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Integrated Immune-Endocrine Biomarker Framework for Differentiating Thyroid Dysfunction in Women

Aveen M. Asaad[1†] and Esmail S. Kakey[2]

[1]Department of Medical Laboratory Technology, Koya Technical Institute, Erbil Polytechnic University, Erbil, Kurdistan Region - F.R. Iraq

[2]Department of Biology, Faculty of Science and Health, Koya University, Koya, Kurdistan Region – F.R. Iraq

***Abstract*—Autoimmune thyroid diseases are the result of multiple interactions among inflammatory cytokines, thyroid autoantibodies, and endocrine dysregulation. However, these biomarkers are commonly studied one by one, and it is not possible to appreciate how immune-endocrine axes collectively discriminate between a hyperthyroid and hypothyroid profile in women, the group at maximum risk. This study has three objectives: To profile endocrine biomarkers (thyroid-stimulating hormone [TSH], T3, and T4) across autoantibody-defined categories; to assess relationships among interleukin (IL)-6, IL-17, and the spectrum of thyroid autoantibodies examined; and to determine whether combined signatures improve distinction of thyroid dysfunction over individual markers. In a cross-sectional study, 103 adult females, 38 euthyroid, 23 hyperthyroid, and 42 hypothyroid, were included. Serum (TSH, T3, T4, anti-thyroid peroxidase [TPO], thyroglobulin [TG], IL-6, and IL-17) was measured using Cobas e411 immunoassay/Enzyme-Linked Immunosorbent Assay kits. Women with hyperthyroidism had a cytokine-dominant pattern with strikingly high IL-6, IL-17, and anti-TPO, whereas hypothyroid ones showed a damage-dominant profile with elevated TSH and TG. IL-6 and anti-TPO were the parameters that showed significant correlation with hormonal imbalances. Anti-TPO and IL-6 had high diagnostic performance for hyperthyroidism. Combination analysis of inflammatory, autoimmune, and endocrine biomarkers provides superior discrimination of hyperthyroidism compared with an individual marker. In this study, a unique multivariate analytical model was used simultaneously for IL-6, IL-17, anti-TPO, TG, and thyroid hormones in order to define immune-endocrine phenotypes in a homogeneous female population.**

***Index Terms*—Anti-thyroid peroxidase, Autoimmune thyroid disease, Cytokines, Thyroglobulin.**

I. Introduction

Thyroid diseases are among the most common endocrinopathies that affect around 5–10% of adults and contribute to metabolic, cardiovascular, reproductive, and neurocognitive morbidities (Taylor, et al., 2018). Mediated by the tightly controlled hypothalamic–pituitary–thyroid axis, thyroid hormones have broad physiological effects on thermogenesis, cellular metabolism and lipid/glucose homeostasis, and cognitive function. In fact, even mild disruption of this axis with a subclinical or borderline derangement has been reported to be associated with infertility, dyslipidemia, depression, and superimposed cardiovascular risk (Kyritsi and Kanaka-Gantenbein, 2020; De Luca, et al., 2021; Bogović Crnčić, et al., 2024).

Autoimmune thyroid diseases (AITDs), including Hashimoto's thyroiditis (HT) and Graves' disease (GD), are the most common cause of acquired thyroid disorders in iodine-sufficient areas (Bedecarratz, 2024; Nautiyal, et al., 2024).

The global prevalence of autoimmune diseases has increased over the past few decades owing to enhancements in diagnostic screening, environmental determinant factors, and changing patterns of autoimmunity susceptibility (Samsami, et al., 2020). The pathogenesis of AITD involves the loss of immune tolerance to thyroid antigens, mainly thyroid peroxidase (TPO) and thyroglobulin (TG), which is eventually associated with the production of anti-TPO and anti-TG autoantibodies, lymphocytic infiltration, correlation with disease severity, and eventual destruction of thyroid tissue (Neupane, 2024).

Recent clinical studies underscore the epidemiological burden of thyroid autoimmunity among females: Anti-TPO positivity reached 21.5% in a Middle Eastern cohort of reproductive-age women (Mirahmad, et al., 2023), and anti-TPO positivity within an Indian cohort with hypothyroidism was up to 79% with a strong 3:1 female-to-male ratio. These patterns have been described elsewhere worldwide (Shah, Singh and Kohli, 2025). Mammen and Cappola (2021) described anti-TPO and anti-TG antibodies as strong immunological surrogates associated with autoimmunity and biochemical thyroid dysfunction.

Increased levels of interleukin (IL)-6 in HT and GD are associated with thyroid-stimulating hormone (TSH) dysregulation as well as higher anti-TPO/TG titers (Hashim and Saeed, 2024). These results indicate that IL-6 can

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†Corresponding author's e-mail: aveen.muhsin@epu.edu.iq

be a marker and an actor in the derangement of thyroid hormones.

Concomitant with IL-6, the Th17/IL-17 axis has recently been recognized as a major player in the local and systemic inflammatory panorama of autoimmune endocrine diseases. IL-17 induces tissue inflammation via the activation of NF-κB and through the production of secondary cytokines such as IL-6 and TNF-α (Lu, et al., 2022). Several studies have shown increased levels of IL-17 in patients with HT and GD, which were positively associated with serum thyroid autoantibody (T-Ab) titer and disease activity (Zaķe, 2021).

In addition, the Th17/Treg imbalance has been suggested as one of the pathogenic mechanisms for the failure of immune tolerance (Ma, et al., 2023). However, the clinical relevance of circulating IL-17 is controversial. The other multicenter Iraqi study showed no statistically significant increase in serum IL-17 level among patients with HT, despite marked biochemical abnormalities (TSH, T3, and T4) (Esfahanian, et al., 2017), which may indicate that IL-17 has a stronger effect at the tissue level or that serum concentrations correlate with disease phase or phenotype.

