# EXPRESSION OF CYTOKINES AND CYTOKINE RECEPTORS-GENES IN PATIENTS WITH DIFFERENT FORMS OF THYROID PATHOLOGY IN UKRAINIAN POPULATION

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# IZRAŽAVANJE CITOKINA I RECEPTORA-GENA CITOKINA KOD BOLESNIKA SA RAZLIČITIM OBLICIMA ŠTITNE PATOLOGIJE U UKRAJINSKOM STANOVNIŠTVU

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## ABSTRACT

*Multiple susceptibility genes can be involved in the development of Hashimoto's thyroiditis. Some of these genes are implicated in other autoimmune diseases, while others are specific to thyroid autoimmune response. 153 patients with thyroid pathology were enrolled in the study (152 women and 1 man, the average age was 46,02±14,3). They were divided into 3 groups: 16 patients with postoperative hypothyroidism; 65 patients with hypothyroidism resulting from autoimmune thyroiditis, and 72 patients with both AIT and elevated serum an anti-thyroglobulin and anti-thyroid peroxidase antibodies. We used a pathway-specific real-time Polymerase chain reaction array to identify and verify cytokines and receptor pathway-associated gene expression in peripheral white blood cells in randomly selected 12 individuals from each group. In the patients with postoperative hypothyroidism and those with hypothyroidism resulting from autoimmune thyroiditis, the expression of Chemokine (C-X3-C motif) receptor 1, Chemokine (C-X-C motif) receptor 4, Interleukin 6, and Interleukin 6 receptor significantly decreased, while the expression of IL6ST and IL10RA increased. In contrast, mRNA levels of Chemokine (C-X3-C motif) receptor 1, Chemokine (C-X-C motif) receptor 4, Interleukin 6, and Interleukin 6 receptor increased in the autoimmune thyroiditis patients with elevated serum anti-thyroglobulin and anti-thyroid peroxidase antibodies, while the expression of Interleukin 6 signal transducer and Interleukin 10 receptor, alpha decreased in this group of patients. The patients with hypothyroidism resulting from autoimmune thyroiditis and patients with elevated serum anti-thyroglobulin and anti-thyroid peroxidase antibodies had significantly lowered expression of Interleukin 10, while the expression of Interleukin 1, beta and Interleukin 1 receptor, type I was elevated. autoimmune thyroiditis and hypothyroidism affect the mRNA-level expression of cytokines and cytokine receptor genes in a gene-specific manner, and these changes to gene expression can be among the triggers of autoimmune inflammation progression in the thyroid gland. Transcriptional activity of cytokines, inducer, and receptor genes in the peripheral white blood cells can be used as an important minimally invasive prognostic marker of the autoimmune thyroid disease severity.*

***Keywords*****:** *autoimmune thyroiditis; cytokines; hypothyroidism; mRNA; receptors.*

## SAŽETAK

*Više gena osetljivosti može biti uključeno u razvoj Hashimotovog tiroiditisa. Neki od ovih gena su umešani u druge autoimune bolesti, dok su drugi specifični za autoimuni odgovor štitne žlezde. U studiju su bila uključena 153 pacijenta sa patologijom štitne žlezde (152 žene i 1 muškarac, prosečna starost je bila 46,02 ± 14,3). Podeljeni su u 3 grupe: 16 pacijenata sa postoperativnim hipotiroidizmom; 65 pacijenata sa hipotireozom uzrokovanom autoimunskim tiroiditisom i 72 pacijenta sa AIT-om i povišenim serumom na antitela protiv tireoglobulina i protiv štitne peroksidaze. Koristili smo niz polimeraznih lančanih reakcija specifičnih za putanju u realnom vremenu za identifikaciju i verifikaciju citokina i ekspresije gena povezanih sa receptorskim putem u perifernim belim krvnim zrncima kod nasumično odabranih 12 pojedinaca iz svake grupe. Kod pacijenata sa postoperativnim hipotiroidizmom i onima sa hipotiroidizmom koji je rezultat autoimunskog tiroiditisa, ekspresija receptora 1 za hemokin (C-Ks3-C motiv), receptor 4 za hemokin (CKSC motiv), interleukin 6 i receptor interleukina 6 značajno se smanjila IL6ST i IL10RA povećani. Nasuprot tome, nivoi mRNK receptora 1 za hemokin (C-Ks3-C motiv), receptor 4 za hemokin (motiv CKSC), interleukina 6 i receptora interleukina 6 povećani su kod pacijenata sa autoimunim tiroiditisom sa povišenim serumskim anti-tiroglobulinom i anti-štitnom peroksidazom antitela, dok se ekspresija signalnog pretvarača interleukina 6 i receptora interleukina 10, alfa smanjila u ovoj grupi pacijenata. Pacijenti sa hipotiroidizmom koji je posledica autoimunog tiroiditisa i pacijenti sa povišenim serumskim antitireoglobulinskim i anti-tiroidnim peroksidaznim antitelima imali su značajno smanjenu ekspresiju interleukina 10, dok je ekspresija interleukina 1, beta i receptora interleukina 1, tipa I bila povišena. autoimuni tiroiditis i hipotiroidizam utiču na ekspresiju citokina i gena za citokinske receptore na nivou mRNA na gen specifičan način, a ove promene u ekspresiji gena mogu biti među pokretačima progresije autoimune upale u štitnoj žlezdi. Transkripciona aktivnost citokina, induktora i receptorskih gena u perifernim belim krvnim zrncima može se koristiti kao važan minimalno invazivni prognostički marker ozbiljnosti autoimune bolesti štitne žlezde.*

