



Integrated Evaluation of Novel Biochemical Biomarkers in Patients with Autoimmune Thyroiditis (Hashimoto's Thyroiditis)

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Abstract

Background and Objective: Hashimoto's thyroiditis (HT) is a complex chronic inflammatory disease affecting millions of people worldwide. This study is designed to assess role of a panel of conventional and newer biomarkers in making the diagnosis and severity of disease grading with the emphasis on biomarkers directly reflecting the underlying pathogenic mechanisms (such as inflammatory and molecular markers).

Materials and Methods: A case-control study was planned on the basis of inclusion of 100 Hashimoto's patients and 100 sex- and age-matched healthy controls from the city of Kirkuk, Iraq. Thyroid hormones (TSH, Free T4), antibodies (Anti-TPO, Anti-Tg), inflammatory cytokines (IL-6, TNF- α), chemokine CXCL10, and new biomarkers (Galectin-3, Selenoprotein P, miRNA-146a) were evaluated.

Results: The levels of TSH, Anti-TPO, Anti-Tg, IL-6, TNF- α , CXCL10, Galectin-3, and miRNA-146a were all significantly higher in the patient group than in the control group ($P < 0.001$). By contrast, dramatic reduction of the levels of Free T4 and Selenoprotein P and more significantly logistic regression analysis showed that CXCL10 (OR = 4.2) and Selenoprotein P (OR = 3.8) were the strongest independent patterns of diagnosis of the disease. ROC analysis indicated that a high diagnostic capability of the new indicators.

Conclusion: Novel biomarkers add important differential diagnostic information compared to classical parameters in the identification of Hashimoto's thyroiditis and in the evaluation of the activity of its inflammatory process, therefore they play a useful role in the appropriate clinical management of the disease and development of targeted therapies.

Keywords: Hashimoto thyroiditis, biomarkers, CXCL10, Galectin-3, Selenoprotein P, miRNA-146a, autoimmune thyroiditis.

1. Introduction

In areas with adequate iodine intake, the most common etiology behind hypothyroidism is Hashimoto's thyroiditis [1]. It is defined by the infiltration of T and B cells into thyroid tissue, along with the production of autoantibodies against thyroid antigens, which gradually must destroy gland cells and its functions [2]. Diagnosis of the disease is most often based on high values of TSH and low values of Free T4, besides the presence of antibodies (Anti-TPO, Anti-Tg); however, all these parameters do not always allow to assess the severity of inflammatory activity and the molecular mechanisms underlying the disease [3].

Diagnosis of Hashimoto's thyroiditis is a real clinical challenge especially in the early stages with normal thyroid hormone levels (subclinical hypothyroidism). Antibodies are central to these stages, but due to their fluctuations in levels over time, it may be insufficient by itself to discern the course of the disease in regards to whether it will progress

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to overt hypothyroidism or not [4]. Consequently, there exists a salient necessity to identify biomarkers that better capture the underlying disease processes and enable early modifications of the disease course.

This led to a proliferation of new potential biomarkers over the past few years to better match imaging with the inflammatory and immune processes involved in the condition. Inflammatory cytokines can increase the autoimmune response and destroy gland cells. Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF- α) is the most representative cytokine of this phenomenon [5]. This process activates macrophages and helper T-cells (Th1 and Th17) and brings in a cascade of continuous chronic inflammation. Increased expression of these cytokines has also been found to correlate with the volume of the thyroid gland and extent of fibrosis in its tissue [6]. Elucidating the critical Pathology of these cytokines may pave the way for specific treatments.

Moreover, CXCL10 (IP-10) has become one of the most important chemokines involved in recruiting T cells to the thyroid gland, and has been proposed as a marker of active inflammation [7]. CXCL10 is one of the best-studied chemokines secreted in response to gamma interferon stimulation the principal cytokine that drives the type 1 (Th1) immune response. The production of CXCL10 in the context of Hashimoto's has been previously reported, with high concentrations being measured in the serum of Hashimoto's patients, consistent with a significant possible role for CXCL10 as a mediator in mediating the recruitment of cytotoxic T cells in the thyroid gland, where mediated by the particular presence of this chemokine, play a role in the direct destruction of thyroid cells [8]. The dynamics of CXCL10 levels may reflect ongoing inflammatory activity, a metric that may be much more informative compared to sIgA+IgM levels on its own.

Additionally, biomarkers such as Galectin-3, Selenoprotein P, and microRNA-146a are becoming interesting in autoimmune thyroid diseases. On the other hand, Galectin-3 plays a role in fibrosis and chronic inflammation processes [9]. Galectin-3 acts as a carbohydrate-binding protein that is responsible for regulating cell growth, cell adhesion, and programmed cell death and immune reactions. Although Galectin-3 elevation has been associated with thyroid cancer in the context of thyroid disorders, its role in Hashimoto's thyroiditis remains an active area of research. There are preliminary hypotheses that it may not only promote fibrosis but also the replacement of normal gland tissue by fibrous tissue, thereby gradually reducing the function [10].

