


RESEARCH ARTICLE

Low levels of serum IL-39 are associated with autoimmune thyroid disease

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Abstract

Background: Interleukin (IL)-39 is a novel member of IL-12 cytokine family, but its role in autoimmune thyroid diseases (AITD) is unclear. The aim of the present study was to determine serum levels of IL-39 in Hashimoto's thyroiditis (HT) and Graves' disease (GD) patients.

Methods: A total of 48 patients with HT, 50 patients with GD, and 45 healthy controls (HCs) were recruited for this study. Levels of serum IL-39 were determined by ELISA.

Results: Compared with HC group, levels of serum IL-39 in patients with HT ($p < 0.05$) and GD ($p < 0.01$) were drastically reduced. Among patients with HT, serum IL-39 levels had a positive correlation with white blood cell count (WBC) count and free triiodothyronine level. Among patients with GD, the levels of IL-39 in serum were positively correlated with WBC count and C-reactive protein levels.

Conclusions: IL-39 may be a new potential predictor for patients with HT and GD.

KEYWORDS

C-reactive protein, Graves' disease, Hashimoto's thyroiditis, interleukin-39, white blood cell

1 | INTRODUCTION

Autoimmune thyroid disease (AITD) is a family of classic autoimmune disorders that mainly consists of Hashimoto's thyroiditis (HT) and Graves' disease (GD).¹ The global prevalence of HT is estimated at 2%.² A significant sign of HT is loss of self-tolerance to thyroid antigens.³ HT is characterized by infiltration of thyroid lymphocytes, circulating thyroid autoantibodies, and apoptosis of thyroid cells leading to the destruction of follicles.^{3,4} GD is an organ-specific thyroid autoimmune disease with anti-thyroid stimulating hormone (TSH) receptor (TSH-R) autoantibodies in circulation that can cause hyperthyroidism. Lack of immune tolerance to thyroid antigens, especially TSH-R, is the cause of GD.⁵ It is estimated that the incidence of AITD in women is significantly higher than that in men. The

prevalence of AITD is 2% in women and 0.2% in men. AITD is most common in adults aged 30–50, and the disease occurrence increases with age.^{6,7}

Cytokines play a crucial role in the induction and action stages of immune and inflammatory responses. Imbalances between pro- and anti-inflammatory cytokines may play an important role in the occurrence and development of AITD. Several cytokines, including interleukin (IL)-17, IL-23, IL-28, and IL-29, have been implicated in the pathogenesis of HT.^{8–14} Additionally, IL-6, IL-21, IL-23, and IL-37 are all involved in the occurrence of GD.^{15–19} IL-12 family plays a key role in immune responses through their functional, unique structural, and immunological characteristics.²⁰ IL-12, IL-23, IL-27, IL-35, and the recently discovered IL-39 comprise the IL-12 cytokine family.²¹ Wang et al.^{22,23} first reported that IL-39 is

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a heterodimeric cytokine composed of Epstein–Barr virus-induced gene (Ebi3) and IL-23p19 subunits in IL-12 family. At present, the research results of IL-39 in humans seem to be contradictory. Two studies showed that IL-39 has no effect on human cells.^{24,25} Because they found that IL-39 chimeric protein could not induce the expressions of IL-6, IL-8, IL-17A, and TNF- α in human leukocytes or phosphorylation of signal molecules.^{24,25} On the other hand, Bastian et al.²⁶ recently found that human T cells can respond to IL-39 and IL-39 concentrations are elevated in patients with acute graft-vs.-host disease (GVHD). Furthermore, several studies confirmed that the serum IL-39 levels were drastically elevated in patients with acute coronary syndrome²⁷ and neuro-myelitis optica spectrum disorders.²⁸ IL-39 also can reduce proliferation and promote apoptosis of human bladder cancer cells,²⁹ but increase growth and inhibit apoptosis of pancreatic cancer cells.³⁰ Many studies indicated that IL-12 family members, especially IL-23 and IL-35, play crucial roles in HT and GD. However, the potential role of IL-39 in AITD, including relationships between IL-39 and HT/GD, is still unknown.

