RESEARCH ARTICLE
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The Association of Antibody Levels with Body Mass Index, Stress Management Ability and Lipid Peroxidation in Patients with Hashimoto's Thyroiditis

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ABSTRACT

Objective: The potential predictive value of body mass index, stress management ability, and thiobarbituric acid reactive substances (TBARS) levels for antibody levels in Hashimoto's thyroiditis (HT) were evaluated.
Materials and Methods: Two hundred patients with HT were included in the study. The patients were allocated into four groups as Group 1, patients with a body mass index (BMI) of 30 or higher (n=54); Group 2, patients with stress management difficulties (n=59); Group 3, patients with a BMI higher than 30 and stress management difficulties (n=11), and Group 4 (control, n=76), those without obesity or stress management issues. The mean age of patients, BMI, stress management abilities, anti-thyroid peroxidase (anti-TPO) antibodies levels, weekly T4 drug dosages, duration of levothyroxine usage, quality of life scores, as well as thiobarbituric acid reactive substances (TBARS) were evaluated.
Results: The antibody levels were significantly associated with body mass index and stress management. The antibody level increased 533 times ($p<0.0001$) in the group without stress management difficulties, and was affected 525 times ($p<0.0001$) in the obesity group.
Conclusion: The findings of our investigation revealed that stress management ability and obesity are the important factors influencing antibody levels.

Keywords: Hashimoto's thyroiditis, thyroid peroxidase antibodies, stress management ability, quality of life scores, TBARS

INTRODUCTION

Hashimoto's thyroiditis (HT), is alternatively referred to as chronic lymphocytic thyroiditis and is classified as an autoimmune disorder characterized by the progressive deterioration of the thyroid gland (1,2). The prevalence of Hashimoto's thyroiditis is significantly higher in females than in males. Typically, the onset of symptoms occurs between ages 30 and 50 (1-4).

HT is considered to arise from a blend of genetic and environmental factors. The risk factors for the development of the syndrome encompass a familial predisposition and the co-occurrence of another autoimmune disorder (3,4). The confirmation of diagnosis typically involves conducting blood tests to measure levels of thyroid-stimulating hormone (TSH), thyroxin (T4), and anti-thyroid autoantibodies, as well as performing a thyroid ultrasound (5-8).

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The symptoms and signs of hypothyroidism are nonspecific. The most common ones are skin dryness, cold intolerance, fatigue, voice alterations, and constipation. Others include bradycardia, slow speech, eye and facial swelling, weight gain, decreased sweating, hair loss, forgetfulness, decreased concentration, depression, menstrual irregularities, and muscle pains and cramps (7-10).

Currently, there is a lack of clinical studies investigating the relationship between antibody increase and the specific characteristics of individuals who have HT. The present study aimed to examine the associations between antibody levels and several factors including age, body mass index (BMI), quality of life, stress management abilities, and length of thyroid hormone drug usage as well as thiobarbituric acid reactive substances (TBARS) levels.

MATERIALS AND METHODS

This study was conducted at the Istanbul University, Istanbul Faculty of Medicine, Department of General Surgery, after obtaining ethical approval from the Bezmialem Vakif University Non-Interventional Research Ethics Committee (Date: 05.03.2019, No:3427) and written informed consent was obtained from each participant prior to the process.

The research comprised 280 individuals who were diagnosed with HT with the presence of thyroid auto-antibodies and/or ultrasound findings of thyroiditis between 2019 and 2022. Male patients (n=12) were omitted from the study due to the small sample size. The study also excluded individuals who were being treated for diabetes, cardiovascular diseases, other autoimmune diseases, previous thyroid surgery, or other diseases interfering with thyroid metabolism.

All patients underwent hormone replacement therapy with Levothyroxine (LT4) due to hypothyroidism. The drug dosage was adjusted by measuring patients' weekly T4 levels. The duration of the disease was determined by calculating the number of months between the date of diagnosis and the date of study enrollment.

In the study, two hundred participants were organized into four separate groups. *Group 1* consisted of patients with a BMI of 30 or higher (n=54), *Group 2* comprised those who experienced stress management difficulties (n=59), *Group 3* included patients with a BMI above 30 and stress management difficulties (n: 11), and *Group 4* (control) included 76 patients who did not have obesity or stress management issues.

Peripheral blood samples from patients with HT were taken into dry tubes. Subsequently, they were centrifuged at 3500 rpm, and sera were separated and preserved at -80 ^{o}C until analyses.

The BMI was determined by measuring the patients' weight and height and calculated by using the kg/height^2 formula.

The SF-36 is a multi-cultural assessment comprising 36 questions and organized into eight domain profiles of scores: physical functioning, general health, role limitation due to physical health, bodily pain, general social functioning, vitality, and role limitations due to emotional and mental health (11). The quality of life was assessed according to patients' answers for SF-36. All questions are rated on a 5-point Likert scale, and the item is scored between 1 and 5.

