Thieme

Helper T Cell 17 and Regulatory T Cell Levels in Peripheral Blood of Newly Diagnosed Patients with Autoimmune Thyroid Disease: A Meta-Analysis

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ABSTRACT

This article aims to explore associated immune indicators of autoimmune thyroid disease (AITD) through a meta-analysis of published case-control studies on newly diagnosed AITD patients, intending to provide some suggestions for research on the mechanisms of AITD. Six electronic databases were searched for case-control studies on newly diagnosed AITD patients from inception to August 15, 2022. A random-effects model was used to calculate the standardized mean difference (SMD), odds ratio (OR), and 95 % confidence interval (95 % CI). A total of 26 articles were included in this meta-analysis. Patients with newly diagnosed AITD had higher levels of helper T cell 17 (Th17) (Hashimoto's disease (HT): SMD = 2.35, 95 % CI: 1.98, 2.72; Graves' disease (GD): SMD = 1.61, 95 % CI: 1.23, 1.98), lower levels of regulatory T cell (Treg) (HT: SMD = –2.04, 95 % CI: –2.67, –1.42; GD: SMD = –1.35, 95 % CI: –2.11, –0.58), and lower levels of forkhead box P3 (FoxP3) mRNA (HT: SMD = –2.58, 95 % CI: –3.12, –2.05; GD: SMD = –2.13, 95 % CI: –2.56, –1.70), compared to the healthy population. In addition, the single nucleotide polymorphism rs3761548 and rs3761549 in the promoter region of FoxP3 showed a higher frequency in the comparison of genotype "CT" only in HT patients than in the healthy population (OR = 1.66, 95 %CI: 1.18, 2.34). In patients with newly diagnosed AITD, the Th17/Treg ratio imbalance may develop AITD. Monitoring Th17 and Treg levels may become an essential tool to assess the organism's immune homeostasis and hopefully guide clinical diagnosis and treatment.

ABBREVIATIONS

AITD:	Autoimmune thyroid disease
GD:	Graves' disease
HT:	Hashimoto's disease
Th17:	Helper T cell 17
Treg:	Regulatory T cells
FoxP3:	Forkhead box P3
TPOAb:	Thyroid peroxidase antibodies
TGAb:	Thyroglobulin antibodies
NOS:	Newcastle-Ottawa scale
SMD:	Standardized mean difference
CI:	Confidence interval
OR:	Odds ratio

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Introduction

Autoimmune thyroid disease (AITD) including Graves' disease (GD) and Hashimoto's disease (HT) [1, 2] is a classic organ-specific autoimmune thyroiditis. Its pathogenesis is complex and unclear and may be related to the living environment, genetic background, and imbalance of immune homeostasis [3]. It is clinically characterized by infiltration of thyroid tissue via different lymphocytes and reactivity to thyroid-associated autoantigens [4]. The lymphocytes that infiltrate the thyroid are T and B lymphocytes. B lymphocytes primarily produce antibodies and cytokines that promote the development of inflammation, and T lymphocytes induce other immune cells' activity by releasing cytokines. These cytokines help to suppress or modulate the immune response [1].

Recent studies have found that helper T cell 17 (Th17) or regulatory T cells (Treg) play an essential role in developing autoimmune diseases [1]. Th17 cells are members of the $CD4^+$T cell lineage. It is generally believed that Th17 cells are developmentally and functionally distinct from Th1 and Th2 cells in that they produce and secrete cytokines such as IL-17 that promote progressive inflammation [5]. Studies have shown that IL-17 and its receptor (IL-17R) can participate in the activation of the NF-κB signaling pathway, which is an important pathway in the human body and is involved in the transmission of tissue damage, stress, cell differentiation, and apoptosis [6]. Esfahanian et al. found elevated serum IL-17 levels in HT patients, which suggested a potential role in the pathogenesis of the disease [7]. Safdari et al. revealed that the expressions of T-bet and GATA3, which were specific transcription factors for Th1 and Th2, respectively, were significantly higher than those in the control group. In addition, elevated RORα and declined forkhead box P3 (FoxP3), which reflect the expression of Th17 and Treg, respectively, were observed, with significant difference [8]. Among severe graves ophthalmopathy patients, moderate graves ophthalmopathy patients, and GD patients, the proportion of IFN-γ producing Th1 cells and IL-17A producing Th17 cells were significantly increased. After steroid pulse therapy, Th1 and Th17 cell were reduced in severe graves ophthalmopathy patients, which indicated that the status of Th1 and Th17 cells were associated with the severity of the disease [9].

Tregs could inhibit autoimmune response and regulate the immune system by secreting cytokines such as IL-10 and TGF-β [10]. And the expression of FoxP3 is essential for the development of Tregs and its role in maintaining autoimmune response and self-tolerance [11]. FoxP3 gene mutation and/or deletion could lead to the loss of regulatory function of Treg, which results in the overactivation of T cells and the occurrence of autoimmune response [12]. Among the FoxP3 polymorphisms, the presence of the FoxP3 rs3761549 "T" allele appears to increase in HT and GD patients. And an increase in CT heterozygous carrier rates has been reported in female GD patients in the Polish population. The association of CT heterozygotes with the pathogenesis of HT and GD can be predicted based on the decrease in FoxP3 transcriptional efficiency, which is likely to impair the regulatory function of Tregs, leading to uncontrolled clonal and amplification of activated T cells in the thyroid environment [13].

The mechanism of Treg dysfunction in AITD and other autoimmune diseases remains to be determined. However, Treg lymphocytes have the potential to transform into pro-inflammatory cells (mainly Th17 and Th1 lymphocytes), which may further the continuation of autoimmune processes. Studies have also shown that the frequency of transformation of FoxP3 to IL-17 or FoxP3 to IFN-γ lymphocytes was increased in psoriasis and type 1 diabetes patients, respectively, and the differentiation of Treg cells into Th17 or Th1 lymphocytes is enhanced [14]. Considering that Th17 cells share a differentiation pathway with FoxP3 Treg, dysregulation of Th17/Treg homeostasis and changes in its associated factors may contribute to autoimmune diseases. Therefore, this review aims to investigate the changes in immune indicators associated with AITD via a meta-analysis of published case-control studies on newly diagnosed AITD. Hopefully, it will provide new directions for studying the mechanisms of AITD.

Materials and Methods

This systematic review and meta-analysis is registered at the International Prospective Register of Systematic Reviews (Number CRD42022353625).

Search strategy

Information retrieval conducted through Pubmed, Web of Science, Cochrane Library, Scopus, China National Knowledge Infrastructure, and Wanfang data. The data were searched on case-control studies of patients with newly diagnosed AITD to compare Th17 and Treg levels and alterations in FoxP3mRNA and FoxP3 (*rs3761548*, *rs3761549*) in peripheral blood of patients with newly diagnosed HT, GD, and healthy subjects, to provide clinical evidence for inferring the etiology of these patients from inception to August 15, 2022. The search keywords were "Autoimmune thyroid disease", "Thyroiditis", "Th17", "Type 17 Helper cell", "Treg", "T lymphocytes, regulatory", and "FoxP3". In addition, we meticulously searched the references of the articles initially included in the systematic search in order not to leave any relevant article behind and to provide comprehensive coverage of Th17, Treg levels, and alterations in FoxP3mRNA and FoxP3 (*rs3761548*, *rs3761549*) in peripheral blood of patients with newly diagnosed AITD.