***Ključne reči*****:** *autoimunski tiroiditis; citokini; hipotiroidizam; mRNA; receptori.*

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## INTRODUCTION

Hashimoto's thyroiditis (HT) is among most common autoimmune thyroid diseases (AITDs). It is a T cell-mediated organ-specific autoimmune disorder that results in thyroid damage and subsequent clinical hypothyroidism. The disorder is mediated by infiltrating and/or locally activated thyroglobulin (Tg)-specific T cells. Surveys indicate that HT is the leading cause of hypothyroidism in iodine-sufficient areas of the world (1).

A significant factor involved in the induction of autoimmune response is a defect in or deficiency of the immune regulation, particularly disequilibrium between the effector T cells (Teff) and regulatory T cells (Treg) which normally prevent the development of autoimmunity (2,3). Multiple susceptibility genes may be involved in the disease development, some of which are also implicated in other autoimmune diseases (4,5), while others are specific to thyroid autoimmunity (6,7). However, data about the expression of cytokines and cytokine receptor pathway-focused genes in patients with different forms of thyroid pathology from Europe are limited, and no such data from Ukraine has been internationally published.

Cytokines can influence the autoimmune process through a number of mechanisms, including the recruitment of inflammatory cells and the activation of molecules required to maintain an inflammatory response in the affected area. Possible direct exposure to cytokines on thyroid function by affecting TFC distribution and modulation of expression and function molecules involved in the synthesis of thyroid hormones (2). Earlier, when studying the type of cytokine response in ATD, it was revealed that in HT, the Th1 response predominates (2).

Cytokines, their receptors, and the signaling pathways they utilize are potentially attractive therapeutic targets in AITDs (8). For instance, T helper (Th1) cytokine is frequently prevalent in HT, as well as in experimental autoimmune thyroiditis as a result of the infiltration of T cells and macrophages into thyroid tissue (9). Furthermore, the thyroid follicular cells can themselves produce several types of cytokines (10).

The aim of the study was to detect changes in the expression of the genes involved in the production of cytokines and their receptors in patients with different forms of thyroid pathology.

## METHODS

153 patients with thyroid pathology were enrolled in the study. They were divided into 3 groups: Group 1 included 16 patients with postoperative hypothyroidism; Group 2 included 65 patients with hypothyroidism resulting from autoimmune thyroiditis (AIT), and Group 3 included 72 patients with both AIT and elevated serum an anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibodies. The control group included 25 healthy individuals, which were recruited randomly, without matching for age or sex. Clinical characteristics of the subjects are shown in table 1.

Hypothyroidism was diagnosed following the recommendations of the American Association of Clinical Endocrinologists, 2012. The diagnosis of AIT was based on detected circulating antibodies to thyroid antigens (Anti-TPO and anti-TG) and reduced echogenicity on the thyroid sonogram in a patient with relevant clinical features (11).

Blood specimens were collected between 8 and 10 AM after an overnight fast. Free thyroxin (fT4, normal range 6.0-13.0 pmol/L for males and 7.0-13.5 pmol/L for females), thyroid-stimulating hormone (TSH, normal range 0.3-4.0 mIU/mL), anti-thyroid peroxidase (anti-TPO, normal range 0-30 IU/mL) and anti-thyroglobulin (anti-TG, normal range 0-65 IU/mL) antibodies levels were determined in every individual using STAT FAX303/Plus analyzer (Awareness Technology Inc, USA).

Patients under the age of 18 or those suffering from malignancy, inflammation associated with rheumatic diseases or acute/chronic infection, diabetes mellitus, cardiovascular or cerebrovascular diseases, chronic hepatic or renal diseases, as well as pregnant women and those taking any drugs that could interfere with thyroid function, were excluded from the study.

