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Roles of Inflammasome and Cytokines in the Pathogenesis of Autoimmune Thyroiditis

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Abstract

Objective: In this study, we aimed to investigate the role of the NLRP3 inflammasome, pro- and anti-inflammatory cytokines, and their correlations in the pathogenesis of HT.

Material and Methods: The pro-inflammatory cytokine tumour necrosis factor alpha (TNF-α), anti-inflammatory cytokine interleukin-10 (IL-10), and NLRP-3 levels were analysed with ELISA method from blood samples taken from healthy controls (Group-1) and patients diagnosed with HT (Group-2). The Student's t-test was used to evaluate quantitative data that showed a normal distribution between the two groups, while the Mann-Whitney U test was used for data sets that did not follow a normal distribution. A significance level of $p<0.05$ was considered acceptable.

Results: In Group-2, a significant increase in NLRP-3 levels was observed compared to the Group-1 ($p<0.05$). A parallel tendency was discernible in TNF-α levels, which demonstrated a significant decline in the Group-2 cohort compared to Group-1 ($p<0.01$). However, IL-10 levels showed a significant decrease in the Group-2 compared to the Group-1 ($p<0.001$). Nevertheless, a statistically significant decrease in IL-10 levels was observed in the Group-2 compared to Group-1 ($p<0.001$).

Conclusion: It has been hypothesised that NLRP-3 may play a role in the pathophysiology of autoimmune diseases, the loss of immune tolerance, and the formation of inappropriate autoreactive immune responses. This may be achieved by increasing the proinflammatory cytokine levels and decreasing the anti-inflammatory cytokine levels.

Keywords Autoimmune disease · Hashimoto's thyroiditis · inflammasome · NLRP3 · cytokine

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INTRODUCTION

Hashimoto's thyroiditis (HT) is an autoimmune disease specific to an organ and it is characterised by inflammation triggered by autoimmunity, lymphocyte infiltration, progressive destruction of the thyroid gland, and the existence of antibodies specific to thyroid antigens such as thyroid peroxidase (TPO) and thyroglobulin (Tg) (1, 2). A high level of serum anti-thyroid peroxidase antibodies (anti-TPO) is widely considered the best serologic marker of HT. It can be detected in approximately 95% of patients with clinically evident disease (3). It is the most common autoimmune disease with a prevalence of 7.5% (4). the pathological autoimmune reaction to the thyroid gland may be triggered by environmental effects such as changes in iodine metabolism, infections, and stress disrupting immune modulation. This leads to chemotaxis of autoimmune-triggered lymphocytes and destruction of the thyroid gland. It is evident that overstimulated T-CD4+ cells and their differentiated cells [T-helper (Th)1, Th2, Th17, T-regulatory (Treg)) are responsible for producing varied pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), interferon and interleukins (IL), which have been shown to cause injury or apoptosis in thyroid follicular cells and to contribute to the pathogenesis of HT (5-7).

A plethora of mechanisms have been postulated to explain the triggering of endogenous pathways and factors that have a role in the disruption of immune tolerance and immune modulation of T cells, which are causative of autoimmunity. Inflammasomes, which have emerged in recent years, have been described as vital players in innate immunity. A number of inflammasome, including nucleotide-binding oligomerization domain (NOD)-like receptor protein (NLRP)1, NLRP3, NLRC4 (NOD-like receptor family CARD-containing 4 protein), and AIM2 (absent in melanoma-2), have been suggested to have an act in the development of autoimmune thyroid disease. Among the various inflammatory mediators, NLRP3 has gained particular prominence as a key player in the detection of both exogenous pathogens and endogenous danger signals (7, 8). It is a crucial link between inflammation and innate immunity. It is recognised that the subject plays a pivotal role in the pathogenesis and prognosis of multiple sclerosis, inflammatory bowel disease, cryopyrin-associated periodic fever syndrome and analogous autoimmune and autoinflammatory diseases (9, 10).

In this study, we investigated the role of the NLRP-3 inflammasome, pro- and anti-inflammatory cytokines, and their correlations in the pathogenesis of Hashimoto's thyroiditis.

MATERIAL AND METHODS

This study was approved by the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 23.02.2024 N0: 04). Following a comprehensive explanation of all procedures applied within the scope of the study, the participants were invited to sign an "Informed Consent" form, thereby indicating that their consent had been obtained.