In the present study, we assessed serum levels of free triiodothyronine (FT3), free thyroxine (FT4), TSH, TSH-R autoantibodies (TRAb), anti-thyroglobulin antibody (TGAb), anti-thyroid peroxidase antibody (TPOAb), C-reactive protein (CRP), and IL-39 as well as white blood count (WBC) count in patients with AITD. We analyzed the correlations between serum IL-39 levels and these parameters.

2 | MATERIALS AND METHODS

2.1 | Study participants

From February 2020 to February 2021, 98 sufferers with AITD were recruited at the Affiliated Lihuli Hospital of Ningbo University (Zhejiang, China), including 48 sufferers with HT and 50 sufferers with GD. Exclusion criteria included pregnancy, hypoalbuminemia, hormone use, infection, trauma, and other diseases (especially autoimmune diseases). Furthermore, 45 healthy controls (HCs) without thyroid disease or other family histories of autoimmune diseases were enrolled. The study was approved by the Medical Ethics Committee of the Affiliated Lihuli Hospital of Ningbo University. All participants provided written informed consent.

The diagnostic criteria of HT were (1) diffuse thyroid enlargement with tough texture; (2) TPOAb and TGAb positive; and (3) thyroid biopsy showing cytological changes in fine-needle aspirates. The diagnostic criteria of GD were (1) diffuse goiter, either soft or tough; (2) abnormal thyroid function as shown by total triiodothyronine >2.45 nmol/L and/or FT3 >5.70 pmol/L, total thyroxine >150.84 nmol/L and/or FT4 >19.05 pmol/L, and TSH <0.35 mIU/L; and (3) exclusion of other causes of hyperthyroidism.

All HT patients were newly diagnosed, excluding patients with overt hypothyroidism. All GD patients were newly diagnosed and

had not received any AITD-related treatment. The patient screening process is shown in Figure 1.

2.2 | Blood samples

A total of 5 ml cubital venous blood was centrifuged at 1500 \times g for 15 min at 4°C for serum separation. The serum was stored in a -80°C low-temperature freezer.

2.3 | Serum IL-39 measurement

Serum IL-39 level was detected by enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Jianglai Biotechnology Co., Ltd) according to the manufacturer's instruction. All measurements were conducted in duplicate.

2.4 | TRAb, FT3, FT4, TSH, TGAb, and TPOAb measurements

The staphylococcal protein A antigen sandwich method was used to quantitate TRAb in human plasma. A two-step immunoassay (Chemiflex, Abbott I-2000 Automatic Chemiluminescence Immunoassay Analyzer; Abbott) was used to quantitate FT3, FT4, TSH, TGAb, and TPOAb in human plasma.

2.5 | Statistical analysis

All statistical analyses were performed with GraphPad Prism 7.0 software (GraphPad Software Inc.). Continuous data were expressed as means \pm standard deviations (SD). Count data were expressed as percentages. One-way ANOVA was used for analysis between three and more groups. An independent sample *t* test was used for analysis between two groups. Chi-square tests were used to compare count data. Pearson correlation analysis was used to assess the correlation between serum IL-39 levels, inflammatory indexes, and AITD-related parameters. Values of $p < 0.05$ were considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics of participants

The baseline demographic and clinical characteristics of patients with AITD and HCs are shown in Table 1. There were no significant differences in TRAb level, WBC, or CRP level among HC, HT, and GD ($p > 0.05$). Patients with HT were slightly older and included more women compared with HCs ($p < 0.05$); moreover, the serum TGAb, TPOAb, and TSH levels were significantly higher than those of HCs

