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Serum Interleukin-23 Levels as inflammatory biomarker in Patients with Thyroid Dysfunction

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Abstract

Autoimmune thyroid diseases are characterized by immune dysregulation and chronic inflammation mediated by cytokine networks. Interleukin-23 (IL-23) is a pro-inflammatory cytokine that plays a key role in the differentiation and maintenance of T helper 17 (Th17) cells, which are involved in autoimmune responses. The present study aimed to evaluate serum IL-23 levels in patients with hypothyroidism (HYPO), hyperthyroidism (HYPER), and healthy controls. Serum IL-23 concentrations were measured using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The results showed that the mean IL-23 levels were 15.33 ± 8.95 pg/mL in the HYPO group, 26.95 ± 23.28 pg/mL in the HYPER group, and 21.43 ± 11.39 pg/mL in the control group. Statistical analysis using one-way ANOVA revealed a significant difference among the study groups ($F = 4.803$, $P = 0.011$). Post hoc Tukey analysis demonstrated a significant increase in IL-23 levels in the hyperthyroid group compared with the hypothyroid group, while no significant differences were observed between the control group and either patient group. These findings suggest that IL-23 may contribute to immune activation associated with hyperthyroidism and may play a role in the immunopathogenesis of thyroid

disorders. The IL-23/Th17 axis could therefore represent a potential biomarker and therapeutic target in autoimmune thyroid diseases.

Keywords: Interleukin-23, IL-23, Thyroid disorders, Hyperthyroidism, Hypothyroidism, Autoimmune thyroid diseas

Introduction

In recent years, increasing attention has been directed toward the role of interleukin-23 (IL-23) and its associated signaling pathways in autoimmune diseases. IL-23 is a heterodimeric cytokine composed of two subunits, p19 and p40, and is primarily produced by activated antigen-presenting cells such as dendritic cells and macrophages. It belongs to the IL-12 cytokine family and plays a crucial role in bridging innate and adaptive immunity (7).

One of the most important biological functions of IL-23 is its involvement in the differentiation, expansion, and maintenance of T helper 17 (Th17) cells, a subset of $CD4^+$ T cells characterized by their production of pro-inflammatory cytokines such as IL-17, IL-21, and IL-22 (2).

The IL-23/Th17 axis has emerged as a key pathway in the pathogenesis of several autoimmune and inflammatory disorders, including multiple sclerosis, rheumatoid arthritis, psoriasis, and inflammatory bowel disease. In thyroid autoimmunity, this pathway contributes to chronic inflammation and immune-mediated tissue damage within the thyroid gland. IL-23 promotes the survival and pathogenic activity of Th17 cells, which in turn produce IL-17, a potent inflammatory cytokine that recruits neutrophils and stimulates the production of other inflammatory mediators, thereby amplifying local inflammation (6).

Several studies have reported elevated levels of IL-23 and IL-17 in patients with autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease. IL-23 enhances Th17 responses, leading to increased inflammatory cytokine production and cytotoxic effects on thyroid epithelial cells. Furthermore, IL-23 may inhibit regulatory T cells (Treg), which normally maintain immune tolerance, thereby contributing to autoimmune processes (1).

Given these immunological roles, IL-23 has been proposed as a potential biomarker for disease activity and therapeutic response in autoimmune disorders. Moreover, targeting the IL-23/Th17 pathway has emerged as a promising therapeutic approach in inflammatory diseases, suggesting potential applications in autoimmune thyroid diseases (8).

Methodology

Measurement of IL-23 by ELISA

Serum IL-23 concentrations were measured using a **quantitative sandwich enzyme-linked immunosorbent assay (ELISA)**.

Principle of the Assay

The ELISA kit uses the sandwich technique, in which microplate wells are pre-coated with a monoclonal antibody specific for human IL-23. Serum samples or standard solutions are added to the wells, allowing IL-23 present in the sample to bind to the immobilized antibody.

Following incubation, a detection antibody specific for IL-23 and conjugated with horseradish peroxidase (HRP) is added. After washing steps to remove unbound components, tetramethylbenzidine (TMB) substrate solution is added to each well.

The enzymatic reaction produces a blue color that turns yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at **450 nm**, and IL-23 concentrations are calculated by comparing the OD values of the samples with those obtained from the standard calibration curve(9).

Assay Conditions

- Sample volume: **50 µL of serum**
- ELISA type: **Quantitative sandwich ELISA**
- Detection target: **Human IL-23**
- Assay duration: **Approximately 120 minutes**
- Kit specificity: **Specific for IL-23 detection**

The assay procedure was performed according to the manufacturer's instructions, and the dilution and preparation procedures were conducted similarly to those used in the TG-Ab ELISA protocol.(10)

Results and Discussion

The present study evaluated serum IL-23 levels in patients with hypothyroidism (HYPO), hyperthyroidism (HYPER), and healthy controls. The results presented in **Table (1-1)** indicate that the mean IL-23 concentration was **15.33 ± 8.95 pg/mL** in the HYPO group, **26.95 ± 23.28 pg/mL** in the HYPER group, and **21.43 ± 11.39 pg/mL** in the control group.

Statistical analysis using **one-way ANOVA** revealed a significant difference among the three groups ($F = 4.803$, $P = 0.011$), indicating that IL-23 levels vary according to thyroid functional status.

Post hoc Tukey analysis demonstrated that there was **no significant difference** between the HYPO and control groups, and no significant difference between the

control and HYPER groups. However, a **significant increase in IL-23 levels was observed in the hyperthyroid group compared with the hypothyroid group**.

Table (1-1): Serum IL-23 levels in the study groups

Groups	Mean	S.D	S.E	F-Value	P-Value
HYPO (n=40)	15.3270[a]	8.9544	1.4158	4.803	0.011*
HYPER (n=26)	26.9527[b]	23.2794	4.5655		
Control (n=24)	21.4263[ab]	11.3879	2.3245		

S.D. = Standard Deviation

S.E. = Standard Error

Means sharing the same letter are **not significantly different** according to Tukey's test. ***Significant at P ≤ 0.05.***

The results indicate that IL-23 levels are significantly altered in thyroid disorders, with higher levels observed in hyperthyroid patients compared with hypothyroid patients. This finding supports the hypothesis that inflammatory pathways, particularly the **IL-23/Th17 axis**, are involved in thyroid dysfunction.

The elevated IL-23 levels in hyperthyroidism may reflect enhanced immune activation associated with autoimmune hyperthyroidism such as Graves' disease. Previous studies have reported increased IL-23 and Th17 activity in autoimmune thyroid diseases, suggesting their involvement in disease pathogenesis (4., 6).

The absence of a significant difference between the control and hyperthyroid groups may be explained by the relatively large variability observed in the hyperthyroid group, as indicated by the high standard deviation. This variability may be related to differences in disease severity, duration, or treatment among patients.

In contrast, the hypothyroid group demonstrated the lowest IL-23 levels, suggesting a lower inflammatory state compared with hyperthyroidism. However, the lack of significant difference between hypothyroid patients and healthy controls indicates that IL-23 may not play a major role in all forms of hypothyroidism, particularly non-autoimmune hypothyroidism. Previous studies have reported that cytokine profiles vary depending on whether hypothyroidism is autoimmune in origin, such as in Hashimoto's thyroiditis (3).

Overall, the significant difference between hypothyroid and hyperthyroid groups highlights the distinct immunological environments associated with these conditions. Thyroid hormones themselves may influence immune responses and cytokine production, thereby modulating inflammatory pathways (5)

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