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# Circulating Th17 cytokine levels are altered in Hashimoto's thyroiditis

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ABSTRACT

The disrupted autoimmune response in Hashimoto's thyroiditis (HT) has long been considered to be dominantly T helper type 1 (Th1) mediated. Recent advances in the field of immunology have introduced a new class of effector T cells, named 'Th17', which plays important roles in autoimmune disorders once thought to be merely Th1 mediated. We aimed to examine the levels of major Th17 cytokines in patients with HT in this study. We studied serum interleukin 17 (IL-17) and interleukin 23 (IL-23) levels in 46 newly diagnosed, untreated patients with HT (40 women and 6 men, aged 40.0 ± 11.8 years) divided into euthyroid (n = 22) and hypothyroid (n = 24) groups and compared them with age and sex matched 26 healthy euthyroid controls without HT (21 women and 5 men; aged 36.0 ± 12.9 years). Serum IL-17 and IL-23 levels were significantly different among euthyroid and hypothyroid HT patients and controls, with highest levels obtained in the euthyroid HT group ($p = 0.041$ for IL-17 and $p < 0.001$ for IL-23). TSH was negatively and FT4 was positively correlated with IL-17 ($p = 0.016$ for TSH and $p = 0.004$ for FT4) and IL-23 ($p < 0.001$ for TSH and $p = 0.003$ for FT4) levels. There were no correlations between thyroid volumes calculated on thyroid ultrasonography and IL-17 ($p = 0.630$) or IL-23 ($p = 0.321$) levels. In conclusion, the levels of IL-17, one of the major effector cytokines of the Th17 system, and IL-23, which had been implicated in the generation, survival and expansion of Th17 cells, are altered in HT. How thyroid hormone status and the course of disease affect Th17 system in chronic autoimmune thyroiditis needs to be determined with further studies.



## 1. Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disorder of the thyroid gland, characterized by the progressive loss of follicular cells and concomitant replacement of the thyroid tissue by lymphoid infiltrates and fibrosis [1]. The disruption of the thyroid architecture and the loss of functionality complicate the course of HT by the development of progressive hypothyroidism. The mechanism behind this disrupted self-immune response seems to be quite complex. T helper type 1 (Th1) and T helper type 2 (Th2) cell imbalance has been accused in the pathogenesis of HT and this model has long been used to explain the mechanism of autoimmune thyroiditis as well as various other autoimmune diseases. However, there were certain phenomena that could not be explained solely by the Th1/Th2 hypothesis [2]. Recently, a new group of effector T cells (Teff), T helper 17 cells (Th17), have been identified and provided a new understanding as to the underlying mechanisms of autoimmunity. They have been reported to play an important role in the development of various autoimmune diseases previously thought to be Th1 mediated, such as multiple sclerosis/experimental autoimmune encephalitis, uveitis, rheumatoid arthritis, Sjogren's syndrome, myasthenia gravis, and psoriasis [3]. Th17 cells mainly produce interleukin 17 (IL-17), interleukin 21 (IL-21) and interleukin 22 (IL-22) and their differentiation is induced by TGF-β, interleukin 6 (IL-6), interleukin 23 (IL-23), and interleukin 1 (IL-1) [4]. In patients with HT, increased number of Th17 cells, increased levels of IL-17 mRNA in peripheral mononuclear leucocytes and a stronger immunohistochemical expression of IL-17 and IL-22 in thyroid tissues have been reported [5]. Similarly, increased numbers of Th17 cells, along with Th1 cells, were observed in spleen and thyroid glands of a mouse model of HT and decreased severity of thyroiditis was observed in IL-17 knockout mice [3].

The aim of the present study was to determine the major cytokine levels of the Th17 cascade in patients with hypothyroid and euthyroid HT and compare them with healthy controls.