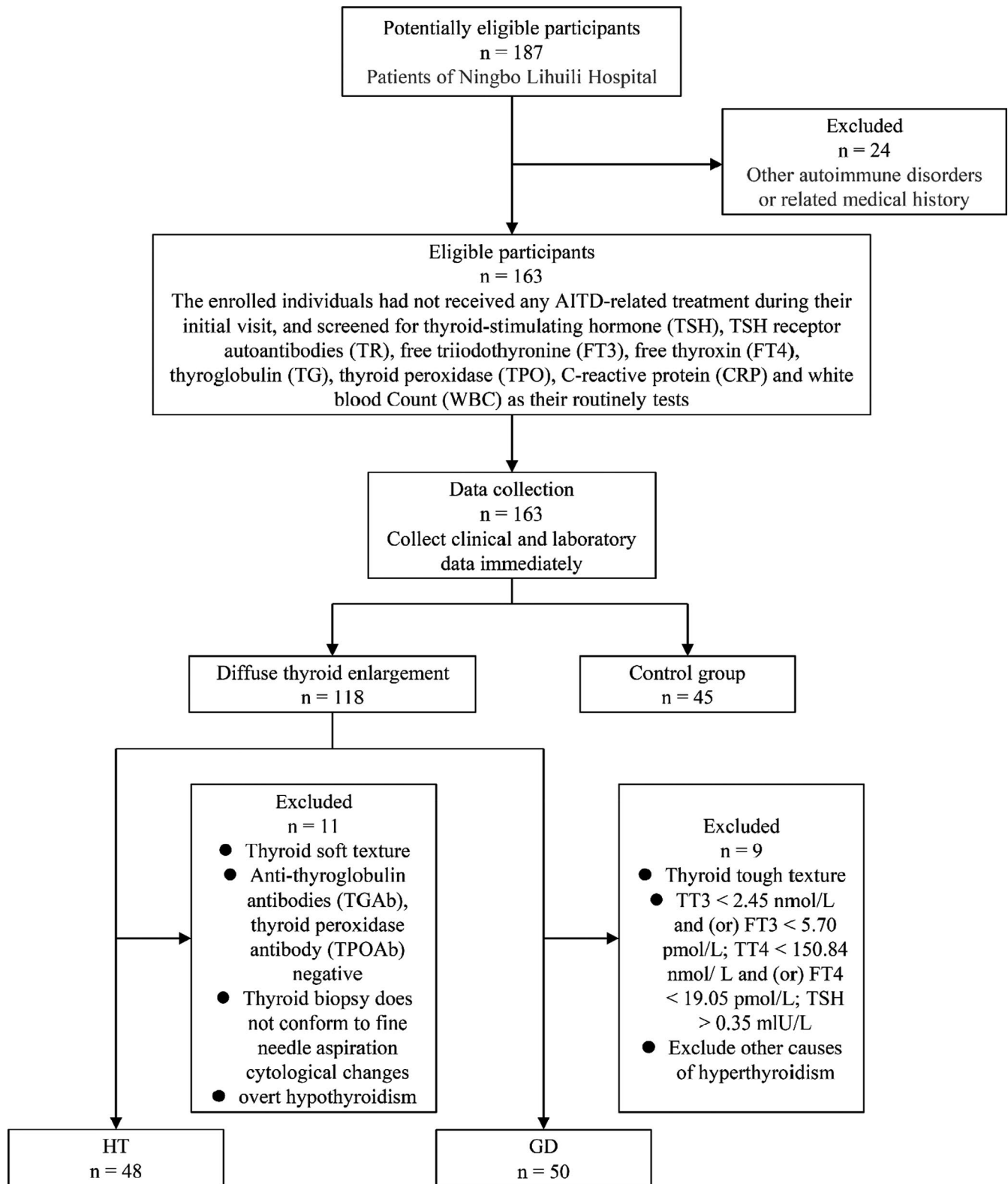


FIGURE 1 Inclusion and exclusion criteria of patients and controls. AITD, autoimmune thyroid disease; HT, Hashimoto's thyroiditis; GD, Graves' disease

($p < 0.0001$). Serum FT3, FT4, TRAb, and TPOAb levels among patients with GD were significantly increased than those of HCs ($p < 0.0001$). Patients with GD included a slightly lower proportion of women compared with HT patients ($p < 0.05$). Serum FT3, FT4, and

TRAb levels were significantly higher among patients with GD compared with those with HT, while serum TSH levels were significantly lower ($p < 0.0001$). These results are in line with the previously established characteristics of AITD.

