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

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Received: 25.01.2021.
Accepted: 30.04.2021.

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UDK:
Eabr 2022; 23(4):299-308
DOI: 10.2478/sjecr-2021-0038



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.*

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

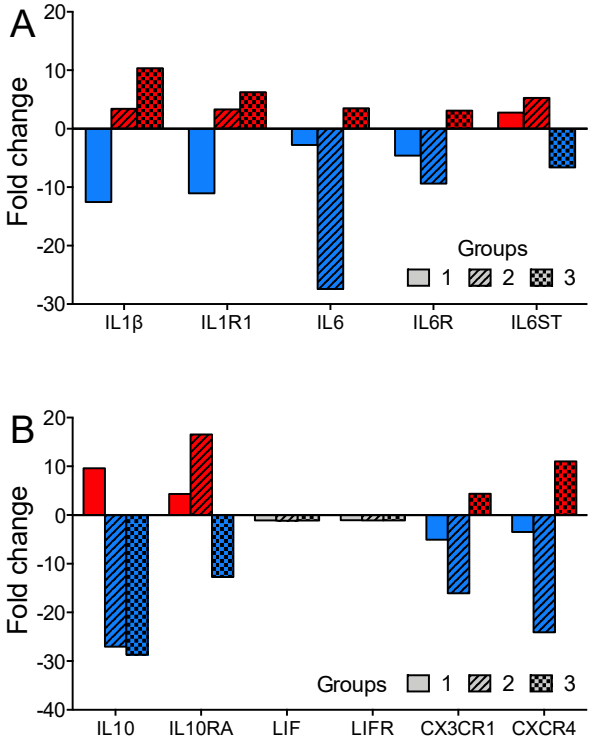
Using the Pathway-Focused PCR Array Profiling (Neurotrophins and Receptors RT2 Profiler PCR Array) we examined the Cytokines and receptor pathway-associated gene expression of patients with primary hypothyroidism as a result of AIT and postoperative hypothyroidism and patients with AIT with rising serum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

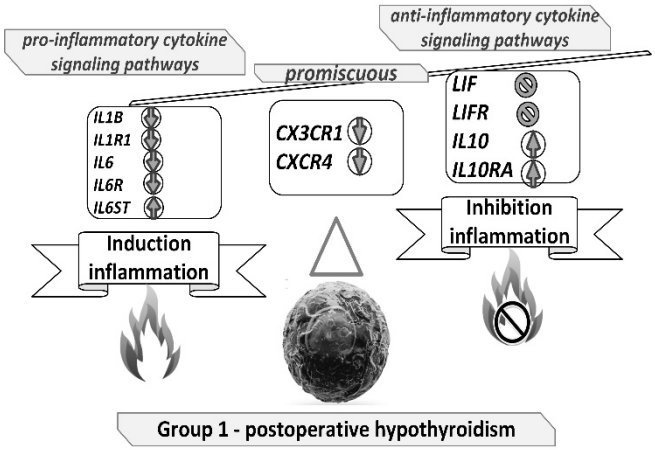


Figure 2B. Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (IL1β, IL1R1, IL6, IL6R, IL6ST); suppressors of pro-inflammatory signals (IL10, IL10RA, LIF, LIFR) and those with multidirectional effects on the inflammatory process (CX3CR1 and CXCR4) in patients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

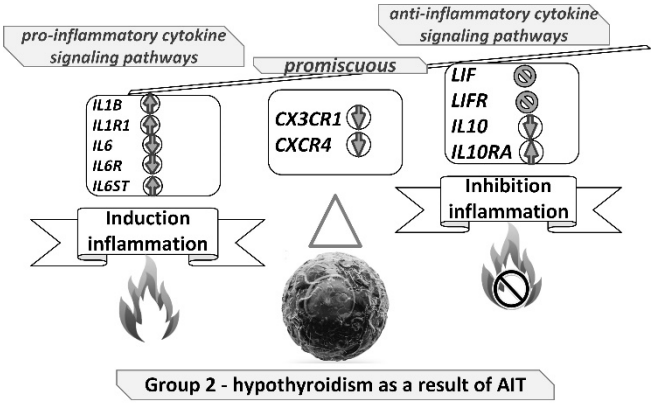
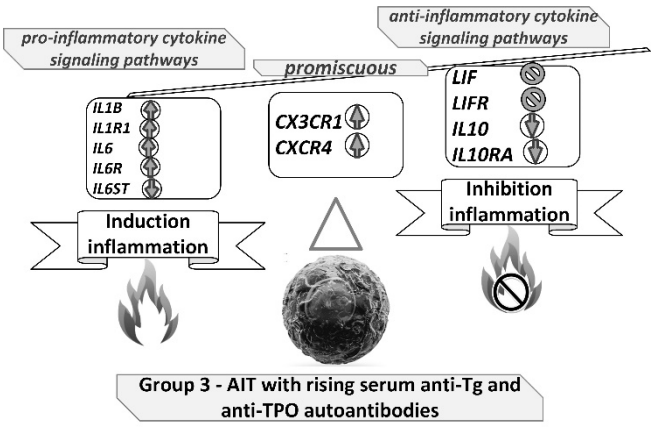