Inclusion and exclusion criteria

Two researchers evaluated the titles and abstracts of the studies from the initial search as per the inclusion and exclusion criteria, respectively. If two researchers disagreed about inclusion in the study, a third researcher would make determinations on whether to include the disputed study on the basis of the opinions of the first two. The inclusion criteria were (1) only a retrospective case-control study was investigated; (2) the research group was patients with newly diagnosed AITD (including HT and GD patients) without any drug intervention, and the healthy population served as the control group; and (3) Th17, Treg, FoxP3mRNA, and FoxP3 (*rs3761548*, *rs3761549*) were recorded in the study in patients and healthy populations. Exclusion criteria (the study would be excluded if any of the following conditions existed): (1) AITD patients of research included those who had performed any pharmacological intervention; (2) the full text of the study was not available, or the required data could not be extracted from the full text; (3) the same trial was repeatedly published; (4) the reported data were incomplete and not available through any credible source; and (5) the

study design was significantly flawed, or the results were reported with significant bias.

Data extraction and quality assessment

Data extraction was implemented by two researchers using a pre-designed data extraction table, respectively. A third researcher checked and solved the problem of the discordance of extracted data. The pre-designed data extraction table included study title, first author of the article, year of publication and journal of publication, type and number of newly diagnosed AITD patients included in the study, subgroup status, age, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), outcome indicators [levels of Th17, Treg, FoxP3mRNA and FoxP3 (*rs3761548*, *rs3761549*)], inclusion and exclusion criteria, outcome indicators of measurements (methods of measurements, markers of Tregs and type of cells for measurement), study design-related indicators.

The Newcastle-Ottawa Scale (NOS) for observational case-control studies was used for quality assessment by two independent researchers [15]. If there was no consensus between the two researchers, a third researcher would join in the discussion forum focusing on the quality score of the disputed study and make a final determination. The assessment items were [1] the appropriateness of the selection of newly diagnosed AITD patients and controls: (1); for example, the strongly positive immunological test of newly diagnosed AITD patients and healthy population and the source of study object selection; (2) the comparability of cases and controls; and (3) the appropriateness of exposure determination. Observational studies were rated as high quality if they scored 6–9, moderate-quality if they scored 4 or 5, and poor quality if they scored 3 or lower.

Statistical analysis

We used Stata 15.1 software to process the extracted literature data [16, 17]. Because the gender composition, the age, TPOAb levels, and TGAb levels of patients with newly diagnosed AITD included in studies were very far apart, it did not have good clinical consistency. Therefore, we could combine the results using a random-effects model to compare the differences in Th17, Treg, FoxP3mRNA, and FoxP3 (*rs3761548*, *rs3761549*) in peripheral blood between patients with newly diagnosed AITD and the healthy population. In this study, the Q-test (chi-square test) and I^2 statistics were combined to evaluate the heterogeneity or homogeneity of the studies. A p-value of the Q-test greater than 0.05 was considered homogeneity. Otherwise, heterogeneity was considered. The I^2-values of less than 50 % and more than 50 % suggested a low and high heterogeneity among studies, respectively [18]. The standardized mean difference (SMD) and odds ratios (OR), and their 95 % confidence interval (95 %CI) were calculated for continuous variables and dichotomous variables, respectively to compare whether the factor is associated with the etiology of AITD. If the number of studies was ≥ 5, Egger's test and funnel plot were used to assess the publication bias of the results. Duval and Tweedie's trim and fill test was used to evaluate the sensitivity of the results [19, 20]. Exact p-values are presented, unless $p < 0.001$. The size of the test was $\alpha = 0.05$.

Results

Literature search, study characteristics, and quality assessment

A search of six database systems yielded 4018 articles, and four articles were obtained after a manual search of references for initial inclusion in the literature. After 1466 duplicate retrieved articles were removed, the titles and abstracts of the accepted articles were screened again, and 2524 articles were excluded for not meeting the inclusion criteria (not related to autoimmune thyroid disease n = 823; review or in vitro/animal studies or letter or editorial or conference paper n = 436; not related to case-control study focusing on the comparison between newly diagnosed AITD patients and healthy population n = 1203; not related to adopted indicators n = 62). Six out of the 32 articles subsequently participating in the full-text assessment were excluded due to repeated population, no data available or could not be translated into valuable data. At last, 26 studies were included for quantitative analysis in this systematic review (▶ **Fig. 1**). A total of 1242 newly diagnosed HT patients and 1,302 newly diagnosed GD patients, and 1815 healthy individuals were included for quantitative analysis. The primary characteristics of the included 26 case-control studies are shown in ▶ **Table 1** [10, 12, 13, 21–43].

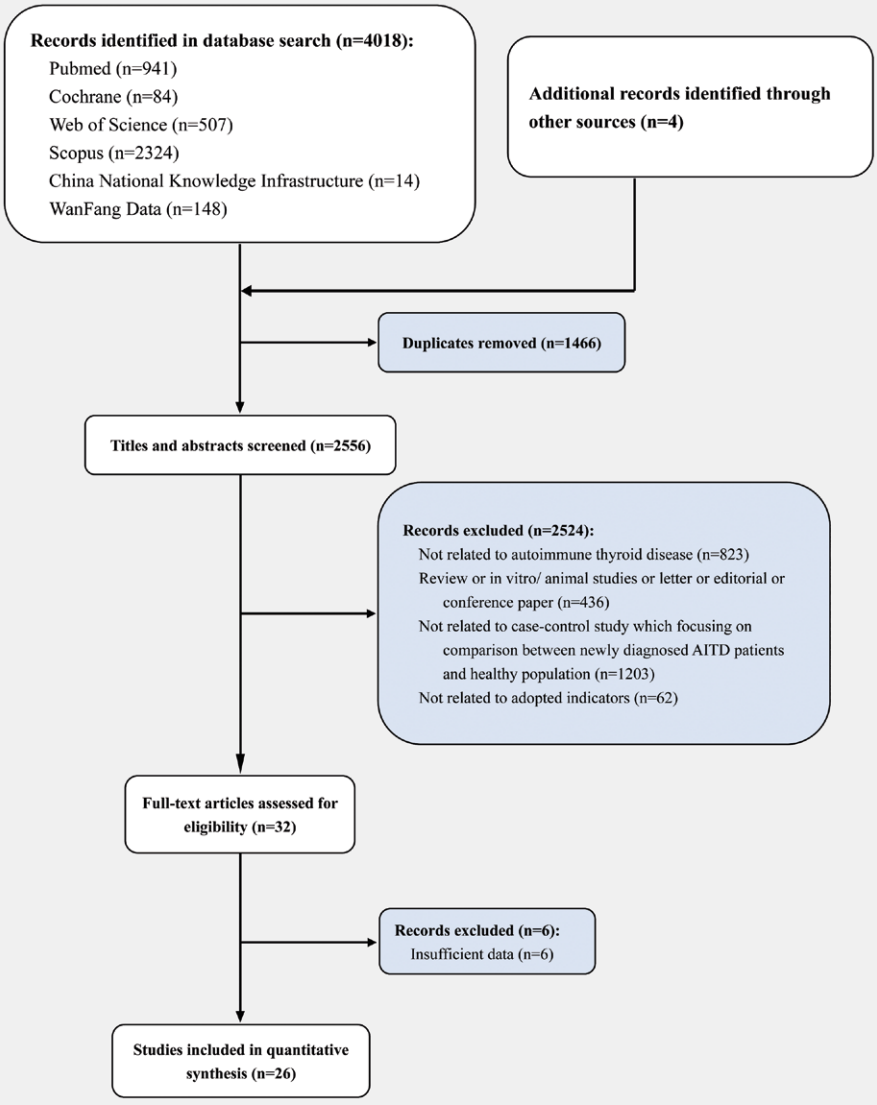
The NOS quality assessment scale scored 22 included studies between 5 and 9, with appropriate selection of cases and controls, and reliable study metric measures. So, the overall quality of studies was considered to be medium to high. The scoring results are displayed in ▶ **Table 2**. Notably, before study initiation, most studies excluded patients with conditions such as (1) comorbid cardiovascular and cerebrovascular systemic disease; (2) medication before examination and a history of related illness; and (3) pregnant and lactating women. The studies thus did not have significant missing data that would have seriously compromised test efficacy but a limited extrapolation of the findings. In summary, the overall quality of the studies included in this meta-analysis was assessed as good quality, and the research results were reliable.

