

Cytokine profiles in papillary thyroid carcinoma, with or without Hashimoto's thyroiditis

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Abstract

The phenomenon that papillary thyroid carcinoma (PTC) is often accompanied by Hashimoto's thyroiditis (HT) raised interest in further study of immune changes caused by thyroid autoimmunity. We aimed to characterize cytokine profiles in the peripheral blood of patients with PTC with or without HT, compared with nodular goiter (NG) and healthy control (HC) patients, in order to determine the autoimmunity-related differences among these groups. A total of 50 PTC patients were divided into two groups, according to whether or not concurrent HT existed. A total of 20 NG patients and 20 HC subjects were also included as controls. All PTC patients and NG patients who underwent surgical thyroidectomy and pathology examination were included. The serum levels of four cytokines, including interferon gamma (IFN- γ), interleukin (IL)-17, IL-10, and IL-35 were measured using AimPlex bead-based immunoassays. Peripheral IFN- γ and IL-35 increased significantly in PTC patients with concurrent HT. IL-10 and IL-35 increased significantly in three groups (PTC with or without HT and NG patients), while IFN- γ increased only in the two PTC groups. IL-17 was highest in the HC group, but significantly lower in the other three groups. IL-17, IL-10, and IL-35 may be involved in tumor onset and progression. Moreover, IFN- γ and IL-35 may play vital roles in the concurrence of PTC and HT, which could warrant further exploration.

Keywords

cytokines, Hashimoto's thyroiditis, immune cells, papillary thyroid carcinoma

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Introduction

Papillary thyroid carcinoma (PTC) accounts for 80%–85% of thyroid carcinomas, tends to invade lymphatic vessels, and displays a high incidence of regional lymph node metastasis. The most common associated condition is Hashimoto's thyroiditis (HT), whose main pathological feature is the breakdown of immune tolerance toward the thyroid gland.¹ Recent studies suggest that PTC follows a more benign course when accompanied by HT. HT in patients with PTC is associated with a low probability of BRAF^{V600E} mutations, which has been correlated with less-aggressive clinical features and inversely related to recurrence.^{2,3} Although the association of HT with PTC was first

reported by Dailey in 1955, there are no distinctive clinical or radiologic features that categorically differentiate HT concurrent with PTC from pure PTC.³ We aimed to throw some lights on the immunologic link between the two entities through

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Table 1. Characterization of patients with thyroid diseases and healthy subjects.

Subjects	N (%)	Gender		Age	
		Male	Female	<45 years	>45 years
Total patients	70	15	55	25	45
PTC+HT	25 (36)	1	24	11	14
PTC	25 (36)	9	16	8	17
NG	20 (28)	5	15	6	14
HC	20	7	14	8	12

PTC: papillary thyroid carcinoma; HT: Hashimoto's thyroiditis; NG: nodular goiter; HC: healthy control.

detecting the difference in serum cytokine levels between PTC and PTC concurrent with HT.

Cytokines are primarily produced by tumor-infiltrating immune cells but are also secreted by thyroid follicular cells. Cytokines play a role in the pathogenesis of autoimmune thyroid disease and contribute to several aspects of thyroid carcinoma initiation and growth. The primary objective of this study was to characterize cytokine profiles in the systemic circulation of PTC patients with or without HT and compare those to nodular goiter (NG) and health control (HC) subjects, in order to study the autoimmunity-related differences between these groups. We also aimed to determine whether or not the anti-tumor immune reaction predominates the anti-autoimmune reaction and provide clues for further research in PTC and HT concurrence.

Materials and methods

Study participants

In total, 50 PTC patients and 20 NG patients were recruited between October 2015 and December 2016 from Tianjin Medical University General Hospital. A total of 20 healthy subjects were also included as controls. The PTC patients and NG patients all underwent surgical thyroidectomy and pathologic examination. Of the 25 patients with PTC and HT, there were 24 female and 1 male, with the sex ratio being statistically lower than the other groups. There was no statistical difference in age distribution among the four groups. The general characteristics of the included subjects are shown in Table 1.