Figure 2C. Gene expression profiles for cytokines and receptors with systemic pro-inflammatory effects (IL1β, IL1R1, IL6, IL6R, IL6ST); suppressors of pro-inflammatory signals (IL10, IL10RA, LIF, LIFR) and those with
multidirectional effects on the inflammatory process (CX3CR1 and CXCR4) in patients 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 postoperative hypothyroidism (Group 1) (n=16)	Patients with hypothyroidism as a result of AIT (Group 2) (n=65)	Patients with AIT with rising serum anti-Tg and anti-TPO autoantibodies (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 differentiation 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 IL-6 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.

ACKNOWLEDGMENTS

The authors thank all the study participants.

CONFLICT OF INTERESTS

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrollment in the study. 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.

FUNDING

None.

LITERATURE

1. Ragusa F, Fallahi P, Elia G, Gonnella D, Paparo SR, Giusti C, et al. Hashimotos' thyroiditis:Epidemiology, pathogenesis, clinic and therapy. Best Pract Res Clin Endocrinol Metab. 2019 Dec;33(6):101367.
2. Sviridenko NY, Bessmertnaya EG, Belovalova IM, Mikheenkov AA, Sheremeta MS, Nikankina LV, Malysheva NM. Autoantibodies, immunoglobulins and cytokine profile in patients with graves' disease and Graves' orbitopathy. Probl Endokrinol (Mosk). 2020;66 (5):15-23
3. Degen A, Krynytska I, Kamyshnyi A. Changes in the transcriptional activity of the entero-insular axis genes in streptozotocin-induced diabetes and after the administration of TNF-α non-selective blockers. Endocrine Regulations. 2020; 54(3):160-171.
4. Kamyshna II, Pavlovych LB, Maslyanko VA, Kamyshnyi AM. Analysis of the transcriptional activity of genes of neuropeptides and their receptors in the