We used a pathway-specific PCR array (Neurotrophins and Receptors RT$^2$ Profiler PCR Array, QIAGEN, Germany) to identify and verify cytokines and receptor pathway-associated gene expression in randomly selected 12 individuals from each group using a real-time PCR following the procedure described below.

Experimental procedures. RNA isolation. Total RNA was isolated from white blood cells using NucleoZOL kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. NucleoZOL is intended for the isolation of total RNA (small and large RNA) in single or separate fractions. White blood cells were lysed and homogenized in NucleoZOL guanidinium thiocyanate and phenol based reagent.

cDNA synthesis. The RNA quality was determined and it was reverse transcribed. The concentration and quality of the isolated total RNA were determined on a NanoDrop spectrophotometer (Thermo Scientific™, USA). For the reverse transcription procedure with a cDNA conversion RT² First Strand Kit (QIAGEN, Germany, Cat. no. 330401), RNA samples with the following parameters were selected: ratio A260/A280 within the range of 1.8-2.2.

The RT$^2$ HT First Strand Kit procedure comprises 2 steps: elimination of genomic DNA contamination, and reverse transcription with an RT master mix, as well as random hexamers and oligo-dT prime reverse transcription to capture more difficult-to-detect genes.

PCR Array. The cDNA was then used with RTI Profiler PCR Array (QIAGEN, Cat. no. PAHS-031Z) in combination with RTI SYBR® Green qPCR Mastermix (QIAGEN, Cat. no. 330504), following the complete RT$^2$ Profiler PCR Array procedure (www.qiagen.com). Samples were assigned to control and study groups. CT (cycle threshold) values were normalized based on the automatic selection from the full panel of reference genes.

Any CT value >35 was considered to be a negative call. The RT$^2$ Profiler PCR Array data analysis software calculates the fold change using the ΔΔCT method. The formula first calculates delta CT between the gene of interest (GOI) and an average of reference genes (HKG). In the second step, delta-delta CT is calculated as (delta CT (Test Group)-delta CT (Control Group)). Fold change is then calculated as 2^(-ΔΔCT). The data analysis report was exported from the QIAGEN web portal at GeneGlobe. The software allows defining the best reference genes for normalization.

A list of cytokines and receptor pathway-associated genes selected for this study is given in table 2.

**Ethics**

The ethical principles contained in the Declaration of Human Rights adopted in Helsinki, in 1975, and revised in 2008, were fully respected in our study. The enrolled subjects, participated in this study voluntarily, and completed and signed a written informed consent. The protocol of the study was approved by the local ethics committees of HSEEU "Bukovinian State Medical University", Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University.

**Statistical analysis of PCR array data**

The RT2 Profiler PCR Array Data Analysis software calculates p-values using a Student's t-test (two-tail distribution and equal variances between the two samples) based on the triplicate 2^(-ΔΔCT) values for each gene in the experimental group compared to the control group. Published results from the Microarray Quality Control (MAQC) confirm that such ranked lists of genes based on fold-change and associated p-value is sufficient to demonstrate reproducible results using the RT$^2$ Profiler PCR Arrays.

## RESULTS

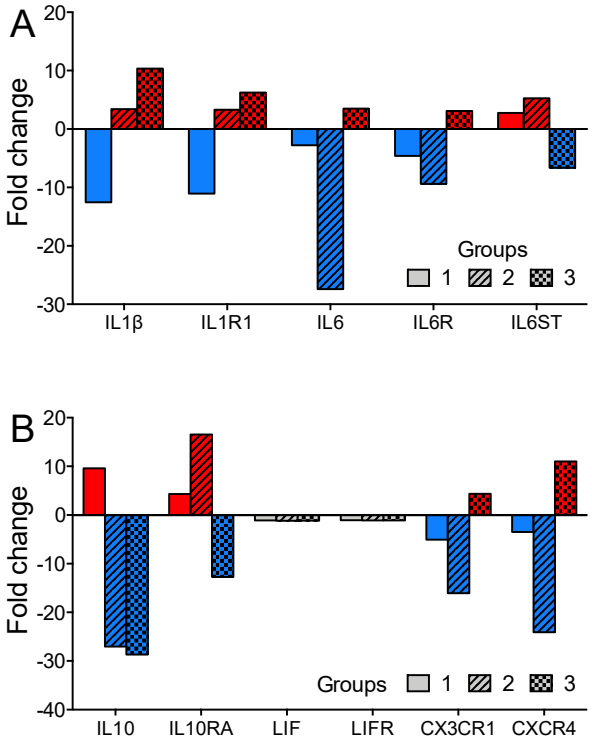
Using the Pathway-Focused PCR Array Profiling (Neurotrophins and Receptors RT2 Profiler PCR Array) we examined the Cytokines and receptor pathway-associated gene expression of patients with primary hypothyroidism as a result of AIT and postoperative hypothyroidism and patients with AIT with rising serum autoantibodies, such as anti-Tg and anti-TPO.