More recently, systems-biology-based approaches have emphasized the fact that thyroid micro autoantibodies, classical indices of TH physiology, and inflammatory cytokines form a dynamic immuno-endocrine network rather than isolated markers (Li, et al., 2024). However, the majority of studies still look at biomarkers one by one, at individual cytokines (Hashim and Saeed, 2024). Certain populations, such as women of reproductive age (Bahrami, et al., 2022), or combined groups with various causes (Bogović Crnčić, et al., 2024). Only rarely do studies investigate TSH, T3, T4, anti-TPO, anti-TG, IL-6, and IL-17 together in the same homogeneous female adult cohort, although women suffer the highest burden of thyroid autoimmunity and inflammatory thyroid dysfunction (Desai and Brinton, 2019; Pitoia and Trimboli, 2024). Limited available data indicate that these multi-marker model profiles can substantially improve the prediction of thyroid dysfunction and disease progression (Li, et al., 2022), and argue for their integration into current research paradigms.

In view of these considerations, the present investigation studied, in a group of 103 adult female participants, different endocrine, immunological, and inflammatory parameters included in an integrated analysis. We (i) characterized the distribution of TSH, T3, and T4 levels across thyroid autoimmunity patterns (PTA); (ii) evaluated relationships between IL-6 or IL-17 and thyroid autoantibodies and changes in hormones; and (iii) determined whether composite biomarker signatures improve discrimination among subgroups at risk for clinically relevant thyroid dysfunction compared with single markers. The objective of this study is to contribute to the comprehension of immuno-endocrine dialogues in women with thyroid disorders by integrating classical thyroid indices, titers of autoantibodies, and profiles of cytokines, which will enable the design of new biomarker-based strategies for risk assessment and therapeutic follow-up. The novelty of this study is the combined multi-marker approach that evaluates at the same time the serum levels of IL-6, IL-17, anti-TPO, TG, and thyroid hormones for discriminating immune-endocrine phenotypes of hyper- and hypothyroidism in a homogeneous population of females.

II. Materials and Methods

A. Study Design and Patient Selection

This research was performed in the Koya district, Erbil Governorate, Kurdistan Region of Iraq, on patients diagnosed with thyroid disorders and referred to Shahid Dktor Khalid Hospital as a cross-sectional study. Institutional ethical approval was obtained from the Faculty of Science and Health, Koya University Ethics Committee (012 Bio) dated December 23, 2024. Blood samples and questionnaires were administered during the period December 2024–April 2025.

B. Patient Population

A total of 115 patients were studied. Candidates must be in a specific age range of 20–60 years. Altogether, 12 patients with co-existing other endocrinopathies, pregnancy, and recent thyroid gland operation or treatment have been excluded from the entire case series. The study group included 65 cases with clearly defined thyroid disease (hypothyroidism and hyperthyroidism) as well as 38 euthyroid controls accompanied by complete clinical and biochemical data, including biochemical markers of the thyroid hormones: TSH, T3, T4, and immunological markers anti-TPO and TG-Ab, along with inflammatory markers IL-6 and IL-17. This study was conducted in female subjects only, as the prevalence of thyroid disease is higher in women. The work was mainly aimed at assessing the variations of biological and immunological indices, in order to determine their diagnostic and prognostic significance in thyroid dysfunction.

The pathophysiology of the thyroid can vary between genders, so we chose to look at females to give a more comprehensive interpretation of biochemical and immunological markers. Participants, according to their clinical diagnostic/hormonal profile and established clinical thresholds, were classified as belonging to Group 1 if they belonged to any of the following groups:

- Euthyroid: Normal serum TSH concentration (0.27–4.2 IU/mL) with normal T3 and T4 concentrations
- Hyperthyroidism: Low TSH levels (<0.27 IU/mL) associated with high serum T3 and/or T4 values
- Hypothyroidism: Increased TSH (>4.2 IU/mL) with low/normal T3 and/or T4 levels.

C. Data Collection and Biochemical Analysis

Fasting blood samples from participants were obtained using plain (non-additive) tubes in a 5 mL quantity. Serum from the samples was separated by centrifugation at 3000 rpm for 10 min. After that, the serum sample was aliquoted into small portions and stored at −20°C for further laboratory examination. Fasting blood samples were collected between 8 and 10 AM, followed by serum assay using the Cobas e411 immunoassay analyzer (Roche Diagnostics,

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Germany) for concentrated TSH, TG, anti-TPO, and anti-TG. The hormonal reference ranges used for this study were: T3 (1.3–3.1 nmol/L), T4 (66–181 nmol/L), and TSH (0.27–4.2 IU/mL). The normal range for anti-TPO in adult women was (normal range: 34 IU/mL, and TG was 1.4–78 ng/mL).

In the current study, serum levels of IL-6 and IL-17 were determined by commercial human sandwich Enzyme-Linked Immunosorbent Assay kits (ELK Biotech, China) following the manufacturer's instructions. Detection of IL-17 was performed using an ELK2610 kit (15.63–1000 pg/mL), and that of IL-6 was done using the ELK1156 kit (7.82–500 pg/mL). All standards and samples were plated in duplicate, and optical density was measured at 450 nm on a microplate reader to determine pg/mL of cytokines in the samples, allowing accurate and consistent quantification throughout all specimens (Hashim and Saeed, 2024). All of the data were placed into an Excel spreadsheet for future analysis.