Indicators

Comparison between newly diagnosed AITD patients and healthy people

Th17 Eight studies compared the differences in Th17 levels of peripheral blood mononuclear cells (PBMC) between newly diagnosed HT patients and the healthy population. Five studies reported differences in Th17 levels of PBMC between newly diagnosed GD patients and the healthy population. The meta-analysis results showed that Th17 levels in PBMC were higher in both HT patients and GD patients than in the healthy population, and the differences were statistically significant (HT: SMD = 2.35, 95 % CI: 1.98, 2.72; GD: SMD = 1.61, 95 % CI: 1.23, 1.98; ▶ **Fig. 2a**). Obviously, the results of the index of Th17 levels in PBMC suggested high heterogeneity in HT and medium heterogeneity in GD (HT: $I^2 = 60.3$ %, $p = 0.014$; GD: $I^2 = 37.7$ %, $p = 0.170$; ▶ **Fig. 2a**). However, the significance of this result needs to be further discussed.

Treg The meta-analysis results reported in ten studies showed that Treg levels marked by $CD4^+CD25^+FoxP3^+$ and measured by flow cytometric were lower in patients with newly diagnosed HT



▶ **Fig. 1** Study selection flowchart of Th17 and Treg levels in peripheral blood of newly diagnosed patients with autoimmune thyroid disease: a systematic review and meta-analysis.

than in the healthy population (Supplemental Table 1S). The difference was statistically significant (HT: SMD = –2.04, 95 % CI: –2.67, –1.42; ▶ **Fig. 2b**). Moreover, the same phenomenon was observed in newly diagnosed GD patients (GD: SMD = –1.35, 95 % CI: –2.11, –0.58; ▶ **Fig. 2b**). It was also noteworthy that the between-study heterogeneity statistic I^2 both suggested that high heterogeneity was observed for this indicator on HT disease and GD disease.

FoxP3 mRNA Eleven studies reported FoxP3 mRNA levels in PBMC of newly diagnosed HT patients, and six studies reported FoxP3 mRNA levels in PBMC of newly diagnosed GD patients (Supplemental Table 1S). The meta-analysis results showed that FoxP3 mRNA levels in PBMC of both HT and GD patients were lower than those in the healthy population, with statistically significant differences (HT: SMD = –2.58, 95 % CI: –3.12, –2.05; GD: SMD = –2.13, 95 % CI: –2.56, –1.70; ▶ **Fig. 3**).

The single nucleotide polymorphisms in the FoxP3 promoter region would likely affect FoxP3 expression, of which *rs3761548* and *rs3761549* were analyzed for comparison. The results are shown in ▶ **Fig. 4**. When the genomes of newly diagnosed HT patients and newly diagnosed GD patients were compared with the control group by PCR amplification, the genotypes "CC," "CA," and "AA" of *rs3761548* were not significantly different from those of the control group ($p > 0.05$). However, when *rs3761549* of HT and GD patients were compared with the controls, the results showed a statistically significant difference between HT patients and the controls in comparing genotype "CT" (OR = 1.66, 95 % CI: 1.18, 2.34; ▶ **Fig. 4c**). While comparing the remaining two genotypes, the results showed no statistically significant differences. In addition, in the comparison between GD patients and the controls, there were

▶ **Table 1** Baseline characteristics of included studies for meta-analysis.