As it is shown in Table 1, there was a probable decrease in fT4 levels in the groups of patients with postoperative hypothyroidism and primary hypothyroidism as a result of AIT in 2.6 and 2.16 times, respectively, compared with the Control group. At the same time, TSH levels were significantly higher in these groups by 3.2 and 2.65 times, respectively. According to our studies, in patients with primary hypothyroidism as a result of AIT, the level of anti-TPO was 11.2 times higher, and the level of anti-TG in this group was 2.16 times higher than in the Control group. Patients in Group 3 had a 9.7-fold increase in anti-TPO and 2.4-fold anti-TG levels compared with the Control group.

The results from RT2 Profiler Neurotrophins & receptors pathway-focused genes expression analysis is given in table 3.

Genes expression analysis showed that in the study Group 1, which included the patients with postoperative hypothyroidism, the expression of IL10 and IL10RA increased 9.6 and 4.3-fold respectively (Figure 1B), while expression of the following genes decreased: CX3CR1 (5.04-fold), CXCR4 (3.5-fold) (Figure 1B), IL1β (12.5-fold), and IL1R1 (11.1-fold) (Figure 1A). Declines in IL6 (2.8-fold) and IL6R (4.6-fold) mRNA levels were also detected in Group 1 (Figure 1A).

**Figure 1.** Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (A) and suppressors of pro-inflammatory signals and those with multidirectional effects on the inflammatory process (B). Fill pattern represents patients of different groups.




Red color shows upregulation, blue – downregulation and grey - no changes.

As it is shown in Table 3, in the patients with hypothyroidism resulting from AIT (Group 2), the expression of the following cytokines and receptor pathway genes increased: IL10RA (16.5-fold), IL1β (3.4-fold), IL1R1 (3.3-fold), and IL6ST (5.3-fold). The expressions of CX3CR1 (16.1-fold), CXCR4 (24.1-fold), IL10 (27.03-fold) (Figure 1B), IL6 (27.4-fold), and IL6R (9.4-fold) (Figure 1A) decreased.

In Group 3, which includes patients with AIT and elevated serum anti-Tg and anti-TPO auto-antibodies, IL10 and IL10RA were down-regulated, 28.6 and 12.7 times respectively (Figure1B). The mRNA levels of IL6 (3.5-fold), and IL6R (3.1fold) significantly increased (Figure1B). IL6ST mRNA levels in the Group 3 were reduced 6.6-fold, while the expression of IL1β (10.4-fold) and IL1R1 (6.2-fold) significantly increased (Table 3). Expression of LIF and LIFR was not different in all groups of patients (Figure 1B).

All the effects are summarized in figure 2. Notably, Group 1 patients solely have up-regulated genes for IL10 and down-regulated for IL1β and its receptor 1L1R1 (Figure 2A).

**Figure 2A.** Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (IL1β, IL1R1, IL6, IL6R, IL6ST); suppressors of pro-inflammatory signals (IL10, IL10RA, LIF, LIFR) and those with multidirectional effects on the inflammatory process (CX3CR1 and CXCR4) in patients with postoperative hypothyroidism.

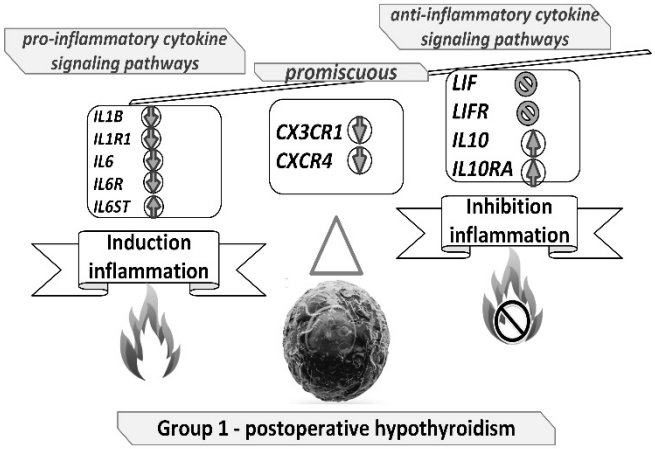



**Figure 2B.** Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (IL1β, IL1R1, IL6, IL6R, IL6ST); suppressors of pro-inflammatory signals (IL10, IL10RA, LIF, LIFR) and those with multidirectional effects on the inflammatory process (CX3CR1 and CXCR4) in patients with hypothyroidism resulting from autoimmune thyroiditis