D. Statistical Analysis

The following statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics, version 25) and GraphPad Prism (version 7.04). For all biomarkers and thyroid-function parameters, descriptive statistics (mean, standard deviation [SD], standard error, 95% confidence interval [CI]) were initially computed to outline the distribution patterns in the population under study. Normality was tested with the Shapiro–Wilk test, and as biomarker levels were not normally distributed, non-parametric methods were chosen. Between-group differences (euthyroid versus hypo- and hyperthyroid participants) were evaluated by the Kruskal–Wallis H test with Dunn's *post hoc* comparisons using Bonferroni correction procedures to compensate for multiple comparisons. Correlation between inflammatory cytokines, autoimmune markers, and thyroid hormones was calculated using two-tailed Spearman's rank coefficients. To measure the ability of IL-17, IL-6, anti-TPO, and TG to distinguish between two thyroid dysfunctions, receiver operating characteristic (ROC) curves were generated, and area under the curve (AUC) estimates were calculated. The association of the presence or absence of thyroid disorders with more than one biomarker was modeled using multinomial logistic regression with likelihood ratio tests and odds ratios; 95% CIs were reported. All analyses were considered statistically significant at $p < 0.05$.

III. Results

A. Descriptive Statistics

Descriptive statistics from 103 female subjects (20–60 years) showed high biochemical variability across thyroid, inflammatory, and autoimmune tests. First, the marked heterogeneity indicated mixed thyroid function: TSH showed a wide range (0.005–45.78 mIU/L) with a high SD. Total T3 and T4 were focused around a narrower distribution, which was mirrored by the narrow CIs indicating stable mean estimates. Second, systemic inflammatory cytokines varied substantially; IL-17 (15.97–124.8 pg/mL) and IL-6 (7.82–98.77 pg/mL) were widely distributed as well, which implied various levels of systemic inflammation among individuals.

Autoimmune markers were the most dispersed. Anti-TPO (5–600 IU/mL) and TG (0.04–171.9 ng/mL) levels revealed broad variation compatible with the presence of mild-to-high-grade autoimmunity. In total, the dataset constitutes a broad spectrum of physiology and will facilitate further investigations in various thyroid and immune-related states. The demographic and clinical characteristics of the study population are summarized in Table I.

B. Descriptive Statistics of Biomarkers Across Thyroid Function Groups

On further stratification of 103 cases as euthyroid (n = 38), hyperthyroid (n = 23), and hypothyroid (n = 42), different biochemistry profiles emerged. The numerical values of the subgroups indicate the geographical distribution of thyroid status within this sequentially selected study population throughout this time frame. These findings are presented in Table II and Fig. 1.

The euthyroid group had a biomarker profile with relatively low intra- and inter-individual variability, which is typical of normal physiology. However, the hyperthyroid group had the highest mean levels of IL-17, IL-6, and anti-TPO, and a lower mean level of TSH, indicating the typical hormonal imbalance of hyperthyroidism and suggesting an

TABLE I
Descriptive Statistics

Parameter	Min.	Max.	Mean±SD	SE	CI limit
TSH (mIU/L)	0.005	45.78	4.208±5.716	0.5632	3.091–5.326
T3 (nmol/L)	1.16	4.62	2.233±0.7395	0.07286	2.089–2.378
T4 (nmol/L)	50.30	181.30	113.2±27.03	2.664	107.9–118.5
IL17 (pg/mL)	15.97	124.80	42.64±27.78	2.737	37.21–48.07
IL6 (pg/mL)	7.82	98.77	24.39±19.56	1.927	20.56–28.21
Anti-TPO (IU/mL)	5.00	600.00	104±137.9	13.58	77.06–130.9
TG (ng/mL)	0.04	171.90	19.14±30.12	2.968	13.25–25.02

SD: Standard deviation, SE: Standard error, TSH: Thyroid-stimulating hormone, IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase

TABLE II
Descriptive Statistics of IL-17, IL-6, Anti-TPO and TG Across Thyroid Function Groups

Biomarker	Group	n	Mean±SD	SE	Min.	Max.
IL-17 (pg/mL)	Euthyroid	38	38.80±34.77	5.64	15.97	124.8
	Hyperthyroid	23	61.70±29.87	6.23	19.19	120.0
	Hypothyroid	42	35.68±9.11	1.41	21.19	62.38
IL-6 (pg/mL)	Euthyroid	38	18.10±15.63	3.01	7.93	78.20
	Hyperthyroid	23	44.95±26.38	5.50	8.23	98.77
	Hypothyroid	42	21.84±8.68	1.36	11.12	45.27
Anti-TPO (IU/mL)	Euthyroid	38	64.72±105.7	17.14	5.00	600.0
	Hyperthyroid	23	255.10±144.6	30.15	28.75	600.0
	Hypothyroid	42	56.80±97.29	15.01	5.67	600.0
TG (ng/mL)	Euthyroid	38	14.17±21.64	3.51	0.04	93.66
	Hyperthyroid	23	5.30±13.23	2.76	0.14	50.94
	Hypothyroid	42	31.21±38.23	5.90	0.04	171.90

SD: Standard deviation, SE: Standard error, IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase

increase in inflammatory and autoimmune response. On the other hand, the mean TSH levels and TG levels were highest in the hypothyroid group, which is a feature of poor thyroid function.

Overall, the descriptive results indicate distinct hormonal, inflammatory, and autoimmune profiles within the thyroid function groups, which are used in the subsequent comparative statistical analyses.