TABLE 1 Demographic, clinical, and biochemical characteristics of participants

Characteristics	HC (n = 45)	HT (n = 48)	GD (n = 50)	p-value
Age (year)	35.71 ± 10.63	43.60 ± 15.24 [*]	37.30 ± 14.76	0.0148
Female (%)	29 ± 64.44	42 ± 87.5 ^{**}	30 ± 60 ^{##}	0.0063
FT3 (pmol/L)	4.44 ± 0.45	4.13 ± 0.63	12.62 ± 7.89 ^{*****##}	<0.0001
FT4 (pmol/L)	13.01 ± 0.93	12.66 ± 1.95	24.6 ± 7.69 ^{*****##}	<0.0001
TSH (mIU/L)	1.88 ± 0.89	6.12 ± 7.09 ^{****}	0.00 ± 0.01 ^{####}	<0.0001
TGAb (IU/ml)	1.10 ± 0.73	828.90 ± 15 ^{****}	322.10 ± 597.90 [#]	0.0001
TPOAb(IU/ml)	0.32 ± 0.24	926.90 ± 10 ^{****}	846.80 ± 971.00 ^{****}	<0.0001
TRAb (IU/ml)	0.35 ± 0.18	0.40 ± 0.43	16.16 ± 15.35 ^{*****##}	<0.0001
WBC (10 ⁹ /L)	5.93 ± 1.34	5.64 ± 1.27	5.63 ± 1.92	0.5686
CRP (mg/L)	0.82 ± 0.54	0.65 ± 0.32	1.26 ± 2.26	0.0830

Note: vs. HCs: ^{*} $p < 0.05$; ^{**} $p < 0.01$; ^{****} $p < 0.0001$; [#] $p < 0.05$; ^{##} $p < 0.01$; and ^{####} $p < 0.0001$.

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

3.2 | Levels of serum IL-39 are decreased in patients with AITD

Levels of serum IL-39 were detected by ELISA. The standard curve of serum IL-39 determined by ELISA is shown in Figure S1. IL-39 levels in patients with HT were slightly lower than those of HCs ($p < 0.05$), while IL-39 levels in patients with GD were significantly lower than those of HCs ($p < 0.01$). There was no significant difference in IL-39 levels between HT and GD patients ($p > 0.05$) (Figure 2). In addition, the level of serum IL-39 had no significant difference with the gender of GD and HT patients and had no correlation with the age of GD and HT patients (Figure S2).

3.3 | Correlational analysis of IL-39 levels and thyroid function parameters

Heat maps were generated to visualize correlations between IL-39 levels and thyroid-related parameters (Figure 3A,B). Pearson correlation analysis was conducted for indicators with high correlation

coefficients (Figure 3C–J). In patients with HT, serum IL-39 level had a positive correlation with WBC count ($r = 0.27$, $p = 0.0031$, Figure 3C) and FT3 level ($r = 0.40$, $p = 0.0026$, Figure 3I). WBC was negatively correlated with FT4 level ($r = -0.27$, $p = 0.0293$, Figure 3E), and CRP was positively correlated with TSH level ($r = 0.30$, $p = 0.0205$, Figure 3G). In patients with GD, serum IL-39 level had a positive correlation with WBC ($r = 0.49$, $p = 0.0002$, Figure 3D) and CRP level ($r = 0.29$, $p = 0.0221$, Figure 3J). Besides, CRP was positively correlated with TSH level ($r = 0.3624$, $p = 0.0048$, Figure 3F) and TRAb level ($r = 0.29$, $p = 0.0203$, Figure 3H).

3.4 | Receiver operating characteristic (ROC) curves

The diagnostic value of IL-39 and IL-39 combined with CRP in HT and GD was evaluated by ROC curve analysis (Figure 4). The area under the curve (AUC) of IL-39 for diagnosis of HT was 0.5454 ($p > 0.05$, Figure 4A), while the AUC of IL-39 combined with CRP for diagnosis of HT was 0.6333 ($p < 0.05$, Figure 4C). The AUC of IL-39 for diagnosis of GD was 0.6107 ($p > 0.05$, Figure 4B), while the AUC of IL-39 combined with CRP for diagnosis of GD was 0.6249 ($p < 0.05$, Figure 4D).