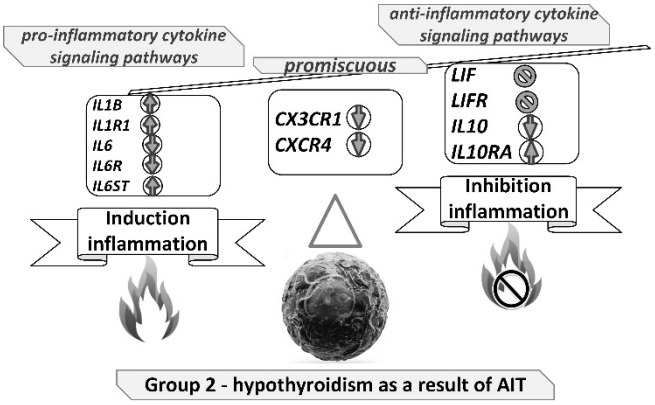
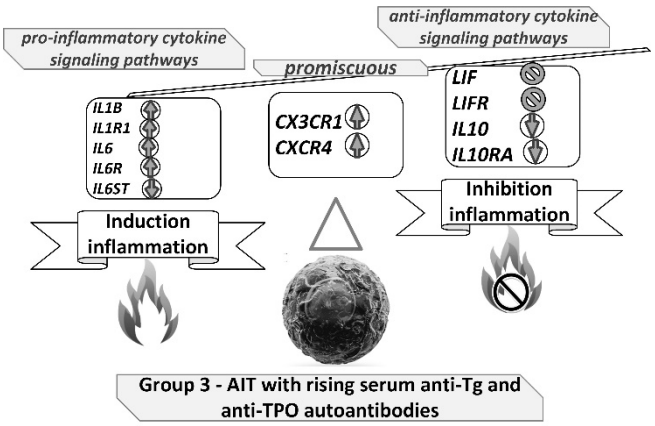



**Figure 2C.** Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (IL1β, IL1R1, IL6, IL6R, IL6ST); suppressors of pro-inflammatory signals (IL10, IL10RA, LIF, LIFR) and those with multidirectional effects on the inflammatory process (CX3CR1 and CXCR4) in patients with AIT and elevated serum anti-Tg and anti-TPO antibodies.




Patients of the Group 3, unlike the others, have up-regulated genes of IL6 and IL6R, while down-regulated IL6ST and IL10RA (Figure 2C). Interestingly, patients of Group 3 have some expression changes similar to those of Group 2 (Figure 2B), but also some unique to Group 3 (Figure 2C).

**Table 1.** Clinical characteristics of the subjects

| | Referent values | Control group (n=25) | Patients with post-operative hypo-thyroidism (Group 1) (n=16) | Patients with hy-pothyroidism as a result of AIT (Group 2) (n=65) | Patients with AIT with rising serum anti-Tg and anti-TPO autoantibod-ies (Group 3) (n=72) |
|---|---|---|---|---|---|
| Age (years) | | 46.1 ± 14.6 | 47.3 ± 12.3 (p>0.05) | 46.7 ± 15.49 (p>0.05) | 45.0 ± 13.7 (p>0.05) |
| fT4 (pmol/L) | 7.0-13.5 | 8.91 ± 0.97 | 3.44 ± 0.31 (p<0.001) | 4.13 ± 0.52 (p<0.001) | 8.51 ± 0.82 (p>0.05) |
| TSH (mIU/mL) | 0.3-4.0 | 2.67 ± 0.52 | 8.61 ± 0.84 (p<0.001) | 7.09 ± 0.50 (p<0.001) | 2.38 ± 0.62 (p>0.05) |
| anti-TPO (IU/mL) | 0-30 | 34.0 ± 3.70 | 36.1 ± 2.78 (p>0.05) | 380 ± 73.4 (p<0.001) | 330 ± 50.2 (p<0.001) |
| anti-TG (IU/mL) | 0-65 | 15.3 ± 1.97 | 15.5 ± 1.90 (p>0.05) | 33.0 ± 4.27 (p<0.001) | 36.4 ± 7.70 (p<0.001) |
| Current dose of L-thyroxine (μg/day) | | None | 111 ± 5.25 | 88.5 ± 1.55 | None |

Data are expressed as mean ± standard deviation
The p values are calculated based on a Student's t-test in the control group and study groups