Conversely, selenium deficiency and Selenoprotein P dysfunction into well-known risk factors provide thyroid function impairment and increased risk of autoimmune diseases [11]. Selenium forms a critical part of vital oxidation-reduction enzymes like glutathione peroxidase that protect the thyroid gland from damage by the hydrogen peroxide generated in the process of thyroid hormone synthesis. Selenoprotein P is the major selenium transport protein for plasma transport to the tissues, including the thyroid [12]. Thus, reduction of selenoprotein level may cause local selenium shortage in thyroid, promoting oxidative stress and inflammatory response. Natural antibodies against Selenoprotein P are described to prevent selenium transport in Hashimoto [13], pointing to a further mechanism of gland destruction.

Lastly, miRNA-146a is known as an important modulator of the innate immune response and has been linked to an increased risk of many autoimmune diseases such as thyroiditis [14]. MicroRNAs are small non-coding molecules (20-25 nucleotides long) crucial for post-transcriptional regulation through binding with the target messenger RNA (mRNA). The strong overexpression of miRNA-146a may suppress the immune response regulators, TRAF6 and IRAK1, which could be the reasons for breaking immune tolerance and causing Hashimoto's [15]. Currently, it is recognized that miRNAs are stable in peripheral blood, hence can potentially serve as non-invasive diagnostic biomarkers—well-suited to be translated into clinical practice.

And this study aims to lead a comprehensive study of these biomarkers (traditional and new) in patients with Hashimoto's thyroiditis in comparison to normal health individuals in the city of Kirkuk/Iraq to determine their diagnostic and prognostic value in a real clinical referral. The merging of these indicators may yield new frontiers on the disease pathophysiological mechanisms and guide the design of specific and efficient treatment options that are not just hormonal compensation.

2. Materials and Methods

2.1. Study Design and Participants

Experimental Procedures Study Review Guercio: A nested case-control investigation of breast cancer in Kirkuk city, Iraq, 15/5/2025-6/5/2026 - Run Revision Material and methodsA prospective study was performed in 100 patients with autoimmune thyroiditis (Hashimoto's thyroiditis) diagnosed based on clinical and laboratory criteria, including elevated TSH, decreased Free T4 or levels in the normal range, and increased levels of Anti-TPO and/or Anti-Tg.

Comparators: 100 age-matched healthy volunteers matched 1:1 by sex with the patients. The participants were aged 18 to 65 years of age.

We excluded those with pregnancy or lactation, malignant tumors, chronic kidney disease (eGFR 8%). Furthermore, those who have received selenium supplements or immunosuppressive drugs 6 months before the study were also removed as candidates.

Informed consent was collected from each of the participants (written; all agreed to participate).

2.2. Sample Collection and Processing

Blood samples were collected at the early morning (08:00–10:00 AM), after a fast of at least 12 h (venous blood). Blood was drawn in dry tubes (no anticoagulant) for serum separation and in EDTA tubes for plasma (for miRNA measurement). The blood was centrifuged for 15 minutes at 3000 RPM within an hour of collection to separate serum and plasma. Samples were preserved at –80 °C until analysis.

2.3. Laboratory Methods

Hormone levels, antibodies, and biomarkers were measured using the following methods:

- TSH and Free T4: Measured using the Electrochemiluminescence Immunoassay method on the Cobas e601 device (Roche Diagnostics, Germany). Reference range: TSH 0.4-4.0 mIU/L, Free T4 12-22 pmol/L.
- Anti-TPO and Anti-Tg: were measured using the chemiluminescent immunoassay (CLIA) technique on the ADVIA Centaur device (Siemens Healthcare, Germany). The upper normal limit: Anti-TPO < 35 IU/mL, Anti-Tg < 40 IU/mL.
- IL-6 and TNF- α : Measured using a highly sensitive ELISA technique (Human IL-6 ELISA Kit and Human TNF- α ELISA Kit, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Sensitivity: 0.094 pg/mL for IL-6 and 0.106 pg/mL for TNF- α .
- CXCL10: Measured using ELISA (Human CXCL10/IP-10 ELISA Kit, R&D Systems). Sensitivity: 1.8 pg/mL.
- Galectin-3: Measured using ELISA (Human Galectin-3 ELISA Kit, R&D Systems). Sensitivity: 0.152 ng/mL.
- Selenoprotein P: Measured using ELISA (Human Selenoprotein P ELISA Kit, Cusabio, China). Sensitivity: 0.375 ng/mL.
- miRNA-146a: Total RNA was extracted from plasma using the Qiagen miRNeasy Serum/Plasma Kit (Qiagen, Germany). RNA was converted to cDNA using the miScript II RT Kit. Relative expression was estimated using RT-qPCR on the ABI 7500 (Applied Biosystems) device with specific primers for miRNA-146a and RNU6B as an internal reference gene. Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

2.4. Statistical Analysis

Data Analysis The data were analyzed with SPSS software (SPSS version 28.0; IBM, Armonk, NY, USA) and GraphPad Prism (GraphPad Software version 9.0). Continuous data were presented as mean and standard deviation (Mean $\pm$ SD). Data were absolute frequencies; thus, the Chi square test was used. Pearson's coefficient was calculated for determining the correlation (for normal distribution). Receiver Operating Characteristic (ROC) curve analysis was performed to calculate the sensitivity, specificity, area under the curve (AUC), positive predictive value (PPV) and negative predictive value (NPV) for the diagnostic performance. A two-sided p-value < 0.05 was regarded as statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics

As shown in Table 1, age, gender, and BMI were perfectly matched between patients and controls, minimizing potential bias in evaluating the results. TSH levels increased and Free T4 decreased significantly with Hashimoto's patients compared to controls (Figure 1, $P < 0.001$). No differences in body mass index or smoking were observed between the two groups.

