

## *Serum levels of IL-18 in the Iraqi Patients with Hashimoto's Thyroiditis*

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### Abstract

Hashimoto's thyroiditis (HT), also recognized as chronic autoimmune thyroiditis or lymphocytic thyroiditis, is an autoimmune disease that targets the thyroid gland causing both dysfunction and tissue destruction. The aim of this case-control study is to investigate the association of human interleukin 18(IL-18), a pro-inflammatory cytokine, serum levels and HT in Iraqi population. The total number of subjects is 100, 50 patients with HT and 50 healthy controls were included. The concentrations of IL-18 serum levels were measured using ELISA. The results of this study revealed that the IL-18 serum levels were significantly elevated in HT patients ( $555.97 \pm 20.51$  pg/ml) comparing to the control which were ( $308.73 \pm 7.18$  pg/ml) regardless of the gender of individual, and the difference between the groups was statistically significant at  $P \leq 0.01$ . The applied statistical tests were the mean, standard error, T-test and P-value. These findings suggest that IL-18 may be involved in the underlying pathogenicity mechanisms of HT.

**Keywords:** Hashimoto's Thyroiditis, Interleukin 18, Autoimmune Diseases, Thyroid Gland.

### Introduction

The thyroid autoimmune disorder Hashimoto's thyroiditis (HT) has genetic basis<sup>1</sup>, and is recognized to be one of the common causes of hypothyroidism<sup>2,3</sup>. It is also alternatively known as chronic autoimmune thyroiditis or lymphocytic thyroiditis<sup>4</sup>.

1–2% of the population is affected by HT globally<sup>5</sup>. one of the main influences is aging since this disease largely affect the middle-aged people specially between 30 and 60, in addition to gender since females are more likely to develop HT by 8-15 times than males<sup>6</sup>.

HT is a thyroid autoimmune disease, in medical, it's defined as a clinical condition, characterized by antibodies, which is normally produced by the body and its function is to provide protection against foreign bodies and has a role in the activation of the immune system, targeting the thyroid normal tissues causing an inflammation response and causing disruption in the thyroid. It will result a negative effect on the thyroid gland and make it incapable to function correctly which lead up to a notable decrease in the levels of secreted hormones production as time passes<sup>7</sup>. All the thyroid hormones work together and consider to have a fundamental roles in the metabolic processes of the human body and any lack in these hormones can have consequence that show as the following symptoms; increased body mass, chilliness, pale skin, issues in fertility, defects in birth and in rare cases of sever condition of hypothyroidism it might result death of the patient<sup>8, 9</sup>. This immune destruction in the thyroid, is linked to HT and irregular high levels of antithyroid antibodies (anti-TAbs)<sup>10</sup>, specifically including antibodies against thyroid peroxidase (TPOAbs) and thyroglobulin antibodies (TgAbs). 90% of the patients diagnosed with autoimmune thyroiditis test positive for TPOAbs and TgAbs<sup>11, 12</sup>, which make it a vital immunological characteristic of this autoimmune disorder, in addition to other characteristics such as, a goiter with lymphocytic infiltration and the circulating T cell population abnormalities<sup>13, 14</sup>.

Cytokines, one of the vital immune substances, play a key role in the activation of the immune system, thus, in autoimmune disorders, such as HT, the malfunction of certain cytokines, specifically the pro-inflammatory cytokines<sup>15, 16</sup>, can affect the regulation of the immune system and start targeting and attacking the thyroid gland. Interleukin-18 (IL-18) is a member of the pro-inflammatory cytokines, and it plays an essential part in the body's defense mechanisms against different infections, also, contribute the innate and adaptive immune responses. Based on this, IL-18 could arguably be involved in the developing of autoimmune diseases since it is capable of inducing inflammatory responses and cytotoxic activities of immune cells<sup>17</sup>.