First author, year [Ref]	AITD type	Research group				Control group				Indicators
		No. of cases (male)	Age	TPOAb	TGAb	No. of cases (male)	Age	TPOAb	TGAb	
Song JZ, 2009 [21]	HT	30 (4)	45.6 ± 11.1	423.3 ± 167.2	1587.9 ± 1210.4	20 (2)	45.7 ± 12.1	15.2 ± 10.4	36.3 ± 26.9	Th17
Jin X, 2018 [22]	HT	40 (7)	28.7 ± 6.2	268.2 ± 106.3	769.5 ± 165.6	40 (6)	28.3 ± 6.2	14.8 ± 8.4	50.4 ± 21.6	Th17, Treg, Th17/ Treg, Treg Foxp3
Li XJ, 2016 [23]	GD	50 (12)	38.3 ± 4.7	–	–	50 (9)	39.4 ± 5.1	–	–	Th17
	HT	50 (11)	37.6 ± 4.2	–	–	–	–	–	–	
Gao ST, 2011 [24]	GD	20 (5)	41.6 ± 12.9	151.0 ± 143.0	117.0 ± 146.0	20 (8)	35.6 ± 10.2	Negative	Negative	Foxp3
	HT	20 (7)	35.8 ± 12.8	565.0 ± 275.0	667.0 ± 780.0	–	–	–	–	
Xue HB, 2012 [25]	HT	40 (6)	28.4 ± 8.3	263.4 ± 160.9	808.5 ± 721.9	31 (4)	31.1 ± 6.4	15.1 ± 9.6	49.9 ± 33.3	Th17, Treg, Foxp3
Zheng LT, 2012 [26]	GD	30 (10)	38.2 ± 3.2	–	–	20 (7)	35.1 ± 1.3	–	–	Treg, Foxp3
	HT	20 (6)	39.1 ± 2.8	–	–	–	–	–	–	
Chen Q, 2012 [27]	GD	59 (19)	31 (22–43)	202.2 ± 164.5	99.7 ± 53.3	55 (22)	32 (16–59)	1.8 ± 1.0	2.2 ± 1.0	Treg, Foxp3
	HT	63 (11)	36 (26–61)	465.3 ± 282.9	233.4 ± 184.2	–	–	–	–	
Zhao JY, 2012 [28]	GD	30 (13)	38.0 ± 11.3	143.5 ± 177.4	270.6 ± 272.3	20 (11)	40.6 ± 11.5	16.3 ± 12.4	55.4 ± 48.9	Th17
	HT	30 (11)	40.2 ± 13.5	342.6 ± 237.3	1146.3 ± 1126.3	–	–	–	–	
Huang GY, 2017 [29]	GD	40 (17)	40.6 ± 9.3	152.2 ± 52.3	169.4 ± 46.4	20 (7)	39.8 ± 9.1	Negative	Negative	Treg, Foxp3
	HT	40 (16)	41.3 ± 10.2	521.3 ± 102.3	643.6 ± 102.3	–	–	–	–	
Hu Y, 2019 [30]	HT	42 (5)	40.3 ± 13.2	238.6 ± 167.7	545.8 ± 432.2	20 (5)	38.9 ± 8.1	13.0 ± 11.2	32.2 ± 20.6	Treg
Mao C, 2011 [31]	GD	77 (12)	41.1 ± 12.7	90.6 ± 65.3	26.1 ± 12.2	74 (14)	40.7 ± 10.5	3.5 ± 2.1	0.5 ± 0.3	Treg
Qin J, 2017 [32]	GD	20 (7)	36.7 ± 11.0	261.0 (10.0–1000.0)	20.0 (20.0–793.0)	20 (7)	32.0 ± 6.1	10.0 (10.0–16.1)	20.0 (20.0–69.6)	Th17, Treg
Xue HB, 2015 [33]	HT	40 (5)	28.9 ± 8.3	200.0 (148.4–299.9)	489.8 (300.7–774.7)	30 (4)	30.9 ± 6.4	14.3 (7.5–24.0)	51.0 (19.8–84.3)	Th17
Şıklar Z, 2016 [34]	HT	32 (4)	13.2 ± 3.7	–	–	24 (6)	12.8 ± 3.4	–	–	Treg, Foxp3
Yang X, 2018 [35]	HT	30 (5)	43.3 ± 0.9	472.5 ± 74.0	278.9 ± 67.6	30 (7)	44.9 ± 0.9	4.1 ± 0.2	4.1 ± 2.6	Treg, Foxp3
Klatka M, 2014 [36]	GD	60 (12)	14.1 ± 1.9	–	–	20 (5)	14.4 ± 2.2	–	–	Treg
Rydzewska M, 2018 [37]	GD	145 (36)	16.5 ± 2.0	331.9 ± 58.1	347.4 ± 86.7	161 (86)	16.3 ± 3.1	66.5 ± 52.7	91.6 ± 30.5	rs3761548, rs3761549
	HT	87 (13)	15.2 ± 2.2	329.9 ± 92.9	620.9 ± 240.3	–	–	–	–	
Fathima N, 2019 [13]	GD	80 (6)	33.9 ± 14.7	–	–	285 (30)	32.1 ± 12.6	–	–	rs3761548, rs3761549
	HT	275 (17)	33.9 ± 11.9	–	–	–	–	–	–	
Kalantar K, 2019 [12]	HT	129 (0)	38.1 ± 12.8	–	–	127	44.4 ± 2.2	–	–	rs3761548, rs3761549
Inoue N, 2010 [38]	GD	65 (8)	35.2 ± 14.7	–	–	71 (10)	44.1 ± 12.2	–	–	rs3761548, rs3761549
	HT	38 (5)	37.3 ± 11.1	–	–	–	–	–	–	

▶ **Table 1** Continued.

First author, year [Ref]	AITD type	Research group				Control group				Indicators
		No. of cases (male)	Age	TPOAb	TGAb	No. of cases (male)	Age	TPOAb	TGAb	
Zheng L, 2015 [39]	GD	308 (91)	39.7 ± 13.8	–	–	306 (107)	41.6 ± 10.1	–	–	rs3761548, rs3761549
Safdari V, 2017 [40]	HT	40 (0)	38.6 ± 10.6	–	–	40 (0)	36.1 ± 11.2	–	–	Foxp3
Li C, 2016 [10]	GD	16 (4)	39.3 ± 11.8	1607.2 ± 1494.3	28.4 ± 19.4	12 (3)	36.9 ± 6.3	7.8 ± 2.0	26.2 ± 12.9	Th17, Treg, Foxp3
	HT	15 (3)	38.0 ± 11.0	2070.5 ± 1345.2	215.3 ± 656.3	–	–	–	–	
Xue HB, 2015 [41]	HT	48 (6)	29.2 ± 8.6	216.2 ± 78.3	619.5 ± 221.4	32 (4)	31.2 ± 6.3	13.4 ± 7.8	46.0 ± 31.2	Th17, Treg, Foxp3
Tan Y, 2019 [42]	GD	28 (7)	–	102.2 ± 73.6	19.3 ± 11.2	24 (8)	–	1.0 ± 0.2	0.1 ± 0.1	Foxp3
Ren X, 2022 [43]	GD	26 (8)	37.7 ± 11.8	971.8 ± 564.4	250.5 ± 193.1	20 (7)	39.3 ± 14.4	37.1 ± 9.8	24.9 ± 9.2	Th17

AITD: Autoimmune thyroid disease; TPOAb: Thyroid peroxidase antibodies; TGAb: Thyroglobulin antibodies; NOS: Newcastle-Ottawa scale; HT: Hashimoto's disease; GD: Graves' disease; Th17: Helper T cell 17; Treg = regulatory T cells; Foxp3 = Forkhead box P3.

no statistically significant differences in the levels of genotypes "CC," "CT," and "TT" ($p > 0.05$).

Publication bias assessment and sensitivity analysis Egger's test was used to detect publication bias for each indicator, and the results showed no significant publication bias for all indexes ($p > 0.05$) (▶ **Table 3**). The funnel plot also reflected the symmetry of indicators Th17, Treg, and FoxP3, and high heterogeneity among studies Supplemental (▶ **Fig. 1S**). The results of Duval and Tweedie's trim and fill test showed that the effect sizes of all quantitative analysis were stable and did not generate significant change before and after trim and fill, with clear guidance (▶ **Table 3**).

Discussion

AITD is an autoimmune disease that manifests primarily as HT and GD. HT is characterized by infiltration of lymphocytes in thyroid tissue and destruction of thyroid follicles, leading to hypothyroidism. On the other hand, GD is characterized by hyperthyroidism due to excessive thyrotropin receptor-specific stimulating autoantibodies (TSAb). GD and HT exhibit different clinical features, but they show similarities in tissue damage, such as lymphocyte infiltration and abnormal cytokine secretion in vivo [44]. Previous studies suggested that the imbalance between Th1/Th2 contributed to the development of AITD. However, recent studies have shown that newly identified subsets of T lymphocytes, such as Th17 and Treg, and their associated cytokines may be associated with autoimmune diseases.

