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Disrupted Immune Balance in Graves' Disease: Evaluation of IL-35, CXCL10, and IL-4

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Abstract

Graves' Disease (GD) is driven by pathogenic TSH receptor antibodies (TRAb), yet the underlying imbalance between inflammatory (Th1/Th2) and regulatory (Treg) immune responses remain complex. Traditionally viewed as a Th2-mediated condition, it is also characterized by lymphocytic infiltration and inflammation in the thyroid and orbit.

The study aims to investigate the immunological profile of patients with autoimmune hyperthyroidism by concurrently measuring IL-35, IL-4, and CXCL10 and to determine their diagnostic utility.

A case-control study with 90 participants—60 newly diagnosed, untreated GD patients and 30 healthy controls matched by age and sex—measured serum IL-4, CXCL10, and IL-35 levels using enzyme-linked immunosorbent assay (ELISA). Nonparametric tests, including the Mann-Whitney U test and ROC analysis, were used to evaluate the diagnostic performance of the biomarkers.

The study demonstrated that patients exhibited significantly elevated levels of IL-4 and CXCL10, with median values of approximately 575.20 ng/L and 193.40 ng/L, respectively, compared to healthy controls. Conversely, IL-35 levels were notably reduced in patients, with a median of 153.30 ng/L, compared to 471.30 ng/L in controls.

Our study reveals a distinct triple immune imbalance in GD patients, characterized by elevated CXCL10 and IL-4 levels and decreased IL-35 levels. These findings enhance understanding of GD's immune pathogenesis and suggest potential therapeutic targets.

Keywords: Graves' Disease; CXCL10, Interleukin-4 Cytokines, Interleukin-35.

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Introduction

Hyperthyroidism is a condition characterized by elevated thyroid hormone levels (triiodothyronine and thyroxine) and low TSH levels, with the most common etiology being Graves' disease (GD), characterized by the formation of thyrotropin receptor antibodies (TRAb) (1,2).

Traditionally, GD has been categorized as a Th2 disease, in which cytokines, including IL-4, IL-5, and IL-13, are secreted, primarily promoting B cell production of autoantibodies against the TSH receptor and causing overstimulation of the thyroid gland. However, recent literature also discussed the lymphocytic infiltration and tissue inflammation often observed in the thyroid gland and orbit (3,4).

CXCL10 or interferon gamma–inducible protein 10 (IP-10) is a proinflammatory chemokine for Th1 cells and serves as the hallmark of Th1 involvement in Graves' disease. CXCL10 is stimulated by IFN-γ and TNF-α, leading to the recruitment of additional Th1 lymphocytes into the thyroid (5,6).

In contrast, IL-35, a novel member of the IL-12 cytokine family, is the immunosuppressive component of the response and comprises two subunits: the p35 (IL-12α) subunit and the Epstein–Barr virus-induced gene 3 (EBI3) protein. (7). IL-35 is secreted by Tregs and Bregs and is central to the maintenance of peripheral tolerance; dysregulation of the IL-35 axis in Tregs has been implicated in many autoimmune diseases (7).

The study aims to investigate the immunological profile of patients with autoimmune hyperthyroidism by concurrently measuring IL-35, IL-4, and CXCL10.

Material and Methods:

Study design

A case-control study was conducted at the University of Fallujah, and samples were collected at the Endocrinology and Diabetes Center in Baghdad, Iraq, and Al-Kindi Teaching Hospital, from August 2025 to October 2025. The study protocols and sample collection were approved by the Institutional Review Board of the University of Fallujah (AS-EC/0001). This study was conducted in compliance with the ethical standards of the Helsinki Declaration. Written informed consent was obtained from all participants before data collection.

Study population

The total study population included 90 participants. The patient group consisted of 60 individuals with confirmed autoimmune hyperthyroidism.

Inclusion Criteria:

Patient Group: Patients were included if they had a new diagnosis of Graves' Disease (GD) and were treatment-naïve (no prior anti-thyroid medications, radioactive iodine, or surgery). The diagnosis was based on thyroid function tests, including suppressed TSH level and elevated thyroid hormone levels, along with positive TRAb status and clinical features consistent with the 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism (8).

Control Group: The control group included 30 age- and sex-matched euthyroid volunteers with no personal or family history of autoimmune disease.

Exclusion Criteria: To maintain the integrity of the cytokine profiles, patients with other known

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autoimmune disorders or chronic inflammatory conditions, individuals with active infections, malignancy, or those currently receiving corticosteroids or immunomodulatory therapies. In addition, pregnant or lactating women were excluded.

