Original Article

The IL-4 Level among Patients with Autoimmune Thyroiditis in Al-Najaf Governorate, Iraq

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Abstract

Background: The study of autoimmune thyroiditis is considered one of the challenges of sustainable development. Autoimmune thyroiditis includes largely cellular and humoral immune responses targeted at the thyroid gland, including Graves thyroiditis and Hashimoto's thyroiditis. **Objectives:** This study aimed to find out the relationship between autoimmune thyroiditis and interleukin-4 (IL-4) levels in infected patients and compare them with healthy individuals by using the enzyme-linked immunosorbent assay (ELISA) technique. **Materials and Methods:** Sixty-two samples were collected from patients with autoimmune thyroiditis in AL-Najaf Governorate and 30 samples from healthy individuals as a control group during the period from March to September 2022. Blood was collected in a gel tube to isolate the serum and measure the IL-4 level using the ELISA technique. **Results:** This study included 46/62 women and 14/62 men with autoimmune thyroiditis, aged between 20 and 65 years. The results showed that the 50 to 59 age group was the most affected. Autoimmune thyroiditis (Graves or Hashimoto's) had a significant effect $P < 0.01$ on the level of IL-4. The type of injury had a significant effect on the level of IL-4, as our results showed that people with Graves disease were more affected by the disease compared to those with Hashimoto's disease, through compared with the control group. **Conclusion:** There is a high level of IL-4 in patients with autoimmune thyroiditis, which means that IL-4 has an important role in the development of the disease in patients with autoimmune thyroiditis when compared to healthy individuals.

Keywords: Autoimmune thyroiditis, ELISA, Graves, Hashimoto, IL-4

Introduction

Autoimmune thyroiditis is the most common organ-specific autoimmune diseases, which affect 5% of the population with significant gender differences (i.e., women 5%–15% and men 5%).[1] Both Graves disease (GD) and Hashimoto thyroiditis (HT) are part of autoimmune thyroiditis. The primary causes of hypothyroidism and hyperthyroidism, respectively, are HT and GD. Both GD and HT share the presence of a cellular and humoral immune responding to the thyroid gland antigens, the reactive infiltration of T and B cells, the formation of autoantibodies, and the subsequent emergence of clinical signs, which reflect the loss of immunological tolerance.[2] The thyroid gland's functionality is changed by lymphocytic infiltration, which also damages surrounding tissue. The thyroid cells are damaged when autoantibodies or sensitized T-lymphocytes interact with them, triggering an inflammatory response and, occasionally, cell lysis.[3]

GD, named for Robert Graves who was the first to identify the connection in 1835, is clinically distinguished by the presence of hyperthyroidism, widespread goiter ophthalmopathy, and dermopathy.[4] GD accounts for up to 80% of instances of hyperthyroidism, making it the most prevalent cause. Ten times more women than men are impacted by it. There has been evidence of a high frequency between the ages of 40 and 60.[5] A higher risk of GD and a younger onset age are linked to a family history

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_250_23

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Submission: 03-Mar-2023 **Accepted:** 23-Apr-2025 **Published:** 28-Jun-2025

How to cite this article: Al-Huchaimi SHK, Alammar MH, Al-Fatlawi SN. The IL-4 level among patients with autoimmune thyroiditis in Al-Najaf Governorate, Iraq. Med J Babylon 2025;22:349-52.

of thyroid illness, particularly in maternal relatives.[6] The presence of circulating thyroid stimulating hormone (TSH) receptor Ab [thyrotropin receptor antibody (TRAb)] causes GD by binding and activating the TSH receptor, causing follicular enlargement and hyperplasia, as well as an increase in thyroid hormone production and the T3 relative proportion to T4 in the blood.[7] In GD, an environmental factor either through bystander activation or molecular mimicry causes an autoimmune reaction to the thyroid. Cytokines are produced, which then activate thyroid-specific T and B lymphocytes to generate TRAb.[8]

HT is the most related cause of hypothyroidism in the region where iodine is abundant. Hakaru Hashimoto was the first to identify it in 1912, when he personified that four ladies have a syndrome, he named Struma Lymphomatosa. The hallmark of HT is a progressive loss of thyroid function, goiter, or both as a result of the thyroid gland being destroyed by the immune system through thyrocyte apoptosis. The HT pathophysiology is thought to be explained by many factors. The first of these is molecular mimicry. It is believed that HT is brought on by an immunological response against an antigen that resembles endogenous proteins structurally.[9] Another option is bystander activation. Virus when enters the thyroid gland activates nonspecific lymphocytes there; then, it may release cytokines that in turn activate thyroid-specific T cells and stimulate an inflammatory response (thyroiditis). Antigen-presenting cells and Th1 cells produce cytokines that cause the development of Fas to attach with the Fas ligand on the thyroid cell, which leads to self-apoptosis in autoimmune thyroiditis.[10]

