

RESEARCH ARTICLE

Decreased serum interleukin-41/Metrnl levels in patients with Graves' disease

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Abstract

Background: Interleukin (IL)-41, also known as Metrnl, is a novel immunomodulatory cytokine, which is involved in the pathogenesis of many inflammatory and metabolic diseases, but its role in thyroid autoimmune diseases is not clear. The aim of this study was to evaluate the serum IL-41 levels in patients with Graves' disease (GD) and its relationship with GD.

Methods: This study included a total of 49 GD patients and 47 age- and sex-matched healthy individuals. All baseline data were obtained by physical examination. Free triiodothyronine 3 (FT3), free triiodothyronine 4 (FT4), thyroid-stimulating hormone (TSH), anti-thyroglobulin antibodies (TgAb), thyroid peroxidase antibody (TPOAb), and thyrotropin receptor antibody (TRAb) levels in plasma of GD patients were measured by chemiluminescence. The high-sensitivity C-reactive protein (CRP) and white blood cell count (WBC) were detected using automated biochemical analyzer. Serum IL-41 levels were measured by enzyme-linked immunosorbent assay.

Results: Serum IL-41 levels in patients with GD were significantly lower than those in healthy controls (201.0 vs. 260.8 pg/mL, $p < 0.05$). There was a significant positive correlation between IL-41 level and CRP ($r = 0.2947$, $p = 0.0385$) and WBC ($r = 0.4104$, $p = 0.0034$) in GD patients. CRP was positively correlated with TRAb ($r = 0.2874$, $p = 0.0452$) and TSH ($r = 0.3651$, $p = 0.0099$) levels in GD patients.

Conclusions: This study demonstrates that GD patients have decreased serum IL-41 levels, and IL-41 plays a potential role in abnormal immune response of GD patients.

KEYWORDS

autoimmune thyroid disease, Graves' disease, interleukin-41, Metrnl

1 | INTRODUCTION

It is well known that Graves' disease (GD) is recognized as a representative disease of clinical hyperthyroidism.¹ The peak incidence of GD is between the ages of 30 and 50 and six times more common in women than in men. The immune disorder of GD leads to

thyroid-stimulating hormone (TSH) receptor antibody (TRAb) to stimulate thyroid, which makes thyroid follicular cells proliferate and increases the secretion of thyroid hormone.² Excessive thyroid hormone production can lead to abnormal metabolism of the body.³ The occurrence of GD is mainly attributed to genetic influences and environmental triggers, as well as the resulting abnormal immune

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function. However, none of these theories can fully explain the pathogenesis of GD, which has also led to limited therapeutic modalities for GD.⁴ Although the pathogenesis of GD remains elusive, it is well accepted that the immune response plays a key role in the development of GD, involving a variety of cytokines.⁵

Cytokines participate in immune response and the pathogenesis of autoimmune diseases. Serum cytokines, including interleukin (IL)-6, IL-10, IL-27, IL-29, IL-35, IL-36, IL-37, and tumor necrosis factor (TNF)- α , have been reported to be abnormally expressed in GD and can influence its severity.⁶⁻⁹ IL-41,^{10,11} also named Metrnl, Cometin, Subfatin, Meteorin (Metrn)-like, or Meteorin- β , is expressed in many tissues, with the most notable expression in subcutaneous white adipose tissue, nervous system, and barrier tissues, such as skin, intestinal, and respiratory epithelium. In addition, IL-41 can also be expressed in immune cells, including activated macrophages. IL-41 was discovered as a neurotrophic factor in 2004.¹² As a newly discovered adipokine, IL-41 contains 311 amino acids, and the N-terminal signal peptide is 45 amino acids. Previous studies have confirmed that IL-41 is involved in the development of metabolic and inflammatory diseases, such as type 2 diabetes (T2D),^{13,14} coronary heart disease,¹⁵ colitis,^{16,17} psoriasis, and arthritis.¹¹ In these diseases, IL-41 has shown to inhibit inflammation, improve metabolism, regulate adipose function, and reduce obesity-induced insulin resistance. In addition to acting as an anti-inflammatory cytokine, IL-41 also plays a role in acquired immunity, IL-41 involved in Th1, Th2, and Th17 immune responses.¹⁸ However, the role of IL-41 in GD is unknown.