Laboratory investigations

All participants underwent venous blood sampling, and the samples were centrifuged and stored at -80 °C until further analysis. Thyroid function tests were measured using an automated chemiluminescence immunoassay ARCHITECT (Abbott, USA). TRAb levels were quantified using a 3rd generation competitive inhibition ELISA kit (RSR Ltd., Cardiff, UK). Serum levels of IL-4, IL-35, and CXCL10 were measured using quantitative sandwich ELISA kits (Elabscience, TX, USA). Following the manufacturers' protocols.

Statistical analysis: Data were analyzed using SPSS version 26.0 (IBM Corp., USA). According to the results of the Kolmogorov–Smirnov and Shapiro–Wilk tests, immunological and hormonal variables exhibited non-normal distributions ($p < 0.05$). Based on these findings, non-parametric statistical tests were considered more appropriate for subsequent analyses. The Mann-Whitney U test was used to compare differences between the patient and control groups, and ROC curve analysis was used to evaluate the diagnostic performance of thyroid hormonal and immunological biomarkers.

Results:

A total of 90 participants were included in the study, comprising 60 untreated, newly diagnosed patients with Graves' disease and 30 healthy controls. The peak age group of patients and controls was 40-59 years (55.0%). The female gender showed a predominance in the patient group (75.0%), with a female-to-male ratio of 3:1 (Table 1).

Patients with untreated GD had lower TSH levels than controls (median 0.10 mIU/L), whereas T3 and T4 were higher (5.22 nmol/L and 187.9 nmol/L, respectively) than in the control group (1.38 nmol/L and 87.5 nmol/L, respectively). Similarly, all TRAb levels were positive for the patient group (median 1.40 IU/L) (Table 1).

Comparison of IL-4, IL-35 and CXCL10 Cytokines Between Patients and Control Groups

Analysis of the cytokine profile showed a significant elevation in IL-4 in the patient cohort (median: 575.20 ng/L) compared with healthy controls (median: 328.90 ng/L). Similarly, CXCL10 concentrations were markedly higher in patients (median: 193.40 ng/L) than in the control group (median: 89.50 ng/L) (Table 2). Conversely, patients exhibited a pronounced reduction in IL-35, with median levels approximately threefold lower (153.30 ng/L) than those observed in healthy controls (471.30 ng/L) (Table 2).

Diagnostic performance of IL-4, IL-35, and CXCL10 Cytokines based on ROC curve analysis

The results of the current study establish an excellent diagnostic accuracy for most parameters. The immunological biomarkers, IL-35 (AUC = 0.997), IL-4 (AUC = 0.977), and CXCL10 (AUC = 0.958) demonstrated excellent diagnostic validity, with sensitivities of 94.28%, 92.11%, and 98.28%, and specificities of 99.67%, 95.37%, and 93.61%, respectively. (Figure 1)

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Table 1: Demographic and hormonal parameters in GD patients and control groups.

	Untreated GD	Healthy Control	P value
N	60	30	
Male/Female	15/45	6/24	0.597
Age [a]	46.8 ±2.8	45.0 ±2.6	0.133
TSH (mIU/L) [b]	0.10 (0.01 - 0.15)	1.8 (0.81-3.24)	<0.001*
T3 (nmol/L) [b]	5.22 (1.24-12.80)	1.38 (1.00-1.90)	<0.001*
T4 (nmol/L) [b]	187.9 (114.5-423.4)	87.5 (64.9-122.0)	<0.001*
TRAb (IU/L) [b]	1.40 (0.50-2.53)	0.31 (0.23-0.40)	<0.001*

a: Data expressed as mean and standard deviation.
b: Data expressed as median and lower and upper percentiles.
**Significant difference between groups (p value < 0.05).*
TSH, thyroid-stimulating hormone; TRAb, TSH receptor autoantibody

Table 2: Comparison of IL-4, IL-35, and CXCL10 Cytokines Between Patients and Control Groups.

	Untreated GD	Healthy Control	P value
IL-4 (ng/L)	575.2 (340.1-983.0)	328.9 (228.8-430.4)	<0.001*
CXCl10 (ng/L)	193.4 (94.1-317.8)	89.5 (41-146.5)	<0.001*
IL-35 (ng/L)	153.3 (64.5-280.2)	471.3 (211.5-877.4)	<0.001*

Data expressed as median (25th–75th percentiles).
** Significant difference between groups (p < 0.05).*
IL, Interleukin; CXCL10, C-X-C motif chemokine ligand 10.

Correlation of IL-4, IL-35, and CXCL10 markers in patients with Graves' disease.