This study was planned according to the ethical guidelines of the Declaration of Helsinki. This project was approved by our institutional ethics committee. All subjects provided their informed consent prior to enrollment in the study.

Cytokine detection

Peripheral venous blood (2–3 mL) was obtained from all subjects in the morning under a fasting state. The supernatant was collected after centrifuging at 1000 g for 10 min; serum was stored at -80°C for further use. Interleukin (IL)-10, IL-17, IL-35, and interferon gamma (IFN- γ) serum levels were determined by AimPlex bead-based immunoassays, according to the manufacturer's instructions (QuantoBio, Beijing, China). All samples were analyzed by a FACSCalibur instrument, and data were collected by CellQuest Pro software (BD Biosciences, San Jose, CA, USA). Data were analyzed by FCAP Array v3.0 software (BD Biosciences).

Data analysis

Data were expressed as the mean \pm standard deviation. For continuous variables, normality was assessed by the Kolmogorov–Smirnov test. Data were analyzed using both parametric and nonparametric tests. Comparison of independent samples was performed by one-way analysis of variance (ANOVA), and post hoc comparisons were carried out using the least significant difference (LSD) and Dunnett's T3 test, respectively. Comparison of sex and age was performed by the chi-squared test. Correlations between clinical examination index and cytokine levels were assessed by calculating the Spearman correlation coefficient (r). Analyses were performed using SPSS version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (San Diego, CA, USA). A P value of less than 0.05 was considered to be statistically significant.

Results

Table 2 demonstrates the clinical test results of all included patients. There was no significant difference

Table 2. Clinical index of patients (mean \pm standard deviation).

Subjects	FT3 (pmol/L)	FT4 (pmol/L)	TSH (mIU/L)	TG (ng/mL)	TGAb (IU/mL)	TPOAb (IU/mL)
PTC + HT	5.08 \pm 0.65	14.86 \pm 2.65	3.56 \pm 1.92	44.59 \pm 84.45	698.55 \pm 1123.20	345.71 \pm 394.05
PTC	4.64 \pm 0.81	15.20 \pm 2.42	2.70 \pm 1.33 ^a	56.99 \pm 85.24	25.14 \pm 23.79 ^b	26.74 \pm 65.51 ^b
NG	4.91 \pm 1.08	16.21 \pm 2.88	2.31 \pm 1.41 ^a	84.48 \pm 88.47	24.51 \pm 19.20 ^b	13.14 \pm 4.30 ^b

FT3: free triiodothyronine; FT4: free tetraiodothyronine; TSH: thyroid-stimulating hormone; TG: thyroglobulin; TGAb: thyroglobulin antibody; TPOAb: thyroid peroxidases antibody; PTC + HT: papillary thyroid cancer and associated Hashimoto's thyroiditis; PTC: papillary thyroid cancer; NG: nodular goiter.

^aCompared with PTC+HT group, $P < 0.05$.

^bCompared with PTC+HT group, $P < 0.01$.

Table 3. Cytokine concentrations in the peripheral blood of patients and control subjects (mean \pm standard deviation).

Subjects	IL-10 (pg/mL)	IL-17A (pg/mL)	IL-35 (pg/mL)	IFN- γ (pg/mL)
PTC + HT	1.63 \pm 0.22 ^c	105.12 \pm 7.08 ^c	195.26 \pm 12.90 ^d	3.87 \pm 0.24 ^d
PTC	1.61 \pm 0.42 ^c	116.27 \pm 8.94 ^c	135.31 \pm 7.88 ^{a,d}	3.32 \pm 0.14 ^{a,c}
NG	1.57 \pm 0.25 ^c	98.96 \pm 6.07 ^d	142.10 \pm 9.21 ^{a,d}	3.17 \pm 0.15 ^a
HC	0.63 \pm 0.11	142.87 \pm 11.09	3.40 \pm 1.16	2.74 \pm 0.42 ^a

IL: interleukin; IFN- γ : interferon gamma; PTC + HT: papillary thyroid cancer and associated Hashimoto's thyroiditis; PTC: papillary thyroid cancer; NG: nodular goiter; HC: health control.

