original article

The relationship between thyroid antibody titer and levothyroxine dose in patients with overt primary hypothyroidism

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Ann Saudi Med 2017; 37(3): 189-193

DOI: 10.5144/0256-4947.2017.189

BACKGROUND: Both excess and insufficient thyroid hormone replacement may produce adverse effects in various target tissues; therefore, understanding factors that affect achievement of target TSH levels is crucial.
OBJECTIVE: Investigate the relationship between antibody titers and levothyroxine dose.
DESIGN: Retrospective, review of data in medical records.
SETTING: Thyroid center of Fatih Sultan Mehmet Education and Research Hospital, Istanbul, Turkey.
PATIENTS AND METHODS: The study population consisted of patients that had been diagnosed as having overt primary hypothyroidism and were taking levothyroxine for at least one year. The serum TSH level for an euthyroid state was between 0.5-4 mIU/L. The levels of anti-thyroid peroxidase (TPOAb) considered positive for antibodies were <5.6 IU/mL and for anti-thyroglobulin (TgAb) autoantibodies <4.10 IU/ mL.
MAIN OUTCOME MEASURE: Daily levothyroxine doses of antibody-positive and negative patients and association of daily drug requirement with antibody titers.
RESULTS: The study population consisted of the 303 patients (273 females and 30 males with the mean [SD] age of 46.6 [13.2] years). In the antibody-positive group (n=210) average daily levothyroxine dose was statistically significantly higher than in the antibody-negative group (n=93) (mean of 78.8 [36.7] vs 64.2 [27.1] mg/day, *P*=.001, respectively). There was a low but statistically significant positive relationship between the TPOAb (r=0.217, *P*<.01) and TgAb levels (r=0.158, *P*<.05) and levothyroxine doses in the antibody-positive group.
CONCLUSION: Antibody titers are positively associated with larger levothyroxine (LT-4) replacement dosing in patients with autoimmune thyroiditis.
LIMITATION: Unknown antibody titers before starting levothyroxine use.

Hypothyroidism, both overt and subclinical forms is one of the most prevalent hormonal disorders in the world, with a prevalence of 4% to 15% in the general population.[1,2] Iodine deficiency in areas of iodine insufficiency and chronic autoimmune thyroiditis in areas of iodine sufficiency are the most frequent causes of primary hypothyroidism.[3] Understanding factors that affect achievement of target TSH levels is crucial since both excess and insufficient thyroid hormone replacement may produce adverse effects in various target tissues.

Chronic autoimmune thyroiditis (Hashimoto thyroiditis) is characterized by polyclonal autoantibodies targeting the thyroid gland, T-cell infiltration and apoptosis of thyroid follicular cells.[4] Typical autoantibodies in the serum of patients with autoimmune thyroiditis represent a response to the ongoing inflammatory reaction rather than playing a direct role in the pathogenesis of the disease.[5] Anti-thyroid peroxidase (TPOAb) and anti-thyroglobulin (TgAb) autoantibodies are often correlated with disease activity.[6] Willms et al reported that markedly decreased echogenicity, heterogeneity, and multifocal pseudonodular hypoechoic infiltration are indicative of a high level of inflammatory activity,

and these sonographic findings were associated with significantly higher TPOAb activity.[7] Also, the presence of TPOAb is associated with an increased risk of overt hypothyroidism.[8]

In the treatment of patients with primary hypothyroidism, the main goal is to normalize thyrotropin (TSH) levels with thyroxine replacement. Since both excess and insufficient thyroid hormone replacement may produce adverse effects in various target tissues, it is crucial to administer levothyroxine (L-T4) in sufficient doses. The optimum daily dose largely depends on the degree of hypothyroidism, body weight and patient age.[9,10] Factors such as low patient compliance or reduced L-T4 absorption resulting from accompanying chronic disease or some drugs used simultaneously affect the daily demand for L-T4.[11-16] As far as we know, the relationship between the levels of thyroid autoantibodies and the daily L-T4 doses has not been investigated. Taking into consideration information on the relationship between thyroid antibodies and disease activity from previous studies, we investigated whether there was a relationship between thyroid antibody level and daily L-T4 doses.

PATIENTS AND METHODS

This retrospective review of medical records included patients with overt hypothyroidism diagnosis who were receiving L-T4 therapy for at least 1 year and who applied to our thyroid polyclinics between January 2015 and December 2015. All patients were treated by the same standard of care and all were euthyroid according to the guidelines with TSH values between with 0.5 to 4 mIU/L. Patients who had been diagnosed with subclinical hypothyroidism and overt hypothyroidism as a result of radioiodine or surgical treatment were excluded. No patients had liver dysfunction, renal failure, diseases of the pituitary gland or hypothalamus such as secondary hypothyroidism, malabsorption diseases, pregnancy, or alcohol abuse. The study protocol was approved by the ethics committee and informed written consent was taken from all participants. The demographic data of the participants including age, gender, weight, body mass index (BMI), and blood pressure were recorded. The disease duration, smoking status and any other drug usage affecting levothyroxine absorption like proton pump inhibitors (PPI) or iron supplements were noted. All patients were receiving levothyroxine sodium tablets. The patients were on different daily dose management rather than a standard fixed dose in order to reach the appropriate TSH levels. For each patient the daily levothyroxine dose by calculated by weekly dose divided into seven.