In the present study, we compared serum levels of IL-41 in Chinese GD patients and healthy controls. Moreover, we analyzed the relationship between serum IL-41 and inflammatory indicators in these GD patients, which may provide new insights into the pathogenesis of GD.

2 | MATERIALS AND METHODS

2.1 | Participants' selection

Serum samples from 49 patients with GD were collected in The Affiliated Lihuili Hospital of Ningbo University from February 2020 to February 2021. GD was defined by: (1) diffuse goiter with soft or tough texture; (2) hyperthyroidism defined as: serum total triiodothyronine (TT3) > 2.45 nmol/L and/or free triiodothyronine 3 (FT3) > 5.70 pmol/L; total thyroxine (TT4) > 150.84 nmol/L and/or free thyroxine 4 (FT4) > 19.05 pmol/L; TSH < 0.35 mIU/L. The healthy control group (HC) was healthy individuals recruited from the Medical Examination Department of the same hospital and ensured that they were matched for sex and age with GD patients and have no history of GD or other autoimmune diseases. Informed consent from each subject and Ethics Committee approval were obtained from The Affiliated Lihuili Hospital of Ningbo University. All baseline data were obtained by physical examination.

2.2 | Measurement of thyroid indicators

Determination of TRAb level in human plasma was detected via *Staphylococcal* Protein A antigen sandwich method using the full-automatic chemiluminescence immunoassay analyzer (MAGLUMI4000plus; Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzhen, China). Thyroid hormones (FT3, FT4), TSH, anti-thyroglobulin antibodies (TgAb), thyroid peroxidase antibody (TPOAb) were measured using automated chemiluminescent immunoassays (i-2000; Abbott, IL, USA).

2.3 | Enzyme-linked immunosorbent assay (ELISA)

Blood samples were drawn from all recruited patients and control persons, centrifuged at 3500 g for 10 min to get serum, and frozen at -80°C . Human IL-41 in serum samples was quantified by ELISA kits (Jianglai Biotechnology Co., Ltd, Shanghai, China) according to the manufacturer's instructions. The procedure is as follows: 50 μl of sample and standard were added sequentially to wells pre-coated with IL-41 antibody, while blank wells were added with sample dilution, then 100 μl of HRP-labeled detection antibody was added to each well. The plates were covered with the adhesive strip provided, incubated for 60 min, and washed five times. Substrates A and B were then added at 50 μl each, incubated at 37°C for 15 min, and protected from light. Finally, 50 μl of stop solution was added, and the absorbance was measured at 450 nm within 15 min. The concentration of IL-41 was calculated from the standard curve. The intra- or the inter-assay coefficients of variation was <9 and <11%, respectively.

2.4 | Statistical analysis

All statistical analyses were performed using GraphPad Prism 9.0 software (La Jolla, CA, USA). Continuous data were presented as mean \pm SD. Count data were expressed as percentages. Differences between the two groups were compared using the *t* test, and the chi-squared test was used to compare count data. Pearson correlation test was used to assess the association between IL-41 levels and inflammatory indicators or thyroid indicators. A value of $p < 0.05$ was regarded as denoting statistical significance in all the analyses.

