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*Original Article*

# Impact of Hashimotoas thyroiditis on clinicopathologic features of papillary thyroid carcinoma associated with infiltration of tumor-infiltrating lymphocytes

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**Abstract:** The association between Hashimoto's thyroiditis (HT) and papillary thyroid carcinoma (PTC) has been a long and ongoing controversy. In the present study, a total number of 322 patients who underwent thyroidectomies were retrospectively studied, and the impact of HT with the incidence, clinicopathologic features, and presence of CD4+, CD8+ tumor-infiltrating lymphocytes (TILs) in PTC patients were analyzed. We further explored the correlation between CD4+, CD8+ TILs and clinicopathologic features in PTC patients with and without HT. The incidence of HT concurrent with PTC was 42.9%, which was significantly associated with a younger age ($P$=0.039) and the presence of hyperthyroid ($P$=0.010). The PTC patients coexistent with HT tended to be more female ($P$=0.001), with diffuse swelling of the thyroid ($P$<0.001), decreased TSH ($P$=0.004), and elevated anti-TgAb ($P$<0.001) and anti-TPOAb ($P$<0.001). The tumor size of PTC with HT was smaller ($P$=0.006) and exhibited more bilateral tumors ($P$<0.001) and less lymph node metastasis ($P$=0.016). Furthermore, CD4+ and CD8+ TILs in PTC with HT were significantly higher than without HT (both $P$<0.001). Both high CD4+ and CD8+ TILs were significantly associated with elevated TSH ($P$=0.019 and $P$=0.023, respectively), anti-TgAb ($P$=0.002 and $P$=0.001, respectively) and anti-TPOAb ($P$=0.001 and $P$=0.003, respectively), and the tumor size was smaller ($P$=0.017 and $P$=0.039, respectively) and with less lymph node metastasis ($P$=0.012 and $P$<0.001, respectively) in PTC with HT. Our study suggests that HT is significantly associated with PTC, which might be ascribed to infiltration of CD4+, CD8+ TILs.

**Keywords:** Hashimoto's thyroiditis, papillary thyroid carcinoma, CD4, CD8, tumor-infiltrating lymphocytes

## Introduction

Hashimoto's thyroiditis (HT) is the most common type of autoimmune thyroid disease [1] and is the leading cause of hypothyroidism and is associated with the development of thyroid malignancy [2]. Papillary thyroid carcinoma (PTC) is the most prevalent thyroid carcinoma worldwide [3]. The incidence of both HT and PTC has rapidly increased in recent years. Numerous studies have concentrated on the relationship between HT and PTC, but this association has been long and ongoing controversial. Some investigations have reported that PTC is significantly associated with HT and PTC coexistent with HT and has favorable clinicopathologic characteristics as well as better prognosis. In contrast, other studies have not observed a positive correlation or distinctive clinicopathologic features of HT concurrent with PTC [4-6].

Studies have long recognized that infiltration of both innate and adaptive immune cells of the immune system in the tumor microenvironment plays an important role in the progression of tumors [7]. Such infiltration may reflect an effort of the immune system in tumor immunosurveillance and eradicating tumor cells. Conversely, increasing evidence suggests that during tumor progression, tumor cells are more prone to tumor immune escape mechanisms [8]. Thus this complex interaction between immune and tumor cells may influence tumor development.

Since HT is a common autoimmune thyroid disease, the thyroid-specific immune response with infiltration of lymphocytes plays an important role in the development of PTC [9]. Ehlers et al. [10] reported that thyroid carcinoma developed due to immune response. Cunha et