Hormone assays

After 8-10 hours overnight fasting blood samples were obtained for TSH, TPOAb and TgAb. TSH, TPOAb and TgAb levels were measured by the same assay using the chemiluminescent microparticle immunoassay (CMIA) method (Architect i2000, Abbott Laboratories). The serum TSH level for a euthyroid state was between 0.5-4 mIU/L. The level of TPOAb was <5.6 IU/mL and TgAb <4.10 IU/ mL, respectively. Values above these levels were considered positive for antibodies.

Statistical analysis

Data was analyzed using SPSS version 22 package (IBM SPSS, Armonk, NY USA). Results were expressed as mean and standard deviation for the parameters showing normal distribution, median and ranges for the parameters showing a nonparametric distribution. The *t* test was used for the comparison of the parameters with a normal distribution. The Mann-Whitney U test was used for data that were not normally distributed. The relationship between the TPOAb and TgAb levels with L-T4 dose and disease duration were analyzed by the Spearman rho test. $P<.05$ was considered statistically significant.

RESULTS

The study population consisted of 303 patients (273 females and 30 males with a mean [standard deviation] age of 46.6 [13.2] years, range 19-73 years). Patients were divided into two groups with respect to the thyroid autoantibody status: antibody-positive group (n=210) and antibody-negative group (n=93). Disease duration in the antibody-positive group ranged from 1 year to 34 years with a mean (SD) of 6.2 (5.4) years and median of 5 years. In the antibody-negative group, disease duration ranged from 1 year to 30 years with an average of 5.3 (5.4), and median 3 years. There were no statistically significant differences in age, weight and body mass index between the groups. There were nonsignificant differences in TSH values of both groups. There was a statistically significant difference in sex distribution between the groups ($P<.01$). In the antibody-negative group the percentage of male patients was 17.2%, while in the antibody- positive group it was 6.7% (**Table 1**).

In the antibody positive group the mean L-T4 dose was significantly higher than in the antibody-negative group (**Table 2**). The male patients had higher but statistically insignificant L-T4 doses than the female patients in both the antibody-negative and antibody-positive group (mean [standard deviation] of 72.6 [34.6], median 67.9 vs 62.5 [25.2], median 50 , $P=.175$ and

mean of 87.1 [46.0], median 87.5 vs 78.3 [34.9], median 75, *P*=0.372, respectively). There was a low but statistically significant positive relationship between the TPOAb levels and L-T4 doses in the antibody positive group (r=0.217; *P*<.01). Similarly, a statistically significant positive relationship was found between the TgAb levels and the L-T4 doses (r=0.158; *P*<.05). There was no statistically significant relationship between TPOAb and TgAb levels and L-T4 doses in the antibody-negative group (*P*>.05) (**Table 3**). There was a weak but statistically significant negative relationship between TPOAb level and disease duration in the antibody-positive group (r=-0,159; *P*<.05). On the other hand, there was no statistically significant relationship between TgAb level and disease duration (*P*>.05) (**Table 4**). There was no statistically significant difference among L-T4 dose and smoking status, and other medications including PPIs and iron supplements (**Table 5**). Similarly, there was no statistically significant difference between smoking, additional drug use and L-T4 dosage in the antibody-negative group. As we expected, there was a weak but a statistically significant relationship between the drug dose and weight (r=0.241, *P*=.008) and BMI (r=0.157, *P*=.024) in the antibody-positive group. There was no statistically significant relationship between the drug dose and weight and BMI in the antibody-negative group.

DISCUSSION

L-T4, which is converted to T3 in peripheral tissues, is the standard treatment option for patients with hypothyroidism. The daily requirement for thyroxine varies from patient to patient. Many factors such as the degree of hypothyroidism, body weight, age, poor patient compliance, accompanying chronic diseases and some drugs used simultaneously affect the daily requirement. Our results suggest that thyroid antibody titer may also be associated with daily replacement need.

It is uncertain whether the typical autoantibodies present in the serum of Hashimoto thyroiditis patients play a direct role in the pathogenesis of the disease. In the early 1990s, some studies showed that experimental autoimmune thyroiditis in mice was induced by using thyroglobulin or thyroid peroxidase antigens and they suggested that these antigens might play a role in the pathogenesis of autoimmune thyroiditis in humans.[17,18] In early studies it has also been reported that TPOAbs fix complement and, at least in vitro, can produce antibody-dependent cell cytotoxicity.[19] Hashimoto thyroiditis the antithyroid immune response begins with the activation of the thyroid antigen-specific helper T cells. Once they are activated, they induce B cells to secrete thyroid antibodies.[20] The autoimmune destruction of the thyroid gland with circulating TgAb and TPOAb may also lead to the fibrosis of the parenchyma. As a result, in patients with subclinical hypothyroidism, high TPOAb levels are associated with progression to overt hypothyroidism.[20] On the other hand, Dussault et al reported that neonates born to mothers with circulating TPOAb had a normal thyroid gland and this result has

Table 1. Demographic characteristics of study population (n=303).