C. Kruskal–Wallis and Dunn's Post hoc Test Results

For all the studied biomarkers (i.e., IL-17, IL-6, and anti-TPO and TG), a significant difference was observed among different groups of thyroid status (euthyroid, hyperthyroidism, and hypothyroidism), as indicated by Kruskal–Wallis test analysis ($p < 0.001$). These results, as summarized in Table III, imply that inflammatory and autoimmune activity are significantly different between the thyroid functional states. With reference to IL-17, *post hoc* analyses showed that hyperthyroid and hypothyroid study subjects presented significantly higher concentrations of this cytokine in comparison with euthyroid individuals, and hyperthyroid samples differed from hypothyroid samples. Likewise, IL-6 was also increased in both thyroid-dysfunction groups when compared with the euthyroid controls, and in hyperthyroid patients it appears to be the most robust inflammatory response.

Autoimmune markers showed an even greater discrepancy. The hyperthyroid group had significantly higher levels of anti-TPO in comparison to both euthyroid and hypothyroid groups, but it was not significantly different between the euthyroid and hypothyroid subgroups. For TG, hyperthyroid subjects again had significantly higher levels than both other groups, with no significant differences between euthyroid and hypothyroid patients.

Euthyroid subjects displayed relatively stable and minimally variable levels of thyroid hormones, indicative of normal physiological regulation. On the contrary, hyperthyroid subjects presented with the typical normal TSH profile with marginally elevated IL-6 and IL-17 levels, indicating a mild inflammatory status related to increased thyroid activity. On the other hand, the hypothyroid group showed significantly raised TSH in association with the highest levels of anti-TPO and TG. In summary, the stratified analysis demonstrates distinct hormonal, inflammatory, and autoimmune differences between thyroid states and justifies group-wise differences-based comparisons in downstream analyses.

The boxplots as illustrated in Fig. 2 highlight distinct alterations of the biomarkers between groups of euthyroid, hyperthyroid, and hypothyroid. There were significantly higher levels of IL-17, IL-6, anti-TPO, and TG in patients with hyperthyroidism in contrast to other groups ($p < 0.05$), expressing more intense inflammatory and autoimmune activation. In euthyroid subjects, serum values were the lowest and represented a steady state of normality. In contrast, the hypothyroid group showed intermediate, relatively high or low levels for both anti-TPO and TG with variable findings (both later on in combination), indicative of partial autoimmune participation.

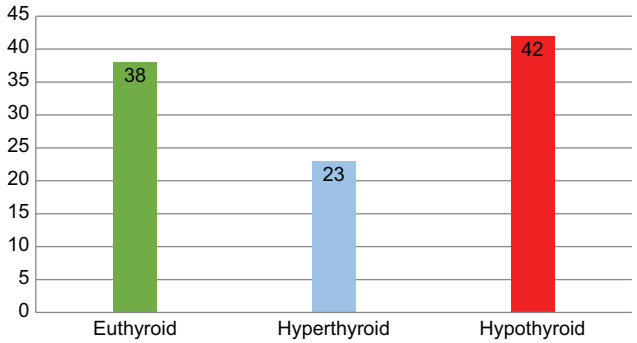

Fig. 1. Descriptive statistics across thyroid function groups.

TABLE III
KRUSKAL–WALLIS H TEST AND DUNN'S *POST HOC* COMPARISONS FOR BIOMARKER DIFFERENCES AMONG THYROID GROUPS

Biomarker	Kruskal–Wallis H	p-value	Pairwise comparison	Adjusted p-value
IL-17	26.20	<0.0001*	Eu versus Hyper	<0.0001****
			Eu versus Hypo	0.0099**
			Hyper versus Hypo	0.0266*
IL-6	29.49	<0.0001*	Eu versus Hyper	<0.0001****
			Eu versus Hypo	0.0190*
			Hyper versus Hypo	0.0027**
Anti-TPO	35.63	<0.0001*	Eu versus Hyper	<0.0001****
			Eu versus Hypo	>0.9999 Ns
			Hyper versus Hypo	<0.0001****
TG	17.14	0.0002*	Eu versus Hyper	0.0178*
			Eu versus Hypo	0.3660 Ns
			Hyper versus Hypo	0.0001***

*, **, ***, and ***indicate significance levels, NS: Non-significant. IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase

In general, these visual patterns match the statistical analyses and suggest that immune-inflammatory markers increase significantly in hyperthyroid dysfunction, whereas decreasing to the lowest levels in euthyroid states, thus strengthening the biological plausibility of group classification.

D. Correlation among Inflammatory Cytokines

The correlation matrix revealed the structured interplay among inflammatory cytokines, autoimmune markers, and thyroid hormones. The results presented in Table IV and Fig. 3 showed no significant correlation was found between IL-17 and other parameters: IL-6 ($p = 0.093$, $r = 0.1665$), anti-TPO ($p = 0.129$, $r = 0.1511$), T3 ($p = 0.06$, $r = –0.1857$), TSH ($p = 0.153$, $r = –0.1418$), and T4 ($p = 0.625$, $r = –0.0488$).

