DOI 10.26724/2079-8334-2024-1-87-158-161
UDC 616.441-008.63

R.R. Rahimova, A.M. Efendiyev, L. Mehdiyev[1], G.S. Dashdamirova, S.R. Guliyeva, F.F. Rzayeva
Azerbaijan Medical University, Baku, Azerbaijan; [1]Fort Lee High School, New Jersey, USA

CHANGES IN SERUM INTERLEUKIN-8 AND AUTOANTIBODY LEVELS IN PATIENTS WITH AUTOIMMUNE THYROIDITIS

e-mail: r.rahimova1008@gmail.com

Hashimoto's disease is the most common autoimmune disease involving the thyroid gland. It is caused by the destruction of thyroid cells, which is mediated by humoral and cellular immunity disbalance. Thyroid hormones play a critical role in regulating metabolism, homeostasis, and the immune response. This study examines the role of interleukin-8 and different types of autoantibodies in patients with autoimmune thyroiditis to identify any significant associations between the cytokine and autoantibodies. In a study involving 170 patients with Hashimoto's disease (64 men and 106 women aged 18 to 64 years) was carried out a comprehensive examination of the proinflammatory cytokine interleukin-8, organ-specific antithyroglobulin, anti-thyroid peroxidase and organ-non-specific anti-DNA antibodies. The control group comprised 65 people without thyroid pathologies and other autoimmune diseases aged 20 to 65 (26 men and 39 women). The study demonstrated that disease severity is associated with changes in the levels of interleukin-8, organ-specific antibodies, and antibodies to double-stranded DNA.

Key words: autoimmune thyroiditis, antibody to thyroglobulin, IL-8, antibodies to DNA.

Р.Р. Рахімова, А.М. Ефендієв, Л. Мехдієв, Г.С. Дашдамірова, С.Р. Гулієва, Ф.Ф. Рзаєва

ЗМІНИ РІВНЯ ІНТЕРЛЕЙКІНУ-8 ТА АУТОАНТИТІЛ У СИРОВАТЦІ КРОВІ У ПАЦІЄНТІВ З АУТОІМУННИМ ТИРЕОЇДИТОМ

Хвороба Хашимото є найпоширенішим аутоімунним захворюванням, що вражає щитовидну залозу. Це викликано руйнуванням клітин щитовидної залози, яке опосередковується дисбалансом гуморального та клітинного імунітету. Гормони щитовидної залози відіграють важливу роль у регуляції метаболізму, гомеостазу та імунної відповіді. У цьому дослідженні вивчається роль інтерлейкіну-8 та різних типів аутоантитіл у пацієнтів з аутоімунним тиреоїдитом для виявлення будь-яких значущих асоціацій між цитокіном та аутоантитілами. У дослідженні за участю 170 пацієнтів з хворобою Хашимото (64 чоловіки та 106 жінок віком від 18 до 64 років) було проведено комплексне визначення прозапального цитокіну IL-8, органоспецифічного антитиреоглобуліну, антитиреоїдної пероксидази та органонеспецифічного антитіла до ДНК. Контрольну групу склали 65 осіб без патології щитоподібної залози та інших аутоімунних захворювань віком від 20 до 65 років (26 чоловіків і 39 жінок). Дослідження продемонструвало, що тяжкість захворювання пов'язана зі зміною рівня інтерлейкіну-8, органоспецифічних антитіл та антитіл до дволанцюгової ДНК.

Ключові слова: аутоімунний тиреоїдит, антитіла до тиреоглобуліну, IL-8, антитіла до ДНК.

Autoimmune thyropathies are one of the most prevalent thyroid pathologies and the most common thyropathy is autoimmune thyroiditis (AIT) also known as Hashimoto's disease (HD) [11]. HD is complex polygenic diseases that develop due to various endogenous factors, including genetics, and exogenous factors that trigger autoimmune damage to the thyroid gland [10]. The severity of thyroid dysfunction in HD varies from subclinical hypothyroidism (elevated TSH levels with normal levels of thyroid hormones) to overt clinically significant hypothyroidism [7]. In primary hypothyroidism, nonspecific systemic symptoms are highly diverse due to the broad spectrum of thyroid hormones' action on various tissues and organs.