Experimental groups

Our study consists of 2 groups: Control (Group-1) (n=17) and Hashimoto Thyroiditis (Group-2) (n=17). Group-1 included healthy individuals between the ages of 18 and 65 years, without any chronic disease. Group 2 included individuals between the ages of 18 and 65 who were diagnosed with HT and without any chronic disease.

Hashimoto diagnosis

The diagnosis of HT was made on the basis of a heterogeneous appearance on thyroid ultrasonography and an increase in at least one of the thyroid antibodies, anti-TPO and anti-thyroglobulin (anti-Tg), measured in the serum. Blood samples were studied in the Biochemistry laboratory with autoanalyzers (Roche Cobas Integra Systems, Roche Diagnostic/Mannheim, Germany).

Samples preparation

Blood samples were collected from Group-1 and Group-2 in 1 dry (yellow) tube for Enzyme-Linked ImmunoSorbent Assay (ELISA) tests. Serum was obtained by centrifuging the blood at 3000 g for 5 min. Serum was stored in aliquots in Eppendorf tubes at −80◦C until the study was conducted.

NLPR3 inflammasome analysis

The ELISA method was used to examine NLRP3 inflammasome levels in the obtained sera. This method involves a biotin-based double antibody sandwich approach, where antibodies are added after serum containing antigenic proteins is added. The antibodies were coated on 96-well, protein-specific kits.

Pro-inflammatory and anti-inflammatory cytokine analyses

The levels of the pro-inflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10 were analysed in the serum samples using the ELISA method with a biotin-based double antibody sandwich technique. This process involves adding antibodies to protein-specific kits coated with antibodies after introducing serum containing proteins with antigenic properties.

Statistical analysis

The statistical analysis of the study's findings was conducted using the Statistical Package for the Social Sciences (SPPS) 25.0 Statistical software (IBM SPSS Corp., Armonk, NY, USA). The Shapiro-Wilk test was employed to assess the data for normality. Conventional descriptive statistical methods, incorporating the mean, standard deviation, and frequency, were then utilised to analyse the data. In instances where the variables exhibited a normal distribution between two groups, the Student's t-test was employed; conversely, in scenarios where the variables did not demonstrate a normal distribution, the Mann-Whitney U test was utilised. The significance level was set as $p<0.05$.

RESULTS

Demographic and laboratory data of the studied groups

The median age of Group 1 was found to be 41.20 years (±4.4 years) with a range from 18 to 65 years, while that of Group 2 was 38.77 years (±3.4 years) with a range from 18 to 65 years.

The Group-1 sample exhibited a female-to-male ratio of 10:7, while the Group-2 sample demonstrated a ratio of 12:5 (Table 1).

Table 1. Demographic and laboratory data of the studied groups (Mean±SEM)

Characteristics	Group-1 (Mean±SEM)	Group-2 (Mean±SEM)
Ages (years)	38.77±3.4	41.20±4.4
Sex (F/M)	10/7	12/5
Anti-TPO (IU/mL)	12±0.8	342±53
Anti-TG (IU/mL)	7.5±0.4	271.8±19
TSH (mIU/L)	1.2±0.02	2.6±0.4
fT3 (pmol/L)	5.49±0.3	4.8±0.1
NLPR3 (ng/mL)	24.01±0.4241	26.10±0.6550
TNF-α (ng/mL)	16.59±0.889	28.30±3.531
IL-10 (ng/mL)	641.2±50.66	327.4±61.23

SEM: Standard Error of Mean, F: Female, M: Male, Anti-TPO: Thyroid Peroxidase Antibody, Anti-TG: Thyroglobulin antibodies, TSH: Thyroid stimulating hormone, fT3: Free triiodothyronine, NLRP3: nucleotide-binding oligomerization domain (NOD)-like receptor protein, TNF-a: Tumour necrosis factor alpha, IL-10: Interleukin-10

Antibodies findings

Anti-TPO levels were found to be 12±0.8 IU/mL in the Group-1 and 342±53 IU/mL in Group-2. Anti-Tg levels were found to be 7.5±0.4 IU/mL in the Group-1, 271.8±19 IU/mL in Group-2. significantly increased in Group-2 compared with Group-1 ($p<0.001$). Statistical analysis revealed a significant increase in the levels of anti-TPO and anti-TG antibodies in Group 2 compared with Group 1 ($p < 0.001$). These antibodies exhibited levels that exceeded the established reference values, that is, antibodies positive, in all participants in Group-2 (Table 1).

