Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Evaluation of Functional and Immunological Biomarkers in Iraqi Patients of Hashimoto Disease

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Received: 10/03/2026
Accepted: 01/06/2026
Published: 30/06/2026

Keywords: Thyroid gland, hypothyroidism, iterleukin-2, Hashimoto disease,

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DOI:10.62472/kjps.v17.i28.587-596

Abstract

Background: -Hashimoto disease, a type of autoimmune thyroid illness, it is featured by chronic lymphocytic inflammation of the thyroid, symptoms include an increase of in thyroid stimulating hormone with low level of free thyroxine and long term of hypothyroidism due to autoantibodies targeting thyroglobulin and thyroid peroxidase. Interleukin 2 is a chemical signal protein used by immune system, this protein plays a significant role in immunological tolerance and have particular application in cancer therapy and primarily instruct immune cells how to grow and behave.

Patients and methods: This case-control investigation consisted of 60 women with Hashimoto disease as case group and 60 healthy individuals as control group. IL-2 measure by using ELISA technic method and anti TPO and thyroid function test (TSH, FT4) measured by ECLIA technic. from serum blood samples.

Results: final results observe that measurement of IL -2 in the patient group was massively higher than controls (p= 0.0001)., and anti-thyroid antibodies (anti TPO) in the Case group was massively higher than controls also elevation of TSH case group more than the control group and clear decrease in FT4 in patient group in comparison to controls, BMI, Age and sex non-significant to link with Hashimoto disease

Conclusion: over all, our results observe that the BMI, sex, Age no clear role on this disease but on the other hand IL- 2 level and anti-thyroid antibodies have an important role in Hashimoto disease.

تقييم المؤشرات الحياتية الوظيفية والمناعية في المرضى العراقيين المصابين بمرض قصور الغدة الدرقية (هاشيموتو)

علي فيصل نصر، تغريد فاضل عباس، حنان ديكان

الخلاصة

المقدمة: مرض هاشيموتو نوع من أمراض الغدة الدرقية المناعية الذاتية، يتميز بالتهاب لمفاوي مزمن في الغدة الدرقية، تشمل الأعراض زيادة في الهرمون المنبه للغدة الدرقية مع مستوى منخفض من الثيروكسين الحر وقصور الغدة الدرقية طويل الأمد بسبب الأجسام المضادة الذاتية التي تستهدف الغلوبولين الدرقي وبيروكسيديز الغدة الدرقية. الإنترلوكين 2 هو بروتين إشارة كيميائي يستخدمه جهاز المناعة، يلعب هذا البروتين دوراً مهماً في التحمل المناعي وله تطبيق خاص في علاج السرطان ويوجه الخلايا المناعية بشكل أساسي حول كيفية النمو والتصرف".

المرضى وطرق العمل: هذه الدراسة (مريض – سليم) تتكون من 60 مريض مصاب بمرض هاشيموتو كمجموعة مرضى و60 فرداً سليماً كمجموعة ضابطة. تم قياس الإنترلوكين 2 باستخدام طريقة تقنية الإليزا وتم قياس مضادات بيروكسيديز الغدة الدرقية (anti TPO) واختبار وظيفة الغدة الدرقية (TSH, FT4) بواسطة تقنية إكليا (ECLIA) من عينات مصل الدم".

النتائج: تلاحظ النتائج النهائية أن قياس الإنترلوكين 2 (IL-2) في مجموعة المرضى كان أعلى بشكل كبير من المجموعة الضابطة($p<0.0001$) ، وأن الأجسام المضادة للغدة الدرقية مضادات (TPO) في مجموعة المرضى كانت أعلى بشكل هائل من المجموعة الضابطة، وكذلك ارتفاع الهرمون المنبه للغدة الدرقية (TSH) في مجموعة المرضى أكثر من المجموعة الضابطة، وانخفاض واضح في الثيروكسين الحر (FT4) في مجموعة المرضى بالمقارنة مع المجموعة الضابطة، كما أن مؤشر كتلة الجسم(BMI) ، والعمر، والجنس غير ذات دلالة للارتباط بمرض هاشيموتو".