**Table 2.** Cytokines and receptor pathways-associated genes

| UNIGENE | REFSEQ | SYMBOL | DESCRIPTION |
|---|---|---|---|
| Hs.78913 | NM_001337 | CX3CR1 | Chemokine (C-X3-C motif) receptor 1 |
| Hs.593413 | NM_003467 | CXCR4 | Chemokine (C-X-C motif) receptor 4 |
| Hs.193717 | NM_000572 | IL10 | Interleukin 10 |
| Hs.504035 | NM_001558 | IL10RA | Interleukin 10 receptor, alpha |
| Hs.126256 | NM_000576 | IL1β | Interleukin 1, beta |
| Hs.701982 | NM_000877 | IL1R1 | Interleukin 1 receptor, type I |
| Hs.654458 | NM_000600 | IL6 | Interleukin 6 (interferon, beta 2) |
| Hs.135087 | NM_000565 | IL6R | Interleukin 6 receptor |
| Hs.532082 | NM_002184 | IL6ST | Interleukin 6 signal transducer (gp130, oncostatin M receptor) |
| Hs.2250 | NM_002309 | LIF | Leukemia inhibitory factor (cholinergic differentia-tion factor) |
| Hs.133421 | NM_002310 | LIFR | Leukemia inhibitory factor receptor alpha |

**Table 3.** Differential expression of mRNA cytokines and receptors pathway-focused genes in patients with different thyroid pathology

| GENE SYMBOL | UP-DOWN REGULATION (COMPARING TO THE CONTROL GROUP) | | |
|---|---|---|---|
| | Patients with postoperative hypothyroidism (Group 1) | Patients with hypothyroidism as a result of AIT (Group 2) | Patients with AIT and rising serum anti-Tg and anti-TPO autoantibodies (Group 3) |
| | Fold change | Fold change | Fold change |
| CX3CR1 | -5.04 (p=0.003) | -16.1 (p=0.0009) | 4.36 (p=0.09) |
| CXCR4 | -3.5 (p=0.001) | -24.1 (p=0.001) | 10.9 (p=0.04) |
| IL10 | 9.6 (p=0.01) | -27.03 (p=0.000001) | -28.6 (p=0.00004) |
| IL10RA | 4.3 (p=0.04) | 16.5 (p=0.04) | -12.7 (p=0.0003) |
| IL1β | -12.5 (p=0.001) | 3.4 (p=0.006) | 10.4 (p=0.04) |
| IL1R1 | -11.1 (p=0.009) | 3.3 (p=0.01) | 6.2 (p=0.04) |
| IL6 | -2.8 (p=0.01) | -27.4 (p=0.001) | 3.5 (p=0.03) |
| IL6R | -4.6 (p=0.005) | -9.4 (p=0.0006) | 3.1 (p=0.05) |
| IL6ST | 2.7 (p=0.01) | 5.3 (p=0.06) | -6.6 (p=0.003) |
| LIF | -1.1 (p=0.37) | -1.17 (p=0.15) | -1.14 (p=0.15) |
| LIFR | -1.06 (p=0.76) | -1.1 (p=0.54) | -1.08 (p=0.45) |

The p values are calculated based on a Student's t-test of the replicate 2^(- Delta CT) values for each gene in the control group and study groups

## DISCUSSION

The cytokines and receptor pathway genes involved in this study can be assigned into 3 functional groups: 1) those with predominantly systemic pro-inflammatory effects (inflammation inducers and regulators of its intensity): IL1β, IL1R1, IL6, IL6R, IL6ST; 2) suppressors of pro-inflammatory signals (inflammation repressors): IL10, IL10RA, LIF, LIFR; and 3) those exerting multidirectional effects on the inflammatory process, depending on the type of cell and tissue in which their expression is studied: CX3CR1 and CXCR4 (Figure 2A, B,C).

For a number of autoimmune and inflammatory diseases it was demonstrated that a key prognostic indicator of their course was the extent of changes to the transcriptional activity in the immune system cells (12, 13). An important task is to find new drugs that can reduce the risk of neurological complications in patients with thyroid disease (14). An important role in AITDs is played by chemokines; for instance, studies showed overexpression of CC and CXC chemokine in both HT and experimental autoimmune thyroiditis (9).

CX3CR1 is a seven-transmembrane G-protein coupled receptor. It has a sole ligand, CX3CL1, also known as fractalkine or neurotactin (15). In the immune system cells, CX3CR1 helps to recognize the CX3CL1 gradient (chemotaxis), which attracts the cells to the inflamed tissue and elicits the innate immune response (16,17). In this study, the expression of CX3CR1 was reduced in the patients with hypothyroidism resulting from AIT as well as with postoperative hypothyroidism. On the other hand, CX3CR1 was significantly up-regulated in the group of patients with elevated serum autoantibodies.