NRLP3 findings

The mean levels of NLRP-3 were found to be 24.01±0.4241 in Group-1 and 26.10±0.6550 in Group-2. A significant increase in NLRP-3 levels was observed in Group-2 compared to Group-1 ($p=0.0116$) (Figure 1).

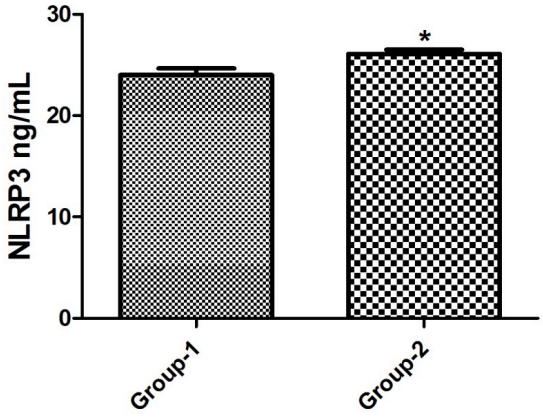

Figure 1. NLRP3 levels for all groups.
*: $p<0.05$ HT group vs. Control group

Cytokines findings

Pro-inflammatory cytokines TNF-α

The mean levels of TNF-α were found to be 16.59±0.8895 in Group-1 and 28.30±3.531 in Group-2. A significant increase in TNF-α levels was observed in Group-2 compared with Group-1 ($p=0.0030$) (Figure 2).

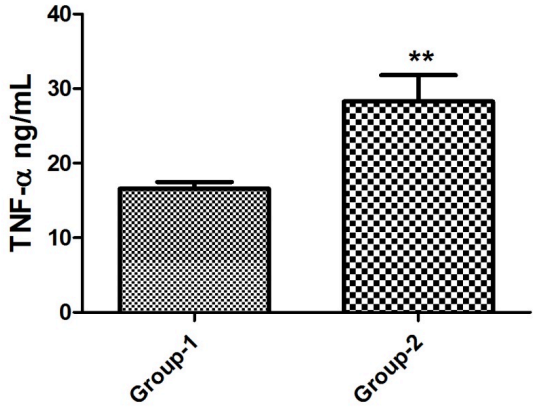

Figure 2. TNF-α levels for all groups.
**: $p<0.01$ HT group vs. Control group

Anti-inflammatory cytokines IL-10

The mean levels of IL-10 were found to be 641.2±50.66 in Group-1 and 327.4±61.23 in Group-2. The IL-10 findings

demonstrated a statistically significant decrease in Group-2 compared to Group-1 (p=0.0004) (Figure3).

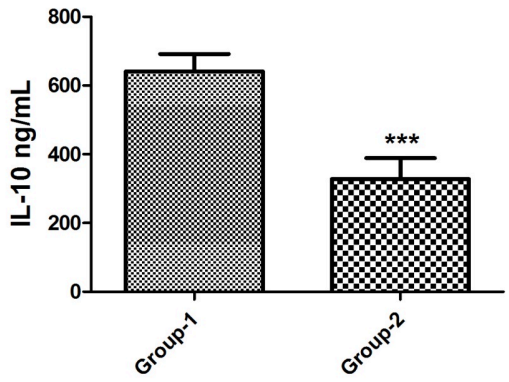

Figure 3. IL-10 levels for all groups.
***: p<0.001HT group vs. Control group

DISCUSSION

Autoimmunity is a spectrum of diseases characterised by immune responses that are misregulated towards self and non-self antigens. such conditions are frequently associated with an inflammatory response, in which inflammatory factors and inflammasome have been demonstrated to play pivotal roles in the process of their progression. In certain individuals who are susceptible to the disease, autoreactive T or B cells can be activated by a foreign antigen due to the similarities between foreign and self peptides (11). The strong association between chronic inflammation and the pathogenesis of autoimmune diseases, typically resulting from aberrant immune responses, has been widely reported. Chronic inflammation is characterised by protracted tissue damage and repair cycles. There is a demonstrable increase in the risk of autoimmune disease because of significant tissue damage. Inflammasomes, vital cytosolic multiprotein complexes that assemble in response to infections and stress, have been shown to activate inflammatory processes in macrophages and stimulate the release of inflammatory mediators. Consequently, therapeutic interventions that target inflammasome and cytokines are of particular relevance in the management of autoinflammatory disorders (12).