Spearman's correlation analysis was performed to examine the relationships between regulatory and pro-inflammatory markers. The cytokine profile shows that IL-4 correlates positively with TRAb ($r = 0.473$, $p < 0.01$) and negatively with IL-35 ($r = -0.558$, $p < 0.01$). Meanwhile, CXCL10 shows a positive correlation with TRAb ($r = 0.622$, $p < 0.01$) and an inverse correlation with IL-35 ($r = -0.611$, $p < 0.01$). A significant positive correlation was found between IL-4 and CXCL10 ($r = 0.585$, $p < 0.001$). (Table 3)

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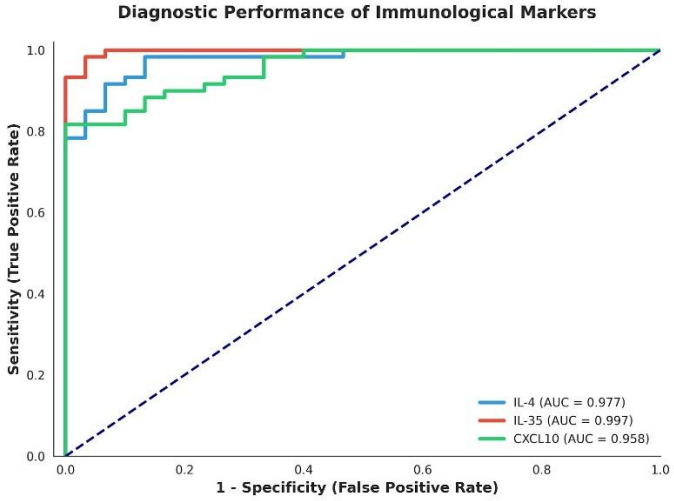

Figure 1: Diagnostic performance of IL-4, IL-35, and CXCL10 Cytokines based on ROC curve analysis.

Table 3: Spearman's correlation coefficients between immunological markers in patients with Graves' disease.

Spearman Correlation	TRAb (IU/L)	IL-4 (ng/L)	IL-35 (ng/L)	CXCL10 (ng/L)
IL-4 (ng/L)	.473**	-	-.558**	.585**
IL-35 (ng/L)	-.562**	-.558**	-	-.611**
CXCL10 (ng/L)	.622**	.585**	-.611**	-
TRAb (IU/L)	-	.473**	-.562**	.622**

Note: IL, Interleukin; CXCL10, C-X-C motif chemokine ligand 10; TRAb, TSH receptor antibody.
***** Correlation is significant at the 0.01 level (2-tailed).***

Discussion

The present study investigated the cytokine imbalance in patients with autoimmune hyperthyroidism (Graves' disease). Our findings revealed a distinct triple imbalance in untreated Graves' disease (GD) patients. The IL-35 levels showed a significant three-fold decrease in the patient group, with the elevated medians of both Th2 cytokine (IL-4) and Th1 cytokine (CXCL10). This triple interaction can reflect the Th1/Th2 pro-inflammatory state, along with regulator failure in compensation, suggesting that the interplay between these pathways plays a central role in disease pathogenesis.

Higher IL-4 levels detected in our cohort support the inflammatory role of this cytokine in promoting B-cell activation, IgG class switching, and production of thyroid-stimulating antibodies, key mediators of hyperthyroidism in GD. The previous studies highlighted that IL-4 contributes to the humoral (Th2) arm of GD pathogenesis (2,4).

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Alongside the positive association between IL-4 and TRAb ($r = 0.473$, $p < 0.01$), this further supports the activation of B-cells by the Th2 cytokines.

Our current study also showed an increased level of CXCL10 chemokine in the patient group compared to the control. CXCL10 is recognized as the hallmark of Th1-driven autoimmune inflammation seen in GD, induced by IFN-γ and TNF-α, and recruits $CXCR3^+$ Th1 lymphocytes into thyroid tissue, and has been shown to decrease upon antithyroid medication uptake (9,10).

On the other hand, the regulatory cytokine IL-35 was markedly depleted in patients with untreated GD. IL-35 is mainly secreted by T regulatory cells (Treg) and B regulatory cells (Breg), exerts an anti-inflammatory effect, such as inhibiting effector T-cell proliferation, and helps maintain the integrity of peripheral tolerance (11). Some studies reported that IL-35 has shown a decrease in several autoimmune diseases, and more recently in GD (12–14). Integrating our findings with prior data suggests that low circulating IL-35 levels may weaken or contribute to reduced regulatory control over Th1 and Th2 cells, thereby sustaining the inflammatory attack and autoantibody production. While the strong negative correlation between IL-35 and TRAb titers suggests that IL-35 suppression is a key driver of autoimmunity

The ROC analysis of our cytokines demonstrated notable diagnostic accuracy for differentiating GD from healthy controls. For example, IL-35 showed a high accuracy (AUC = 0.997) and a high specificity (99.67%), suggesting that suppressed IL-35 levels are characteristic of the active autoimmune state in GD. Furthermore, CXCL10 exhibits high sensitivity (98.28%) in the early inflammatory phase, making it a reliable marker for screening active thyroiditis.