الاستنتاج:" بشكل عام، تلاحظ نتائجنا أن مؤشر كتلة الجسم، والجنس، والعمر ليس لها دور واضح في هذا المرض، ولكن من ناحية أخرى، فإن مستوى الإنترلوكين 2 (IL-2) والأجسام المضادة للغدة الدرقية لها دور مهم في مرض هاشيموتو".

1. Introduction

Hashimoto thyroiditis (HT), It is an expression derived by Haraku Hashimoto's 1912 description, referring to oversized thyroid gland deposited with lymphocytes. The prevalence of the disease has been detected at 0.3–1.5 instances per thousand individuals, with a male-female ratio of 7–10:1. It is chronic autoimmune thyroiditis is a form of autoimmune disease which leads to inflammation than death of thyroid follicular cells through immune-mediated mechanisms. This form of autoimmune disease is a major etiology of hypothyroidism in developing countries (Klubo-Gwiezdzinska and Wartofsky, 2022). Hashimoto's disease is often the result of an interplay of hereditary predisposition and risk factors from the environment; this can result in plasmocytic as well as lymphocytic infiltration(Kostoglou-Athanassiou, Athanassiou and Athanassiou, 2022). As a consequence, it has been characterized by the high conc. of autoantibodies towards thyroid peroxidase (anti TPO) and to thyroglobulin (anti TG), which induce thyroid dysfunction (Luo *et al.*, 2025). Ninety percent 90% of patients with thyroiditis caused by Hashimoto's disease show circulatory anti TPO compared to anti TG demonstrated in 60–80% of cases (Majid *et al.*, 2024), Diagnostic by elevation autoantibodies (anti TPO and anti TG) and thyroid stimulating hormone (TSH) while decrease thyroid hormone (Thyroxine (T4), triiodothyronine T3, Free thyroxine (FT4), and Free triiodothyronine (FT3). (Kaur and Jialal, 2026). Interleukin 2, It is a crucial cytokine for T cell peripheral tolerance and immune response. current paper examines the role of IL-2 interacting with the highly affinity corresponding receptor (IL -2R) in promoting growth and proliferation of T regulatory cells, as well as its contribution to the proliferation of helper, cytotoxic, and memory T cells (Shouse, LaPorte and Malek, 2024). Human cytokine producing and stimulation is regulated by genetic factor. Thus, polymorphisms may affect the rate at which different cytokines are produced. In this study, the aim is to evaluate the effect functional and immunological markers (TSH, FT4) and TPO Ab, IL 2 respectively in Iraqi Hashimoto patients.

2. Materials, Patients and Method

2.1.Patients' Constant and Enrollment

sixty patients suffer autoimmune hypothyroidism represent the case group in this case-control study, and sixty healthy persons as group of control. patients aged 11 to 69 who had autoimmune hypothyroidism made up the case group. survey form was used to collect data from the two groups health history and their style of live. All of the participants elected for this study were live in Karbala. for ethical purposes, all contributors were told about the study for their approval.

2.2.Inclusion Criteria patients aged 11to 69 years diagnosed with Hashimoto disease,

2.3.Exclusion Criteria: Patients with a treatment duration of less than three months, Patients under 70 years old, Patients suffer from other autoimmune diseases or cancer and Patients who had undergone thyroidectomy.

2.4.Blood Collection

5 ml of blood was drawn from each patient via vein puncture. The samples were collected in gel tube for serum extraction.

2.5.Hormonal Detection Assay

Put the sample at room normal temperature for clotting after it has been collected. Usually, this spends fifteen min. then Centrifuge at 3,000-4000 rpm to 15 minutes to obtain clear serum and separate the clot. Use the Bio Tek 800 TS ELISA reader and the BioTek 50 TS ELISA washer. and BT lab, Human Interleukin 2 (IL-2) ELISA Kit, diluent standard (150 µl) are added to dilute the standard to 180 pg /ml, 120 pg /ml, 60 pg /ml, 30 pg/ ml, and 15 pg/ml. ELISA- Sandwich is the technique used with this ELISA kit Using spectrophotometer, the optical density is determined at a wavelength of 450nm ,and detection of TPO Ab and TSH,FT4 by ECLIA method in COBAS e411 instrument.

2.6. Statistical Analysis

The statistical package for the social sciences (SPSS) software (version 21) was used to do the statistical analysis of the collected data. The corelation between the examined IL-2 and Hashimoto risk was examined using logistic regression. The threshold for statistical significance was set at $p<0.05$.