**Table 1.** Demographics and clinicopathologic features of the patients

| | HT (n=116) | HT with PTC (n=87) | PTC (n=119) |
|---|---|---|---|
| Gender, n (%) | | | |
| Male | 4 (3.4) | 6 (6.9) | 28 (23.5) |
| Female | 112 (96.6) | 81 (93.1) | 91 (76.5) |
| Age, n (%) | | | |
| <45 | 32 (27.6) | 36 (41.4) | 59 (49.6) |
| ≥45 | 84 (72.4) | 51 (58.6) | 60 (50.4) |
| Diffuse swelling of thyroid, n (%) | | | |
| No | 51 (44.0) | 50 (57.5) | 115 (96.6) |
| Yes | 65 (56.0) | 37 (42.5) | 4 (3.4) |
| TSH, n (%) | | | |
| Normal | 71 (61.2) | 65 (74.7) | 108 (90.7) |
| Elevated | 13 (11.2) | 13 (14.9) | 4 (3.4) |
| Decreased | 32 (27.6) | 9 (10.4) | 7 (5.9) |
| Anti-TgAb, n (%) | | | |
| Elevated | 52 (44.8) | 43 (49.4) | 100 (84.0) |
| Normal | 64 (55.2) | 44 (50.6) | 19 (16.0) |
| Anti-TPOAb, n (%) | | | |
| Elevated | 81 (69.8) | 52 (59.8) | 101 (84.9) |
| Normal | 35 (30.2) | 35 (40.2) | 18 (15.1) |
| Tumor size (M ± SD, cm) | - | 1.518 ± 1.101 | 1.924 ± 0.993 |
| Number of tumors, n (%) | | | |
| Unilateral | - | 14 (16.1) | 85 (71.4) |
| Bilateral | - | 73 (83.9) | 34 (28.6) |
| Lymph node metastasis, n (%) | | | |
| No | | 43 (49.4) | 39 (32.8) |
| Yes | | 44 (50.6) | 80 (67.2) |
| TNM stage, n (%) | | | |
| I/II | - | 71 (81.6) | 86 (72.3) |
| III/IV | - | 16 (18.4) | 33 (27.7) |

al. [11] demonstrated that infiltration of immune cells plays an important role in immune-escape mechanisms of PTC and results in a decreased immune response which may lead to a favorable prognosis. In addition, Muzza et al. [12] found that a mixture of lymphocytes is frequently found within and surrounding PTC concurrent with thyroiditis. However, the precise impact of HT on infiltration of the immune cells in PTC and its subsequent impact on progression of PTC remain to be clarified.

Considering the above, we investigated the impact of HT on incidence and clinicopathologic features of PTC patients. We further explored the presence of CD4+, CD8+ tumor-infiltrating lymphocytes (TILs) and their correlation with clinicopathologic features in PTC patients with and without HT.

## Materials and methods

### *Patients*

This was a retrospective study of a series of 322 patients who underwent thyroidectomies between 2013 and 2015 in Tianmen First People's Hospital. Inclusion criteria for the present study were patients: (1) underwent bilateral thyroidectomies; (2) diagnosed pathologically with HT, concurrent with and without PTC; (3) diagnosed pathologically with PTC, all of whom without HT; (4) all of whom with neck dissections. Exclusion criteria were patients: (1) with unilateral thyroidectomies; (2) those diagnosed pathologically of HT with benign thyroid nodules; (3) those without neck dissections. The histological diagnosis of HT was the presence of diffuse lymphocytic infiltration in the thyroid parenchyma and stroma, with formation of reactive germinal centers and lymphoid nodules and presence of oxyphilic cells [13]. Clinicopathologic features of each patient were obtained from the Department of Thyroid Breast Surgery, Tianmen First People's Hospital (**Table 1**). TNM staging of PTC was ascertained according to the 7$^{th}$ edition of AJCC/UICC TNM system for differentiated thyroid carcinoma [14]. The study was approved by the Scientific Research Ethics Committee of Tianmen First People's Hospital, and the informed consent for the use of tissues for *ex vivo* experimentation was obtained from each patient.

### *Immunohistochemical staining*

A conventional immunohistochemical (IHC) staining protocol was used in this study. Briefly,