NLRP3 is a multimeric protein complex that has the capacity to detect both exogenous and endogenous irritants and danger signals. The principal function of the NLRP3 inflammasome is the promotion of proptosis by the stimulation of the secretion of specific pro-inflammatory cytokines and pro-apoptotic molecules (7).

It has been documented that elevated iodine intake can augment the process of proptosis in thyroid follicular cells, with this effect being transmitted through a pathway involving ROS, NF-KB, and NLRP3. This phenomenon might be instrumental in the genesis of HT. Also, in a study conducted on rats, it has been reported that autoimmune thyroiditis can be alleviated by down-regulating the NLRP3 inflammasome with a traditional Chinese herbal mixture (Yanghe herbal formulation) and regulating the Th17/Treg ratio (13).

Studies have shown that there is overexpression of inflammasome in tissue cells in autoimmune disorders such as multiple sclerosis, psoriasis and lupus nephropathy (9, 10, 14). In addition, in a study conducted on Hashimoto patients, increased expression levels of NLRP-1 and ACP inflammasome and their correlations with anti-TPO and anti-Tg levels were observed (6, 9). Another study suggested that this overexpression, which causes autoimmune disease, may be associated with single-nucleotide polymorphisms in inflammasome (15).

Guo et al. conducted immunohistochemistry studies to investigate whether the high expression of autoimmune thyroiditis inflammasome is concomitant with the infiltration of mononuclear cells. The results demonstrated that the production of NLRP1, NLRP3, NLRC4, and AIM2 was primarily localised in the thyroid follicular cells in close proximity to the lymphatic infiltration. The study further suggests that the secretion of inflammatory cytokines by infiltrating mononuclear cells and lymphocytes may be a contributing factor to the observed expression of these inflammasome components. This is supported by the rarity of thyroid follicle cells expressing inflammasome proteins in the thyroid tissue without lymphocytic infiltration (6).

In our study, the levels TNF-α were considerably elevated in Group-2 compared with Group-1. Conversely, IL-10 levels were found to be lower in Group-2 than in Group-1.

The imbalance in the ratio of Th17 cells and regulatory T cells (Treg) plays an essential role in the pathophysiology of autoimmune diseases. Th17 cells have been shown to initiate immune responses by releasing inflammatory cytokines such as IL-17 and TNF-α, while Treg cells have been observed to suppress such responses by secreting immunosuppressive cytokines, including IL-10 and TGF-β. Research has identified an imbalance in the Th17/Treg ratio in various autoimmune thyroid disease subtypes, including Hashimoto's thyroiditis, Graves' disease, and Graves' ophthalmopathy (5, 16). Treatments that target this imbalance have demonstrated protective efficacy in experimental autoimmune thyroiditis models by regulating the Th17/Treg cell ratio (17). Recent

studies have reported that NLRP-3 is a negative regulator of TREG differentiation (18).

It has been shown that the activation of the NLRP3 inflammasome caused by the Th17/Treg imbalance is realised by inducing the IL-17/NF-κB pathway and as a result, increasing pro-inflammatory cytokine release and suppressing anti-inflammatory cytokines (19).

In an experimental study, it was determined that feeding a high-fat diet increased NOD-1 levels and, in relation to this, T4 hormone levels in mice with Graves' disease, one of the autoimmune thyroid diseases. As seen in this study, inflammasome have been shown experimentally to have inducing roles in the development of autoimmune thyroiditis (20).

In parallel with the literature, in our study, NLRP-3 levels in Group-2, significantly increased compared to Group-1. Furthermore, there is an observable correlation between the rise in these measurements and the levels of TNF-α, as well as the existence of anti-TPO and anti-Tg antibodies. On the other hand, we investigated that IL-10 levels decreased in parallel with this increasing.

In conclusion, it is imperative to comprehend the pathogenesis of Hashimoto's thyroiditis, an autoimmune disease that affects a substantial proportion of the population, to ascertain the precise treatment target. The present study hypothesises that increased inflammasome expression, attributable to environmental and genetic factors, may promote the pathogenesis of autoimmune diseases and serve as a viable therapeutic target. This assertion is based on the premise that inflammasome activation enhances the inflammatory response, impedes the anti-inflammatory response, and contributes to the dissolution of immune tolerance.

Ethics Committee Approval	This study was approved by the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 01.02.2024, No: 02/2024-262).
Informed Consent	Written informed consent was obtained from all participants.
Peer Review	Externally peer-reviewed.
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