It was evident from the results of the meta-analysis that both newly diagnosed HT and newly diagnosed GD patients had statistically significant elevated levels of Th17 in their peripheral blood compared to healthy controls. Even though there was high heterogeneity in the meta-analysis results for the indicator Th17, it was believed to be related to the included patients' essential characteristics, such as gender differences and mean age. However, the high heterogeneity of the results did not affect our assertion that HT patients and GD patients had significantly higher levels of Th17 in their peripheral blood than healthy controls under them. It was observed from the forest plot (▶ **Fig. 2a**) that the newly diagnosed AITD patients in each study exhibited higher Th17 levels than the healthy control population, and the differences were all statistically significant. Moreover, we did not observe substantial publication bias in the indicator Th17 from Egger's test and instability in Duval and Tweedie's trim and fill test. Therefore, there was no doubt about the assertion that peripheral blood levels of Th17 were significantly higher in newly diagnosed AITD patients than in healthy controls. Th17 lymphocytes are essentially pro-inflammatory and mainly produce cytokines such as IL-17A/F, and IL-21 which could act as a causative agent in many chronic inflammatory and autoimmune diseases such as various asthma, allergies, and many other diseases [1]. Zake et al. found that IL-17 immunopositivity is observed in thyroid cells and inflammatory infiltrates in patients with HT and that IL-17-positive thyroid follicles frequently showed impaired integrity and destruction of follicular cells [45]. In summary, the meta-analysis results provide clinical evidence that Th17 plays a possible key role in developing AITD.

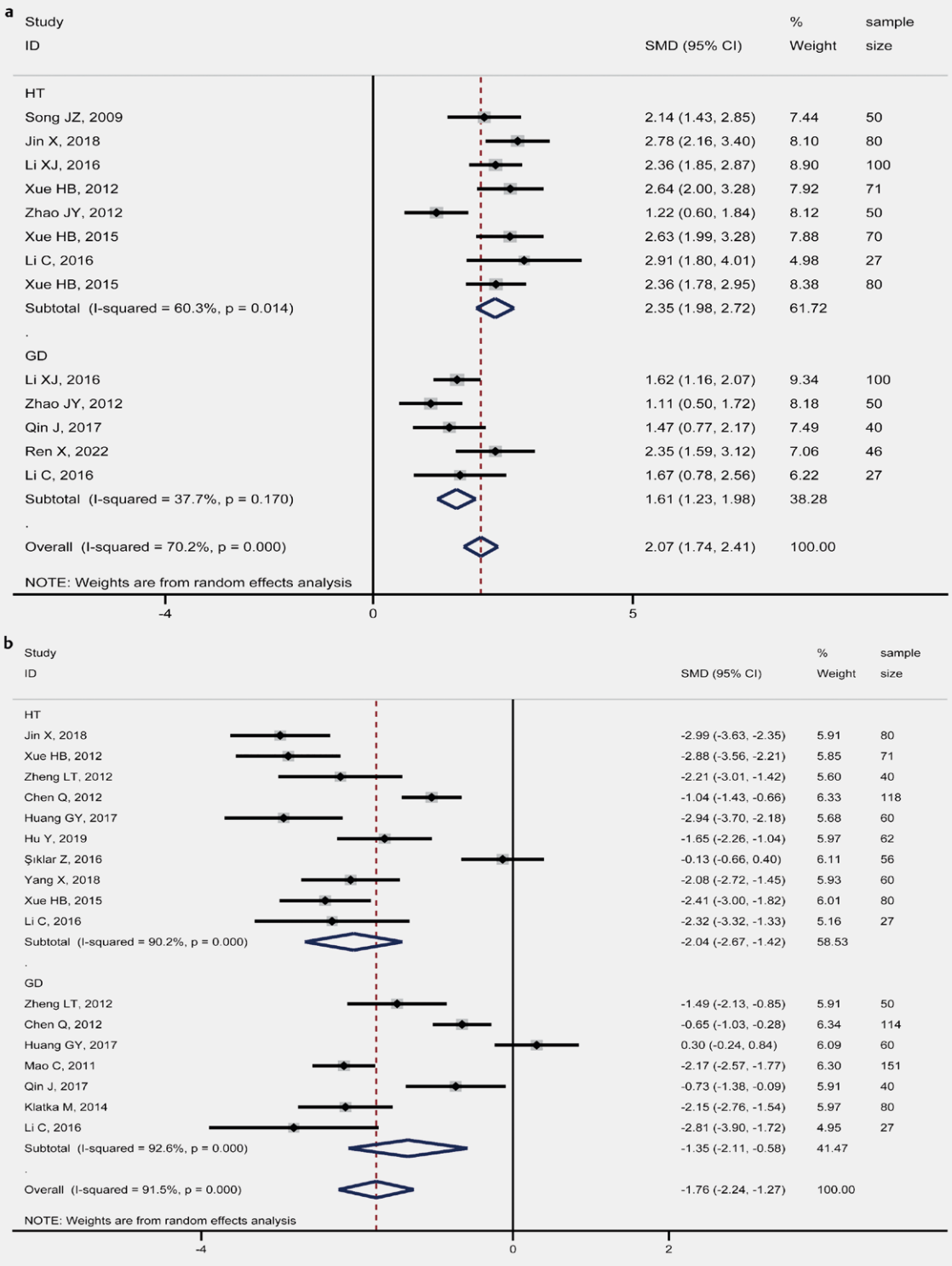
Likewise, after considering both forest plots, publication bias, and outcome stability tests for FoxP3 mRNA and Treg indicators in

▶ **Table 2** Quality assessment of Newcastle-Ottawa Scale for case-control studies.

Study [Ref]	Selection				Comparability of cases and controls	Exposure			Scores
	Adequate definition of cases	Representativeness of the cases	Selection of Controls	Definition of Controls		Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate	
Song JZ, 2009 [21]	1	1	1	1	1	1	1	1	8
Jin X, 2018 [22]	1	1	1	1	1	1	1	1	8
Li XJ, 2016 [23]	1	1	0	1	1	1	1	0	6
Gao ST, 2011 [24]	1	1	0	1	1	1	1	1	7
Xue HB, 2012 [25]	1	1	1	1	2	1	1	1	9
Zheng LT, 2012 [26]	1	1	0	1	0	1	1	0	5
Chen Q, 2012 [27]	1	1	0	1	1	1	1	0	6
Zhao JY, 2012 [28]	1	1	0	1	0	1	1	0	5
Huang GY, 2017 [29]	1	1	0	1	0	1	1	0	5
Hu Y, 2019 [30]	1	1	0	1	1	1	1	1	7
Mao C, 2011 [31]	1	1	0	1	0	1	1	0	5
Qin J, 2017 [32]	1	1	1	1	1	1	1	1	8
Xue H, 2015 [33]	1	1	0	1	1	1	1	0	7
Şıklar Z, 2016 [34]	1	1	1	1	1	1	1	1	8
Yang X, 2018 [35]	1	1	1	1	2	1	1	1	9
Klatka M, 2014 [36]	1	1	0	1	1	1	1	0	6
Rydzewska M, 2018 [37]	1	1	1	1	1	1	1	1	8
Fathima N, 2019 [13]	1	1	1	1	1	1	1	1	8
Kalantar K, 2019 [12]	1	1	0	1	0	1	1	0	5
Inoue N, 2010 [38]	1	1	1	1	2	1	1	1	9
Zheng L, 2015 [39]	1	1	1	1	1	1	1	1	8
Safdari V, 2017 [40]	1	1	0	1	1	1	1	0	6
Li C, 2016 [10]	1	1	1	1	1	1	1	1	8
Xue HB, 2015 [41]	1	1	1	1	1	1	1	1	8
Tan Y, 2019 [42]	1	1	0	1	1	1	1	0	6
Ren X, 2022 [43]	1	1	1	1	1	1	1	1	8