**Table 2.** Correlation of HT with clinicopathological features of PTC patients

| | HT with PTC (n=87) | PTC (n=119) | *P*-value |
|---|---|---|---|
| Clinical Features | | | |
| Gender, n (%) | | | |
| Male | 6 (6.9) | 28 (23.5) | 0.001 |
| Female | 81 (93.1) | 91 (76.5) | |
| Age, n (%) | | | |
| <45 | 36 (41.4) | 59 (49.6) | 0.244 |
| ≥45 | 51 (58.6) | 60 (50.4) | |
| Diffuse swelling of thyroid, n (%) | | | |
| No | 50 (57.5) | 115 (96.6) | <0.001 |
| Yes | 37 (42.5) | 4 (3.4) | |
| TSH, n (%) | | | |
| Normal | 65 (74.7) | 108 (90.7) | 0.004 |
| Elevated | 13 (14.9) | 4 (3.4) | |
| Decreased | 9 (10.4) | 7 (5.9) | |
| Anti-TgAb, n (%) | | | |
| Elevated | 43 (49.4) | 100 (84.0) | <0.001 |
| Normal | 44 (50.6) | 19 (16.0) | |
| Anti-TPOAb, n (%) | | | |
| Elevated | 52 (59.8) | 101 (84.9) | <0.001 |
| Normal | 35 (40.2) | 18 (15.1) | |
| Pathological features | | | |
| Tumor size (M ± SD, cm) | 1.518 ± 1.101 | 1.924 ± 0.993 | 0.006 |
| Number of tumor, n (%) | | | |
| Unilateral | 14 (16.1) | 85 (71.4) | <0.001 |
| Bilateral | 73 (83.9) | 34 (28.6) | |
| Lymph node metastasis, n (%) | | | |
| No | 43 (49.4) | 39 (32.8) | 0.016 |
| Yes | 44 (50.6) | 80 (67.2) | |
| TNM stage, n (%) | | | |
| I/II | 71 (81.6) | 86 (72.3) | 0.120 |
| III/IV | 16 (18.4) | 33 (27.7) | |

paraffin-embedded tumor tissue blocks were cut into sections (4 μm thick), dried, deparaffinized, and dehydrated in a graded series of ethanol. Tissue sections were treated with 1% hydrogen peroxide for 10 min to block endogenous tissue peroxidase activity, followed by treatment with bovine serum for 30 min to reduce nonspecific binding. Antigen retrieval was then accomplished using citrate buffer (pH 6.0) as follows: high heat microwave processing for 5 min followed by low heat microwave processing for 20 min. All the slides were incubated with monoclonal mouse anti-human CD4 antibody (Ready-to-use, 1F6, Gene Tech. Co. Ltd., Shanghai, China) and monoclonal rabbit anti-human CD8 antibody (Ready-to-use, SP16, Gene Tech. Co. Ltd., Shanghai, China) overnight at 4°C, followed by a 30-min incubation in Ultra-Sensitive S-P Kit (Maixin-Bio, Fuzhou, China). Slides were then rinsed with phosphate-buffered saline before color development using 3,3'-diaminobenzidine substrate kit, and counterstained with hematoxylin.

Slides were read by two senior pathologists who were blinded to the clinicopathologic data. Membranous or cytoplasmic staining with CD4 and CD8 antibodies in TILs was defined as positive. IHC staining of CD4 and CD8 proteins were assessed in terms of staining intensity and percentage of positive cells as follows: 0 (negative, ≤5% of cells staining positive), 1+ (weak staining, 6-25% of cells staining positive), 2+ (moderate staining, 26-50% of cells staining positive), and 3+ (strong staining, >50% of cells staining positive). The final score for each slide was represented by the average of three representative high-power fields (hpf, ×400). Scores ≤1+ were defined as low expression and scores ≥2+ were described as high expression.

*Statistical analysis*

Statistical analysis was performed using SPSS 21.0 software. The risk factors associated HT