CXCL12 is constitutively expressed by various cells and tissues. Interacting with CXC chemokine receptor 4, CXCL12 can elicit several responses, such as migration of inflammatory cells across the endothelium and leukocyte mobilization (18,19). The principal source of CXCL12 in the thyroid are thyrocytes; this is in contrast to its receptor

CXCR4, which is mainly expressed by T and B cells (18). Similarly, thyroid epithelial cells produce cytokines CCL2, CXCL9, and CXCL10. Various factors can up-regulate production of these cytokines in the thyrocytes. For instance, iodine-induced thyrocyte necrosis stimulates production of IL1 and TNFα by the resident macrophages. This prompts synthesis of CXCL12 by the nearby thyrocytes (20). In mice with autoimmune thyroid diseases, the expression of both CXCL12 and CXCR4 is elevated. In a study of HT patients Armengol et al. showed up-regulated CXCL12 mRNA expression and elevated protein levels in the thyroid glands compared to non-autoimmune thyroid glands; the predominant source of CXCL12 in AIT was thyrocytes (20). In this study we found that expression of CXCR4 was reduced in the patients with hypothyroidism resulting from AIT as well as postoperative hypothyroidism. In contrast, CXCR4 was up-regulated in the group of patients with elevated serum autoantibodies. These results suggest that a high level of serum autoantibodies, such as anti-Tg and anti-TPO, can be linked to up-regulation of CX3CR1 and CXCR4.

Thyroid cells synthesize several cytokines, including IL-1, IL-6, IL-8, IL-12, IL-13, and IL-15 (21). The IL-1 family consists of IL-1α, IL-1β, and two receptors, IL-1 receptor 1 (IL1R1) and IL-1 receptor 2 (IL1R2); these receptors are found to be associated with thyroid carcinogenesis (22). IL-1β is found to be produced in the thyroid gland of HT patients by infiltrating monocytes and macrophages, activated endothelium, fibroblasts, and thyrocytes. In normal thyrocytes, it can induce Fas expression, resulting in massive thyrocyte apoptosis and tissue destruction (23). In this study, IL-1β was significantly down-regulated in the patients with postoperative hypothyroidism. In contrast, IL-1β was up-regulated in the patients with elevated serum autoantibodies anti-Tg and anti-TPO and the patients with hypothyroidism resulting from AIT. This corresponds to the findings of higher IL-1β mRNA expression in the peripheral blood mononuclear cells (PBMCs) of HT patients compared to Graves' disease (GD) patients, while the normal controls had the lowest level of IL1β expression (25). Similar trend was also detected in the thyroid tissues of the same groups of patients and normal controls. These results suggest IL-1β involvement in pathogenesis of AITDs and its utility as a biological marker to distinguish HT from any other AITDs (24). We found that expression of IL1R1 increased in the patients with elevated serum anti-Tg and anti-TPO antibodies and the patients with hypothyroidism resulting from AIT. In contrast, IL1R1 was down-regulated in the group of patients with postoperative hypothyroidism.

IL-10 is a Treg cytokine, crucial for maintaining and controlling inflammation. This cytokine is also shown to increase antibody production, in particular of anti-thyroid peroxidase antibodies (TPOAbs) that play an important role in the development of HT (25). Moreover, the complex interactions within the cytokine network can switch it from producing typical anti-inflammatory responses to pro-inflammatory ones. This complex cytokine interaction, resulting in the addition of IL-10 pathogenic effect to B-cell autoantibody production, could be mediating the IL-10 role in HT susceptibility (26). An IL10 polymorphism IL10−592A/C (27) and let-7e, a miRNA that regulates sIL10 expression (28), are both associated with HD severity. We found that patients with postoperative hypothyroidism had significantly lower expression of IL10 compared to the control group. On the other hand, high levels of serum anti-Tg and anti-TPO antibodies were associated with increased expression of IL10.

IL10RA (Interleukin-10 receptor alpha) is a subunit of IL-10 receptor heterotetramer which belongs to the family of IFNR-like receptors (28, 29). In humans, IL-10 and its receptors together contribute to controlling intestinal mucosal immune responses. Genome-wide association studies linked nucleotide sequence polymorphisms in the IL10 gene to the elevated risk of developing inflammatory bowel disease (IBD), as reported in (30). In this study, decrease in IL10RA mRNA was found in the patients with elevated serum autoantibodies anti-Tg and anti-TPO, while in the patients with postoperative hypothyroidism and with hypothyroidism resulting from AIT, the expression of IL10RA increased.