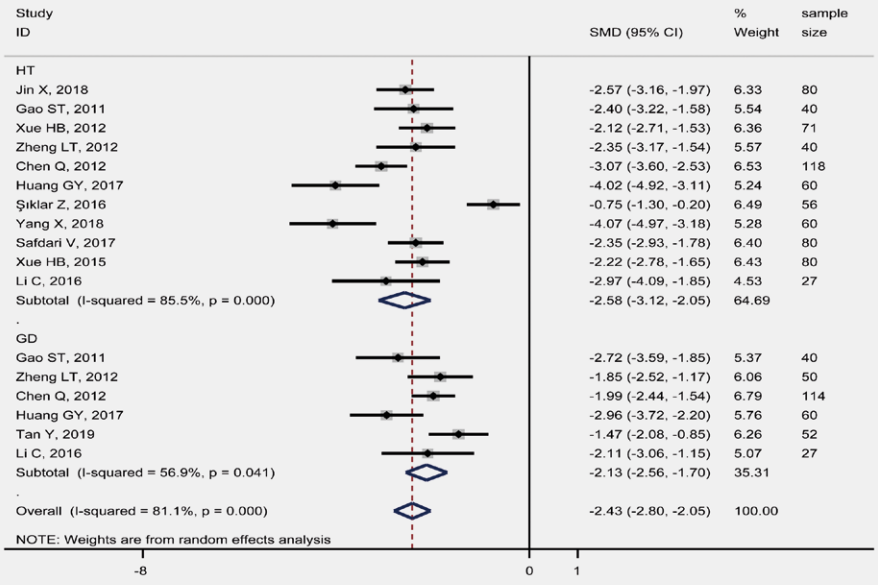
the meta-analysis results, we could confirm that FoxP3 mRNA and Treg results in newly diagnosed AITD patients were consistent, and the patients' peripheral blood levels were lower than the healthy control populations. The differences were statistically significant. The high heterogeneity of the results for both metrics may guide more profound studies but will not impact the conclusions of this meta-analysis. Th17 cells share a differentiation pathway with FoxP3 and Treg and transforming growth factor β is involved in the development of Th17 and Treg and plays a crucial role in maintaining the subpopulation of T cells involved in the pathogenesis of AITD. Immune tolerance is generated by stimulating Treg and suppressing cells [1]. Regulatory T cells (Treg) are characterized by the expression of the transcription factor FoxP3, a Treg-specific marker [46]. Loss-of-function mutations in the FoxP3 gene are associated with immune dysregulation, multiple endocrine disorders, inflammatory bowel disease, and severe allergies [47]. This study showed that Treg levels and FoxP3 mRNA levels were reduced in peripheral blood of newly diagnosed AITD patients, indicating a regulatory role of FoxP3 for Treg. In other words, its expression was not lost in either physiological or inflammatory responses, providing a theoretical basis for its role in the pathogenesis of autoimmune thyroid.

In the experiments of Cao et al. rat model, the measurement of Th17 and Treg levels after successful induction of HT model and bone marrow mesenchymal cell intervention in vitro showed that the percentage of Th17 decreases and Treg levels increase significantly after the intervention, further confirming the possible relevance of Th17/Treg to the development of AITD disorders [48]. Zhang et al. suggested that the Th17/Treg ratio imbalance may positively correlate with TGAb and TPOAb [49]. Thus, the Th17/Treg ratio imbalance may be involved in the development of AITD.

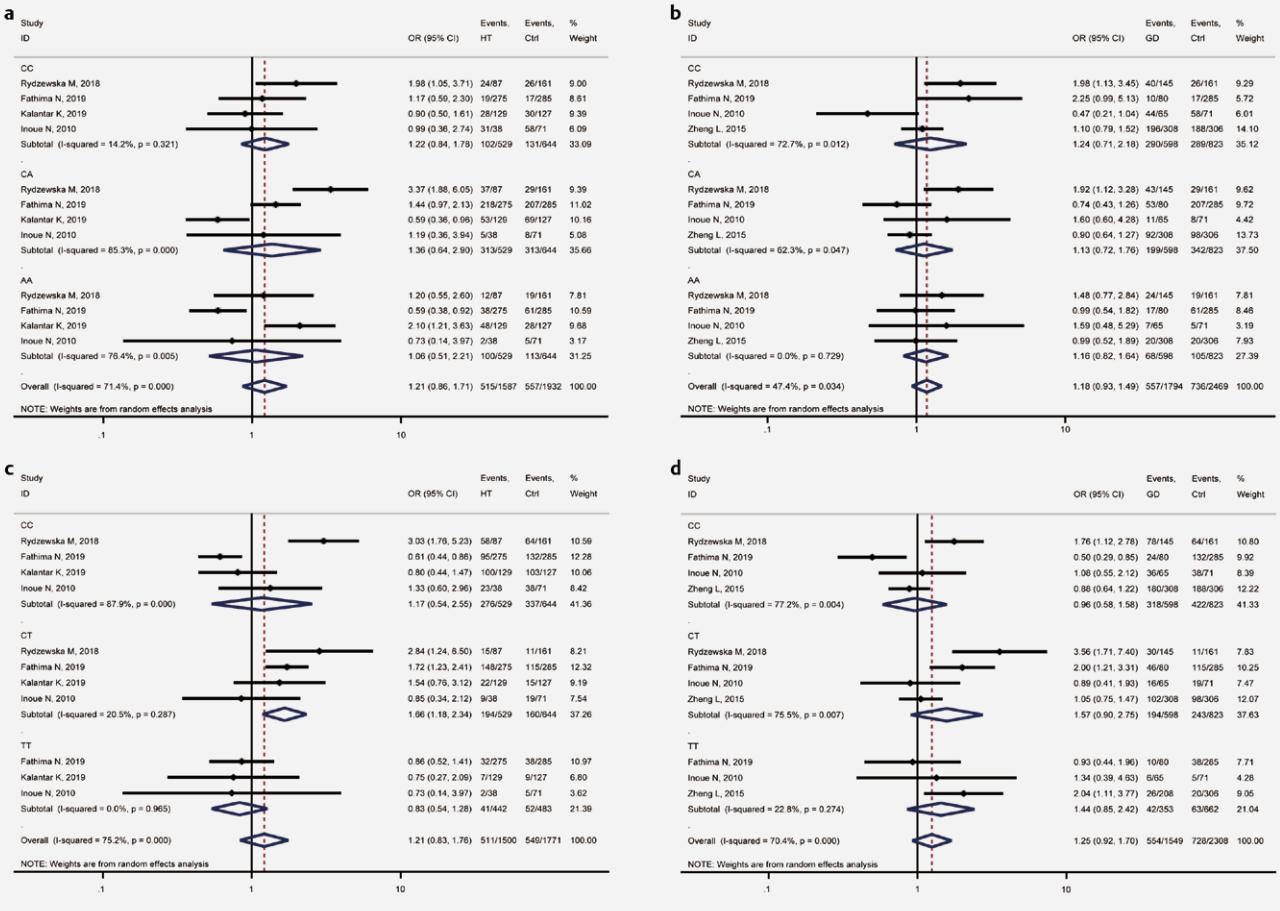


▶ **Fig. 2** Comparison of newly diagnosed AITD patients and healthy people: **A**: Th17; **B**: Treg.