The ability of patients to control their stress was evaluated using the perceived stress scale, which is frequently used to measure stress sensitivity (12). Two questions from the scale were translated into Turkish and utilized: "How often have you felt that you were unable to control the important things in your life?" and "How often have you found that you could not manage all the things that you had to do?". The answer options were: "never" (score=1), "almost never" (score=2), "sometimes" (score=3), "fairly often" (score=4), and "very often" (score=5).

Anti-thyroid peroxidase (anti-TPO) antibody levels were determined by chemiluminescent assay using DPP Modular System manufactured by Roche Diagnostics (Mannheim, Germany). Lipid peroxidation was carried out by determination of thiobarbituric acid reactive substances (TBARS) levels (13). The Buege-Aust reagent was combined with the homogenates, and the mixture was subjected to incubation in a boiling water bath for 15 minutes, followed by cooling and subsequent centrifugation at 1000xg. The absorbances of supernatants were then recorded at 532 nm and the results were calculated using the extinction coefficient ($1.56x10^{-5}M^{-1}cm^{-1}$) and expressed as nmol/mL

Statistical analyses

Statistical analysis was conducted using the SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA) program. The data for all variables were presented as mean ± standard deviation (SD). Comparisons of data were done by student's t, Spearman correlation, the chi-squared test, and logistic regression analysis. Results were considered statistically significant when p value of the two-tailed was less than 0.05.

RESULTS

The mean age was 38.6±8 years (20-60 years), quality of life score was 4.2±1 (1-5), duration of drug usage was 52±46 months (2-240 months), and drug dosage was 470±324 µg/week (87.5-1200 µg/week) for all patients in the study.

The demographic characteristics of patients in each group are demonstrated in Table 1. Statistical significant variation was not found among groups in terms of age, BMI, quality of life score, duration of drug usage, and LT4 dose.

Significant increases were detected in anti-TPO antibody (4.4, 14, 11 fold; p<0.001) and TBARS (1.76, 1.6, 2.3 fold; p<0.001) levels in Group 1, 2 and 3, respectively, as compared to patients without obesity or stress management issues in group 4 (Table 2).

No correlation was found between antibody levels and the patients' age, duration of drug usage, and weekly drug doses. However, body mass index (r=0.215; p= 0.002), stress control (r=0.700; p= 0.0001), and TBARS levels (r=0.543; p= 0.0001)

Table 1: Demographic data of the patients with Hashimoto's thyroiditis

	Group 1 BMI >30 (n=54)	Group 2 Stress management difficulty (n=59)	Group 3 BMI >30 and stress management difficulty (n=11)	Group 4 Control (n=76)
Mean age (years)	36±8	38±8	35.7±9	40±9
BMI (kg/height^2)	36.4±11	26.2±0.8	35.7±9	35.7±9
Quality of life score	4.1±0.9	4±1	4.6±0.8	4.1±1.1
Duration of drug usage (months)	62±59	53±50	47±48	50±42
LT4 dose (mcg/week)	455±327	495±392	602±266	483±338

*p< 0.001 as compared to the control group

Table 2: Anti-thyroid peroxidase antibodies (anti-TPOAb) and thiobarbituric acid reactive substances (TBARS) levels of the patients with Hashimoto's thyroiditis

	Group 1 BMI >30 (n=54)	Group 2 Stress management difficulty (n=59)	Group 3 BMI >30 and stress management difficulty (n=11)	Group 4 Control (n=76)
Anti-TPOab (IU/mL)	**563±505***	1782±1290*	1443±792*	127±74
TBARS (nmol/mL)	9.7±2.9*	9±2.8*	12.7±3.6*	5.5±1.4

*p<0.001 compared with control group

were found to be positively correlated with antibody levels (Figure 1).

Considering the parameters affecting the antibody level, the antibody level increases 533 times (p<0.0001) in the group struggling with stress management, and it is affected 525 times (p<0.0001) in the obesity group.

DISCUSSION

HT is characterized by the immune system's T-cells directly attacking the thyroid gland. This is demonstrated through histological observations, which reveal the infiltration of lymphocytes and plasma cells, the development of fibrosis, the formation of lymphatic follicles, and the atrophy of the thyroid tissue (9,10,14,15).

The autoimmune manifestation of Hashimoto's thyroiditis (HT) is influenced by the interaction of environmental factors and genetic predisposition, including variations in human leukocyte antigen, T lymphocyte-associated 4, protein tyrosine phosphatase, non-receptor type 22 genes, and patterns of X chromosome inactivation. This interplay results in an impaired balance between self-tolerance mechanisms mediated by regulatory T and B lymphocytes (3-7).

The prevalence of HT is estimated to range from 0.3 to 1.5 cases per 1000 individuals, with a higher occurrence in females compared to males, with a ratio of 7 to 10 females for every male. The prevalence of the condition tends to rise with advancing age, particularly among those who have been diagnosed with additional autoimmune disorders, such as myasthenia gravis, systemic sclerosis, and other connective tissue diseases. Other ailments that are associated with an increased prevalence include Sjögren's syndrome, pernicious anemia, autoimmune liver disease, and celiac disease (2-8).