blood of patients with thyroid pathology. J Med Life. 2021;14(2): 243-249.
5. Bilous II, Korda MM, Krynytska IY, Kamyshnyi AM. Nerve impulse transmission pathway-focused genes expression analysis in patients with primary hypothyroidism and autoimmune thyroiditis. Endocr Regul. 2020;54(2):109-18.
6. Bilous I., Pavlovych L., Krynytska I., Marushchak M., Kamyshnyi A. Apoptosis and Cell Cycle Pathway-Focused Genes Expression Analysis in Patients with Different Forms of Thyroid Pathology. Open Access Macedonian Journal of Medical Sciences. 2020;8(B): 1-9.
7. Bilous I., Pavlovych L, Kamyshnyi A. Primary hypothyroidism and autoimmune thyroiditis alter the transcriptional activity of genes regulating neurogenesis in the blood of patients. Endocr Regul. 2021 Jan; 55(1):101–111.
8. Cheng CW, Wu CZ, Tang KT, Fang WF, Lin JD. Simultaneous measurement of twenty-nine circulating cytokines and growth factors in female patients with overt autoimmune thyroid diseases. Autoimmunity. 2020;53(5):261-269.
9. Fallahi P, Ferrari SM, Ragusa F, Ruffilli I, Elia G, Paparo SR, Antonelli A. Th1 Chemokines in Autoimmune Endocrine Disorders. J Clin Endocrinol Metab. 2020;105(4):dgz289.
10. Martin TC, Ilieva KM, Visconti A, Beaumont M, Kiddle SJ, Dobson RJB, et al. Dysregulated Antibody, Natural Killer Cell and Immune Mediator Profiles in Autoimmune Thyroid Diseases. Cells. 2020;9(3):665.
11. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults:cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012;18(6):988-1028.
12. Koval HD, Chopyak VV, Kamyshnyi OM, Kurpisz MK. Transcription regulatory factor expression in T-helper cell differentiation pathway in eutopic endometrial tissue samples of women with endometriosis associated with infertility. Cent Eur J Immunol. 2018;43(1):90-6.
13. Zherebiatiev A. Kamyshnyi A. Expression levels of proinflammatory cytokines and NLRP3 inflammasome in an experimental model of oxazolone-induced colitis. Iranian Journal of Allergy, Asthma and Immunology. 2016;15(1):39-45.
14. Nosulenko IS, Voskoboynik OY, Berest GG, Safronyuk SL, Kovalenko SI, Kamyshnyi OM, Polishchuk NM, Sinyak RS, Katsev AV. Synthesis and Antimicrobial Activity of 6-Thioxo-6,7-dihydro-2H-[1,2,4]triazino[2, 3-c]-quinazolin-2-one Derivatives. Sci Pharm. 2014;82 (3):483-500.
15. Wu W, Ren F, Guo M, Yang J, Xiao Y, Liu W. Increased expression of CX3CL1 and CX3CR1 in papillary thyroid carcinoma. Histol Histopathol. 2020;35(10): 1189-1196.
16. Lee M, Lee Y, Song J, Lee J, Chang SY. Tissue-specific Role of CX3CR1 Expressing Immune Cells and Their Relationships with Human Disease. Immune Netw. 2018 Feb;18(1):e5.
17. Hamon P, Loyher PL, Baudesson de Chanville C, Licata F, Combadiere C, Boissonnas A. CX3CR1-dependent endothelial margination modulates Ly6C(high) monocyte systemic deployment upon inflammation in mice. Blood. 2017 Mar 9;129(10):1296-307.
18. Mousavi A. CXCL12/CXCR4 signal transduction in diseases and its molecular approaches in targeted-therapy. Immunol Lett. 2020;217:91-115.
19. García-Cuesta EM, Santiago CA, Vallejo-Díaz J, Juarranz Y, Rodríguez-Frade JM, Mellado M. The Role of the CXCL12/CXCR4/ACKR3 Axis in Autoimmune Diseases. Front Endocrinol (Lausanne). 2019 Aug 27;10:585.
20. Armengol MP, Cardoso-Schmidt CB, Fernandez M, Ferrer X, Pujol-Borrell R, Juan M. Chemokines determine local lymphoneogenesis and a reduction of circulating CXCR4+ T and CCR7 B and T lymphocytes in thyroid autoimmune diseases. J Immunol. 2003 Jun 15;170(12):6320-8.
21. Luty J, Ruckemann-Dziurdzińska K, Witkowski JM, Bryl E. Immunological aspects of autoimmune thyroid disease - Complex interplay between cells and cytokines. Cytokine. 2019;116:128-133.
22. Xiong Z, Sun Y, Wu J, Niu F, Jin T, Li B. Genetic polymorphisms in IL1R1 and IL1R2 are associated with susceptibility to thyroid cancer in the Chinese Han population. J Gene Med. 2019;21(6):e3093.
23. Yan H, Hong Y, Cai Y. Association between FAS gene -670 A/G and -1377 G/A polymorphisms and the risk of autoimmune diseases:a meta-analysis. Biosci Rep. 2020;40(1):BSR20191197.
24. Sun L, Zhang X, Dai F, Shen J, Ren C, Zuo C, et al. Elevated interleukin-1beta in peripheral blood mononuclear cells contributes to the pathogenesis of autoimmune thyroid diseases, especially of Hashimoto thyroiditis. Endocr Res. 2016;41(3):185-92.
25. Kristensen B, Hegedus L, Lundy SK, Brimnes MK, Smith TJ, Nielsen CH. Characterization of Regulatory B Cells in Graves' Disease and Hashimoto's Thyroiditis. PLoS One. 2015;10(5):e0127949.
26. Gerenova J, Stanilova S. IL-12B and IL-10 gene polymorphisms in the development of Hashimoto's thyroiditis. Int J Immunogenet. 2016;43(6):397-403.
27. Inoue N, Watanabe M, Wada M, Morita M, Hidaka Y, Iwatani Y. IL-10 -592A/C polymorphism is associated with severity of Hashimoto's disease. Cytokine. 2013;64(1):370-4.
28. Kagawa T, Watanabe M, Inoue N, Otsu H, Saeki M, Katsumata Y, et al. Increases of microRNA let-7e in peripheral blood mononuclear cells in Hashimoto's disease. Endocr J. 2016 Apr 25;63(4):375-80.
29. Stożek K, Grubczak K, Marolda V, Eljaszewicz A, Moniuszko M, Bossowski A. Lower proportion of $CD19^{+}IL\text{-}10^{+}$ and $CD19^{+}CD24^{+}CD27^{+}$ but not $CD1d^{+}CD5^{+}CD19^{+}CD24^{+}CD27^{+}$ $IL\text{-}10^{+}$ B cells in children with autoimmune thyroid diseases. Autoimmunity. 2020;53(1):46-55.