FoxP3 promoter region polymorphism can affect the expression of this transcription factor. Moreover, in this meta-analysis, the association of FoxP3 *rs3761548* with *rs37615489* gene single nucleotide polymorphism in newly diagnosed GD patients was evaluated, revealing no significant difference in genotype frequency with this single patient nucleotide polymorphism, suggesting that this polymorphism is not associated with GD susceptibility. Kalantar et al. reported that FoxP3 *rs3761548* "CC" is significantly correlated with the TPOAb level of HT and is associated with the disease's activity [12]. However, it requires further clarification by a large num-



▶ **Fig. 3** Comparison of newly diagnosed AITD patients and healthy people. Foxp3 mRNA.



▶ **Fig. 4** Comparison of newly diagnosed AITD patients and healthy people: **a**: the genotypes "CC," "CA," and "AA" of *rs3761548* in Hashimoto's disease; **b**: the genotypes "CC," "CA," and "AA" of *rs3761548* in Graves' disease; **c**: the genotypes "CC," "CT," and "TT" of *rs3761549* in Hashimoto's disease; **d**: the genotypes "CC," "CT," and "TT" of *rs3761549* in Graves' disease.

▶ **Table 3** Evaluation of publication bias and sensitivity analysis.

Index	Egger's regression		Duval and Tweedie's trim and fill		
	Intercept	*p*	Original effect size	Studies trimmed	Adjusted effect size
HT					
Th17	1.715	0.621	2.35 (1.98, 2.72)	2	2.23 (1.89, 2.57)
Treg	−6.944	0.145	−2.04 (−2.67, −1.42)	0	−2.04 (−2.67, −1.42)
Foxp3	−5.637	0.112	−2.58 (−3.12, −2.05)	0	−2.58 (-3.12, −2.05)
GD					
Treg	−2.342	0.667	−1.35 (−2.11, −0.58)	0	−1.35 (−2.11, −0.58)
Th17	1.339	0.650	1.61 (1.24, 1.98)	0	1.61 (1.24, 1.98)
Foxp3	−2.482	0.367	−2.13 (−2.56, −1.71)	0	−2.13 (−2.56, −1.71)

Abbreviation: HT = Hashimoto's disease; GD = Graves' disease; Th17 = helper T cell 17; Treg = regulatory T cells; Foxp3 = Forkhead box P3.

ber of experimental studies. For the present results, the "CT" of *rs37615489* was different in HT patients compared to the controls, suggesting that this change in genotype frequency may predispose to HT. However, due to the lack of relevant literature, it is still necessary to find more relationships between FoxP3 gene polymorphism and AITD to understand better the immune regulation mechanism, pathogenesis, and prognosis of the disease.

The main limitations of this meta-analysis are as follows: 1. Some of the literature did not provide data on relevant indicators, or the data provided were not extractable, and these non-included studies might have a slight impact on the results; and 2. The literature on some indicators was small, and the sample size was insufficient. So, the results were unstable. Therefore, the current results still need a large sample of studies to elucidate further.

Conclusion

In conclusion, the Th17/Treg ratio imbalance in AITD patients may develop AITD. The monitoring of Th17 and Treg levels may become a critical tool to evaluate the immune homeostasis of the body to guide clinical diagnosis and treatment, contributing to the disease assessment. However, more studies are required to explore the specific pathogenesis of AITD.

Authors' Contributions

Aizhi Chen, Liang Huang: Critical revision of the manuscript; Aizhi Chen, Liang Huang, Liqin Zhang: Substantial contribution to the conception and design, manuscript drafting; Aizhi Chen, Liang Huang, Liqin Zhang: Acquisition, analysis, and interpretation of the data; Aizhi Chen, Liang Huang, Liqin Zhang: Revising the manuscript critically for final approval of the version to be published. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Luty J, Ruckemann-Dziurdzińska K, Witkowski JM et al. Immunological aspects of autoimmune thyroid disease – complex interplay between cells and cytokines. Cytokine 2019; 116: 128–133

[2] Inoue Y, Watanabe M, Inoue N et al. Associations of single nucleotide polymorphisms in precursor-microRNA (miR)-125a and the expression of mature miR-125a with the development and prognosis of autoimmune thyroid diseases. Clin Exp Immunol 2014; 178: 229–235

[3] Cogni G, Chiovato L. An overview of the pathogenesis of thyroid autoimmunity. Hormones (Athens) 2013; 12: 19–29

[4] Guo Y, Zynat J, Xing S et al. Immunological changes of T helper cells in flow cytometer-sorted CD4 + T cells from patients with Hashimoto's thyroiditis. Exp Ther Med 2018; 15: 3596–3602

[5] Gerenova J, Manolova I, Stanilova S. Serum levels of interleukin – 23 and interleukin – 17 in Hashimoto's thyroiditis. Acta Endocrinol (Buchar) 2019; 5: 74–79

[6] Zhang N, Wang Q, Tian Y et al. Expressions of IL-17 and TNF-α in patients with Hashimoto's disease combined with thyroid cancer before and after surgery and their relationship with prognosis. Clin Transl Oncol 2020; 22: 1280–1287

[7] Esfahanian F, Ghelich R, Rashidian H et al. Increased levels of serum interleukin-17 in patients with Hashimoto's thyroiditis. Indian J Endocrinol Metab 2017; 21: 551–554

[8] Safdari V, Alijani E, Nemati M et al. Imbalances in T cell-related transcription factors among patients with Hashimoto's thyroiditis. Sultan Qaboos Univ Med J 2017; 17: e174–e180

[9] Fang S, Zhang S, Huang Y et al. Evidence for associations between Th1/Th17 "Hybrid" phenotype and altered lipometabolism in very severe Graves orbitopathy. J Clin Endocrinol Metab 2020; 105: dgaa124

[10] Li C, Yuan J, Zhu YF et al. Imbalance of Th17/Treg in different subtypes of autoimmune thyroid diseases. Cell Physiol Biochem 2016; 40: 245–252

[11] Zhang D, Qiu X, Li J et al. MiR-23a-3p-regulated abnormal acetylation of FOXP3 induces regulatory T cell function defect in Graves' disease. Biol Chem 2019; 400: 639–650

[12] Kalantar K, Khansalar S, Eshkevar Vakili M et al. Association of foxp3 gene variants with risk of Hashimoto's thyroiditis and correlation with anti-TPO antibody levels. Acta Endocrinol (Buchar) 2019; 15: 423–429

[13] Fathima N, Narne P, Ishaq M. Association and gene-gene interaction analyses for polymorphic variants in CTLA-4 and FOXP3 genes: role in susceptibility to autoimmune thyroid disease. Endocrine 2019; 64: 591–560.