3. Results

3.1.Assessment of AGE, SEX, BMI in Patients and Control

The mean of the ages of cases as in Table 1 was 35.140±9.856 in compression to the mean age of control (37.360±8.175, Value =0.33491) There was no significant statistical variations in patients and control group, also the sex has P value 0.46738 and also non-significant statistically, Table1.

The mean of BMI of control is close to mean of case (27.562±3.087 vs 28.276±3.624) there was no significant difference in BMI between the groups (P. value =0.40219). Table2, Fig.1.

Table1: Distribution of Ages Characteristic of Patients and Control According To The Study Subjects

Parameter	Characteristic	Control	Patient	P value
Age	**Mean ±SD**	37.360±8.175	35.140±9.856	0.33491
	Ranges	19 -50 Year	16 - 57 Years	† NS
SD: standard deviation; †: Independent T test; HS: Highly significant at P ≤ 0.001; NS: not significant at P > 0.05.				

Table2: The Compared Between the Study Groups Regarding BMI Levels.

Parameter	Characteristic	Control	Patients	P value
Body mass index (BMI)	Mean ±SD	27.562±3.087	28.276±3.624	0.40219
	Range	22.863 - 36.391	20.281 - 35.003	† NS

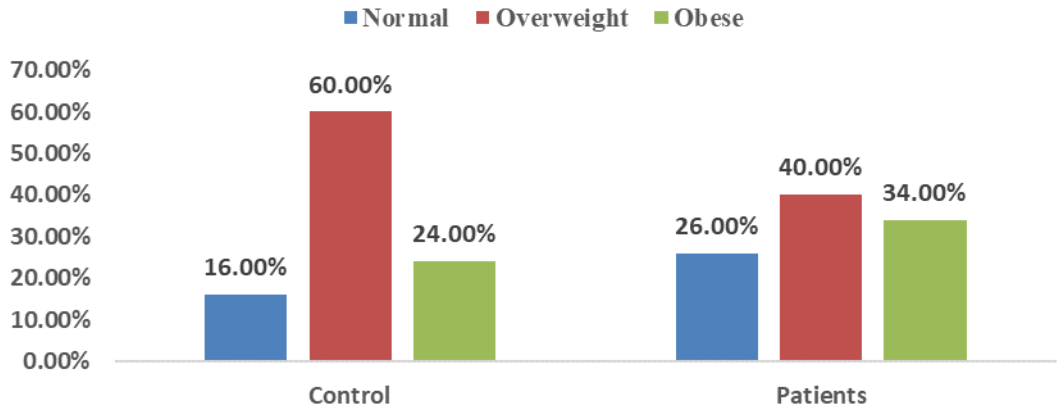

Figure1: Distribution of Body Mass Index (BMI) Categories Among the Control and Patient Groups. The figure illustrates the percentage of participants classified as having normal weight, overweight, and obesity in the control and patient groups. Overweight was the most prevalent BMI category in both groups (60.0% in controls and 40.0% in patients), while obesity was more common among patients (34.0%) than controls (24.0%). Normal weight was observed in 16.0% of controls and 26.0% of patients.c

3.2.Thyroid Function Tests and Autoantibodies

The current results show significantly elevation in thyroid peroxidase antibodies in patients mean: 359.767 whereas in controls 3.935 as in Table 3 and Fig.2, also elevation in thyroid stimulating hormone TSH mean in case group 7.612 comparing to control group 1.761, and decrease in free thyroxine FT4 that indicate decrease activity of the thyroid gland (hypothyroidism) the mean was lower in-patient group then control group 8.056 VS 14.958.

Table3: Mean Level of Hormones Levels and TPO Antibodies in Patients with Hashimoto's Disease and Control Individuals.