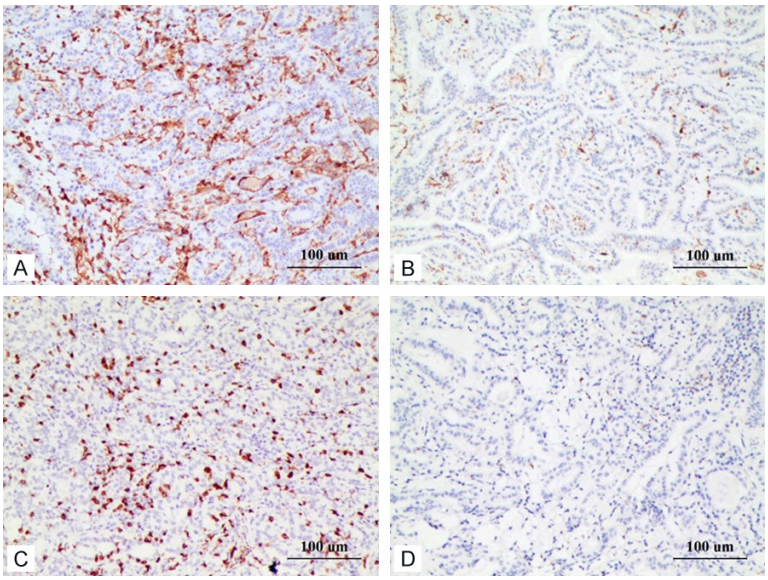


**Figure 1.** Imumunohistochemical staining of CD4 and CD8 protein in PTC tissues with HT. Staining of CD4 and CD8 protein was mainly located in the membrane or cytoplasm of lymphocytes (×200): High CD4+ (A) and CD8+ (C) TILs infiltration in PTC with HT; Low CD4+ (B) and CD8+ (D) TILs infiltration in PTC with HT.

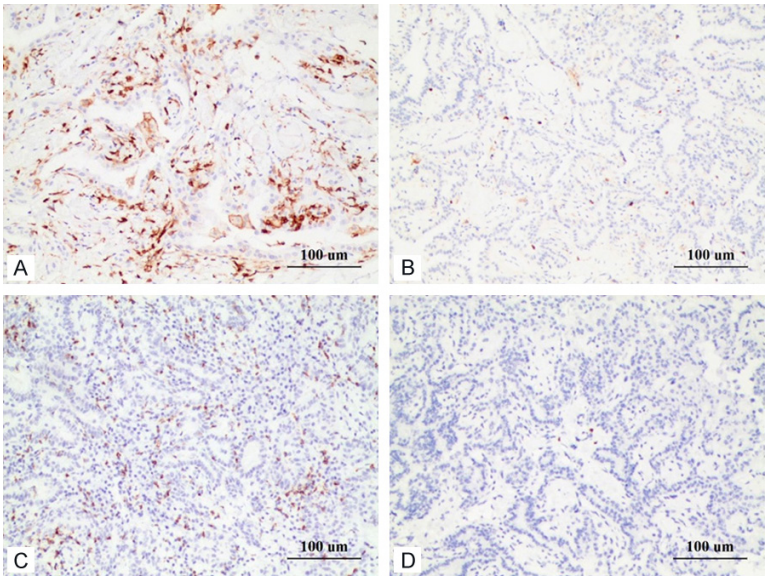


**Figure 2.** Imumunohistochemical staining of CD4 and CD8 protein in PTC tissues without HT. The staining of CD4 and CD8 was mainly located in the membrane or cytoplasm of lymphocytes (×200): High CD4+ (A) and CD8+ (C) TILs infiltration in PTC without HT; Low CD4+ (B) and CD8+ (D) TILs infiltration in PTC without HT.

with PTC were analyzed by Chi-square test and multivariate logistic regression analysis. Correlation of HT with the clinicopathological features of PTC patients were compared by Chi-square test and Mann-Whitney U tests. Furthermore, the presence of CD4+, CD8+ TILs and correlation with clinicopathologic features of PTC patients with and without HT were analyzed using Chi-square and Mann-Whitney U tests analysis. All two-sided *P*-values <0.05 were considered statistically significant.

## Results

### *Risk factors for the incidence of PTC in HT patients*

Among 203 patients who diagnosed with HT, 87 (42.9%) patients were coexistent with PTC, 73 (83.9%) of whom were bilateral PTC and the other 14 (16.1%) were unilateral PTC. The risk factors for the incidence of PTC in HT patients were analyzed by univariate and mutivariate analysis. Univariate analysis found that HT patients concurrent with PTC were significantly associated with a younger age (*P*=0.039) and a presence of hyperthyroid (*P*=0.010), while they had no association with gender, diffuse thyroid swelling, anti-TgAb level, and anti-TPOAb (*P*> 0.05 for all) (Supplementary Table 1). Multivariate logistic regression analysis showed that age [HR=0.512, 95% CI (0.276-0.948); *P*=0.033] and hyperthyroid [HR=0.306, 95% CI (0.130-0.720); *P*=0.007] were independent factors for incidence of PTC in HT patients (Supplementary Table 2).