3 | RESULTS

3.1 | Characteristics of the GD and control groups

As shown in Table 1, a total of 96 subjects were enrolled in this study: 49 GD patients and 47 HC persons. There was no statistically significant difference between the two groups in terms of age, sex ratio, and also their inflammatory parameters such as WBC, CRP ($p > 0.05$). Compared with HC, GD patients had significantly higher

TABLE 1 Demographic and clinical characteristics of the participants

Parameter	HC (n = 47)	GD (n = 49)	t/χ^2	<i>p</i>
Female (n, %)	31, 65.9%	29, 59.1%	0.4696	0.4931
Age (years)	36.34 ± 11.85	36.84 ± 14.54	0.1829	0.8553
TSH (mIU/L)	1.79 ± 0.86	0.003 ± 0.007	14.56	<0.0001****
FT3 (pmol/L)	4.43 ± 0.44	12.59 ± 8.58	6.509	<0.0001****
FT4 (pmol/L)	12.83 ± 1.01	24.72 ± 7.75	10.43	<0.0001****
TgAb (IU/mL)	1.089 ± 0.71	328.6 ± 602.3	3.727	0.0003***
TPOAb (IU/mL)	0.33 ± 0.25	863.5 ± 973.8	6.075	<0.0001****
TRAb (IU/mL)	0.36 ± 0.20	15.43 ± 16.41	6.294	<0.0001****
WBC (10 ⁹ /L)	5.88 ± 1.36	5.59 ± 1.92	0.8283	0.4096
CRP (mg/L)	0.85 ± 0.54	1.28 ± 2.28	1.266	0.2086

Note: Compared with controls. ****p* < 0.001; *****p* < 0.0001, *p* values were calculated by *t* test, and chi-squared test.

Abbreviations: CRP, C-reactive protein; GD, Graves' disease group; HC, healthy control group; FT3, free triiodothyronine 3; FT4, free triiodothyronine 4; TgAb, anti-thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TRAb, TSH-receptor antibody; TSH, thyroid stimulating hormone; WBC, white blood cell count.

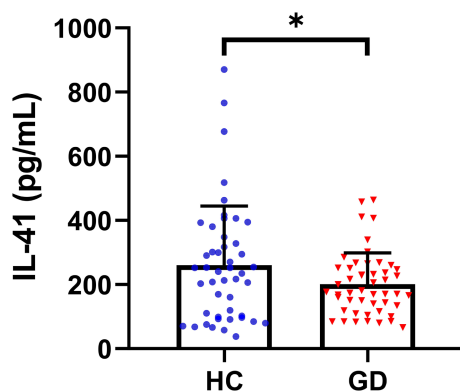


FIGURE 1 Serum IL-41 levels of GD and HC. Serum IL-41 was significantly lower in GD than in HC. **p* < 0.05. HC, healthy control group; GD, Graves' disease group

levels of FT3, FT4, TgAb, TPOAb, and TRAb, and significantly lower levels of TSH (*p* < 0.001).

3.2 | The serum concentration of IL-41

As shown in [Figure 1](#), serum IL-41 levels were significantly lower in GD patients (260.8 ± 183.9) compared with HC (201.0 ± 97.33, *p* < 0.05). The standard curve for IL-41 measured by ELISA is shown in [Figure S1](#).

3.3 | Correlation of serum IL-41 with inflammatory and thyroid markers

We used correlation heat maps to show the correlation between IL-41 and inflammatory indicators or thyroid markers and then analyzed those with significant correlations using Pearson analysis

([Figure 2A](#)). The results showed that IL-41 was positively correlated with CRP (*r* = 0.2947, *p* = 0.0385, [Figure 2B](#)) and WBC (*r* = 0.4104, *p* = 0.0034, [Figure 2C](#)). However, there was no significant correlation between IL-41 levels and serum TRAb, FT3, or FT4 levels (*p* > 0.05, [Figure 2D–F](#)). Additionally, CRP, an indicator of inflammation, was positively correlated with the thyroid markers TSH (*r* = 0.3651, *p* = 0.0099, [Figure 2G](#)) and TRAb (*r* = 0.2874, *p* = 0.0452, [Figure 2H](#)).

4 | DISCUSSION

In the present study, we found that serum levels of IL-41 were significantly lower in patients with GD than in HC. Correlation analysis showed that IL-41 level in GD patients was positively correlated with both inflammatory indicators CRP and WBC, while there was no correlation with thyroid hormones (FT3, FT4) and thyroid autoantibody TRAb. Meanwhile, both thyroid indicators TRAb and TSH in GD patients were positively correlated with CRP.