Parameters	Characteristic	Control	Patients	P value
Thyroid peroxidase (TPO)	**Mean ±SD**	3.935±1.743	359.767±150.346	0.00002
	Range	1.020 - 8.220	41.950 - 998.000	† NS
Thyroid-Stimulating Hormone (TSH)	**Mean ±SD**	1.761±1.203	7.612±3.383	0.00003
	Range	0.270 - 4.110	3.670 - 15.750	† NS
Free Thyroxine (FT4)	**Mean ±SD**	14.958±2.039	8.056±2.440	0.00001
	Range	12.090 - 19.550	5.990 - 16.130	† NS

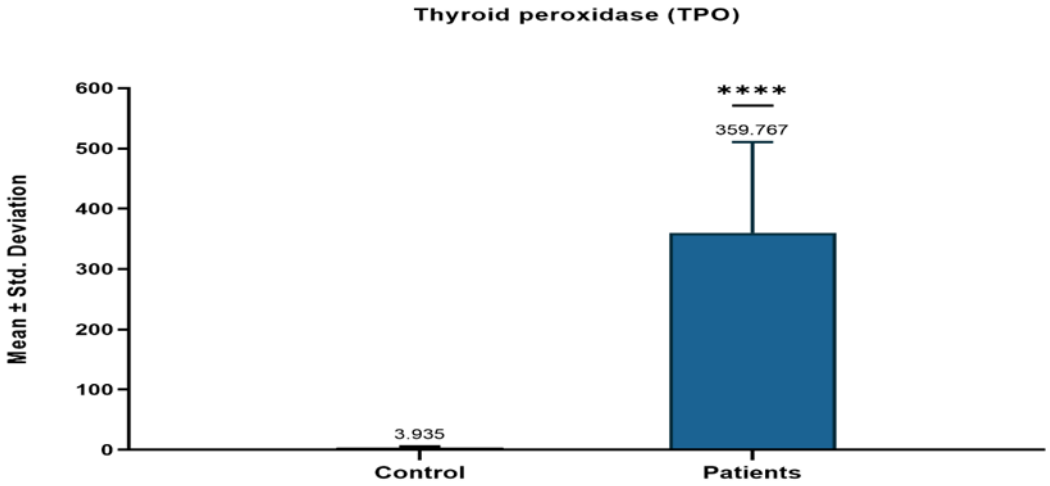

Figure2: Comparison of Serum Thyroid Peroxidase (TPO) Levels Between the Control and Patient Groups. Bars represent the mean ± standard deviation (SD) of serum TPO concentrations in the two study groups. Patients exhibited significantly higher serum TPO levels than healthy controls (****P < 0.0001).

3.3.Distribution of IL-2 in Patients and Control

IL-2 mean in patient higher than control (404.274 vs 276.263) significant p=0.00001.

Results show the IL-2 important predictive or risk factor in Hashimoto disease. Table3, Fig.3.

Table3: The Comparisons Among Investigation Marker (IL-2) In Patients and Controls.

Parameter	Groups	Means	SD	P. value
IL-2	Control	276.263	84.577	0.00001**
	Patients	404.274	130.554	

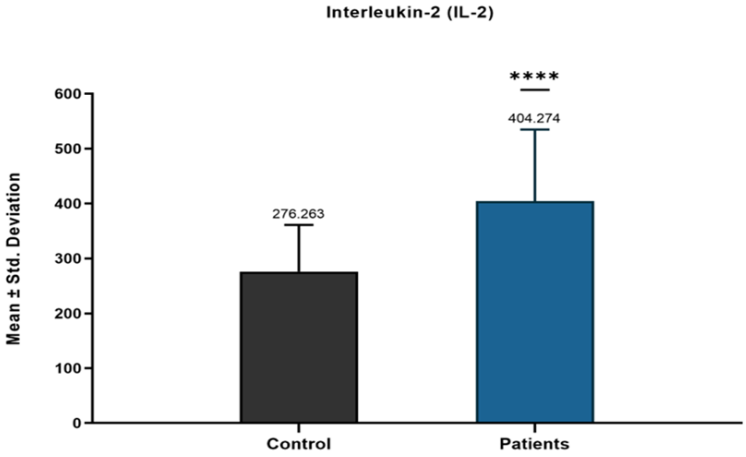

Figure3: Comparison of Serum Interleukin-2 (IL-2) Levels Between the Control and Patient Groups. Bars represent the mean ± standard deviation (SD) of serum IL-2 concentrations in the control and patient groups. Patients exhibited significantly higher serum IL-2 levels than healthy controls (****P < 0.0001).