Significant contributions from cytokines are made to inflammation and immunity. In 1980, interleukin (IL)-4 was first identified. Eosinophils, basophils, mast cells, and Th2 lymphocytes all generate this cytokine. In numerous cells, such as macrophages, lymphocytes, endothelial cells, epithelial cells, and fibroblasts, IL-4 helps to regulate cell division, gene expression, and prevent apoptosis. Along with other cytokines, such as IL-15, IL-13, and IL-10, IL-4 helps CD4+ lymphocytes differentiate and become T helper 2 cells. Also, it inhibits T helper 1 and lowers IFN-production by Th1 cells. The synthesis of IgE and IgG4 in B cells as well as the regulation of immunoglobulin class alterations are two additional functions of IL-4.[11] The aim of this study is to identify the relationship between autoimmune thyroiditis and IL-4 levels in AL-Najaf patients.

Materials and Methods

Samples collection

A total of 62 samples were collected from patients with autoimmune thyroiditis of the patients in Najaf Governorate and 30 samples from healthy individuals as a control during the period from March to September 2022. The samples were collected by drawing 3 mL of blood and placing it in a gel tube. The serum was isolated and kept at a temperature of −20°C for the purpose of measuring the IL-4 level using the enzyme-linked immunosorbent assay (ELISA) technique (Sunlong Biotech Co., China).

Patients

A standardized questionnaire was developed after a study of the literature on HT and Graves thyroiditis (GT) and had its validity examined by three experts, the socio-demographic data. The data collection was carried out through face-to-face meeting between the researcher and the subjects. The expert doctors conducted a thorough medical history and diagnosis before referring the patient to the laboratory for testing. The control group (No. 30) consisted of people who appeared to be in good health, had no signs of chronic illness, were thyroidally normal based on thyroid function testing, and had no antithyroidal antibodies at the time of sample collection.

Cytokines detection

Serum levels of IL-4 were quantitatively determined in patients and control subjects by means of a sandwich ELISA test using commercially available kits (SunLong Biotech Co., LTD). Catalog number: (SL0997Hu).

Statistical analysis

Statistical analyses are carried out with the aid of the SPSS program (version 22, IBM Corp., Armonk, NY, USA). As descriptive statistics, the SD mean (minimum–maximum) for metric variables and the percentage for categorical variables were also used. Statistical significance was identified as a P value < 0.05.

Inclusion criteria

1. Patients newly diagnosed with HT or GT by physicians were included in this study, according to the criteria of the Najaf Center for Diabetes and Endocrinology, and were adults of both genders.
2. Presence of clinical features of HT or GT plus confirmatory thyroid function tests in addition to serum autoantibodies against thyroid antigens (thyroid anti-TPO, anti-Tg, and TRAB).
3. Thyroid sonogram.

Ethical approval

Samples were collected according to procedural methods, including human participants, in accordance with the ethical standards set by the Ministry of Health in Iraq. Verbal and written consent were obtained regarding the study that aims to find the causes of the disease.

Results

This study included 62 pathological samples from people with autoimmune thyroiditis, distributed into

two groups: the first group, GD (32 samples), and the second group, HT (30 samples), as shown in Table 1. Ages ranged from 20 to 69 years, distributed into age groups, as shown in Table 2. The majority of the studied sample was women (48/62), compared to men (14/62), as shown in Table 3.

This study proved that the incidence of autoimmune thyroiditis had a significant effect ($P < 0.01$) on the IL-4 levels in patients, compared to the control group, while the patient's gender or age had no significant effect on the level of IL-4 in patients, as shown in Table 4. It was found that the level of IL-4 was elevated in all types of autoimmune thyroiditis, except for Graves or Hashimoto's, where the equal value was $P < 0.01$.

The type of injury also had a significant effect on the IL-4 level, as our results showed that people with GD were more affected by the disease compared to those with Hashimoto's disease, though compared with the control group [Tables 5–7].