### *Correlation of HT with clinicopathological features of PTC patients*

Among 206 patients who were diagnosed with PTC in this study, HT was present in 87 patients (42.2%) and absent in 119 (57.8%). The male to female ratios of the PTC patients with and without HT were 1:13.5 and 1:3.25, respectively

**Table 3.** Expression of CD4 and CD8 in PTC patients with and without HT

| | HT with PTC (n=87) | PTC (n=119) | *P*-value |
|---|---|---|---|
| CD4, n (%) | | | |
| Low | 36 (41.4) | 86 (72.3) | <0.001 |
| High | 51 (58.6) | 33 (27.7) | |
| CD8, n (%) | | | |
| Low | 43 (49.4) | 89 (74.8) | <0.001 |
| High | 44 (50.6) | 30 (25.2) | |

(*P*=0.001). Comparison of the clinical features of PTC without HT, PTC with HT tended to exhibit diffuse swelling of thyroid (*P*<0.001), more hypothyroid (*P*=0.004), and elevated level of anti-TgAb (*P*<0.001) and anti-TPOAb (*P*<0.001). The tumor size of PTC with HT was smaller vs. without HT (1.518 ± 1.101 vs. 1.924 ± 0.993, *P*=0.006), and PTC with HT exhibited more bilateral tumors (*P*<0.001) and less lymph node metastasis (*P*=0.016). However, there were no statistically significant differences in age (*P*=0.244) or TNM stage (*P*=0.120) (**Table 2**).

*Presence of CD4+, CD8+ TILs and correlation with clinicopathologic features in PTC with and without HT*

IHC of 87 cases of PTC with HT and 119 PTC samples was performed. As shown in **Figures 1**, **2**, positive staining of CD4 and CD8 protein was mainly localized to the membrane or cytoplasm of lymphocytes in PTC tissues. The results of IHC staining of CD4 and CD8 proteins in PTC with HT and PTC are shown in **Table 3**. CD4 and CD8 expression in PTC with HT were significantly higher than that in PTC (both *P*<0.001).

The correlation between CD4+, CD8+ TILs and clinicopathologic features in PTC with and without HT were also analyzed (**Table 4** and Supplementary Table 3). It was found that in PTC with HT, both high CD4 and CD8 expression were significantly associated with elevated TSH (*P*=0.019 and *P*=0.023, respectively), anti-TgAb (*P*=0.002 and *P*=0.001, respectively), and anti-TPOAb (*P*=0.001 and *P*=0.003, respectively). Furthermore, high expression of CD4 and CD8 in PTC with HT, correlated with smaller tumor sizes (*P*=0.017 and *P*=0.039, respectively) and with less lymph node metastasis (*P*=0.012 and *P*<0.001, respectively). Expression of CD4 and CD8 were not associated with any clinicopathologic features in PTC without HT (*P*>0.05 for all).

## Discussion

HT is a most common form of autoimmune thyroid disease in which the immune system reacts against a variety of thyroid antigens and produced a variety of autoantibodies, most commonly like anti-thyroglobulin antibodies (anti-TgAb) and anti-thyroid peroxidase antibodies (anti-TPOAb). It is the leading cause of hypothyroidism and associated with the development of thyroid malignancy as well. PTC is the most common histological subtype of thyroid carcinoma, which represents 75%-85% of all thyroid cancer. Since Dailey [15] first described the relationship between HT and PTC in 1995, an increasing number of studies explored this association but conflicting results were reported. In contrast to studies showing an association of PTC with HT [4, 5, 16], there are some other studies show a lack of correlation. In a study including 919 patients with 1321 PTC lesions, among which 317 had (34.5%) coexistent HT, indicated that none of the ultrasonography features, fine-needle aspiration biopsy (FNAB), or histopathological findings were influenced by the presence of HT [17]. Actually, the association of PTC and HT has been less understood [18]. Hence, clarifying the relationship between HT and PTC is urgently required.