In the view of IL-18's potent role in the immune responses, IL-18 might contribute to the pathogenesis of HT. IL-18 increased expression has been proved in thyroid gland in HT patients, suggesting that IL-18 could act as secreted immune factor in HT<sup>18</sup>. There is potential that IL-18 might consider a biomarker for HT since IL-18 can play a role in clinical diagnosis and monitoring, especially in medical conditions related to the immune system such as diagnosis of hemophagocytic lymphohistiocytosis, macrophage activation syndrome<sup>19</sup>.

In the consideration of the several study that has been done cytokines and their contribution to autoimmune diseases<sup>20, 21</sup>, this study aimed to investigate the correlation between the interleukin-18 serum levels and Hashimoto Thyroiditis in Iraqi population.

## Materials and Methods

### Subjects:

This case-control study had included 100 subjects (50 samples as HT patients and 50 samples as healthy control), this number was determined by the research guidelines with an age range of (20-75) years old collected from the National Diabetes Center, Al- Mustansiriyah University. The Ethics Committee of

[Al-Nahrain University/ the College of Biotechnology] had reviewed and approved of the protocol used in this study. The diagnosis of HT was ensured through several laboratory tests to evaluate the thyroid function which involved hormonal tests as main indicators for the hypothyroidism, which is strongly associated to HT, in addition to immunological tests to determine the presence of Anti-TAbs which are crucial markers for the diagnosis of HT. The controls were selected depending on their clinical history and normal tests results.

### Estimation Serum Level of Interleukin 18

For the immunoassay of the IL-18 in the collected serum samples, the enzyme-linked immunosorbent assay (ELISA) technique was applied. The analysis was conducted using the Elabscience Human IL-18 ELISA kit, China. This technique is very sensitive and specific for the estimation of IL-18 serum level. The implemented procedure was followed based on the instructions of the kit to be certain of the reliability and accuracy of the results. The steps included: Sample preparation, Reagent addition, Incubation, Washing and finally the measurement of optical density to get the results of the serum level of IL-18 in the samples.

### Statistical Analysis:

The Statistical Packages of Social Sciences -SPSS (2019) program was used to do the statistical calculation of all the samples. The mean, standard error, T-test and P-value were the main statistical tests to be applied.

### Results and Discussion

The results of the serum level of IL-18 in Iraqi patients is presented in table (1) and the figure (1). The shown data clearly determined that IL-18 serum level is significantly higher in patients with HT than the healthy individuals. The observed normal range is (268.442 - 431.869 pg/ml). The (mean±SE.) of IL-18 levels in the patients of HT was (555.97 ±20.51) whereas it was 308.73 ±7.18 in the healthy individuals. The statistical difference between the sample groups is noticeably significant at  $P \leq 0.01$ .

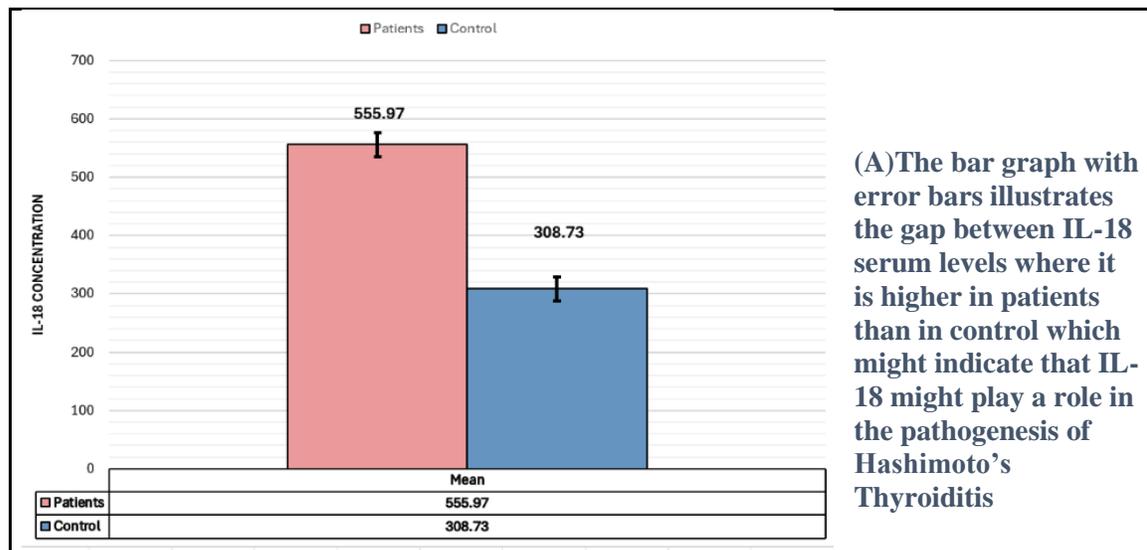
**Table 1. The level of the serum of Interleukin-18 in Hashimoto's thyroiditis patients and control**