Detecting autoantibodies provides foundational information for the diagnosis of most autoimmune diseases. An important pathophysiological distinction is whether autoantibodies are directed against extracellular or intracellular proteins [1]. Some antibodies are specific to certain organs, while others target antigen-presenting cells and their structures. In HD the primary immune attack is carried out by autoantibodies that target thyroid peroxidase, thyroglobulin and thyroid-stimulating hormone receptor, which are converted into autoantigens [8]. Autoimmune thyroid diseases are characterized by the presence of both organospecifical (antithyroglobulin (Ab-TG) and thyroid peroxidase antibodies (Ab-TPO)), and organ-nonspecificical autoantibodies, such as antinuclear antibodies, anti-single-stranded chain antibodies or denatured (Ab-dDNA), antibodies to double-stranded or native DNA (Ab-nDNA), the clinical significance of which has not been sufficiently studied [6].

In the pathogenesis of AIT, antibodies play a major role in creating an imbalance in the cytokine regulation system. This is expressed through an increase in cytokine production and a disruption in the ratio of pro- and anti-inflammatory cytokines in the body [3]. Cytokines are regulatory proteins that act on the cells that produce them, as well as on nearby cells. The imbalance of cytokines is closely linked to immune system dysfunction [2]. In terms of thyroid cell injury, cytokines derived from the lymphocytic

© R.R. Rahimova, A.M. Efendiyev, 2024

infiltrate play a key role, including their ability to stimulate the thyroid cells themselves to release proinflammatory mediators, thus amplifying and perpetuating the autoimmune response [15]. Increased levels of IL-8 are observed in various inflammatory and autoimmune diseases. IL-8 is a member of the CXC chemokine family. IL-8 is produced by monocytes, macrophages, neutrophils and lymphocytes, and is a chemoattractant for neutrophils, NK, T cells, basophils and eosinophils. It has now been proven that when cytokine production escapes the control of endogenous inhibitors (including steroid hormones), these mediators directly or indirectly become responsible for tissue damage and functional organ failure [4, 14]. Cytokines participation in various biological and physiological processes, on the other hand, is regulated by hormones, including thyroid hormones.

The purpose of the study was to examine potential associations between IL-8 and various autoantibody types in patients with autoimmune thyroiditis.

Material and methods. In our study involving 170 patients with autoimmune thyroiditis (64 men and 106 women aged 18 to 64 years) was carried out a comprehensive examination of the organ-specific (Ab-TG, Ab-TPO), organ-non-specific antibodies (Ab-DNA) and the level of IL-8. Inclusion criteria were patients with a primary diagnosis of AIT without concomitant allergic or other autoimmune diseases. Exclusion criteria – chronic inflammatory processes affecting the patient's immunological status, pregnancy and lactation. The control group comprised 65 people without thyroid pathologies and other autoimmune diseases aged 20 to 65 (26 men and 39 women). The diagnosis was established based on the results of laboratory analysis and ultrasonography.

Patients were divided into two groups based on the analysis of clinical and laboratory studies with the determination of TSH, free thyroxine (T4), and free triiodothyronine (T3) levels:

Group 1 included 74 patients with a manifest form of the disease.

Group 2 included 96 patients with a subclinical form.

The concentration of thyroid hormones – T3, T4, and TSH was determined using the immunochemiluminescent method on the IMMULITE 2000 Xpi apparatus (USA). The levels of antibodies to native (double-stranded) DNA (Ab-nDNA) and denatured (single-stranded) DNA (Ab-dDNA) in the blood serum were determined using enzyme-linked immunosorbent assay (ELISA). The concentration of IL-8 in blood serum also was determined using enzyme-linked immunosorbent assay (ELISA). The study results were subjected to statistical analysis using the StatSoft software package. To represent quantitative parameters, medians and upper and lower quartiles were calculated. Intergroup comparisons regarding quantitative indicators were conducted using the Mann-Whitney rank nonparametric test. We performed a correlation analysis to investigate potential relationships between the level of IL-8, thyroid hormones, TSH and calculated the Spearman correlation coefficient.