	Antibody-positive	Antibody-negative	*P* value
Age (years)	47.20 (12.58)	45.12 (14.51)	.207[a]
Weight (kg)	73.83 (14.39)	73.91 (12.37)	.961[a]
BMI (kg/m²)	28.47 (5.55)	27.95 (4.43)	.432[a]
Female	196 (93.3%)	77 (82.8%)	.007[b]
Male	14 (6.7%)	16 (17.2%)	

Data are mean (standard deviation) or number (percentage).
[a]*t* test; [b]Fisher exact test

Table 2. Relationship of drug dose and antibody groups.

Drug dose	Mean (SD)	Median	*P*
Antibody-positive	78.83 (35.66)	75	.001
Antibody-negative	64.22 (27.013)	50	

Mann-Whitney U test

Table 3. The relationship between anti-thyroid peroxidase (TPOAb) and anti-thyroglobulin (TgAb) autoantibody levels with levothyroxine doses.

Drug dose	TPO Ab level		TgAb level	
	r	*P*	r	*P*
Antibody-positive	0.217	.002[a]	0.158	.023[b]
Antibody-negative	0.158	.219	0.178	.093

Spearman rho test
[a]*P*<.01 [b]*P*<.05

Table 4. The relationship between anti-thyroid peroxidase (TPOAb) and anti-thyroglobulin (TgAb) autoantibody levels and disease duration in the antibody-positive group.

Antibody positive	Disease duration	
	r	*P*
TPOAb	-0.159	.021[a]
TgAb	-0.021	.765

Spearman rho test
[a]*P*<.05

Table 5. The relationship between smoking status, use of proton-pump inhibitors (PPI), iron and other medications with levothyroxine dose in the antibody-positive group.

Antibody positive		n	L-T4 dose (µg/day)		*P*
			Mean (SD)	Median	
Smoking	None	176	77.05 (32.48)	75	.179
	Use	34	88.07 (48.54)	93	
PPI	None	177	79.07 (36.44)	75	.956
	Use	33	77.55 (31.64)	75	
Iron	None	190	78.08 (34.79)	75	.594
	Use	20	86.04 (43.44)	75	
Additional medication	None	156	80 (37.3)	75	.732
	Use	54	75.46 (30.52)	75	

Mann-Whitney U test

changed the view of the pathogenic role of circulating thyroid antibodies in autoimmune thyroiditis.[21] Later, many authors reported that TPOAb correlated with the amount of lymphocytic infiltration and disease activity.[6] Currently, it is accepted that thyroid autoantibodies indicate a response to an ongoing inflammatory reaction rather than a direct pathogenic role.

The degree of hypothyroidism is an important factor in determining the need for thyroid hormone replacement therapy. Residual thyroid tissue that functions is an important factor in determining the degree of hypothyroidism. Some studies have shown that the degree of damage in the thyroid tissue is associated with antibody titers. Antibody-negative autoimmune thyroiditis has a milder course,[22] while the presence of TPOAb is associated with an increased risk of overt hypothyroidism.[8] From all these data we can expect that patients with higher antibody levels may need more hormone replacement. Our study showed that the hormone replacement requirement to achieve target TSH levels was higher in antibody-positive patients than in antibody-negative patients and there was a positive correlation between antibody titers and L-T4 doses. This result is consistent with the conclusion that the antibody titer is associated with disease activity. Inequality in gender distribution can be seen as a confounding factor. Since the number of male patients was higher than females in the antibody-negative group, the thyroid replacement dose should be higher in the antibody-negative group than the positive group if we consider the average replacement dose of a male patient was higher than in a female patient in both antibody-negative and positive groups. Therefore, we can say that the difference between the two groups is related to autoantibody presence and titer. We can also say that the presence of thyroid peroxidase antibodies is not only related to the risk of progression to overt hypothyroidism,[8] but also to the need for more hormone replacement. The results of the present study would not indicate a need to change the clinical practice of bringing the level of TSH to normal in the treatment of hypothyroidism, but we can assume that we need a higher dose of hormone replacement to reach the target TSH level.

This study has some limitations. First, it involved patients who were already under replacement therapy. There are many studies examining the long-term course of thyroid antibodies and their levels in chronic autoimmune thyroiditis with or without L-T4 treatment, but they have conflicting results. A wide variability has been reported in antibody levels. Some studies show that L-T4 treatment can modulate thyroid status by reducing the lymphocyte infiltration and progression of the disease process.[23,24] Thus, present antibody titers may not reflect the baseline titers. In our study, a negative correlation between antibody titers and the duration of the disease also suggests that the titer of antibody decreases over time, either with levothyroxine effect or independently of the drug. Second, there were no histopathological findings. Our results suggest that thyroid antibody titer may be associated with daily replacement need. The relationship between antibody titers and thyroid hormone requirement should be investigated in patients with newly diagnosed autoimmune thyroiditis by prospective, large-scale and long-term studies.

Funding

This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

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