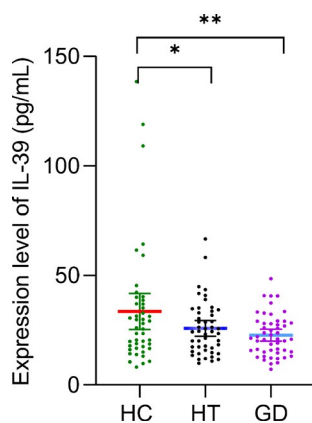


FIGURE 2 Levels of serum IL-39 in AITD patients. HT, Hashimoto's thyroiditis group; GD, Graves' disease group; HC, healthy control group. ^{*} $p < 0.05$; ^{**} $p < 0.01$

4 | DISCUSSION

To our knowledge, this is the first study on the role of serum IL-39 in AITD. We demonstrated that IL-39 levels were significantly lower in patients with AITD than those in HCs. Serum IL-39 levels among women, who accounted for most patients with AITD, showed a similar trend. Pearson correlation analysis indicated that serum IL-39 level had a positive correlation with WBC count and FT3 level in patients with HT, while WBC count had a negative correlation with FT4 level and CRP level had a positive correlation with TSH level. In patients with GD, CRP levels and WBC count

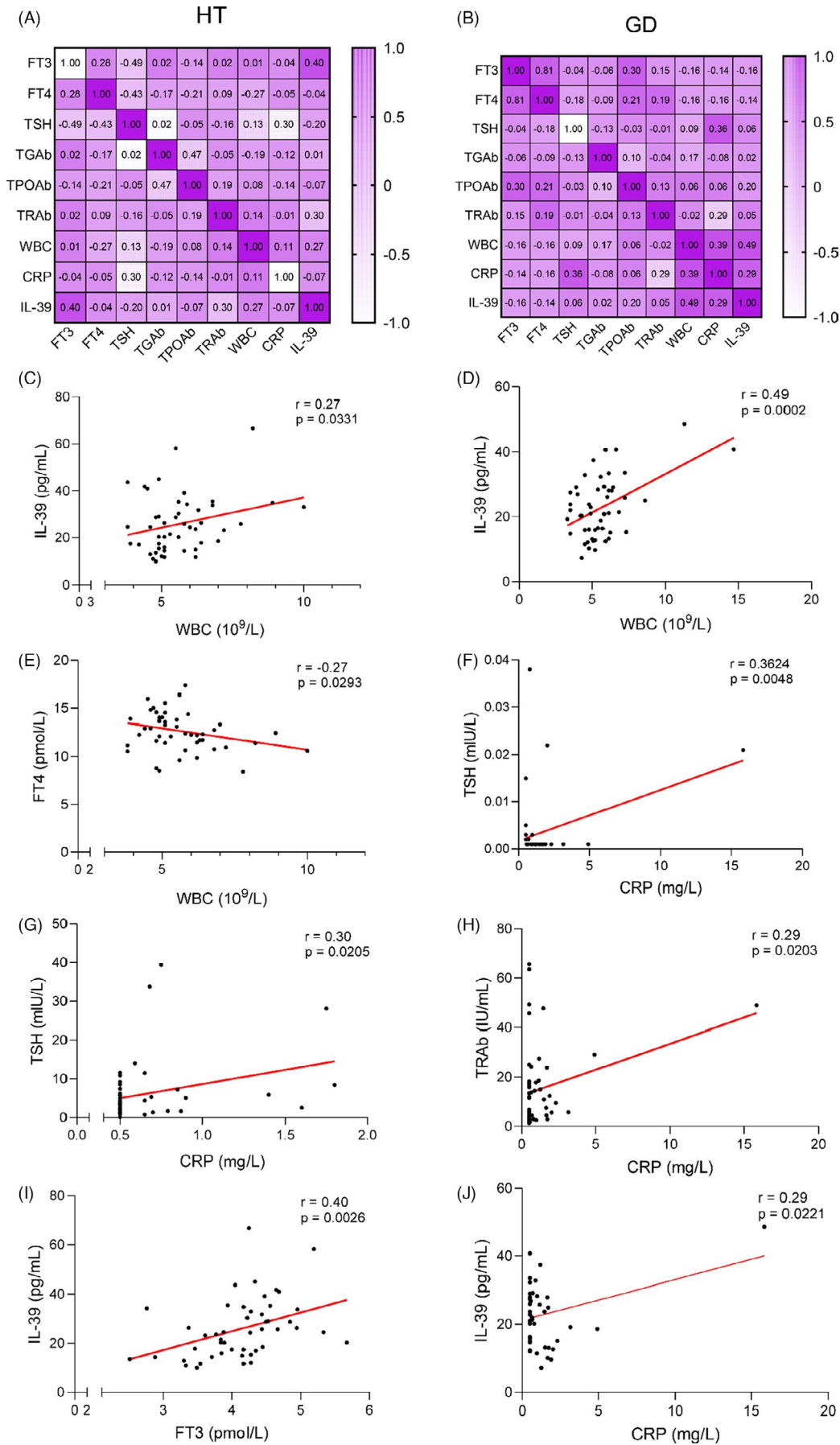


FIGURE 3 Correlation analysis of IL-39 levels and thyroid-related parameters

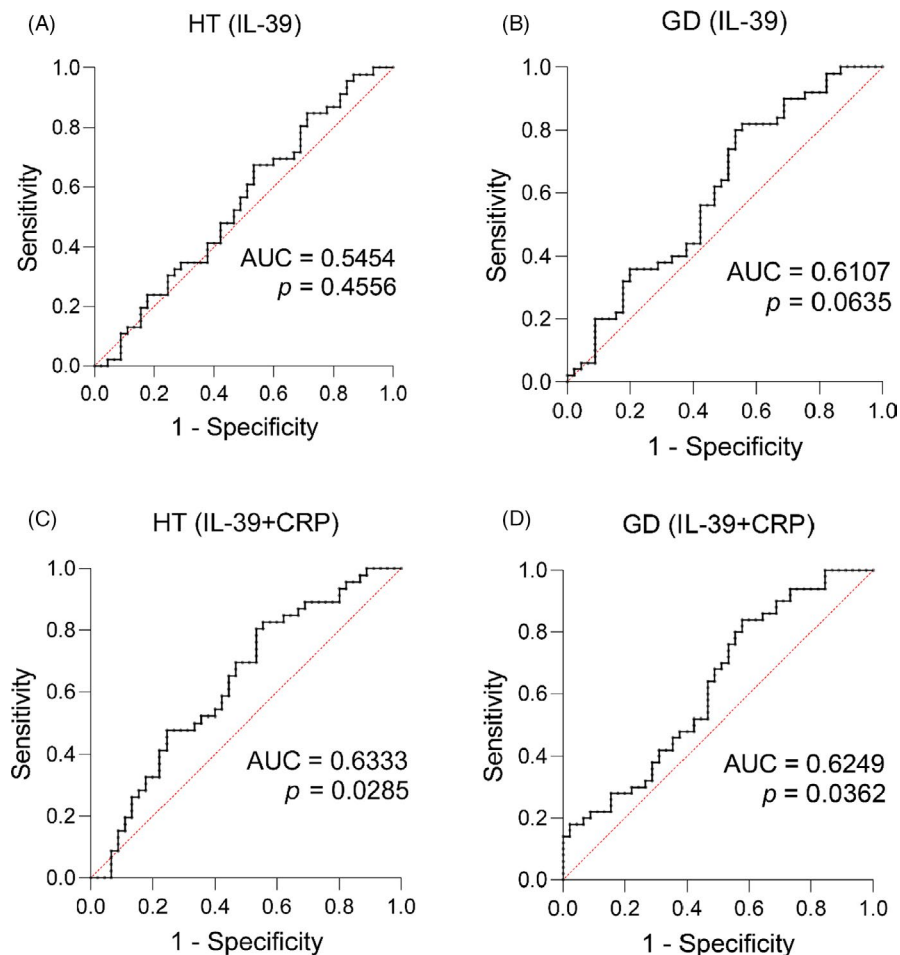


FIGURE 4 ROC curves of IL-39 and IL-39 combined with CRP for diagnosis of AITD

had a positive correlation with IL-39 level, while CRP level was positively correlated with TRAb and TSH levels. ROC curve analysis showed that IL-39 level alone had limited diagnostic value for AITD but combined with CRP level had high utility for diagnosis of HT and GD.