However, IL-6 showed the most noticeable profile with a low positive correlation with T3 ($p \leq 0.001$, $r = 0.3887$), whereas the correlation weakened toward anti-TPO ($p \leq 0.001$, $r = 0.3190$) and was lowest toward TSH ($p = 0.042$, $r = –0.2007$). This sequence of events implies that IL-6 is enhanced with increased metabolism in hyperthyroidism. Anti-TPO exhibited significant correlations,

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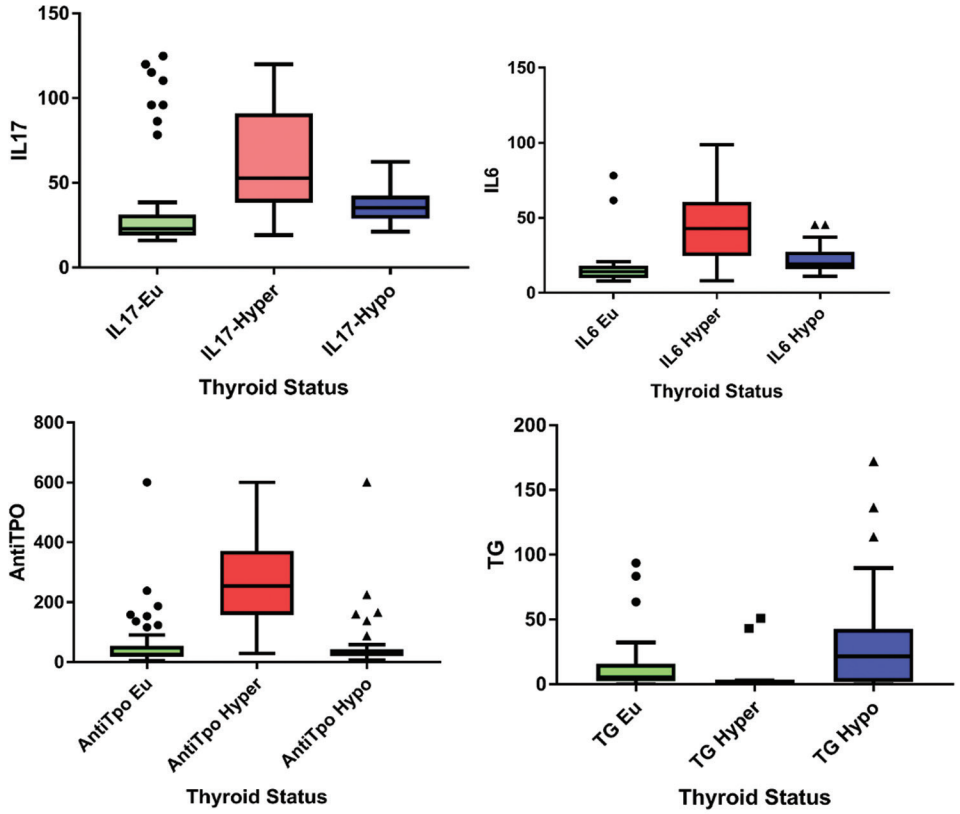

Fig. 2. The boxplots of the biomarkers between groups of euthyroid, hyperthyroid, and hypothyroid.

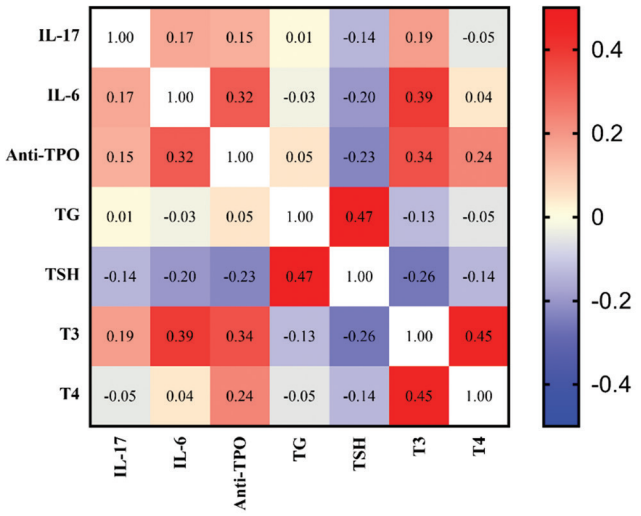

Fig. 3. Correlation between parameters.

TABLE IV
CORRELATION BETWEEN PARAMETERS

Parameter	IL-17	IL-6	Anti-TPO	TG	TSH	T3	T4
IL-17							
r	1	0.1665	0.1511	0.0132	−0.1418	0.1857	−0.0488
p-value		0.093	0.129	0.895	0.153	0.06	0.625
IL-6							
r	0.1665	1	0.3190	−0.0306	−0.2007	0.3887	0.0449
p-value	0.093		<0.001	0.758	0.042	<0.001	0.652
Anti-TPO							
r	0.1511	0.3190	1	0.0471	−0.2302	0.3393	0.2362
p-value	0.129	<0.001		0.637	0.019	<0.001	0.016
TG							
r	0.0132	−0.0306	0.0471	1	0.4699	−0.1343	−0.0534
p-value	0.895	0.758	0.637		<0.001	0.176	0.593
TSH							
r	−0.1418	−0.2007	−0.2302	0.4699	1	−0.2581	−0.1407
p-value	0.153	0.042	0.019	<0.001		0.008	<0.001
T3							
r	0.1857	0.3887	0.3393	−0.1343	−0.2581	1	0.4520
p-value	0.06	<0.001	<0.001	0.176	0.008		<0.001
T4							
r	−0.0488	0.0449	0.2362	−0.0534	−0.1407	0.4520	1
p-value	0.625	0.652	0.016	0.593	<0.001	<0.001	

TSH: Thyroid-stimulating hormone, IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase

which were positive with T3 ($p \leq 0.001$, $r = 50.3393$) and T4 ($p = 0.016$, $r = 0.2362$), and negative with TSH ($p = 0.019$, $r = –0.2302$), confirming the involvement of autoimmune thyroid dysfunction in hormone secretion. TG provided the strongest association with TSH ($p \leq 0.001$, $r = 0.4699$), which would suggest that TG synthesis was prompted by TSH stimulation, then demonstrated poor relationships with IL-6 ($p = 0.758$, $r = −0.0306$), IL-17 ($p = 0.895$, $r = 0.0132$), and anti-TPO ($p = 0.637$, $r = 0.0471$). The anticipated endocrine associations were found, with TSH significantly related negatively to T3 ($p = 0.008$, $r = –0.2581$) and to T4 ($p = < 0.001$, $r = −0.1407$). T3 and T4 correlated moderately ($p \leq 0.001$, $r = 0.4520$), indicating that they share the same biosynthesis as well as metabolic pathways.