Table 1: Distribution of study samples based on type of disease

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Graves	32	34.4	51.6	51.6
	Hashimoto	30	32.3	48.4	100.0
	Total	62	66.7	100.0	

Table 2: Distribution of study samples depended on the age of the patients

		Frequency	Percent	Valid percent	Cumulative percent
Valid	20–29	4	4.3	6.5	6.5
	40–49	19	20.4	30.6	37.1
	50–59	29	31.2	46.8	83.9
	60–69	10	10.8	16.1	100.0
	Total	62	66.7	100.0	

Table 3: Distribution of study samples depended on the sex of the patient

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Male	14	15.1	22.6	22.6
	Female	48	51.6	77.4	100.0
	Total	62	66.7	100.0	

Discussion

Glycoproteins called cytokines serve as adjuvant immune system regulators. One of the most significant cytokines in the body, IL-4, has a function in both inflammatory and immunological responses. It is released by white blood cells.[12]

A total of 62 blood samples from autoimmune thyroiditis patients were taken for this investigation, and the results showed that females were more likely than males to have the disease. Our findings indicated that individuals aged between 50 and 59 were more likely to have autoimmune thyroiditis, and these findings are in line with the study. In general, autoimmune thyroiditis patients had significantly higher levels of IL-4 than healthy individuals, which was consistent with the findings published by Lichtiger *et al*.[13] Our study found that patients with GD had higher IL-4 levels than patients with Hashimoto's disease, and both groups were compared to the healthy group. This result is similar to that reported by Al-Hammami *et al*.,[14] who found that IL-4 levels were higher in Graves than in Hashimoto. The most prevalent autoimmune cause of hyperthyroidism is GD, which is ultimately connected to the production of IgG antibodies that stimulate the thyrotropin receptor. The tolerance mechanisms, in both systemically (peripheral blood) and locally (tissues), are most likely what led to GD.[15] These include T regulatory cell activity failure, autoreactive T and B cell proliferation, and improved TSH-R presentation. The

Table 4: The correlation between sex, type of disease, level of IL-4, and age of patient

			Sex of patient	Type of disease	Level of IL-4	Age of patient
Spearman's rho	Sex of patient	Correlation coefficient	1.000	0.060	−0.130	−0.026
		Sig. (1-tailed)		0.322	0.158	0.421
		N	62	62	62	62
	Type of disease	Correlation coefficient	0.060	1.000	−0.361**	0.000
		Sig. (1-tailed)	0.322	–	0.002	0.500
		N	62	62	62	62
	Level of IL-4	Correlation coefficient	−0.130	−0.361**	1.000	−0.170
		Sig. (1-tailed)	0.158	0.002	–	0.093
		N	62	62	93	62
	Age of patient	Correlation coefficient	−0.026	0.000	−0.170	1.000
		Sig. (1-tailed)	0.421	0.500	0.093	–
		N	62	62	62	62

**Correlation is significant at the 0.01 level (1-tailed)

Table 5: The comparison of some statistical parameters of IL-4 level according to the type of autoimmune thyroiditis

	Type of group	*N*	Mean	Standard deviation	Standard error mean
Level of IL-4	Graves	32	17.2625	6.87097	1.21463
	Hashimoto	30	15.0293	11.13954	2.03379

Table 6: The comparison of some statistical parameters of IL-4 level in patients with Graves

	Type of group	*N*	Mean	Standard deviation	Standard error mean
level of IL-4	Graves	32	17.2625	6.87097	1.21463
	Control	30	3.1733	1.50949	0.27559

Table 7: Comparison of some statistical parameters of IL-4 level in patients with Hashimoto

	Type of group	*N*	Mean	Standard deviation	Standard error mean
Level of IL-4	Hashimoto	30	15.0293	11.13954	2.03379
	Control	30	3.1733	1.50949	0.27559

skew in Th1-Th2 cytokines may be explained by the fact that TRAb is an IgG antibody. The final stage is when TRAb continues to activate the TSH receptor that is found on thyroid follicular cells. IgG4 antibodies are required for extended immunization, that is, stimulated by Th2 cytokines (such as IL-4).[16,17] Hyperthyroidism is brought on by this continuously dysregulated thyroid stimulation, which also raises IL-4 levels. To show the prevalence of IgG4-related thyroiditis, Kawashima tested serum total IgG levels in HT patients. The titers of anti-TPO and anti-TG antibodies are strongly associated with the levels of IgG4 and IgG. This finding suggests that IgG4-related thyroiditis may at least a small proportion of HT patients with high anti-thyroid antibody titers. It is well recognized that excessively activated T-lymphocytes CD4+ are the primary factor in the development of HT.[18,19] T cells contribute to the pathophysiology of HT in two ways. T helper type 2 Th2 cells cause thyroiditis by overstimulating the development of plasmatic and B cells, which in turn create antibodies against thyroid antigens. IL-4 is produced by T helper 1 and IFN-gamma produced by T helper 2 cells, respectively.[20] These studies explain the reasons why samples from people with Graves and Hashimoto's diseases have high amounts of IL-4.

Conclusion

There is a high level of IL-4 in patients with autoimmune thyroiditis, which means that IL-4 affects the development of the disease in patients with autoimmune thyroiditis when compared to healthy people.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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