IL-12 family is a key regulator of immunity, and its members participate in the pathogenesis of a variety of diseases, including autoimmune diseases. IL-12 family contains five cytokines: IL-12, IL-23, IL-27, IL-35, and the newly discovered IL-39. Previous studies reported that IL-12 family plays a key role in AITD. Serum IL-23 levels in HT were markedly higher than those of HCs.^{11,14} Moreover, levels of serum IL-35 in HT patients were decreased compared with HCs.³¹ Levels of serum IL-35 were elevated in patients with new-onset GD.³² Several reports have suggested that IL-39 may play key roles in various diseases, stimulating extensive discussion among researchers. Serum IL-39 levels were significantly elevated in patients with acute coronary syndrome.²⁷ Bastian et al.²⁶ showed that serum IL-39 levels in transplant recipients had a positive correlation with the occurrence of GVHD in experimental animals and clinical settings, suggesting that IL-39 possibly be concerned in the pathogenesis of acute GVHD. Yang et al.²⁸ found that IL-39 may have a pro-inflammatory influence in the pathogenesis of neuromyelitis optica spectrum disorders and that levels of this cytokine may be associated with the severity of the disease.

Our recent results revealed that IL-39 can exacerbate concanavalin A-induced hepatitis and may represent a therapeutic target in inflammatory liver disease.³³ Nevertheless, the potential impact of IL-39 in AITD has not been explored. In the current study, we measured the levels of serum IL-39 in patients with HT and GD and found that they were significantly lower than those of HCs. Furthermore, abnormal plasma CRP levels and WBC count are indicators of inflammation, and elevated FT3 levels are indicators of abnormal thyroid function and often indicate AITD progression. We found that the levels of serum IL-39 in patients with AITD had a positive correlation with CRP level, WBC count, and FT3 level, suggesting that IL-39 may be related to AITD.

Members of the IL-12 family are composed of two subunits (α subunit: IL-23p19, IL-12p35, and IL-27p28; β subunit: Ebi3 and IL-12p40) that form the α/β chains. IL-39 represents a novel complex formed by subunit p19 and Ebi3. In vitro studies confirmed that IL-39 could be secreted by LPS-stimulated B cells.^{22,23} Lymphocyte infiltration in thyroid tissue is the main feature of AITD. T cells and B cells infiltrate the thyroid gland during AITD development. Autoantibodies and B-cell dysfunction are considered to be the main immune responses in AITD.³⁴⁻³⁷ Therefore, we speculate that B cells may produce IL-39 in AITD and IL-39 may be involved in the pathogenesis of AITD. Furthermore, IL-35 consists of two subunits (p35/Ebi3). Yilmaz et al.³¹ showed that the levels of serum IL-35 in

HT were lower than those of HCs, in agreement with the results of our study. We infer that decreased serum IL-35 levels may relate to sharing of a common subunit between IL-39 and IL-35 (Ebi3).

C-reactive protein is an acute phase reactive protein and non-specific inflammatory factor produced when the internal and external environment changes. Studies showed that CRP is related to autoimmune diseases.³⁸ WBC can indicate the presence of diseases involving inflammatory immune responses. FT3 and FT4 have important roles in the diagnosis of hyperthyroidism and hypothyroidism, evaluation of disease severity, and monitoring of therapeutic effects. Elevated TSH is one of the primary symptoms of clinical hypothyroidism in HT. TRAb is a specific biomarker of GD. These measurements help in the differential diagnosis of hyperthyroidism and enable accurate and rapid diagnosis of GD. In this study, we discovered that WBC count and FT3 levels were positively correlated with IL-39 levels in patients with HT, while WBC count and CRP levels had a positive correlation with IL-39 levels in patients with GD. Additionally, CRP level had a positive correlation with TSH level in patients with GD and HT, and CRP level was positively correlated with TRAb level in patients with GD. Thus, IL-39 and CRP may be valuable for the diagnosis of HT and GD and the evaluation of disease activity. We assessed the diagnostic value of these parameters through ROC curve analysis and found that IL-39 alone showed limited diagnostic value for HT and GD. However, the combination of IL-39 and CRP levels showed clinical significance in guiding the diagnosis of these diseases. These findings demonstrated that IL-39 has potential value in assessing disease severity in AITD patients.

In conclusion, we found reduced levels of serum IL-39 in AITD patients. Serum IL-39 levels were positively correlated with thyroid-related and inflammatory indexes, suggesting that IL-39 may serve as a biomarker for assessing the severity of AITD. Furthermore, IL-39 may represent a new therapeutic target for AITD. The specific roles of IL-39 in AITD require further study.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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 may be found in the online version of the article at the publisher's website.

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