In conclusion, IL-6 and anti-TPO were the closest biomarkers to thyroid hormonal changes, whereas IL-17 had minimal effect. These data reveal the relation between inflammatory and autoimmune mechanisms in the regulation of thyroid function, where TSH and TG reflect the degree of glandular stimulation and compensatory downregulation.

E. ROC Analysis for IL-17, IL-6, anti-TPO, and TG

ROC analysis indicated that the assessed biomarkers have different diagnostic applications in various thyroid dysfunction states, and the AUC was calculated. In hyperthyroidism, the AUC values for IL-17 (AUC = 0.787), IL-6 (AUC = 0.854), and particularly anti-TPO (AUC = 0.908) were very good for discriminating between cases, with higher specificity toward hyperthyroid-associated immune–inflammatory markers being observed. TG, in contrast, showed a reverse distribution (AUC = 0.236) and might be more useful to rule out hyperthyroidism than to rule it in.

Overall diagnostic accuracy for hypothyroidism was low. IL-17 (AUC = 0.545) and IL-6 (AUC = 0.571) could not predict it, whereas anti-TPO had an inverse relationship (AUC = 0.373). TG alone was moderately discriminative (AUC = 0.680), providing only some value in detecting hypothyroidism. In general, these results indicate that the distinction of hyperthyroidism is better achieved by immune-inflammatory biomarkers, whereas the separation on the basis of biomarker profiles in hypothyroidism was less pronounced and may be mirrored by a less homogeneous immunological state. These data are presented in Table V and Fig. 4.

Diagnostic ranks were reported without cutoffs, and these are summarized in the table below. For all of the markers, Anti-TPO demonstrated the greatest diagnostic ability and performed excellently for hyperthyroidism (AUC 0.908). IL-6 followed with high accuracy (AUC 0.854), and IL-17 showed good performance (AUC 0.787), all for hyperthyroid identification alone. In the case of hypothyroidism, TG had a fair discriminatory ability (AUC 0.680); all other markers, including anti-TPO, IL-6, and IL-17, showed bad/no diagnostic utility.

F. Multinomial Logistic Regression

Likelihood ratio tests demonstrated that all four biomarkers (IL-17, IL-6, anti-TPO, and TG) were statistically significant and conditionally independent predictors of thyroid status, as summarized in Table VI. IL-6 showed the greatest improvement in model performance, being one of the strongest drivers of discrimination of thyroid disease. Furthermore, IL-17 and anti-TPO showed significant boosting of the model, indicating their activity in inflammation and autoimmunity. TG was also a significant contributor, especially regarding hypothyroid classification. The intercept was highly significant, suggesting that baseline category probabilities differed prior to biomarkers being included.

TABLE V
Diagnostic Performance of IL-17, IL-6, Anti-TPO, and TG for Hyperthyroidism and Hypothyroidism

Biomarker	Thyroid status	AUC	p-value	Advantage usage
IL-17	Hyperthyroidism	0.787	<0.001	Useful biomarker
IL-17	Hypothyroidism	0.545	0.442	No diagnostic value
IL-6	Hyperthyroidism	0.854	<0.001	Strong diagnostic marker
IL-6	Hypothyroidism	0.571	0.223	Not useful
Anti-TPO	Hyperthyroidism	0.908	<0.001	Best biomarker for hyperthyroidism
Anti-TPO	Hypothyroidism	0.373	0.029	Inverse association
TG	Hyperthyroidism	0.236	<0.001	Inverse association
TG	Hypothyroidism	0.680	0.002	Moderately useful

IL: Interleukin, TG: Thyroglobulin, AUC: Area under the curve, TPO: Thyroid peroxidase

The regression coefficients indicated unique biomarker profiles among the thyroid groups as presented in Table VII. Among hyperthyroidism, IL-17, IL-6, and anti-TPO had a significant positive association, such that higher concentrations of these markers are associated with higher odds of being hyperthyroid. TG had a negative and significant effect, indicating that lower TG is characteristic of those with a hyperthyroid condition. For hypothyroidism, IL-6 and TG were significant positive predictors, indicating that high values of each TBI lead to a diagnosis of hypothyroidism. Anti-TPO had borderline significance (p = 0.052), and IL-17 was not an independent predictor in this analysis. IL-6 and TG were identified as stable predictors in both prevailing states, whereas IL-17 and anti-TPO had state-dependent effects on the development of either disease, mainly hyperthyroidism.

IV. Discussion

The present study revealed significant variances in inflammatory cytokines, thyroid autoantibodies, and thyroid hormones among euthyroid, hyperthyroid, and hypothyroid women. These findings support a concept of immune-endocrine interactions in AITD and indicate that a combined evaluation of these biomarkers may aid in differentiating various thyroid functioning states. This explanation aligns with prior publications detailing connections between inflammatory and endocrine pathways in AITD.

Hyperthyroidism patients in this study showed significantly higher levels of IL-17, IL-6, and anti-TPO, with an average of (mean ± SD) (61.70 ± 29.87, 44.95 ± 26.38, and 255.10 ± 144.6), respectively. High levels of TG were observed in hypothyroidism patients, with 31.21 ± 38.23.

