.075

* Spearman's rho p<0.05, ** Spearman's rho p<0.01. †The time passed from the diagnosis to treatment.

salt developed thyroid disease. In contrast, individuals who consumed excessive amounts of iodized salt developed thyroid disease to a much lesser extent (31). Since the legal regulation of salt iodization in 1997, following World Health Organization guidelines (32), iodine deficiency is no longer present in Croatia. According to Kolja Poljak et al. (33), the median urinary iodine excretion in school children in Split-Dalmatia County was 23.6 μg/dL and in the Osijek-Baranja County 28.1 μg/dL. According to the International Council for the Control of Iodine Deficiency Disorders guidelines, a value above 10 μg/dL of excreted iodine in urine indicates sufficient iodine intake (34).

In the present study, euthyroid patients had significantly lower concentrations of all measured hormones TSH, FT3, and FT4, as well as TPO-Ab, compared to hypothyroid patients. Hypothyroid patients started the treatment immediately, whereas euthyroid patients began treatment after eight years. The study showed that the higher the TPO-Ab concentration, the shorter the time for patients to start receiving therapy.

In the Whickham (7) and Busselton (35) studies, higher TSH levels in the first survey increaesd the likelihood of developing clinical hypothyroidism and this likelihood increased further when TPO-Ab were present. As in our study, patients with increased TSH

concentrations were not initially excluded from the study. Our conclusions would be more robust if we had analyzed only patients with TSH levels within the typical reference values. A study by Roos et al. (36) indicated that variations in TSH concentrations within the normal range are essential for predicting the development of clinical hypothyroidism.

The pathophysiological processes in the context of positive TPO-Ab and conditions for future thyroid disease development are complex and have not been fully elucidated. TPO is the primary enzyme synthesizing thyroid hormones and it is a major autoantigen (37, 38). Moreover, it is hypothesized that TPO-Ab should not be viewed as a single entity since their pathogenic potential depends on the epitope of thyroid peroxidase to which they bind (39). TPO-Ab presence is characteristic of autoimmune thyroid diseases, both Hashimoto's thyroiditis and Graves' disease (40). Therefore, the etiology of Hashimoto's thyroiditis is most likely a combination of genetic predisposition and environmental factors (41). Brčić et al. (42) confirmed that the genetic variations within or near the *TPO*, *ATXN2*, and *RASGRP1* genes and TPO-Ab concentration are associated with Hashimoto's thyroiditis. Lymphocytes mainly produce antibodies in the thyroid gland (42). The combination of TPO-Ab presence and the resulting increase in TSH concentration suggest that TPO-Ab presence can be considered a biomarker for an increased risk of clinical hypothyroidism (43). The presence of TPO-Ab causes a compensatory increase in TSH concentration to maintain the euthyroid state. Although this finding should be confirmed by studies with larger sample sizes of patients, the results suggest that measuring the concentration of TPO-Ab and TSH can be used as a marker to identify individuals at risk of developing hypothyroidism in the general population.

Incidences of clinical hypothyroidism and subclinical hypothyroidism are remarkably higher when individuals have high levels of TPO-Ab (44). TPO-Ab in the serum is present in about 95% of patients with Hashimoto's thyroiditis. In addition, TPO-Ab has been identified as a risk factor for progression to hypothyroidism in the general population (22). There is little evidence to suggest a role for TPO-Ab in the pathogenesis of autoimmune thyroid disease. TPO-Ab can fix complement, and it binds and kills thyrocytes *in vitro*. However, no correlation between disease severity and serum TPO-Ab concentration has been observed in humans. Nevertheless, a positive serum TPO-Ab concentration is know to be associated with the active phase of the disease (24).

In recent years, controversial views have been stated regarding normal TSH concentrations. Subclinical hypothyroidism, i.e., elevated TSH levels combined with normal FT4 levels, was associated with ischemic heart disease (45, 46). However, there is a lack of randomized clinical trials demonstrating that levothyroxine (LT4) therapy in subclinical hypothyroidism improves survival or reduces cardiovascular disease (47, 48).

Clinical hypothyroidism generally develops slowly, and symptom recognition is often complex. Thus, many guidelines advise screening people in case of vague symptoms (5). The screening is especially recommended in pregnant women, women aged over 60, and others at high risk for thyroid dysfunction, although a complete consensus on the screening strategy is lacking (36, 49). It is unknown how long only the average TSH value can be reliable as a sustained euthyroid status parameter.