The diagnosis of HT relies on the evaluation of clinical manifestations associated with hypothyroidism and the detection of TPO antibodies (6-9).

The utilization of ultrasonic imaging in assessing the morphology of the thyroid gland can be beneficial in distinguishing between various medical conditions. Approximately 95% of patients exhibit the presence of serum anti-TPO antibodies, while positive anti-thyroglobulin antibodies are observed in 60% to 80% of cases (1-5). TPO antibodies are acknowledged as potential risk factors for the development of overt hypothyroidism in the general population as time progresses (3,4).

The relationship between anti-TPO antibodies and quality of life score has been investigated in two recent studies. The first study unveiled a negative correlation between quality of life scores in HT patients and the levels of thyroglobulin and anti-TPO antibodies. However, no evidence of correlation emerged between autoantibody levels and thyroid function tests within this study (16). In the latter one, these autoantibodies have demonstrated a positive correlation with hypothyroidism symptoms. However, no correlation was present between thyroid hormones and thyroglobulin antibodies (17).

Although there are studies investigating the relation between the symptoms of thyroid antibodies in patients, there is no study examining the factors affecting the height of antibodies. In our study, no correlation was found between antibody levels and age, hormonal status, quality of life, and complaints, which was in accordance with the literature. TBARS, which is by product of lipid peroxidation, serves as a biochemical indicator that can effectively signify the presence of cellular stress. The study

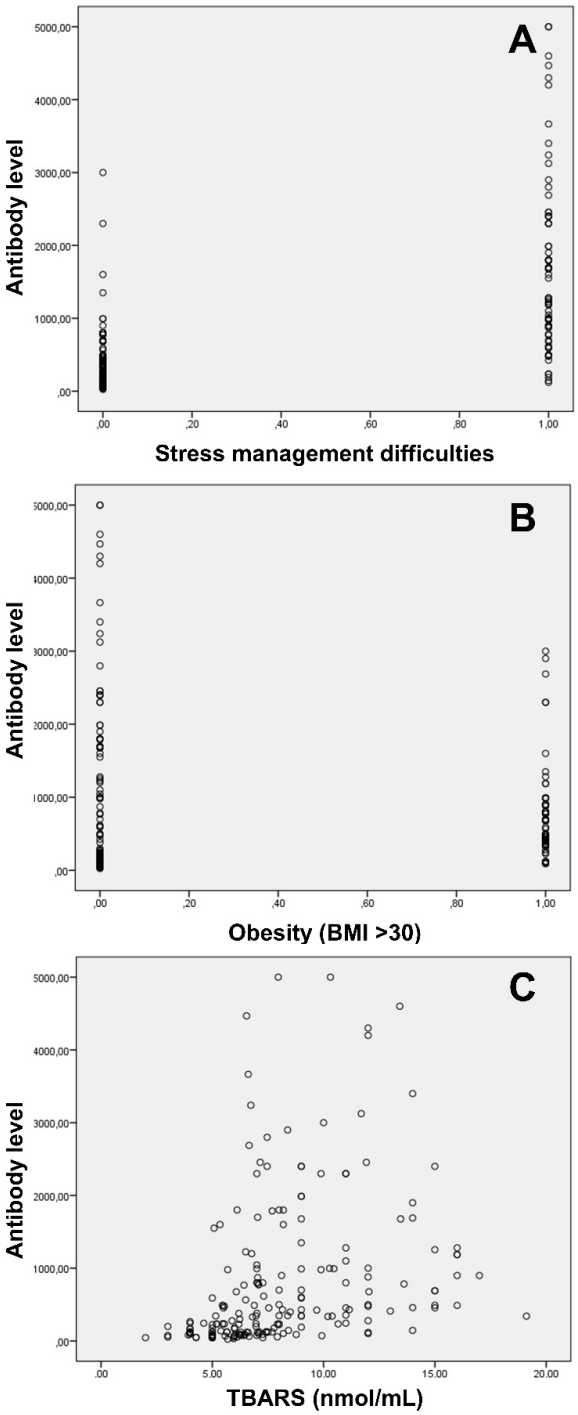

Figure 1: Relationship between antibody levels and stress management difficulties (A); and body mass index (BMI), (B); and thiobarbituric acid reactive substances (TBARS) levels (C) in Hashimoto disease.

revealed an important positive correlation between elevated levels of antibodies and indicators of stress. Similarly, TBARS levels were found to be high in obesity group patients. A significant positive link was seen between obesity and elevated levels of antibodies. The present study indicated that both stress and obesity have an impact on high antibody levels. However, our findings revealed that stress exerted a more significant influence on antibody elevation compared to obesity.

In conclusion, these findings demonstrate that stress management ability and obesity are the important factors influencing antibody levels. Further functional investigations based on a larger sample size are required in order to clarify the relationship between additional biochemical parameters and high antibody levels in patients with HT.

Ethics Committee Approval: This study was approved by Bezmialem Vakif University Non-Interventional Research Ethics Committee (Date: 05.03.2019, No: 05/54).

Informed Consent: Written informed consent was obtained.

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