The reported incidence in a series of studies of HT with PTC ranges from 0.5% to 43.8% [19]. Some investigations have reported that PTC is significantly associated with HT and has favorable clinicopathologic characteristics as well as a better prognosis, whereas other studies have not observed such phenomena [4-6]. Ahn et al. [20] observed that HT concurrent with PTC had a greater female preponderance and patients were younger at presentation. In the present study, the incidence of HT concurrent with PTC was 42.9%, which matched with the literature. We also found that HT concurrent with PTC was associated with a younger age and a presence of hyperthyroid. HT also had great effect on clinicopathologic features of PTC. HT concurrent with PTC had a greater female preponderance, and tended to exhibit diffuse thyroid swelling, elevated level of TSH, anti-TgAb, and anti-TPOAb. The tumor size was smaller in PTC with HT, and exhibited more bilateral tumors with less lymph node metastasis. These data

**Table 4.** Correlation of CD4 and CD8 expression with clinicopathologic features in PTC with HT

| | CD4 | | *P*-value | CD8 | | *P*-value |
|---|---|---|---|---|---|---|
| | Low (n=36) | High (n=51) | | Low (n=43) | High (n=44) | |
| Gender, n (%) | | | | | | |
| Male | 4 (11.1) | 2 (3.9) | 0.192 | 4 (9.3) | 2 (4.5) | 0.381 |
| Female | 32 (88.9) | 49 (96.1) | | 39 (90.7) | 42 (95.5) | |
| Age, n (%) | | | | | | |
| <45 | 15 (41.7) | 21 (41.2) | 0.964 | 18 (41.9) | 18 (40.9) | 0.928 |
| ≥45 | 21 (58.3) | 30 (58.8) | | 25 (58.1) | 26 (59.1) | |
| Diffuse swelling of thyroid, n (%) | | | | | | |
| No | 21 (58.3) | 29 (56.9) | 0.891 | 27 (62.8) | 23 (52.3) | 0.321 |
| Yes | 15 (41.7) | 22 (43.1) | | 16 (37.2) | 21 (47.7) | |
| TSH, n (%) | | | | | | |
| Normal | 32 (88.9) | 33 (64.7) | 0.019 | 37 (86.0) | 28 (63.6) | 0.023 |
| Elevated | 1 (2.8) | 12 (23.5) | | 2 (4.7) | 11 (25.0) | |
| Decreased | 3 (8.3) | 6 (11.8) | | 4 (9.3) | 5 (11.4) | |
| Anti-TgAb, n (%) | | | | | | |
| Elevated | 11 (30.6) | 33 (64.7) | 0.002 | 14 (32.6) | 30 (68.2) | 0.001 |
| Normal | 25 (69.4) | 18 (35.3) | | 29 (67.4) | 14 (31.8) | |
| Anti-TPOAb, n (%) | | | | | | |
| Elevated | 14 (38.9) | 38 (74.5) | 0.001 | 19 (44.2) | 33 (75.0) | 0.003 |
| Normal | 22 (61.1) | 13 (25.5) | | 24 (55.8) | 11 (25.0) | |
| Tumor size (M ± SD, cm) | 1.850 ± 1.428 | 1.284 ± 0.723 | 0.017 | 1.767 ± 1.365 | 1.275 ± 0.694 | 0.039 |
| Number of tumor, n (%) | | | | | | |
| Unilateral | 4 (11.1) | 10 (19.6) | 0.288 | 6 (14.0) | 8 (18.2) | 0.592 |
| Bilateral | 32 (88.9) | 41 (80.4) | | 37 (86.0) | 36 (81.8) | |
| Lymph node metastasis, n (%) | | | | | | |
| No | 12 (33.3) | 31 (60.8) | 0.012 | 13 (30.2) | 30 (68.2) | <0.001 |
| Yes | 24 (66.7) | 20 (39.2) | | 30 (69.8) | 14 (31.8) | |
| TNM stage, n (%) | | | | | | |
| I/II | 27 (75.0) | 44 (86.3) | 0.181 | 33 (76.7) | 38 (86.4) | 0.247 |
| III/IV | 9 (25.0) | 7 (13.7) | | 10 (23.3) | 6 (13.6) | |

indicate that HT is associated with the incidence and clinicopathologic features of PTC, and HT could be a risk factor for PTC.