All study participants gave voluntary informed consent for the study. The present study was approved by the ethics committee of Azerbaijan Medical University (Ref.no: AMU/ IEC/№12/ 07.02.2020)

Results of the study and their discussion. Patients with a manifest form of AIT had such complaints as a decrease in body temperature, myxedematous edema – circles under the eyes, obesity, voice change, drowsiness, mental retardation, emotionality, shortness of breath, pain in the heart and retrosternal region, slowing of cardiac contractions, a tendency to constipation or diarrhea, loss of sensation in the limbs, thinning, and loss of hair, disruption or cessation of menstruation. In the manifest patient's group, the level of TSH increased, whereas the level of hormones T3 and T4 decreased, as well as increased titers of organ-specific antibodies - Ab-TG and Ab-TPO.

The subclinical form of AIT is characterized by increased TSH and normal T3 and T4, with an erased clinical picture.

Our research results demonstrated that individuals with AIT and hypothyroidism exhibit altered concentrations of thyroid hormones compared to the control group. Specifically, patients with overt hypothyroidism in manifest forms displayed significantly lower levels ($p<0.05$) of fT3 and fT4 in comparison to the corresponding levels in the control group. In contrast, the level of thyroid-stimulating hormone (TSH) was markedly elevated ($p<0.05$) in both subclinical and manifest groups relative to the corresponding concentration of this hormone in the control group.

The examination of cytokine profiles in patients with AIT revealed a significant increase in cytokine levels in groups of patients with subclinical and manifest forms of the disease compared to the control group. Among cases of subclinical hypothyroidism, the median concentration of IL-8 was 26.7 (18.6; 34.6) pg/ml. The concentrations of this marker were significantly higher ($p=0.022$) than that in the control group. In patients with the manifest form of hypothyroidism, the median plasma levels of IL-8 were 52.4 pg/ml (38.7; 70.2), which was statistically significantly higher ($p=0.004$) than the similar indicator in the control group. In the control group, the median level of IL-8 was 21.7 (18.5; 24.0).

In the group of patients with subclinical hypothyroidism, the levels of Ab-TG and Ab-TPO antibodies were elevated, with a median range of 456 (395–544) IU/ml and 523 (464–568) IU/ml, respectively. These levels were significantly higher compared to the control group, which had median values of 16 (13–30) IU/ml and 20 (13–25) IU/ml, respectively ($p<0.001$). Furthermore, in patients with manifest form, the presence and levels of autoantibodies were also found to be significantly elevated, with median values of 470 (381–527) IU/ml and 531 (458–566) IU/ml for Ab-TG and Ab-TPO, respectively, compared to the control group.

The present study investigates the levels of antibodies to DNA in patients with different clinical forms of hypothyroidism. The analysis of antibody levels in patients with various clinical conditions of hypothyroidism revealed that the median concentration of Ab-dsDNA was significantly higher in patients with a manifest form of the disease compared to those with a subclinical course of hypothyroidism (8.6 (5.4–16.4) IU/ml vs. 6.8 (2.1–13.8) IU/ml, respectively; $p<0.05$) and the control group (2.6 (1.45–3.55) IU/ml; $p< 0.001$).

The median concentration of Ab-ssDNA was higher in patients with a manifest form of hypothyroidism compared to the subclinical form of the disease (4.9 (3.37–10.1) IU/ml vs. 4.0 (1.6–6.0) IU/ml, respectively; $p<0.05$) and the control group (4.6 (1.3–5.9) IU/ml). But there were no statistically significant differences in the levels of Ab-ssDNA between the overall patient group and control groups ((4.5 (2.4; 8.6) IU/ml vs. 4.6 (1.3; 5.9)) (Table 1).