IL-41 is a novel immunomodulatory cytokine produced by activated macrophages that plays an important role in the inflammatory system. Rao et al.¹⁹ reported that IL-41 exerts anti-inflammatory effects via IL-4 released by eosinophils. Recent studies showed that serum IL-41 levels were reduced in many diseases, especially in patients with inflammation-related diseases. Cai et al.²⁰ found that serum IL-41 levels were lower in chronic heart failure patients when compared to control subjects. Several studies demonstrated that serum IL-41 levels were decreased in T2D patients compared with the healthy controls.^{21–23} Moreover, serum IL-41 concentrations were considerably lower in patients with coronary artery disease¹⁵ and ulcerative colitis and Crohn's disease²⁴ in comparison with healthy controls. In this study, we also observed a decrease in serum IL-41 levels in GD patients. However, other studies showed that serum IL-41/Metnrl levels were higher in T2D patients compared with healthy controls.^{13,25,26} Bridgwood et al.¹¹ examined

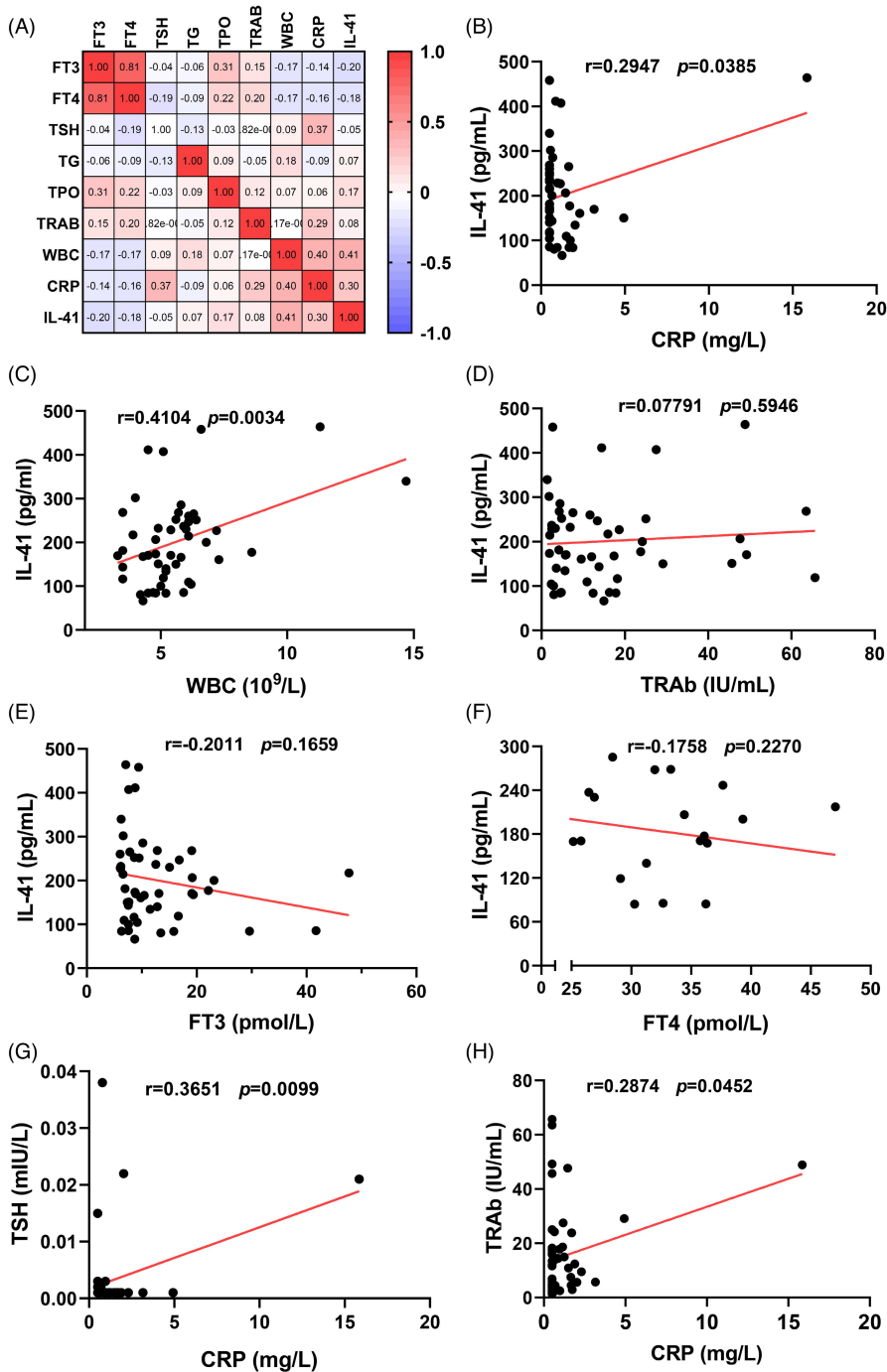


FIGURE 2 Correlation analysis of serum IL-41 levels, thyroid indicators, and inflammatory indicators in patients with GD. Correlations were determined by Pearson correlation test. $p < 0.05$ means statistically significant. CRP, C-reactive protein; GD, Graves' disease group; FT3, free triiodothyronine 3; FT4, free triiodothyronine 4; TSH, thyroid stimulating hormone; TgAb, anti-thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TRAb, TSH-receptor antibody; WBC, white blood cell count

IL-41 level in the synovial tissue of rheumatoid arthritis and psoriatic arthritis and found that IL-41 was compensatory upregulated in both diseases. These results suggest that the expression level of IL-41 in serum varies with different diseases, and even there are contradictory results of the expression level of IL-41 in serum of the same disease (such as T2D), which needs further study.