A major mediator of the host response to injury and infection, IL-6 is a key interleukin contributing to inflammation, inflammation-associated cancers, and autoimmune processes (31). Graves' disease, an autoimmune thyroid disorder, is characterized by elevated IL-6 levels (32, 33). IL-6 is involved in regulation of thyroid cells growth and differentiation, while its expression in these cells regulates infiltration of the lymphocytes (32). In this study, we found reduced IL-6 expression in the patients with hypothyroidism resulting from AIT as well as with postoperative hypothyroidism. In contrast, IL-6 was significantly up-regulated in the group of patients with elevated serum autoantibodies. These results suggest that the high level of serum autoantibodies, including anti-Tg and anti-TPO, can up-regulate IL-6. Previous studies support the correlation between increased IL-6 levels and Hashimoto's thyroiditis (33, 34). For instance, El-Shenawy et al. found high IL-6 levels in different groups of individuals with HT (33). IL-6 has been proposed as the immune system mediator in the pathogenesis of HT disease, and the association between the levels of IL-6 and inflammation severity in HT points out to a direction for further studies (33, 34).

An IL6 receptor locus polymorphism (Asp358Ala) is correlated with changes in serum IL6R levels and to a lesser extent, with IL-6 levels. The presence of IL6R 358Ala allele increased the expression of soluble ILR isoform in functional studies, but reduced the levels of membrane-bound isoform in CD4 T cells and monocytes, reducing overall IL6R response (35). In our study, IL6R was down-regulated in the patients with hypothyroidism resulting from AIT and in patients with postoperative hypothyroidism. Conversely, in the group of patients with AIT and elevated serum autoantibodies, the expression of IL6R did not change. These results suggest that hypothyroidism results in suppressed expression of IL6R.

IL-6 transducer (IL6ST or gp130), is the signal-transducing subunit of the IL-6 cytokine family receptors activated by the IL-6/IL6Rα complex. IL6R is composed of two different proteins: IL6Rα, or ligand-binding subunit, and IL6ST/gp130, or signal-transducing subunit. IL6ST/gp130 is expressed in almost all organs. IL6Rα can be released as a soluble receptor (sIL6Rα); binding IL-6 it then interacts with a gp130 located on the surface of any cell. This trans-signaling process is likely the chief mechanism of IL-6 signaling in humans, because the sIL6R/IL-6 complex can exert an agonist effect. The soluble receptor form appears to be essential in the regulation of IL-6 action (36). Several studies linked increased sIL6R levels to different autoimmune diseases as well as their severity (36, 37). In this study, IL6ST expression increased in the patients with hypothyroidism resulting from AIT as well as with postoperative hypothyroidism. In contrast, IL6ST was significantly down-regulated in the group of patients with elevated serum anti-Tg and anti-TPO antibodies. These results suggest that a high level of serum autoantibodies will down-regulate the expression of IL6ST.

These results indicate that even in the patients which currently do not present clinical manifestations (those with AIT and elevated serum anti-Tg and anti-TPO autoantibodies), there is a significant transcriptional induction of pro-inflammatory cytokine genes and their receptors IL1β, IL1R1, IL6, IL6R against the background of suppressed expression of cytokine inhibitors of inflammation IL10 and IL10RA.

Leukemia inhibitory factor (LIF) belongs is a member of the IL-6 family with neuroprotective and anti-inflammatory properties (38). Pro-inflammatory cytokines IL-6 and tumor necrosis factor stimulate production of LIF. The beneficial role of LIF was demonstrated on both histological and functional models of different cell types and maturity. LIF receptor (LIFR) is activated after an injury, binding LIF, and is a type 1 cytokine receptor located in the nuclei of neuronal cells (39).

In this study, we did not detect changes in the transcriptional activity of LIF and LIFR genes in patients with primary hypothyroidism and AIT. However, while there are only a few studies on the association of HT with LIF and LIFR, some indications of TSH involvement in the mediation of LIF signaling exist. For instance, TSH up-regulated LIF expression in monkey thyroid tissue (40). A study of the LIF signaling pathway in the culture of endometrium tissue showed that in stromal cells TSH elevated LIF expression. Furthermore, TSH promotes the mRNA expression of LIFR (41). In an animal study, high levels of TSH affected the LIF/STAT3 pathway, resulting in poor fetus implantation outcomes (42).

## CONCLUSION

This study demonstrated that autoimmune thyroiditis and hypothyroidism can affect the mRNA-level expression of cytokines and cytokine receptor genes in a gene-specific manner and that these changes to genes expression can be among the triggers of autoimmune inflammation progression in the thyroid gland. Transcriptional activity of cytokines, inducer, and receptor genes in the peripheral white blood cells can be used as minimally invasive prognostic marker of autoimmune thyroid disease and its severity.

Data Sharing Statement. The data set generated and/or analyzed during this study are included in this submitted manuscript and is available from the corresponding author on reasonable request.

## CONSENT TO PUBLISH

All authors approved the submitted manuscript.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## DISCLOSURE

All authors have no conflicts of interest to declare.

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