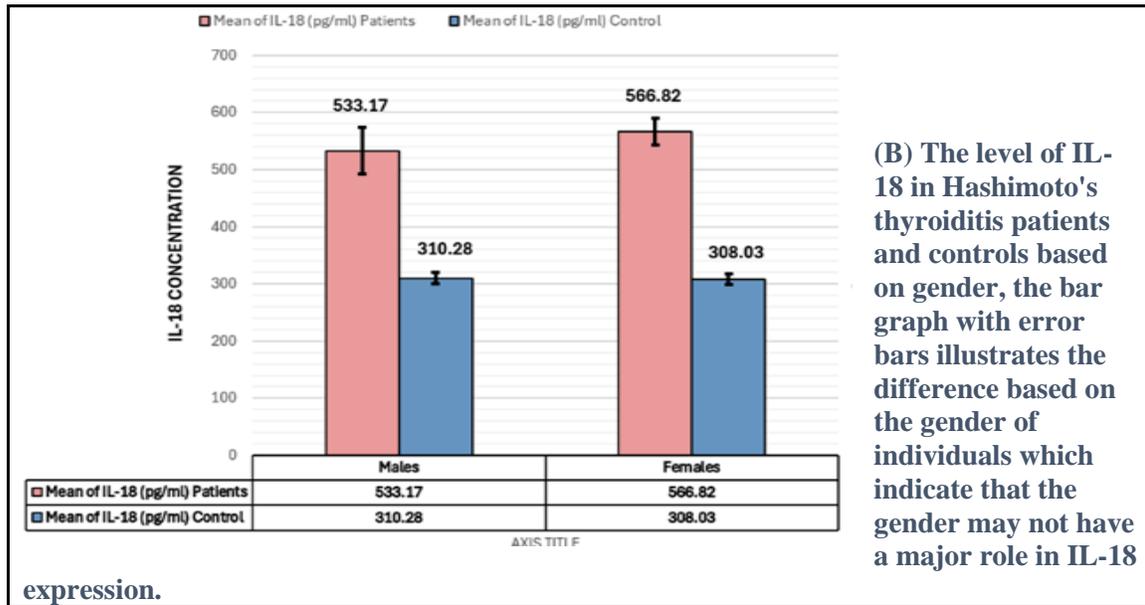
Group	Mean ±SE of IL-18 (pg/ml)
Patients	555.97±20.51
Control	308.73 ±7.18
T-Test	44.694
P-value	0.0001

The results in table (2), figure (1), show data of IL-18 serum level between patients and control according to the gender. The (mean±SE.) of IL-18 levels in the male patients of HT was (533.17 ±40.81) while in females (566.82 ±23.59) whereas in the males was (310.28 ±9.73) and in females (308.03 ±9.61) in the healthy individuals. This result shows a non-significant difference between the IL-18 concentration and the gender of the individual which possibly suggests that the gender does not have a major role in affecting IL-18 expression in the examined population.