In their study, Okuroglu et al. (50) found a positive correlation between the antibody concentration and higher levothyroxine dosing in patients with autoimmune thyroiditis (50).

Given the studies that confirm the association between TPO-Ab and TSH concentrations and the review article by Toulis et al. (51), dietary selenium supplementation is suggested because selenium-dependent enzymes have several modifying effects on the immune system. In patients with Hashimoto's thyroiditis who participated in four studies and took specific doses of selenium for three months, a decrease in TPO-Ab concentration and subjective improvement was demonstrated. Patients continued to take the same amount of selenium with the addition of levothyroxine, with no changes in the thyroid parenchyma observed on ultrasound (51).

The present study has several limitations. It included a relatively small sample size of patients with chronic lymphocytic thyroiditis, most of whom were women. Also, the only database used was the thyroid diseases database, which contained no information on other biochemical parameters and comorbidities that could affect the study results. Patients with TSH values below the reference values were excluded because we

wanted to examine the likelihood of the development of hypothyroidism, and lower TSH would indicate hyperthyroidism. Future studies should include more male patients and analyze other anamnestic comorbidities that could affect the study results. In female patients, the status of sex hormones should also be determined to gain insight into their impact on autoimmune thyroid diseases.

In conclusion, a positive association was found between the presence of TPO-Ab and TSH concentration. The result is consistent with the hypothesis that the presence of TPO-Ab causes a compensatory increase in TSH concentration to maintain the euthyroid state, even at normal thyroid hormone levels. Furthermore, independent indicators of future thyroid damage – the presence of TPO-Ab in combination with TSH concentration – suggest that TSH levels would be lower in patients with TPO-Ab presence if they negatively correlated with TPO-Ab. Although there is a statistically significant positive correlation between TPO-Ab and TSH concentration, it is unfortunately not strong enough to have a predictive value for developing clinical hypothyroidism in our study. A higher level of TSH is a criterion for initiating therapy. However, as TSH correlates with TPO-Ab, TPO-Ab is also a possible indication for initiating treatment. Nevertheless, the cut-off value of TPO-Ab for introducing therapy is unknown due to the wide range of TPO-Ab. Therefore, it should be further investigated in a large sample of patients, and multicenter studies should be carried out to accurately examine the predictive value of the correlation between TPO-Ab and TSH.

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Sažetak

KORELACIJA KONCENTRACIJE ANTI-TPO SA TIREOIDNIM HORMONIMA KAO PREDIKTOR RAZVITKA KLINIČKE HIPOTIREOZE

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Cilj rada bio je utvrditi u uzorku bolesnika s kroničnim limfocitnim tireoiditisom odnos koncentracije protutijela koje blokira tireoidnu peroksidazu (anti-TPO) s koncentracijama tireotropina (TSH, engl. *thyroid-stimulating hormone*), slobodnog tiroksina (FT4, engl. *free thyroxine*) i slobodnog trijodtironina (FT3, engl. *reverse triiodothyronine*). Ovo presječno ispitivanje obuhvatilo je 144 bolesnika s dijagnozom kroničnog limfocitnog tireoiditisa čija je prosječna dob bila 46 ± 15 godina u vrijeme postavljanja dijagnoze. Uključeni su eutireoidni i hipotireoidni bolesnici procijenjeni prema koncentraciji TSH unutar laboratorijskih referentnih vrijednosti. Nakon što je isključeno 59 bolesnika zbog razine TSH ispod referentnog raspona, nedostatka nalaza anti-TPO, supstitucijske terapije, imunosne hipertireoze ili razina anti-TPO i TSH izvan granica razlučivanja laboratorijskih pretraga, u ispitivanju je ostalo 85 bolesnika. U ovom istraživanju opažena je pozitivna korelacija koncentracije anti-TPO s koncentracijom TSH unutar eutireoidnog i hipotireoidnog raspona i negativna korelacija koncentracije anti-TPO s koncentracijom FT4. Rezultati ovog istraživanja pokazuju da bi se koncentracija TSH i anti-TPO mogla koristiti kao alat za prepoznavanje osoba kojima prijeti razvoj hipotireoze u općoj populaciji.

Ključne riječi: *Slobodni tiroksin (FT4); Slobodni trijodtironin (FT3); Hipotireoza; Tireoglobulin; Antitijela na tireoiduperoksidazu (anti-TPO); Tireotropin (TSH)*