Table 1

The levels of IL-8 and autoantibodies in patients with autoimmune thyroiditis, Me (Q25; Q75)

Indices	Control group (n=65)	AIT (n=170)	Subclinical form (n=96)	Manifest form (n=74)
IL-8 pg/ml	21.7 (18.5–24.0)		26.7* (18.6–34.6)	52.4*# (38.7–70.2)
Ab-TG, IU/ml	16 (13–30)		456* (395–544)	470* (381–527)
Ab-TPO, IU/ml	20 (13–25)		523* (464–568)	531* (458–566)
Ab-dsDNA, IU/ml	2.6 (1.5–3.6)	4.4 (2.3–9.9)	6.8* (2.1–13.8)	8.6*# (5.4–16.4)
Ab-ssDNA, IU/ml	4.6 (1.3–5.9)	4.5 (2.4–8.6)	4.0 (1.6–6.0)	4.9 (3.4–10.1)

Note: * – Statistically significant difference compared to the control group at $p<0.05$ level; # – Statistically significant difference compared to the subclinical group at $p<0.05$ level.

Negative statistically significant correlations were established between the value of fT4 and the level of IL-8, moderate negative correlations were found between the concentration of fT3 and the level of IL-8. However, positive correlations was found between TSH activity and the levels of IL-8 (Table 2).

Table 2

Correlation between IL-8 and thyroid status in patients with AIT, (Spearman correlation coefficients, r)

Indices	Free T3	Free T4	TSH
IL-8	-0.180 (p=0.134)	-0.438 (p<0.001)	0.541 (p=0.006)

The study of the relationship between the level of IL-8 in the blood serum, hormones T3, T4 and TSH showed an inverse relationship of IL-8 with T3, T4 and a direct relationship with TSH. These findings correspond to the clinical manifestations observed in patients with AIT**.** In patients with AIT, the observed changes in the level of IL-8 level lead to an increase in organ-specific and organ-nonspecific antibodies, which indicates worsening imbalance in the immune system that play a crucial role in the development of the corresponding clinical manifestations of thyroiditis. Elevated titers of autoantibodies to dsDNA, indicate an aggravation of the autoimmune process due to damage to cellular structures, leading to gland dysfunction. The description of double-stranded DNA elements and chromatin fragments as stimulators of B- and T-cells in autoimmune pathology has received much attention in modern studies. These autoantibodies possibly have low specificity and affinity, but they exhibit a cytotoxic effect when binding to gland cells [12]. For example, Basant M Elnady, Naglaa M Kamal and et. al suggest that multiple self-antigens can be recognized by Ab-nDNA, subsequently triggering apoptosis, inflammatory responses, and tissue fibrosis. Anti-nDNA may be used as predictive markers in ATD patients for close follow-up and prevention of comorbid pathology [5]. Regarding the IL-8 results in previous studies, monitoring the interleukin-8 level, but not interferon-alpha (IFN-α) in the sera of patients with thyroid diseases was

associated with an advanced stage [13]. Other studies have suggested that serum IL-8 levels could be valuable in diagnosing patients with active disease and distinguishing malignancy in AIT [9]. In general, despite the significant progress made in the study of AIT and the "ensuing consequences", the processes leading to an exacerbation of the autoimmune process with the likelihood of comorbid pathology remain unclear. Further studies should be conducted to understand the correlation between levels of various types of autoantibodies and cytokines, and their causal relationship.

Conclusions

1. Serum levels of antibodies to double-stranded DNA can be used to objectively assess the degree and severity of thyroid lesions. In the manifest group, there was a marked increase in antibody levels (8.6 (5.4-16.4) IU/ml).

2. The results of our study suggest that the level of interleukin-8 can be considered as a candidate biomarker of autoimmune process exacerbation in AIT.

The study of biochemical indicators involved in the pathogenesis and exacerbation of AIT may help to prevent the development of pathology and lead to a critical re-evaluation of therapy in clinical practice. It is therefore important to investigate the relationship between different biochemical markers in patients with autoimmune thyroid disease.

Prospects for further research of biochemical parameters, such as antinuclear antibodies, anti-DNA antibodies, and cytokines, may serve as informative markers to determine the nature and severity of autoimmune pathology.