4. Discussion

Serum concentrations of FT4, TSH, and TPO Ab were quantified for each of the persons under research. According to the current observations, HT patients' levels of TSH values were massively higher (P <0.00003) than of the controls. The mean TSH conc. in the patient group were larger than control group, it is at 7.612±3.383 versus 1.761±1.203 IU/mL, respectively and about that we have agreement with (Al-Zamali *et al.*, 2025), and (Xue *et al.*, 2025). Patients' levels of FT4 concentrations were massively lower than those of the healthy participants, also these observations in the same line with this done by (Huwiler *et al.*, 2024) and that suggested by (Andreas *et al.*,2024). In Compression to the controls, Hashimoto's cases had significantly larger levels of thyroid TPO Ab (table 3). As in our observations, the mean of TPO Ab in patients were found to be largely than in healthy controls, 359.767±150.346 versus 3.935±1.743, respectively (P <0.00002). these findings align with these performed by (Ali *et al.*, 2025) and (Tripolino *et al.*, 2024)**.** Serum levels of TSH and TPO Ab were significantly higher in Hashimoto's patients in compression to a group of control, according to our study. However, the patients' FT4 levels were lower than those of a normal, healthy control group. Clinical diagnostic laboratories frequently use anti-TPO antibodies because they are readily accessible

(Fairweather, Frisancho-Kiss and Rose, 2008). These antibodies are the main ones that attack the thyroid in Hashimoto's disease. Researchers have found that elevated concentrations of anti-TPO antibodies are associated with advanced symptoms of the condition (Acar *et al.,* 2013). In addition, other study (Siriwardhane *et al.,* 2019) found that blood anti-TPO concentrations can be used as indicator to predict the early beginning of thyroid autoimmune disease. They also suggest performing these assays to the battery of thyroid screen tests that already includes T3, T4, and TSH, FT4, FT3.

According to some research, patients who have Hashimoto disease have high levels of inflammatory cytokines and one of these is IL 2 that found to be elevated levels than controls in many studies in AITD such (Yao *et al.,* 2024**).** Also, another study found that Interleukin-2 is an enhancing factor and crucial for HT development, (Hu *et al.,* 2020). substantially this results in the same line with our results which show the level of interleukin 2 in patients is greater than that of control. One of several factors elucidating the function of Interleukin-2, cytokine in T cell biology, is its dual role: it enhances the activity of effector T cells and natural killer (NK) cells, thereby intensifying inflammation, while concurrently facilitating the proliferation and functionality of regulatory T cells (Tregs), which are crucial for sustaining immunotolerance. The critical role of IL-2 positions it as a catalyst for auto immunity and a prospective gaol for immunotherapy (Li, Lin and He, 2025). BMI of control and cases is closely related (27.562±3.087 vs 28.276±3.624) this result shows there was no significantly relationship between the BMI and the Hashimoto disease. And these results agree with (Zhou et al., 2024) who tell there no relationship between BMI and autoimmune hypothyroidism, but disagreed with other study (Škrlec et al., 2025), that observe that worsens of disease may increase with obesity that may lead to decrease the efficiency of treatment and increase progress of disease.

The non-compatible results studies may be caused by variations in research designs and the demographic factor; size of the specimen may differ hugely between cross-sectional and long term study, which influence statistics and generality; many research's take into many losses, variations of populations may have a role; elements like genetics, healthcare assessment, and food can have a clear effects on results and may clarify why BMI is represent a threat factor in some cases but not in others .

age, there were no-significant statistical variations among patients and control group (37.360±8.175 vs 37.360±8.175), this result agrees with many studies that noticed age non-significant with Hashimoto disease (Korevaar et al., 2018) and (Ríos-Prego, Anibarro and Sánchez-Sobrino, 2019). The opposite findings across researches can be caused by differences in demographic characteristics as well as research designs. The power and generality of a study are affected by the sample size, which could vary greatly between prolonged and cross-sectional studies. Furthermore, some studies include two or more losses, while others ask for three. The fact that age is considered a risk factor in certain settings but not others may be attributable to population

variances; factors such as genetic background, availability of healthcare, and nutrition all significantly affect outcomes

Conclusion

this research showed that in participants in Kerbala City, IL-2 levels and thyroid auto antibodies was linked to Hashimoto disease risk. BMI and Age non-significant to related with the disease. overall, larger number of studies on different areas and larger number samples number is recommended in order to assess the involvement of BMI, age, IL -2 on the disease in order to good understanding the linking with disease.

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