Patients with hyperthyroidism demonstrated markedly elevated serum levels of IL-6, IL-17, and anti-TPO compared to euthyroid controls. These findings align with those of Lu, et al. (2023), who reported increased levels of IL-6 and IL-17 in Graves' disease, indicating the activation of Th17-associated inflammatory pathways. He, et al. (2025) similarly established that IL-17 serves a role in the inflammatory signaling correlated with thyroid autoimmunity. The elevated anti-TPO levels identified in this study agree with the research findings of Hussein, et al. (2025), who documented increased humoral autoimmune responses in individuals with autoimmune hyperthyroidism. Collectively, these results support the correlation between inflammatory activation and autoimmune thyroid dysfunction.

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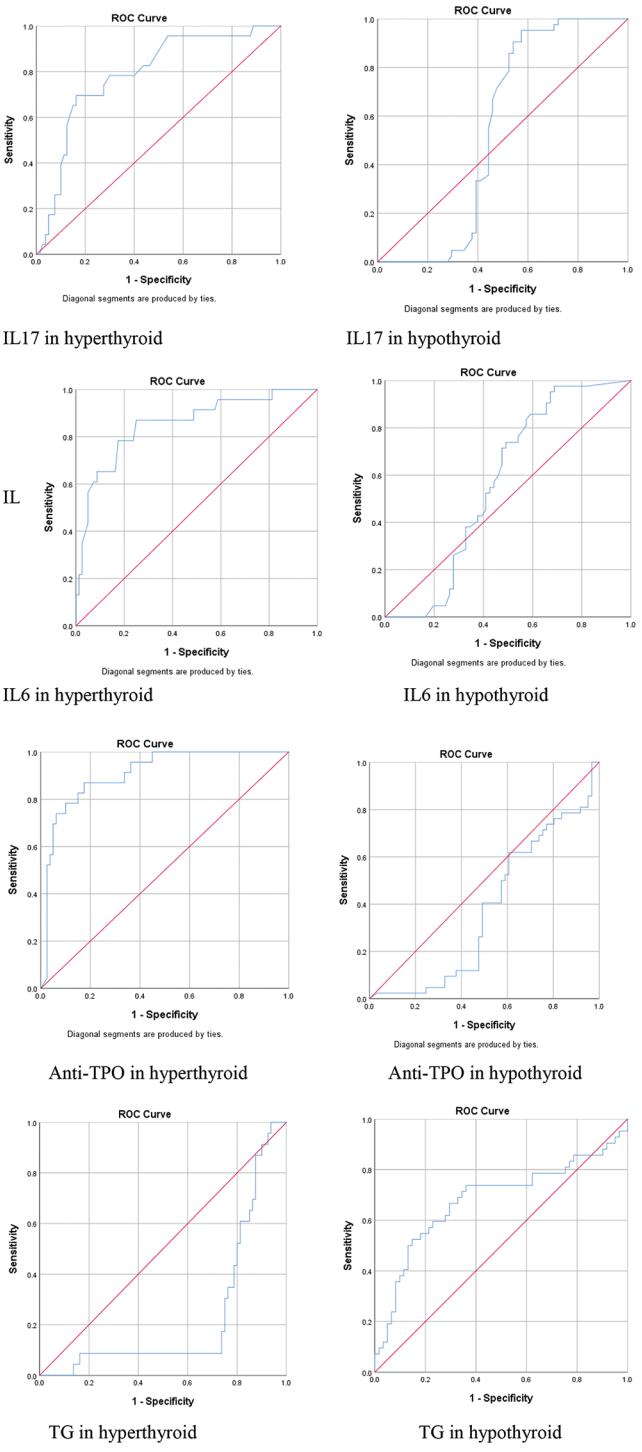

Fig. 4. Receiver operating characteristic analysis for interleukin (IL)-17, IL-6, anti-thyroid peroxidase, and thyroglobulin.

TABLE VI
Likelihood Ratio Tests for Each Predictor

Predictor	−2Log likelihood	$\Delta\chi^2$	p-value
Intercept	171.413	48.774	<0.001***
IL-17	138.634	15.995	<0.001***
IL-6	150.339	27.700	<0.001***
Anti-TPO	144.249	21.610	<0.001***
TG	148.900	26.261	<0.001***

IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase

On the other hand, hypothyroid patients have highly increased levels of TG (mean ± SD) (31.21 ± 38.23), which are a consequence of a destructive autoimmune process seen in Hashimoto thyroiditis, where follicles become disrupted, causing hormone deficiency with antigen leakage into the circulation and eventual pituitary activation implications (Hashim and Saeed, 2024). These descriptive profiles together portray two different immunological phenotypes of the AITD spectrum: A cytokine-dominant, stimulatory profile as seen in G and a damage-dominant, degenerative profile observed in hypothyroidism. Therefore, the immune and endocrine signatures discovered in our work not only differentiate the two thyroid functional states but also reflect the mechanistic pathways to develop the main clinical subtypes of AITDs.

By contrast, these findings are in concordance with clinical and mechanistic reports of Hashimoto thyroiditis, in which TPO–directed autoimmunity is accompanied by progressive structural damage associated with reduced T3 and T4 levels (Hashim and Saeed, 2024).

The significant variations revealed by the Kruskal–Wallis test demonstrate that inflammatory and endocrine biomarkers varied systematically among thyroid function categories. Naser and Mahmood (2024) have found analogous findings, indicating increased IL-6 levels in patients with AITDs. Our results reinforce prior research indicating that IL-17 levels are much higher in hyperthyroidism compared to euthyroid people (He, et al., 2025).