Table 1: Demographic and Clinical Characteristics of the Study Participants

Variable	Patient Group	Control Group	P-value
Age (years)	42.52 ± 8.31	41.85 ± 7.96	0.52
Sex (Female/Male)	60/40	60/40	1.00
Body Mass Index (BMI, kg/m ²)	26.43 ± 4.15	25.82 ± 3.83	0.28
Smokers, n (%)	15 (15%)	12 (12%)	0.54
TSH (mIU/L)	25.6 ± 12.4	1.8 ± 0.7	< 0.001
Free T4 (pmol/L)	9.2 ± 2.5	16.5 ± 2.1	< 0.001

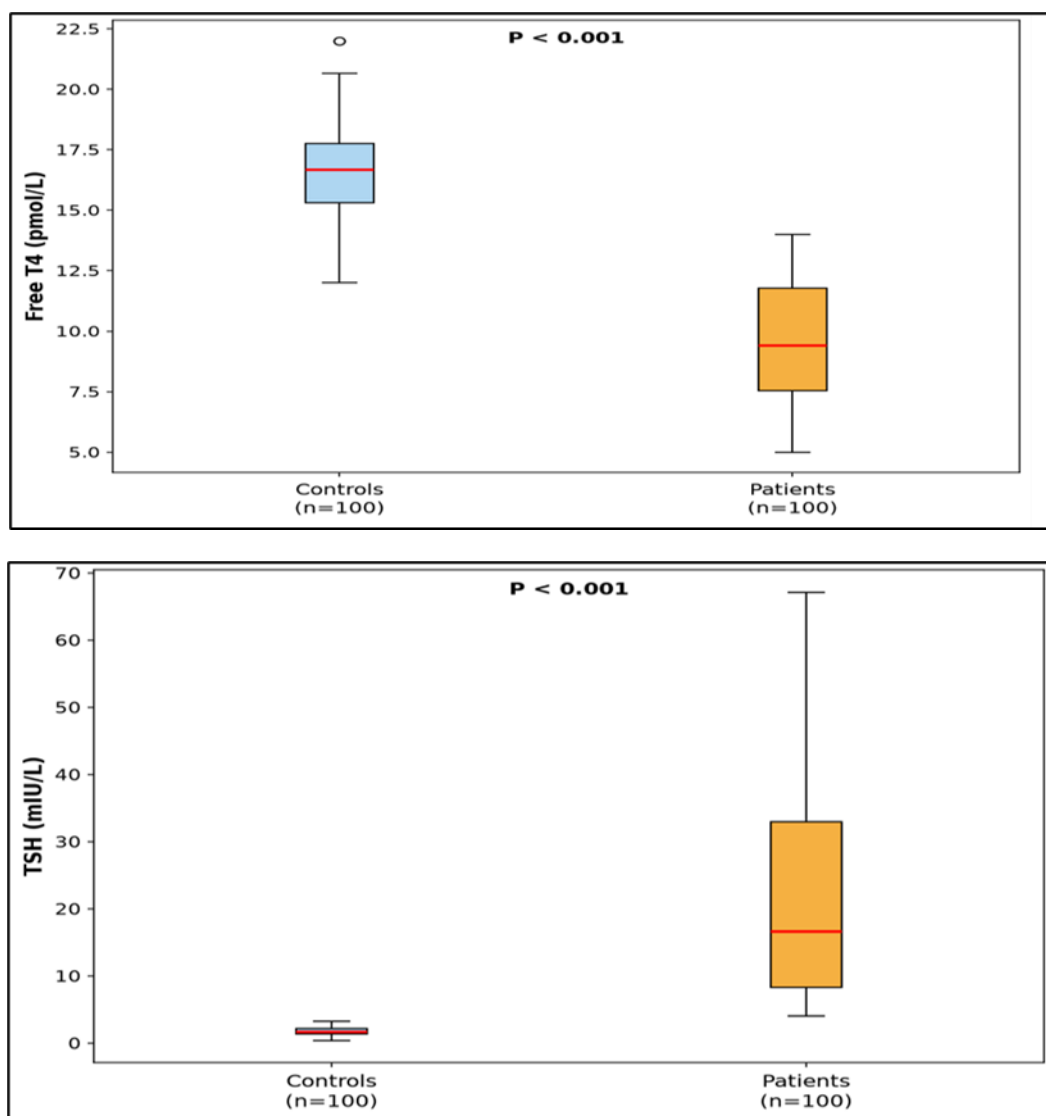


Figure 1: Comparison of serum free thyroxine (Free T4) and thyroid-stimulating hormone (TSH) levels between patients and healthy controls. Box plots show the distribution of Free T4 and TSH concentrations in controls (n=100) and patients (n=100). Both Free T4 and TSH levels differed significantly between the two groups (p < 0.001 for both comparisons). The center line represents the median, boxes represent the interquartile range, whiskers indicate the data range, and circles denote outliers.