^aCompared with PTC+HT group, $P < 0.05$.

^bCompared with PTC+HT group, $P < 0.01$.

^cCompared with HC group, $P < 0.05$.

^dCompared with HC group, $P < 0.01$.

in serum free triiodothyronine (FT3), free tetraiodothyronine (FT4), and thyroglobulin (TG) levels between groups. Thyroid-stimulating hormone (TSH), TGAb, and TPOAb increased markedly in the group of patients with PTC and concurrent HT.

IL-10, IL-17, IL-35, and IFN- γ levels in the peripheral blood of two groups of patients with PTC (with or without HT), a group of patients with NG, and HC subjects were determined by multiplex immunoassays. These results are shown in Table 3 and Figure 1.

The mean levels of IL-10 and IL-35 in the PTC with HT, PTC, and NG groups were significantly higher in the HC group ($P < 0.05$), while IL-17 levels were significantly lower in the HC group ($P < 0.05$). The expression of IFN- γ increased significantly in the two PTC patient groups compared with HC subjects. The slight increase of IFN- γ in the NG group showed no statistical significance. There was no difference in IL-10 levels among the three patient groups (Figure 1(a)). IL-17 levels were slightly lower in the NG group compared with PTC patients, whether or not HT was present. However, there was no significant difference between IL-17 levels between the PTC group and the PTC with HT group (Figure 1(b)). IFN- γ contents in the peripheral blood of patients with PTC

and HT was significantly higher in the group of patients with PTC without HT, or NG patients (Figure 1(c)). Furthermore, IFN- γ levels positively correlated with the contents of TGAb ($r = 0.398$, $P = 0.001$). Similarly, IL-35 levels in the peripheral blood showed the same changing trend with IFN- γ levels. IL-35 levels increased significantly in the PTC with HT group, compared with the PTC without HT group and the NG group (Figure 1(d)).

Discussion

We investigated four cytokines, including IFN- γ , IL-17A, IL-10, and IL-35, which represent Th1/Th17/Treg, respectively. The imbalance among these lymphocyte subgroups were correlated with tumor occurrence and HT concurrence in thyroid cancer. Studies have shown that HT is a Th1-mediated autoimmune disease which also involved the participation of other subsets of CD4⁺T cells, such as Th17 cells and Treg cells.⁴ IFN- γ is produced primarily by Th1 cells and plays an important role in tumor immune surveillance. A prior study showed that peripheral blood cells from patients with PTC and concurrent HT produced more IFN- γ than in patients with PTC without HT.⁵ There is an increased ratio of Th17 to Treg in HT