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## 2. Materials and methods

### 2.1. Patient population

46 patients with newly diagnosed HT (40 women and 6 men; aged 40.0 ± 11.8 years) were included in the study. Twenty-six age and sex-matched healthy subjects (21 women and 5 men; aged 36.0 ± 12.9 years) were included as controls. All subjects underwent a complete thyroid evaluation including physical examination, thyroid ultrasonography and measurement of serum free T4 (FT4), free T3 (FT3), TSH, *anti*-thyroid peroxidase (*anti*-TPO) and *anti*-thyroglobulin (*anti*-T) autoantibodies. The diagnosis of HT was based on the presence of elevated thyroid autoantibodies and a reduced echogenicity on thyroid ultrasound. Subjects with acute or chronic systemic illness (recent infection/inflammation, malignancy, autoimmune diseases, chronic renal or liver failure, heart failure, chronic obstructive pulmonary disease or other systemic condition) or on treatment with drugs known to interfere with immune system or thyroid function (corticosteroids, nonsteroidal antiinflammatory drugs, interferon, amiodarone and lithium) were excluded. Pregnant women and women lactating over the previous 6 months were not included. Control group consisted of healthy volunteers and the exclusion criteria for control group were the presence of abnormal values of serum FT4, FT3, TSH or *anti*-TPO/*anti*-T positivity or thyroiditis on thyroid ultrasound. There was no history of thyroid operation and prior exposure to radioiodine or external radiation in any of the participants included in the study. According to their thyroid function, patients were classified into 2 groups as "euthyroid HT" (normal FT4, FT3 and TSH; n = 22) and "hypothyroid HT" (low FT4 or FT3 and elevated TSH; n = 24). Informed consent was obtained in all subjects and the study was approved by the local ethics committee. The study was conducted according to the declaration of Helsinki.

### 2.2. Laboratory evaluation

Blood samples were drawn after an overnight fast and were immediately centrifuged at 4500*g* for 5 min at +4 °C and stored at −80 °C. Samples from hypothyroid patients were obtained before initiation of the levothyroxine treatment. Serologic assessment was performed in all cases. Serum concentrations of TSH (normal ranges: 0.35–4.94 μU/mL), FT4 (normal range: 0.7–1.48 ng/dL), FT3 (normal range: 2.3–4.2 pg/mL), *anti*-T and *anti*-TPO antibodies were determined by chemiluminescence immunoassay using Abbott-Architect analyzer (Chicago, IL). *anti*-TPO or *anti*-T antibody titer <60 U/mL was considered as negative. Serum levels of IL-17 and IL-23 were analyzed using enzyme linked immunosorbent assay (ELISA) kits (Human IL-17A Platinum ELISA- BMS2017 and Human IL-23 Platinum ELISA-BMS2023/3, eBioscience, San Diego, CA) and the results were expressed as pg/mL. The lower detection limit for IL-17 was 0.5 pg/mL and for IL-23 was 4.0 pg/mL.

### 2.3. Thyroid ultrasonography

An ultrasonographic investigation of the thyroid gland was performed using a GE Logiq 5 Pro system equipped with an 8–12 MHz linear probe (GE Medical Systems, Milwaukee, WI). Patients were examined in supine position with the neck slightly hyperextended. Transverse and longitudinal scans of the thyroid gland were performed, and various measures were recorded. The volume of each lobe was calculated by the formula: V (ml) = 0.479 × depth × width × length (cm). Total thyroid volume (Thyroid $Vol_T$) was the sum of volume of two lobes [6].

### 2.4. Statistical analysis

Normally distributed data were reported as mean ± SD. Data that did not show a Gaussian distribution were expressed as median (interquartile range). The categorical variables were represented as number and percentage. Comparisons of various numeric parameters among patient and control groups were analyzed with the ANOVA and Kruskal Wallis tests, as necessary. For two-group comparisons, Mann Whitney *U* test with Bonferroni correction was used to test for statistical significance at the 5% level after adjustment that was based on the number of groups that were compared (Bonferroni-corrected $p < 0.05/3 = 0.0167$). The correlations of serum interleukin levels with other parameters were carried out using Spearman's Rho test. Analyses were performed using SPSS version 15.0 and GraphPad Prism 6 for Windows. Differences were considered to be statistically significant if $p$ values were < 0.05.