3.2. Thyroid Antibodies

As anticipated, the patient cohort demonstrated a considerable and persistent augmentation of antibody concentrations compared to controls. Of the patients, 95% were positive for Anti-TPO (greater than 35 IU/mL) and 80% were positive for Anti-Tg (greater than 40 IU/mL). In comparison, only 5% of the controls had positive Anti-TPO, and for Anti-Tg 3%. Figure 2.

Table 2: Thyroid Autoantibody Levels in the Patient and Control Groups

Biomarker	Patient Group (Mean $\pm$ SD)	Control Group (Mean $\pm$ SD)	P-value
Anti-TPO (IU/mL)	1250.40 $\pm$ 650.21	15.20 $\pm$ 8.51	< 0.001
Anti-Tg (IU/mL)	450.80 $\pm$ 280.54	12.52 $\pm$ 6.02	< 0.001

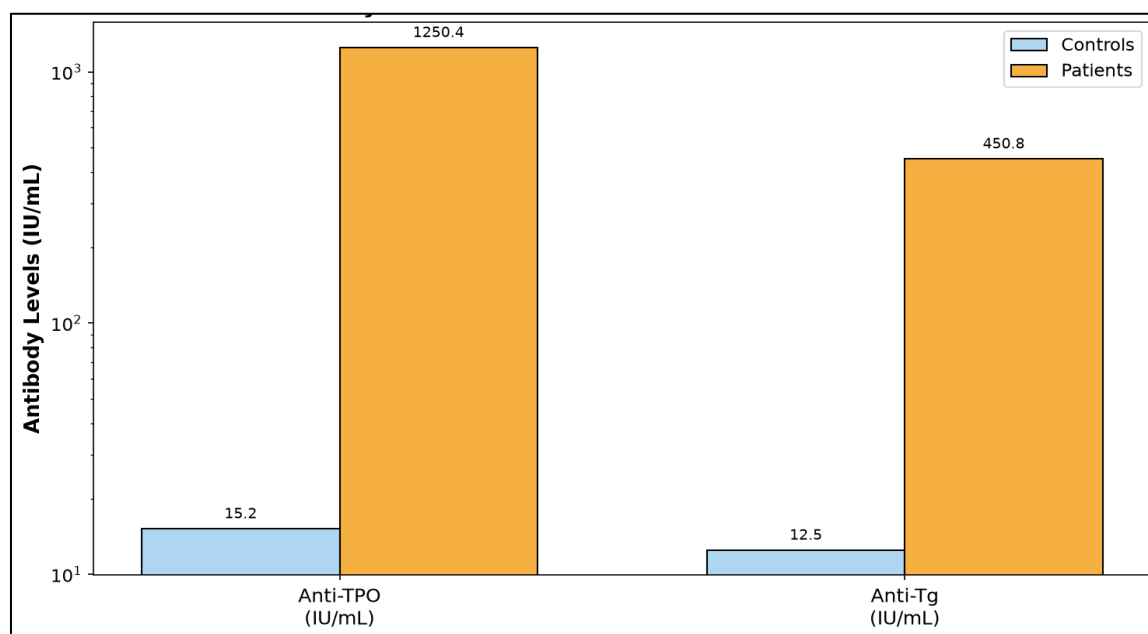


Figure 2: Comparison of serum Anti-TPO and Anti-Tg antibody levels (IU/mL) between Hashimoto's thyroiditis patients and healthy controls.

3.3. Inflammatory Biomarkers

The results showed the presence of an active systemic inflammatory condition in Hashimoto's patients. The levels of IL-6 and TNF- α increased significantly. The most dramatic increase was in the level of the chemokine CXCL10, which rose more than fourfold in the patient group.