[14] González-Amaro R, Marazuela M. T regulatory (Treg) and T helper 17 (Th17) lymphocytes in thyroid autoimmunity. Endocrine 2016; 52: 30–38

[15] Wells GA, Shea B, O'Connell D et al. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

[16] Harris RJ, Deeks JJ, Altman DG et al. Metan: fixed- and random-effects meta-analysis. Stata J 2008; 8: 3–28

[17] Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Archiv Pub Health 2014; 72: 39

[18] Higgins JPT, Thomas J, Chandler J et al. Cochrane handbook for systematic reviews of interventions version 6.0 (updated July 2019) 2019

[19] Egger M, Davey Smith G, Schneider M. Bias in meta-analysis detected by a simple, graphical test. Br Med J 1997; 315: 629–634

[20] Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000; 56: 455–463

[21] Song JZ, Wu HN, Qian W. Significance of the alternation of Th17 cells in patients with chronic lymphocytic thyroiditis. Chin J Cell Mol Immunol 2009; 25: 927–931

[22] Jin X, Shang XM, Chen HB et al. Study on dynamic changes and significance of the peripheral blood regulatory T cells (Treg) and Th17 cells in patients with Hashimoto's thyroiditis. J Clin Exp Med 2018; 17: 2092–2094

[23] Li XJ, Zhang L. Th3, Th17 and its related influential factors in autoimmune thyroid diseases and its significance. J North Pharm 2016; 13: 129–130

[24] Gao ST, Zhu TN, Zhang YN et al. The frequency and function of CD4 + CD25 + regulatory T cells in autoimmune thyroid diseases. Chin J Immunol 2011; 27: 269–273

[25] Xue HB, Ma L, Li YB et al. Correlation between Treg/Th17 cells imbalance and autoimmunity in Hashimoto thyroiditis. Chin J Modern Med 2012; 22: 67–71

[26] Zheng LT, Yu SP, Wang N et al. The role of regulatory T cells and TGF-β1 in autoimmune thyroid disease. Shandong Med J 2012; 52: 81–83

[27] Chen Q, Niu J, Zheng BJ et al. Application of regulatory T cells and thyroid autoantibody detection in autoimmune thyroid diseases. Curr Immunol 2012; 32: 144–147

[28] Zhao JY, Wen Y, Wang Q. Detection and significance of the alteration of Th17 cells in patients with Hashimoto's thyroiditis and Graves' disease. Chin J Lab Diagn 2012; 16: 637–640

[29] Huang GY, Xuan WT, He LJ. Tregs role of CD4 + CD25 + Foxp3 + Tregs in autoimmune thyroid diseases. Med Innov China 2017; 23: 16–20

[30] Hu Y, Zhang L, Chen H et al. Analysis of regulatory T cell subsets and their expression of helios and PD-1 in patients with Hashimoto thyroiditis. Int J Endocrinol 2019; 5368473:

[31] Mao C, Wang S, Xiao Y et al. Impairment of regulatory capacity of CD4 + CD25 + regulatory T cells mediated by dendritic cell polarization and hyperthyroidism in Graves' disease. J Immunol 2011; 186: 4734–4743

[32] Qin J, Zhou J, Fan C et al. Increased circulating Th17 but decreased CD4 + Foxp3 + Treg and CD19 + CD1dhiCD5 + Breg subsets in new-onset Graves' disease. Biomed Res Int 2017; 8431838:

[33] Xue HB, Yang Y, Zhang Y et al. Macrophage migration inhibitory factor interacting with Th17 cells may be involved in the pathogenesis of autoimmune damage in Hashimoto's thyroiditis. Mediators Inflamm 2015; 621072:

[34] Şıklar Z, Karataş D, Doğu F et al. Regulatory T cells and vitamin D status in children with chronic autoimmune thyroiditis. J Clin Res Pediatr Endocrinol 2016; 8: 276–281

[35] Yang X, Lun Y, Jiang H et al. SIRT1-regulated abnormal acetylation of FOXP3 induces regulatory T-cell function defect in Hashimoto's thyroiditis. Thyroid 2018; 28: 246–256

[36] Klatka M, Grywalska E, Partyka M et al. Th17 and Treg cells in adolescents with Graves' disease. Impact of treatment with methimazole on these cell subsets. Autoimmunity 2014; 47: 201–211

[37] Rydzewska M, Góralczyk A, Gościk J et al. Analysis of chosen polymorphisms rs2476601 a/G – PTPN22, rs1990760 C/T – IFIH1, rs179247 a/G – TSHR in pathogenesis of autoimmune thyroid diseases in children. Autoimmunity 2018; 51: 183–190

[38] Inoue N, Watanabe M, Morita M et al. Association of functional polymorphisms related to the transcriptional level of FOXP3 with prognosis of autoimmune thyroid diseases. Clin Exp Immunol 2010; 162: 402–406

[39] Zheng L, Wang X, Xu L et al. Foxp3 gene polymorphisms and haplotypes associate with susceptibility of Graves' disease in Chinese Han population. Int Immunopharmacol 2015; 25: 425–431

[40] Safdari V, Alijani E, Nemati M et al. Imbalances in T cell-related transcription factors among patients with Hashimoto's thyroiditis. Sultan Qaboos Univ Med J 2017; 17: e174–e180

[41] Xue H, Yu X, Ma L et al. The possible role of $CD4^+CD25$(high)$Foxp3^+$/ $CD4+IL\text{-}17A^+$ cell imbalance in the autoimmunity of patients with Hashimoto thyroiditis. Endocrine 2015; 50: 665–673

[42] Tan Y, Chen W, Liu C et al. Effect of IL-21 on the balance of Th17 cells/ Treg cells in the pathogenesis of Graves' disease. Endocr Res 2019; 44: 138–147

[43] Ren X, Chen H. Changes in Th9 and Th17 lymphocytes and functional cytokines and their relationship with thyroid-stimulating hormone receptor antibodies at different stages of graves' disease. Front Immunol 2022; 13: 919681

[44] Li Q, Wang B, Mu K et al. The pathogenesis of thyroid autoimmune diseases: New T lymphocytes – Cytokines circuits beyond the Th1-Th2 paradigm. J Cell Physiol 2019; 234: 2204–2216

[45] Zake T, Skuja S, Kalere I et al. Upregulated tissue expression of T helper (Th) 17 pathogenic interleukin (IL)-23 and IL-1β in Hashimoto's thyroiditis but not in Graves' disease. Endocr J 2019; 66: 423–430

[46] Zhao N, Liu X, Wu C et al. Changes in Treg numbers and activity in papillary thyroid carcinoma with and without Hashimoto's thyroiditis. J Int Med Res 2020; 48: 300060520919222

[47] Lee DJ. The relationship between TIGIT + regulatory T cells and autoimmune disease. Int Immunopharmacol 2020; 83: 106378

[48] Cao Y, Jin X, Sun Y et al. Therapeutic effect of mesenchymal stem cell on Hashimoto's thyroiditis in a rat model by modulating Th17/Treg cell balance. Autoimmunity 2020; 53: 35–45

[49] Zhang D, Qiu X, Li J et al. MiR-23a-3p-regulated abnormal acetylation of FOXP3 induces regulatory T cell function defect in Graves' disease. Biol Chem 2019; 400: 639–650

Notice

This article was changed according to the erratum on July 06, 2023.

Erratum

In the above-mentioned article the authors Aizhi Chen and Liang Huang contributed equally.