30. Huang H, Fang M, Jostins L, Umicevic Mirkov M, Boucher G, Anderson CA, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature. 2017;547(7662):173-8.
31. Zhang GQ, Jiao Q, Shen CT, Song HJ, Zhang HZ, Qiu ZL, Luo QY. Interleukin 6 regulates the expression of programmed cell death ligand 1 in thyroid cancer. Cancer Sci. 2021;112(3):997-1010.
32. Kaur S, Bansal Y, Kumar R, Bansal G. A panoramic review of IL-6:Structure, pathophysiological roles and inhibitors. Bioorg Med Chem. 2020;28(5):115327.
33. Kristensen B. Regulatory B and T cell responses in patients with autoimmune thyroid disease and healthy controls. Dan Med J. 2016 Feb;63(2):B5177.
34. Sieminska L, Wojciechowska C, Kos-Kudla B, Marek B, Kajdaniuk D, Nowak M, et al. Serum concentrations of leptin, adiponectin, and interleukin-6 in postmenopausal women with Hashimoto's thyroiditis. Endokrynol Pol. 2010;61(1):112-6.
35. Ferreira RC, Freitag DF, Cutler AJ, Howson JM, Rainbow DB, Smyth DJ, et al. Functional IL6R 358Ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. PLoS Genet. 2013;9(4):e1003444.
36. Ferrari SM, Fallahi P, Elia G, Ragusa F, Camastra S, Paparo SR, Giusti C, Gonnella D, Ruffilli I, Shoenfeld Y, Antonelli A. Novel therapies for thyroid autoimmune diseases:An update. Best Pract Res Clin Endocrinol Metab. 2020;34(1):101366.
37. Marinou I, Healy J, Mewar D, Moore DJ, Dickson MC, Binks MH, et al. Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. Arthritis Rheum. 2007;56(8):2549-56.
38. Davis SM, Collier LA, Leonardo CC, Seifert HA, Ajmo CT, Jr., Pennypacker KR. Leukemia Inhibitory Factor Protects Neurons from Ischemic Damage via Upregulation of Superoxide Dismutase 3. Mol Neurobiol. 2017;54(1):608-22.
39. Davis SM, Collier LA, Goodwin S, Lukins DE, Powell DK, Pennypacker KR. Efficacy of leukemia inhibitory factor as a therapeutic for permanent large vessel stroke differs among aged male and female rats. Brain Res. 2019;1707:62-73.
40. Ren SG, Seliktar J, Li X, Hershman JM, Braunstein GD, Melmed S. In vivo and in vitro regulation of thyroid leukemia inhibitory factor (LIF):marker of hypothyroidism. J Clin Endocrinol Metab. 1999;84(8):2883-7.
41. Aghajanova L, Stavreus-Evers A, Lindeberg M, Landgren BM, Sparre LS, Hovatta O. Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology. Fertil Steril. 2011;95(1):230-7.
42. Shan L, Zhou Y, Peng S, Wang X, Shan Z, Teng W. Implantation failure in rats with subclinical hypothyroidism is associated with LIF/STAT3 signaling. Endocr Connect. 2019;8(6):718-27.