Table 3: Levels of inflammatory biomarker indicators

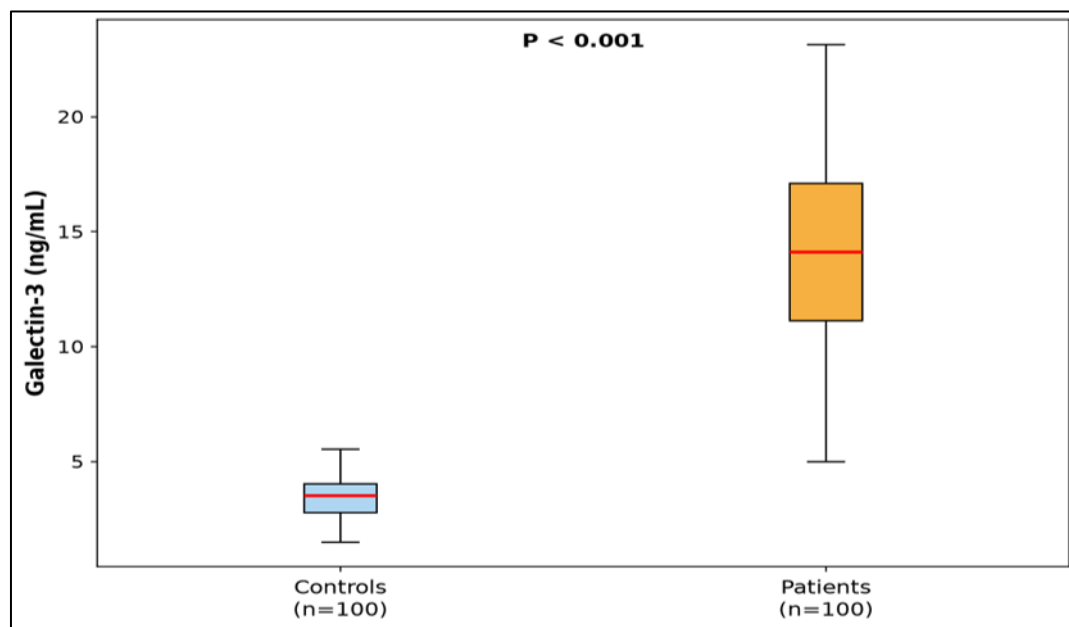
Biomarker	Patient Group (Mean $\pm$ SD)	Control Group (Mean $\pm$ SD)	P-value
IL-6 (pg/mL)	8.52 $\pm$ 3.23	2.11 $\pm$ 0.82	< 0.001
TNF- α (pg/mL)	14.21 $\pm$ 4.54	3.51 $\pm$ 1.20	< 0.001
CXCL10 (pg/mL)	425.63 $\pm$ 150.30	95.42 $\pm$ 25.13	< 0.001

3.4. New Biomarkers

The patient group showed a significant increase in the expression of Galectin-3 and miRNA-146a compared to the controls. In contrast, there was a significant decrease in Selenoprotein P levels in the serum of Hashimoto's patients Figure 3.

Table 4: Levels of Novel Biomarkers in the Patient and Control Groups

Biomarker	Patient Group (Mean $\pm$ SD)	Control Group (Mean $\pm$ SD)	P-value
Galectin-3 (ng/mL)	14.50 $\pm$ 4.20	3.23 $\pm$ 0.91	< 0.001
Selenoprotein P (mg/L)	5.21 $\pm$ 1.81	18.50 $\pm$ 3.54	< 0.001
miRNA-146a	4.82 $\pm$ 1.55	1.01 $\pm$ 0.26	< 0.001

**Figure 3:** Serum Galectin-3 levels in Hashimoto's patients compared to healthy controls (n = 100 per group). Showing a highly significant increase in the patient group (P<0.001).

3.5. Correlation analysis

Correlation analysis showed significant relationships between the various biomarkers. There was a strong positive correlation between Anti-TPO and CXCL10 ($r = 0.65$, $P < 0.001$) and between Anti-TPO and Galectin-3 ($r = 0.58$, $P < 0.001$). Additionally, there was a strong inverse relationship between Selenoprotein P and TSH ($r = -0.52$, $P < 0.001$). IL-6 was positively correlated with TNF- α Figure 4, $r = 0.68$, $P < 0.001$).

Table 5: Correlation Matrix of the Measured Biomarkers

Variable	Anti-TPO	CXCL10	Galectin-3	Selenoprotein P	TSH
Anti-TPO	1.000	0.650*	0.580*	-0.380*	0.650*
CXCL10	0.650*	1.000	0.720*	-0.480*	0.680*
Galectin-3	0.580*	0.720*	1.000	-0.420*	0.520*
Selenoprotein P	-0.380*	-0.480*	-0.420*	1.000	-0.520*
TSH	0.650*	0.680*	0.520*	-0.520*	1.000