TSH is the major growth factor for thyroid cells, and elevated serum TSH served as a risk factor for thyroid cancer development. Elevated levels of TSH in hypothyroid patients with HT may stimulate follicular epithelial proliferation, thereby promoting development of papillary carcinoma [21]. Fiore et al. [22] found that TSH levels also correlated strongly with the presence of PTC. Herein, we found that HT concurrent with PTC was significantly associated with the level of TSH, and that decreased TSH tended to suppress development of PTC.

It is generally believed that infiltration of immune cells plays an important role in the progression of tumor through immunosurveillance and eradicating tumor cells in tumor microenvironment [7]. Tamimi et al. [9] reported a significantly higher rate of lymphocytic infiltrate in patients with PTC compared to patients with follicular adenoma and suggested a link between chronic lymphocytic infiltration and PTC. HT is a most common autoimmune thyroid disease, in which the thyroid-specific immune response that infiltration of lymphocytes plays an important role in the development of PTC [9]. PTC concurrent with HT was found associated with intratumoural infiltration of lymphocytes, suggesting that HT may modulate the tumour microenvironment, hence increasing an antitumour immune response [11]. In addition, Muzza et al. [12] indicated that a mixture of lymphocytes is frequently found within and surrounding PTC concurrent with thyroiditis. However, the precise impact of HT in lympho-

cyte infiltration and the subsequent impact on PTC progression remains to be clarified.

T lymphocytes are the major subgroup of tumor-infiltrating immune cells, among which CD8+ T cells and CD4+ T cells comprise the primary immune cells responsible for anti-tumor immunity [23]. Our results showed that CD4+ and CD8+ TILs infiltration in PTC with HT tissues were significantly higher than in PTC tissues. Both high infiltration of CD4+ and CD8+ TILs were significantly associated with elevated TSH, anti-TgAb and anti-TPOAb, and the tumor size was smaller and with less lymph node metastasis in PTC with HT. Furthermore, CD4+ TILs, CD8+ TILs, anti-TgAb, and anti-TPO could have predictive performance associated HT with PTC. These data suggest a role for infiltration of CD4+ and CD8+ TILs in affecting the clinicopathologic features of HT with PTC.

In conclusion, our study provides evidence that HT is significantly associated with the incidence and clinicopathologic features of PTC, which might be correlated with infiltration of CD4+ and CD8+ TILs. Therefore, HT can be considered as a risk factor for PTC. However, further studies are required to elucidate the mechanism through which HT participates in the progression of PTC.

## Disclosure of conflict of interest

None.

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**Supplementary Table 1.** Univariate analysis of risk factors for the incidence of PTC in HT patients

| | HT (n=116) | HT with PTC (n=87) | *P*-value |
|---|---|---|---|
| Gender, n (%) | | | |
| Male | 4 (3.4) | 6 (6.9) | 0.261 |
| Female | 112 (96.6) | 81 (93.1) | |
| Age, n (%) | | | |
| <45 | 32 (27.6) | 36 (41.4) | 0.039 |
| ≥45 | 84 (72.4) | 51 (58.6) | |
| Diffuse swelling of thyroid, n (%) | | | |
| No | 51 (44.0) | 50 (57.5) | 0.075 |
| Yes | 65 (56.0) | 37 (42.5) | |
| TSH, n (%) | | | |
| Normal | 71 (61.2) | 65 (74.7) | 0.010 |
| Elevated | 13 (11.2) | 13 (14.9) | |
| Decreased | 32 (27.6) | 9 (10.3) | |
| Anti-TgAb, n (%) | | | |
| Elevated | 52 (44.8) | 43 (49.4) | 0.598 |
| Normal | 64 (55.2) | 44 (50.6) | |
| Anti-TPOAb, n (%) | | | |
| Elevated | 81 (69.8) | 52 (59.8) | 0.136 |
| Normal | 35 (30.2) | 35 (40.2) | |