Several reports have confirmed that GD is a T-cell-dominated autoimmune disease characterized by increased thyroid hormone (FT3, FT4), which inhibit the synthesis and release of TSH.²⁷ FT4 combined with TSH can be used to detect thyroid dysfunction.⁴ Furthermore, GD diagnosis is based on the presence of hyperthyroidism and positive TRAb title. IL-41 plays an important role in inflammation and

various autoimmune diseases, but its role in thyroid diseases is not clear. In the present study, we found that IL-41 were decreased significantly in GD patients ($p < 0.05$), while there was no significant correlation between IL-41 and thyroid hormone ($p > 0.05$). We hypothesized that IL-41 is involved in the development of GD and may be related to macrophage polarization. Macrophages can be divided into two phenotypes: classically activated macrophages (M1), which promotes inflammation, and the other alternatively activated macrophages (M2), which inhibits inflammation.²⁸ Th1 cytokine, which is closely related to GD, also induces macrophages to polarize towards M1. Imam et al.²⁹ found that in patients with GD, NK cells are activated, and macrophages exhibit an M1-like phenotype. The

upregulation of the M1 phenotype is accompanied by a decrease in the M2 phenotype. IL-41 has been shown to be produced by M2-like macrophages.³⁰ Therefore, we hypothesize that the decrease in serum IL-41 in GD patients may be related to M1/M2 imbalance.

In order to explore the potential relationship between GD and inflammation, we further analyzed the relationship between TSH, TRAb, and inflammatory marker (CRP). Interestingly, we observed that the inflammatory CRP was positively correlated with TSH and TRAb. Our results also demonstrated a positive correlation between serum IL-41 concentrations and inflammatory indices (CRP, WBC) in patients with GD.

In conclusion, our study shows that IL-41 is downregulated in GD and serum IL-41 is correlated with GD, suggesting that IL-41 may play a potential role in abnormal immune response of GD patients. Further studies are needed to expand the current findings, especially to determine IL-41 expression in thyroid tissue and explore the exact mechanism of IL-41 in the pathogenesis of GD.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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