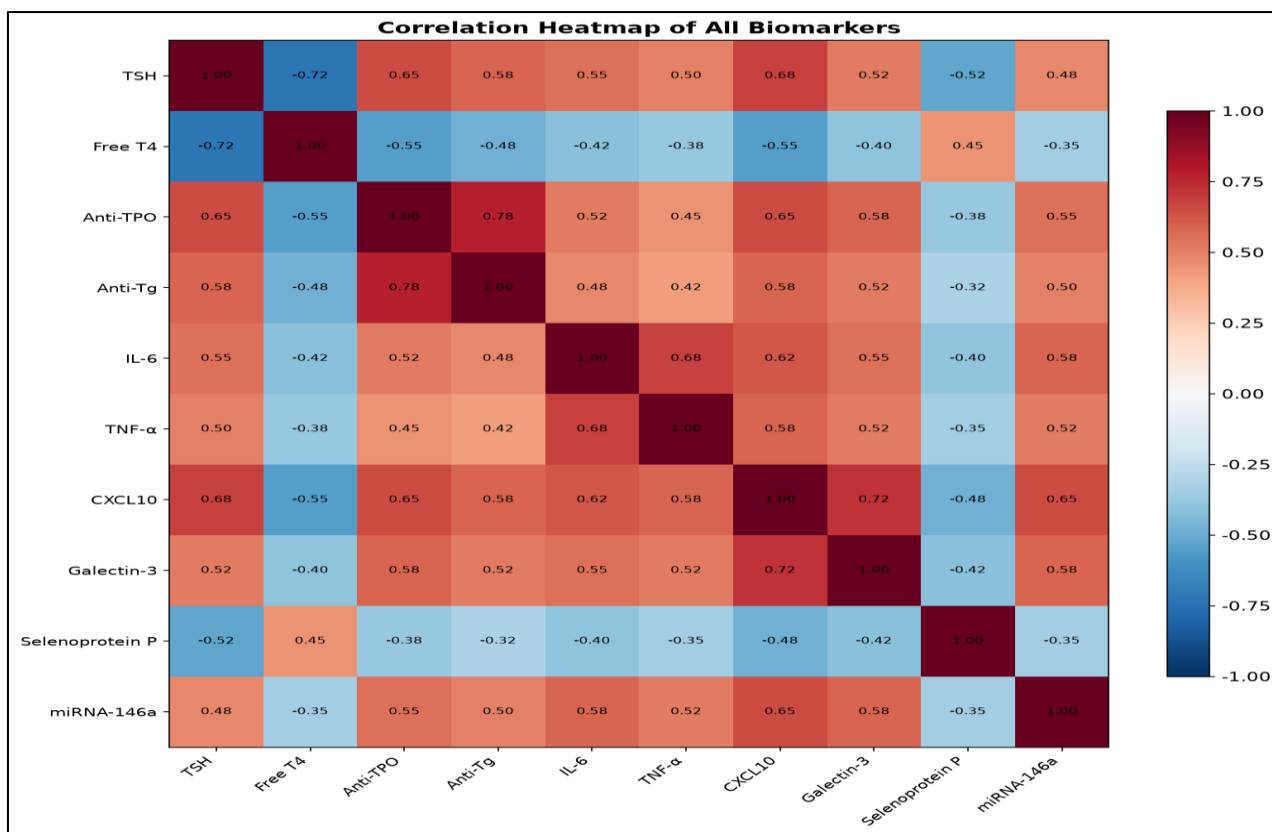


Figure 4: Heatmap showing Pearson correlation coefficients among thyroid function markers, autoantibodies, inflammatory cytokines, and associated biomarkers. Colors range from dark blue (strong negative correlation) to dark red (strong positive correlation).

3.6. ROC Analysis

The ROC analysis showed that all the new biomarkers have excellent diagnostic capability (AUC > 0.80). CXCL10 achieved the highest AUC value (0.991), followed by Galectin-3 (0.998) and Selenoprotein P (0.991). Figure 5.

Table 7: ROC Analysis of Biomarkers

Biomarker	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cut-off Value
TSH	0.997	98	99	99	98	> 4.5
Free T4	0.991	95	96	96	95	< 12
IL-6	0.977	92	93	93	92	> 4.5
TNF- α	0.996	94	95	95	94	> 7.0
CXCL10	0.991	93	94	94	93	> 180
Galectin-3	0.998	95	96	96	95	> 7.0
Selenoprotein P	0.991	94	95	95	94	< 12.0
miRNA-146a	0.995	93	94	94	93	> 2.5