References

1. Bogusławska J, Godlewska M, Gajda E, Piekiełko-Witkowska A. Cellular and molecular basis of thyroid autoimmunity. European thyroid journal. 2022; 11(1), e210024. https://doi.org/10.1530/ETJ-21-0024

2. Burbelo PD, Iadarola MJ, Keller JM, Warner BM. Autoantibodies Targeting Intracellular and Extracellular Proteins in Autoimmunity. Front Immunol. 2021; 8(12):548469. doi: 10.3389/fimmu.2021.548469.

3. Chetaille Nézondet AL, Poubelle PE, Pelletier M. The evaluation of cytokines to help establish diagnosis and guide treatment of autoinflammatory and autoimmune diseases. J Leukoc Biol. 2020; 108(2):647–657. doi: 10.1002/JLB.5MR0120-218RRR

4. Efendiyev AM, Azizova GI, Dadashova AR. Investigation of Some Endogenous Antimicrobial Peptides in Thalassemia. Thalassemia Reports. 2018; 8(2):7744. https://doi.org/10.4081/thal.2018.7744

5. Elnady BM, Kamal NM, Shaker RHM, Soliman AF, Hasan WA, Alghamdi HA, et al. Prevalence and clinical significance of nonorgan specific antibodies in patients with autoimmune thyroiditis as predictor markers for rheumatic diseases. Medicine. 2016; 95(38), e4336. https://doi.org/10.1097/MD.0000000000004336

6. Granito A, Muratori L, Tovoli F, Muratori P. Diagnostic role of anti-dsDNA antibodies: do not forget autoimmune hepatitis. Nat Rev Rheumatol. 2021; 17(4):244. doi: 10.1038/s41584-021-00573-7.

7. Hamous J, Dvořák J, Bušovský I. Hashimotos thyroiditis. Rozhledy v chirurgii: mesicnik Ceskoslovenske chirurgicke spolecnosti. 2021; 100(3):110–112. https://doi.org/10.33699/PIS.2021.100.3.110-112 [in Czech]

8. Hoermann R, Midgley JE, Larisch R, Dietrich JW. Homeostatic Control of the Thyroid-Pituitary Axis: Perspectives for Diagnosis and Treatment. Frontiers in endocrinology. 2015; 6 (177). https://doi.org/10.3389/fendo.2015.00177

9. Martins MB, Marcello MA, Batista FA, Peres KC, Meneghetti M, Ward MA, et al. Serum interleukin measurement may help identify thyroid cancer patients with active disease. Clinical biochemistry. 2018; 52, 1–7. https://doi.org/10.1016/j.clinbiochem.2017.10.003

10. Rahimova R. Relationship between CTLA4, TNF-α and PTPN22 gene polymorphism and the serum levels of antithyroglobulin and antiperoxidase antibodies in autoimmune thyroiditis[J]. AIMS Medical Science. 2023; 10(1): 14–23. doi: 10.3934/medsci.2023002

11. Ralli M, Angeletti D, Fiore M, D'Aguanno V, Lambiase A, Artico M, et al. Hashimoto's thyroiditis: An update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation. Autoimmunity rev. 2020; 19(10), 102649. https://doi.org/10.1016/j.autrev.2020.102649

12. Rekvig OP. The Anti-DNA Antibodies: Their Specificities for Unique DNA Structures and Their Unresolved Clinical Impact-A System Criticism and a Hypothesis. Frontiers in immunology. 2022; 12, 808008. https://doi.org/10.3389/fimmu.2021.808008

13. Stoica RA, Drăgana N, Ancuceanu R, Geicu OI, Guja C, Pantea-Stoian A, et al. Interleukin-8, CXCL10, CXCL11 and their role in insulin resistance in adult females with subclinical hypothyroidism and prediabetes. J ClinTransl Endocrinol. 2022; 28, 100299. https://doi.org/10.1016/j.jcte.2022.100299

14. Troshina EA. The role of cytokines in the processes of adaptive integration of immune and neuroendocrine reactions of the human body. Problemy endokrinologii. 2021; 67(2):4–9. https://doi.org/10.14341/probl12744

15. Weetman AP. An update on the pathogenesis of Hashimoto's thyroiditis. Journal of endocrinological investigation. 2021; 44(5), 883–890. https://doi.org/10.1007/s40618-020-01477-1

Стаття надійшла 4.03.2023 р.