Limitations of the study

A limitation of this study is the use of strictly healthy, euthyroid controls rather than diseased controls (such as patients with toxic multinodular goiter). This spectrum contrast may overestimate the diagnostic accuracy (AUC) of the cytokines. Future studies incorporating hyperthyroid disease controls are required to better define the clinical specificity of IL-35 and CXCL10.

Conclusion

In conclusion, our current study identifies a distinct and complex immune imbalance in GD patients characterized by elevated levels of measured CXCL10 and IL-4, along with reduced regulatory levels of IL-35. These findings can help elucidate the immune pathogenesis of GD and highlight its potential as a therapeutic target.

Ethical considerations:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The Ethics Committee of the University of Fallujah, College of Applied Science, approved the study protocol (AS-EC/0001).

Author contributions

M.N.A: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft. R.J.A: Supervision, Validation, Writing, Review & Editing.

Conflict of Interest: The authors declare that they have no competing interests.

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References:

1. Chaker L, Cooper DS, Walsh JP, *et al.* Hyperthyroidism. *Lancet.* 2024 Feb 24;403(10428):768-80. https://doi.org/10.1016/S0140-6736(23)02016-0
2. Yao P, Xie Y, Wang Y, *et al.* Comparison between thyroid-stimulating immunoglobulin and TSH-receptor antibodies in the diagnosis and management of Graves' disease. *Front Endocrinol* (Lausanne). 2024;15:1487490. https://doi.org/10.3389/fendo.2024.1487490
3. Li Q, Wang B, Mu K, Zhang JA. The pathogenesis of thyroid autoimmune diseases: New T lymphocytes – Cytokines circuits beyond the Th1−Th2 paradigm. *J Cell Physiol*. 2018 Mar;234(3):2204-16. https://doi.org/10.1002/jcp.27180
4. Kustrimovic N, Gallo D, Piantanida E, *et al*. Regulatory T Cells in the Pathogenesis of Graves' Disease. *Int J Mol Sci.* 2023;24(22):16432. https://doi.org/10.3390/ijms242216432
5. Ferrari SM, Paparo SR, Ragusa F, *et al*. Chemokines in thyroid autoimmunity. *Best Pract Res Clin Endocrinol Metab*. 2023;37(2):101773. https://doi.org/10.1016/j.beem.2023.101773
6. Antonelli A, Ferrari SM, Giuggioli D, *et a*l. Chemokine (C–X–C motif) ligand (CXCL)10 in autoimmune diseases. *Autoimmun Rev*. 2014;13(3):272-80. https://doi.org/10.1016/j.autrev.2013.10.010
7. Ye C, Yano H, Workman CJ, Vignali DAA. Interleukin-35: Structure, Function and Its Impact on Immune-Related Diseases. *J Interferon Cytokine Res*. 2021;41(11):391-406. https://doi.org/10.1089/jir.2021.0147
8. Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. *Thyroid*. 2016;26(10):1343-421. https://doi.org/10.1089/thy.2016.0229
9. Hiratsuka I, Itoh M, Yamada H, et al. Simultaneous measurement of serum chemokines in autoimmune thyroid diseases: possible role of IP-10 in the inflammatory response. *Endocr J.* 2015;62(12):1059-66. https://doi.org/10.1507/endocrj.EJ15-0448
10. Fallahi P, Ferrari SM, Elia G, et al. Cytokines as Targets of Novel Therapies for Graves' Ophthalmopathy. *Front Endocrinol (Lausanne).* 2021;12:654473. https://doi.org/10.3389/fendo.2021.654473
11. Li Q, Yang C, Liu C, et al. The circulating IL-35+ regulatory B cells are associated with thyroid-associated ophthalmopathy. Immun *Inflamm Dis.* 2024 May;12(5):e1304. https://doi.org/10.1002/iid3.1304
12. Su LC, Liu XY, Huang AF, et al. Emerging role of IL-35 in inflammatory autoimmune diseases. *Autoimmun Rev*. 2018;17(7):665-73.https://doi.org/10.1016/j.atrev.2018.01.017
13. Saeed MH, Kurosh K, Zahra A, et al. Decreased serum levels of IL-27 and IL-35 in patients with Graves' disease. *Arch Endocrinol Metab.* 2020;64(5):521-7. https://doi.org/10.20945/2359-3997000000227
14. Ye Z, Jiang Y, Sun D, et al. The Plasma Interleukin (IL)-35 Level and Frequency of Circulating IL-35+ Regulatory B Cells are Decreased in a Cohort of Chinese Patients with New-onset Systemic Lupus Erythematosus. *Sci Rep.* 2019;9(1):13210. https://doi.org/10.1038/s41598-019-49748-z