**Table 2. The level of the serum of Interleukin-18 in Hashimoto's thyroiditis patients and control**

Group	Mean ±SE of IL-18 (pg/ml)	
	Patients	Control
Males	533.17 ±40.81	310.28 ±9.73
Females	566.82 ±23.59	308.03 ±9.61
T-Test	42.667	29.571 NS
P-value	0.1673	0.584
Non-Significant		





**Figure 1.** The level of the serum of Interleukin-18 in Hashimoto's thyroiditis patients and controls.

These higher levels of IL-18 regardless of the gender may determine an ongoing inflammatory response that is possibly associated with the thyroid gland, since IL-18 is defined to be a pro-inflammatory cytokine that makes it able to contribute to the activation of the immune system<sup>22</sup>. Involving the activation of immune cells, specifically natural killer (NK) cells. It was found in a study that even at very low concentrations, IL-18 can still work synergistically with other types of cytokines to stimulate antibody-Dependent Cellular Cytotoxicity (ADCC) response which plays a role in the destruction of thyroid cells in HT patients<sup>23, 24</sup>. Several papers have observed the role of IL-18 in the Th1 immune response<sup>25, 26</sup>. The Th1 immune response can trigger a severe inflammatory infiltrate of the lymphocytes in the thyroid gland, leading to thyroiditis and a major destruction in the gland by damaging the follicular cells due to the stimulation of functional Fas receptors aside with Fas ligands expression that play a vital part in regulating programmed cell death which can lead to self-apoptosis<sup>27</sup>. All these immunopathological events are fundamentally affected by IL-18, that explains why the elevated levels of this pro-inflammatory cytokine may be crucial in the pathogenesis of HT.

The results of the ELISA test align with the results of several other studies that were conducted in 2024 and it also show the significant gap among the IL-18 level in patients with HT in comparison to the healthy individuals, A study by *Khalaf et al* in Diyala city, Iraq which concluded that there are an increase in the level of IL-18 in the patients with HT<sup>28</sup>. Similarly, another study by *Naser et al* in Najaf city, Iraq found that the IL-18 level was increased in the patients with autoimmune thyroid disorders specifically in the ones with HT<sup>29</sup>. In addition to a study that was done by *Xiao et al* that noted mRNA expression of IL-18 to be at low levels in the healthy group with normal thyroid tissues comparing to the thyroid tissues of HT patients which

were found to have a significantly higher level of mRNA expression<sup>30</sup>. in all the mentioned studies it was reported how the IL-18 levels significantly elevated in the patients with HT.

There were some limitations in this study including the small sample size, a multivariate analysis wasn't performed to indicate if there are any other potential effective variables and finally the study was conducted in a single group of population.

## **Conclusion**

In conclusion, the findings of this study has revealed that the serum level of IL-18 were significantly elevated in patients with Hashimoto Thyroiditis compared to the healthy control group regardless of the gender of the individual, this elevation may suggest the potential of IL-18 role in inflammation and in thyroid autoimmune diseases. Further investigation is recommended to explore the role of IL-18 in Hashimoto disease, a study on a larger, more diverse population and conduct a long-term study to investigate if IL-18 can potentially play a role as biomarker in the diagnosis of HT.

## **Acknowledgment**

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## **Authors' Declaration**

-We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.

-We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

-Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare

## **Authors' Contribution Statement**

[First Author]: Conducted experiments required for this research, in addition to the interpretation of the results and writing the manuscript.

[Second Author]: Had a role in supervising this research, providing guidance, and designing the study.

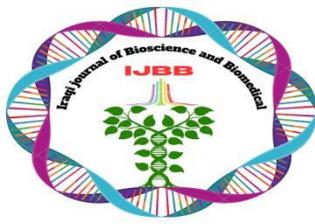
[Third Author]: Provided technical support during some of the laboratory work and handling procedures.

All authors reviewed the manuscript

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