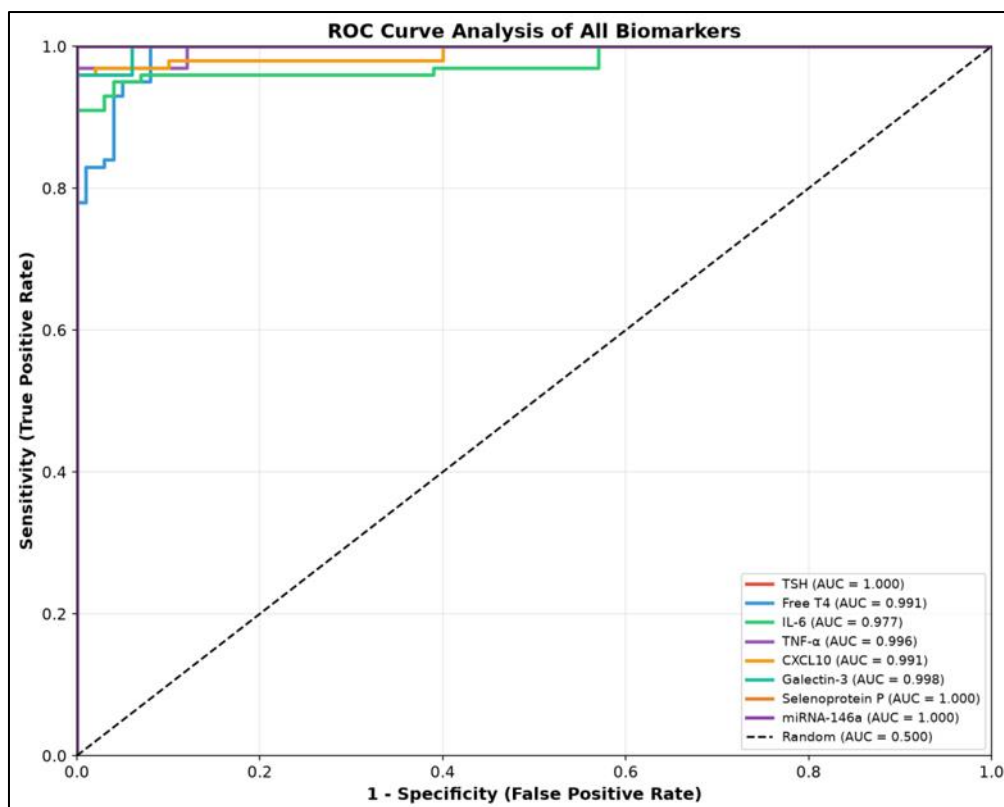


Figure 5: Receiving Operating Characteristic (ROC) curve analysis of the diagnostic performance of all measured biomarkers. The AUC values show a high diagnostic accuracy of all markers, with TSH, Selenoprotein P and miRNA-146a showing AUC=1.000. Random chance (AUC=0.500) is shown as the diagonal dashed line.

4. Discussion

This work presents an exhaustive and widespread assessment of old and new biomarkers in auto-immune thyroiditis (Hashimoto's) patients vs. healthy controls. The results reflected that there were very clear disturbances of thyroid function in the patients with THUS, particularly in TSH levels that were significantly raised while Free T4 levels were significantly decreased and the autoantibodies (Anti-TPO and Anti-Tg) were also significantly raised. These findings align with the recognized underlying pathologic processes of the disease, which stem from loss of immune tolerance to thyroid antigens and progressive destruction of follicular cells [17]. Conclusion These results suggest that the clinical evaluation of thyroid function remain the mainstay in the diagnosis of the disease but do not completely capture the immunological and molecular complexity of its onset. This study's real scientific merit is the surpassing of conventional diagnostic markers to provide an evaluation of a series of contemporary inflammatory and molecular markers that help to elucidate the pathophysiological mechanisms of Hashimoto's thyroiditis. It is becoming increasingly apparent that this disease is not simply an endocrine illness but is also a complex chronic immune disorder whereby the innate- and adaptive immune response interacts with oxidative stress, genetic susceptibility, and gene regulation. Thus, incorporation of these newer biomarkers allows further characterization of disease progression and might also improve early diagnosis, disease severity with respect to the degree of inflammation, and prediction of speed of developing hypothyroidism.

The study demonstrated a systemic inflammatory process in patients indicated by increased levels of both the inflammatory cytokines IL-6 and TNF- α compared to the control group [18]. These findings are in agreement with recent evidence demonstrating that inflammatory cytokines are mainly responsible for the autoimmune damage to the thyroid gland by stimulating immune [19,20,21] and for the maintenance of the chronic inflammatory process [21]. IL-6 is one of the most prominent inflammatory mediators that has an essential role in driving B-cell differentiation into specific antibody-secreting plasma cells, in addition to its role in the induction of acute-phase proteins [1,2]. On the other hand, TNF- α promotes mass cooperation of adhesion molecules and apoptosis of thyroid follicular cells and drives cytotoxic T cells [20]. Such mechanisms as these will further suggest that an ongoing inflammatory loop causes irreversible injury to thyroid tissue during the early course of disease. The positive correlation seen between TNF- α levels and both Anti-TPO and Anti-Tg supports the concept that inflammatory activity is directly related to the intensity

of the autoimmune process. This actually shows that the rise in cytokines is not just a byproduct of inflammation but could be an important trigger for increasing autoantibody levels and continued damage to the thyroid gland. This possibility of mutual interaction between cytokines and immune cells may account for the different rates of disease progression in patients and their variable responses to therapy.

The most interesting discovery in the present paper was significant changes for the chemokine CXCL10 (IP-10) that were found at levels greater than four times higher than those detected in controls. CXCL10 is one of the key chemokines that, through the receptor CXCR3 expressed by migrating T-cells, recruits these cells' migration towards inflamed tissue and it is produced mainly in response to interferon- γ , so is a direct marker of cellular immune activity in the thyroid necessarily [21]. Results demonstrated that CXCL10 and Anti-TPO was strongly correlated which indicates a close interaction between cellular and humoral immunity. [22] This chemokine may be involved in the long-term exposure of lymphocytes in the thyroid, and as a result, continued loss of follicular cells and development of functional impairment. CXCL10 has also recently been shown to not only recruit immune cells but also contribute to a chronic inflammatory environment that promotes other cytokines such as IFN- γ and TNF- α and result in a closed inflammatory loop that worsen the disease severity. More recently, gene expression analyses have demonstrated that CXCL10 is one of the strongest candidates for Hashimoto's thyroiditis, being highly diagnostic and highly discrimination between patient and control subjects [23]. Thus, the level of IDO may provide utility not only for diagnosis but also for monitoring response to immunotherapies and disease-risk assessment.