## 3. Results

The age ($p = 0.195$) and sex ($p = 0.611$) distributions were similar among the patient groups and controls (Table 1). The cases with hypothyroid HT had a significantly higher TSH and lower FT4 concentrations than euthyroid HT cases and controls ($p < 0.001$ for TSH and $p = 0.001$ for FT4), whereas FT3 levels did not differ among the groups ($p = 0.230$) (Table 1). Both euthyroid and hypothyroid HT patients had higher thyroid autoantibody titers than controls ($p < 0.001$ for both *anti*-T and *anti*-TPO). Thyroid $Vol_T$ was significantly different among the groups, with the lowest volumes in hypothyroid patients ($p = 0.021$) (Table 1). However, in two-group comparisons, statistical significance between groups was lost when Bonferroni-adjusted $p$ value was used (euthyroid vs hypothyroid $p = 0.024$; hypothyroid vs controls $p = 0.018$; euthyroid vs control $p = 0.552$; adjusted $p < 0.0167$). The characteristics of the patient and control groups are given in Table 1.

Serum IL-17 levels were 6.13 (4.37) pg/mL for euthyroid HT patients, 4.16 (1.62) pg/mL for hypothyroid HT patients and 4.04 (4.17) pg/mL for the controls and the difference was significant among the three groups ($p = 0.041$) (Table 1). After the Bonferroni correction for subgroup analyses, the significances between groups were lost when the adjusted $p$ value was used (euthyroid vs hypothyroid $p = 0.018$; hypothyroid vs controls $p = 0.614$; euthyroid vs control $p = 0.043$; adjusted $p < 0.0167$) (Fig. 1). Serum IL-23 levels were significantly different among the three groups, with

**Table 1**
Demographic and laboratory parameters of study groups.

| | Euthyroid HT (n = 22) | Hypothyroid HT (n = 24) | Controls (n = 26) | $p^*$ |
|---|---|---|---|---|
| Age (years)[a] | 37.7 ± 11.0 | 42.1 ± 12.3 | 36.0 ± 12.9 | 0.195 |
| Sex (F/M) | 20/2 | 20/4 | 21/5 | 0.611 |
| TSH (μU/mL)[b] | 2.28 (1.85) | 10.69 (11.02) | 2.02 (1.04) | *<0.001* |
| FT4 (ng/dL)[b] | 1.07 (0.13) | 0.91 (0.27) | 1.05 (0.21) | *0.001* |
| FT3 (pg/mL)[b] | 3.14 (0.27) | 3.10 (0.58) | 3.25 (0.66) | 0.230 |
| *anti*-TPO (U/mL)[b] | 1300.0 (1343.5) | 1300.0 (2179.8) | 43.0 (22.7) | *<0.001* |
| *anti*-T (U/mL)[b] | 146.7 (107.2) | 183.5 (392.0) | 25.9 (17.6) | *<0.001* |
| Thyroid $Vol_T$ (mL) | 11.6 (7.1) | 8.1 (7.8) | 11.8 (12.8) | *0.021* |
| IL-17 (pg/mL)[b] | 6.13 (4.37) | 4.16 (1.62) | 4.04 (4.17) | *0.041* |
| IL-23 (pg/mL)[b] | 766.10 (776.76) | 207.53 (87.42) | 220.89 (103.41) | *<0.001* |

F: females, M: males, TSH: thyroid stimulating hormone, FT4: serum free T4, FT3: serum free T3, *anti*-TPO: *anti*-thyroid peroxidase antibody, *anti*-T: *anti*-thyroglobulin antibody, Thyroid $Vol_T$: Total thyroid gland volume, IL-17: Interleukin 17, IL-23: Interleukin 23.

[*] p value is for comparison of 3 groups; One-way ANOVA test for normally distributed data and Kruskal-Wallis test for not-normally distributed data; $p$ values < 0.05 were considered to be statistically significant.

[a] Presented as mean ± SD.

[b] Presented as median (interquartile range).