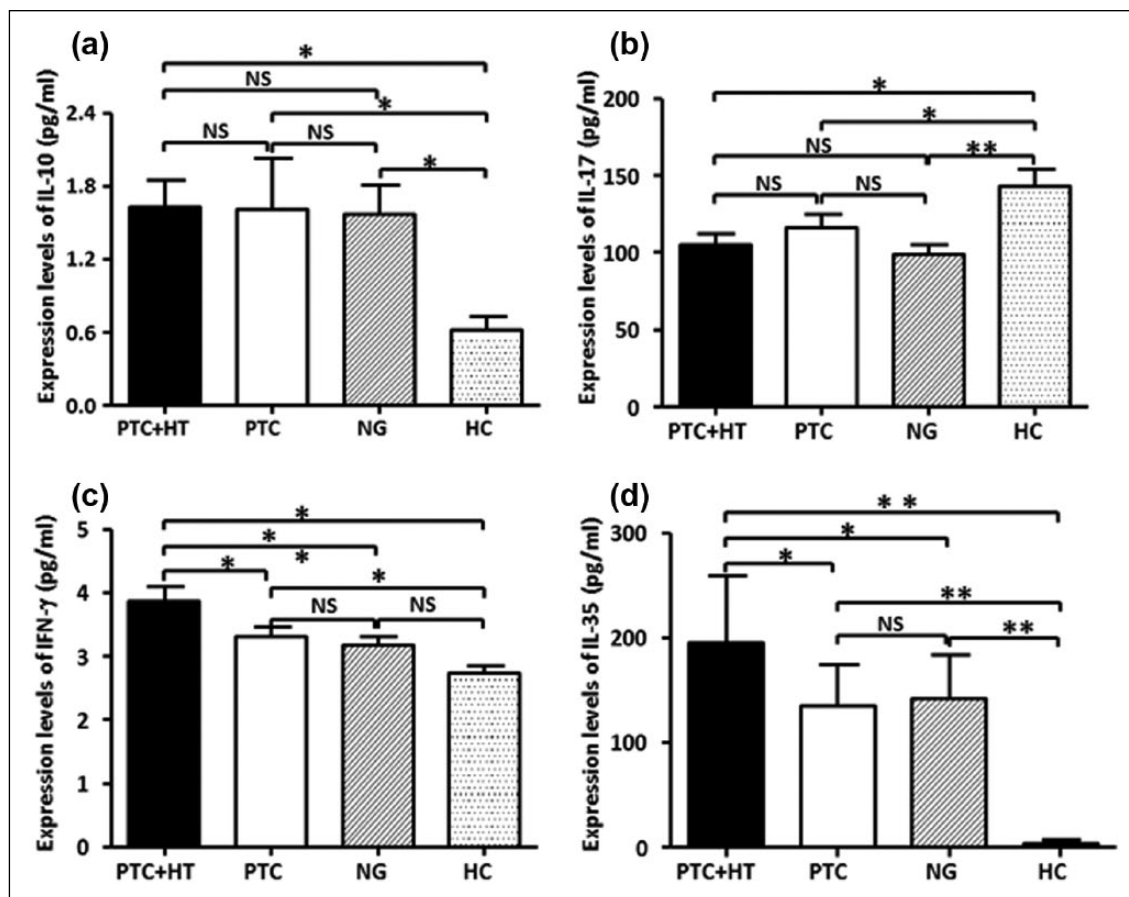


Figure 1. The expression levels of cytokines in the peripheral blood. Mean concentrations of IL-10, IL-17, IL-35, and IFN- γ in the peripheral blood of patients with papillary thyroid cancer accompanied by Hashimoto's thyroiditis (PTC+HT, $n=25$), papillary thyroid cancer (PTC, $n=25$), nodular goiter (NG, $n=20$), and healthy control subjects (HC, $n=20$).

patients and a positive correlation with auto-antibodies.^{6,7} Peripheral IL-17 levels in patients with HT was significantly higher than in the control group.⁸ IL-17 may play a pathological role in the early stage and may be one cause for the massive stromal fibrosis that helps distinguish this disease from other benign thyroid diseases.⁹ Furthermore, plasma IL-35 levels and IL-35 expression in the tumor-infiltrating lymphocytes have been shown to correlate with progression and poor prognosis.^{10,11} The serum levels of IL-35 in thyroid cancer patients was significantly lower than that of thyroid adenoma patients.¹² Our research suggested that IFN- γ , IL-17A, IL-10, and IL-35 are involved in the pathogenesis of thyroid tumors. Specifically, IL-10 and IL-17 have no relation to the concurrent phenomenon of PTC and HT, whereas IFN- γ and IL-35 of patients with PTC and HT were significantly higher than in those with PTC without HT, and NG patients. Furthermore, IFN- γ levels were positively correlated with TGAb levels. The

increased secretion of IFN- γ and IL-35 might be beneficial, considering the good prognosis in patients with PTC accompanied by HT.

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F.L. and C.W. are co-first authors.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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