This evidence is consistent with findings that IL-17 promotes T-cell activation and autoantibody production, which contribute to maintaining a state of thyroid overactivity (He, et al., 2025, Hussein, et al., 2025). In contrast, the disparities observed between hypothyroid and euthyroid prepared individuals were largely due to rises in TG and TSH reflecting structural gland damage secondary to rather than macrophage-triggered proinflammatory stimulation. This discrepancy between hyperthyroid and hypothyroid is therefore an immunological dichotomy: in one case, dominant stimulatory cytokine signaling and antibody-mediated activation; in the other, destructive autoimmunity leading to gland failure.

In this study, the correlation analyses additionally delineate how immune markers mechanistically relate to thyroid function. The positive correlation between IL-6 and T3 and the negative association with TSH imply that IL-6 may participate in the development of a hypermetabolic status observed in autoimmune hyperthyroidism. Although the correlation with IL-17 is not an independent predictor in hypothyroidism, the increase in its levels in hyperthyroidism and reported capacity to induce IL-6 production as well as B-cell differentiation may suggest that this cytokine influences a cascade mechanism contributing to both inflammation and hormonal derangement (Wrońska, Hałasa and Szczuko, 2024). The correlation of anti-TPO and TG with IL-6 and IL-17, which were found between cytokines and autoantibodies, suggests the more likely interpretation that a differentiation in B-cell activation through cytokines is associated with the production of autoantibodies in

TABLE VII
MULTINOMIAL LOGISTIC REGRESSION COEFFICIENTS FOR THYROID STATUS

Predictor	Hyperthyroid versus euthyroid			Hypothyroid versus euthyroid		
	B	p	OR (95% CI)	B	p	OR (95% CI)
IL-17	0.060	**0.008****	1.06 (1.02–1.11)	−0.012	0.280	0.99 (0.97–1.01)
IL-6	0.166	**0.001*****	1.18 (1.07–1.30)	0.071	**0.021***	1.07 (1.01–1.14)
Anti-TPO	0.012	**0.010****	1.01 (1.00–1.02)	−0.007	0.052†	0.99 (0.99–1.00)
TG	−0.121	**0.045***	0.89 (0.79–1.00)	0.026	**0.014***	1.03 (1.01–1.05)

*,**,***: Significant levels. Reference category: Euthyroid, †borderline significant. OR: Odds ratio, CI: Confidence interval, IL: Interleukin, TG: Thyroglobulin, TPO: Thyroid peroxidase.

AITD. This corroborates previous data demonstrating that proinflammatory cytokines participate in the generation of thyroid autoantibodies and also modulate disease activity (Saheb and Mahmood, 2025).

Although analyses of ROC were not the main focus in this study, these ROC results suggest that IL-6 and IL-17 could have diagnostic utility to differentiate thyroid functional statuses. These results are consistent with new data indicating that cytokine profiling might represent an additional diagnostic tool in assessing autoimmune thyroid involvement and in revealing early or indeterminate cases. In light of their involvement in disease mechanisms, IL-6 and IL-17 may also be useful as markers to determine disease activity or shift between hyperfunctional and hypofunctional states.

The results in combination support a comprehensive mechanistic model where hyperthyroidism is characterized as a cytokine-driven, hyperinflammatory state, and hypothyroidism is a damage-dominant phenotype with structural destruction and increased TG but decreased hormone synthesis. These findings support the notion that AITD may represent a continuum characterized by immune-endocrine interactions, and that combined profiling of cytokines, autoantibodies, and hormonal markers provides a more complete overview of disease dynamics. This research has several strengths, including the simultaneous assessment of multiple biomarkers and the application of appropriate statistical tests for non-normally distributed biological data.

V. Conclusion

This study shows that thyroid dysfunction in women is associated with an integrated pattern of biochemical changes, including inflammatory, autoimmune, and endocrine markers, and not just one biochemical change.

By combining IL-6, IL-17, Anti-TPO, TG, and thyroid hormone cytometric data into a unique model-based analysis, we were able to identify two well-separated immune-endocrine phenotypes. Hyperthyroid patients exhibited a cytokine-dominant pattern with high IL-6, IL-17, and anti-TPO, indicative of stimulatory autoimmune activation and an increase in inflammatory signaling. Conversely, hypothyroid patients demonstrated a damage-dominant phenotype characterized by significantly higher TSH and TG levels; these results are consistent with follicular damage and Hashimoto's disease.

The overall set of correlation, ROC, and multinomial regression analyses confirmed together that multi-marker profiling provides additional discriminatory power compared to single biomarkers, and reveals the anti-TPO/IL-6 discriminative relevance in hyperthyroidism as well as the strong relationship between TG and hypothyroid condition. These results further support the notion that AITD is a continuum characterized by integrated immune and hormonal responses, and emphasize the importance of understanding these interactions for better understanding the cause-and-effect nature of our measured outcomes.

Finally, integrated measurement of biomarkers improves contemporary knowledge on thyroid autoimmunity in women, to provide a more accurate basis for differential diagnosis, powerful stratification, and also potential clinical follow-up. Validation of these patterns and translation into risk stratification and clinical applications will require future longitudinal studies integrating broader cytokine panels with genetics.

VI. Limitations and Future Work

There are a number of limitations to this study. Causal inference is limited by the cross-sectional design, and Th17/Treg immunophenotyping was not carried out. Furthermore, the results may not be entirely applicable to men because the study sample was predominantly female, which reflects the higher prevalence of autoimmune thyroid problems in females. Future longitudinal research should include genetic analysis of the IL-6 and IL-17 pathways, broader immunological profiling (including IL-23 and IL-1β), serial cytokine measures, and assessment of the impact of body mass index and other metabolic parameters on thyroid autoimmunity and cytokine regulation.

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