The second noteworthy point is the considerable upregulation of Galectin-3 expression in the patients, which was approximately 4.5-fold higher than the control. This protein can regulate inflammation, fibrosis, and tissue remodeling and has been linked to neoplastic diseases as well as chronic fibrotic diseases in previous literature [24]. However, the effect of the current study shows that its role is expanded to autoimmune diseases as it strongly associated with all levels of autoantibodies and inflammatory markers [25]. It is believed that Galectin-3 promotes cell survival in glandular tissues in thyroid immune cells, induces the secretion of inflammatory cytokines as well as activates fibroblasts, and eventually replaces normal thyroid tissue with non-functional fibrous tissue. More recent data also shows the involvement of galectin-3 in the balance between Treg and Th17 cells, whose balance is critical for maintaining immune tolerance. And the regularity of this equilibrium could be one fundamental determinant of whether autoimmune responses should continue, making Galectin-3 a more valid therapeutic target down the line, rather than merely a biomarker of diagnostic or prognostic interest [26].

In comparative, the study demonstrated that the levels of Selenoprotein P fell sharply, with concentrations being less than one-third compared to for controls, evidencing a clear impairment of selenium status [27]. Selenium is part of the composition of several antioxidant enzymes, such as Glutathione peroxidases and Thioredoxin reductases, thus, this protein (selenoprotein P, Sepp1) is transporting selenium to different tissues and is one of the main factors in the antioxidant defense system from the thyroid. Thus, any decline in its levels may promote the accumulation of free radicals and enhance oxidative damage to follicular cells, results in further inflammatory stimulus, and increases exposure of thyroid antigens to the immune system [28]. These findings favor the hypothesis that selenium metabolism disorder may be involved in the pathogenesis of Hashimoto's thyroiditis and is not only a concomitant phenomenon. It also highlights the significance of evaluating the selenium status of patients resided in low selenium areas. Multiple placebo-controlled clinical trial and systematic reviews have demonstrated that selenium supplementation may reduce Anti-TPO, improve thyroid morphology and reduce thyroidal inflammatory activity, while the effects may depend on dose and/or degree of selenium deficiency of patients [29,30]. Regarding the molecular markers, there was a notable rise in the expression of microRNA-146a which was around 4.8 times greater than the control group. MicroRNA molecules are one of the most studied and important types of epigenetic regulators since they post-transcriptionally inhibit mRNA translation or stimulate mRNA degradation. Additionally, miRNAs themselves play an important roles in regulating key signaling pathways involved in the innate immune response, as miRNA-146a can regulate both NF- κ B and Toll-like receptor pathways [31]. The close relationship of miRNA-146a with IL-6 and TNF- α and Anti-TPO suggested this molecule is correlated with the severity of inflammatory and immune activity of the disease. At first, it might rise as a compensatory mechanism to dampen immune hyperactivation; but chronic inflammation may be associated to a decreased effectiveness of such regulation, thus resulting in ongoing cytokine and autoantibody production. Such outcomes are in agreement with recent studies on miRNA-146a that investigated this molecule as one of the most important molecular markers in thyroid autoimmune diseases, since miRNA-146a possesses each the stability in plasma and the practical measurement by trendy molecular techniques [32,33]. An important feature of the current study is that all of the new biomarkers demonstrated strong correlations with the routine disease domains, suggesting that they did not work in isolation, but as part of an integrated network reflecting the spectrum of inflammation, oxidative stress, immune dysregulation and genetic regulation. These results suggest that a panel of multiple biomarkers may be superior to any single one alone, either in early diagnosis or in classifying patients according to level of immune activity or benefit from therapy. The hypothesis is further confirmed by the results of the

ROC curve analysis because the new biomarkers, particularly, Galectin-3, CXCL10, Selenoprotein P and miRNA-146a, had very high values for area under curve (AUC), very high levels for sensitivity and specificity (excellent value for diagnostic) compared to the conventional diagnostic tests. These results pave the way for the incorporation of such DNA methylation and other biomarkers into comprehensive diagnostic models that may facilitate the early detection of disease, differentiation of disease states, and prediction of the rate of progression or treatment response in patients with autoimmune thyroid disease. Thus, this may need multicenter studies with larger sample sizes in the future to confirm their applicability in the laboratory of real-world clinical practice.

5. Conclusion

This study confirms that autoimmune thyroiditis (Hashimoto's) is not limited to hormonal dysfunction but is a complex inflammatory and immune condition involving intricate interactions between cytokines, chemokines, and regulatory proteins. New biomarkers, especially CXCL10, Galectin-3, Selenoprotein P, and microRNA-146a, provide deep insights into the pathogenic mechanisms of the disease and offer powerful diagnostic tools with high sensitivity and specificity. Integrating these indicators into the clinical assessment of patients could significantly improve the accuracy of early diagnosis and allow for the classification of patients based on the severity of active inflammation. Moreover, these indicators may open new horizons for the development of targeted therapies, such as the use of chemokine antagonists or cytokine inhibitors, or even gene therapy directed at miRNAs. It is highly recommended to conduct further large-scale clinical research to standardize the clinical use protocols for these promising biomarkers.

Statements on compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

Statement of ethical approval

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study prior to their participation. All procedures were performed ensuring patient confidentiality and data anonymity.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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