**Supplementary Table 2.** Multivariate logistic regression analysis of risk factors associate HT with PTC

| Variables | B | Wald value | OR (95% CI) | *P*-value |
|---|---|---|---|---|
| Gender (Male/Female) | -1.067 | 2.275 | 0.344 (0.086-1.376) | 0.131 |
| Age (<45/≥45) | -0.67 | 4.529 | 0.512 (0.276-0.948) | 0.033 |
| Diffuse swelling of thyroid (No/Yes) | -0.367 | 1.485 | 0.693 (0.384-1.250) | 0.223 |
| TSH (Elevated/Normal) | -1.185 | 7.356 | 0.306 (0.130-0.720) | 0.007 |
| Anti-TgAb (Elevated/Normal) | 0.118 | 0.12 | 1.125 (0.576-2.198) | 0.729 |
| Anti-TPOAb (Elevated/Normal) | -0.395 | 1.218 | 0.674 (0.334-1.359) | 0.270 |

**Supplementary Table 3.** Correlation of CD4 and CD8 expression with clinicopathologic features in PTC

| | CD4 | | *P*-value | CD8 | | *P*-value |
|---|---|---|---|---|---|---|
| | Low (n=86) | High (n=33) | | Low (n=89) | High (n=30) | |
| Gender, n (%) | | | | | | |
| Male | 18 (20.9%) | 10 (30.3%) | 0.281 | 18 (20.2%) | 10 (33.3%) | 0.143 |
| Female | 68 (79.1%) | 23 (69.7%) | | 71 (79.8%) | 20 (66.7%) | |
| Age, n (%) | | | | | | |
| <45 | 46 (53.5%) | 13 (39.4%) | 0.169 | 47 (52.8%) | 12 (40.0%) | 0.225 |
| ≥45 | 40 (46.5%) | 2060.6%) | | 42 (47.2%) | 18 (60.0%) | |
| Diffuse swelling of thyroid, n (%) | | | | | | |
| No | 84 (97.7%) | 31 (93.9%) | 0.312 | 87 (97.8%) | 28 (93.3%) | 0.245 |
| Yes | 2 (2.3%) | 2 (6.1%) | | 2 (2.2%) | 2 (6.7%) | |
| TSH, n (%) | | | | | | |
| Normal | 78 (90.7%) | 30 (90.9%) | 0.445 | 81 (91.0%) | 27 (90.0%) | 0.417 |
| Elevated | 2 (2.3%) | 2 (6.1%) | | 2 (2.3%) | 2 (6.7%) | |
| Decreased | 6 (7.0%) | 1 (3.0%) | | 6 (6.7%) | 1 (3.3%) | |
| Anti-TgAb, n (%) | | | | | | |
| Elevated | 13 (15.1%) | 6 (18.2%) | 0.683 | 15 (16.9%) | 4 (13.3%) | 0.649 |
| Normal | 73 (84.9%) | 27 (81.8%) | | 74 (83.1%) | 26 (86.7%) | |
| Anti-TPOAb, n (%) | | | | | | |
| Elevated | 12 (14.0%) | 6 (18.2%) | 0.564 | 13 (14.6%) | 5 (16.7%) | 0.785 |
| Normal | 74 (86.0%) | 27 (81.8%) | | 76 (85.4%) | 25 (83.3%) | |
| Tumor size (M ± SD, cm) | 1.966 ± 0.955 | 1.812 ± 1.091 | 0.450 | 1.996 ± 0.965 | 1.710 ± 1.058 | 0.174 |
| Number of tumor, n (%) | | | | | | |
| Unilateral | 61 (70.9%) | 24 (72.7%) | 0.846 | 64 (71.9%) | 21 (70.0%) | 0.841 |
| Bilateral | 25 (29.1%) | 9 (27.3%) | | 25 (28.1%) | 9 (30.0%) | |
| Lymph node metastasis, n (%) | | | | | | |
| No | 27 (31.4%) | 12 (36.4%) | 0.605 | 27 (30.3%) | 12 (40.0%) | 0.329 |
| Yes | 59 (68.6%) | 21 (63.6%) | | 62 (69.7%) | 18 (60.0%) | |
| TNM stage, n (%) | | | | | | |
| I/II | 63 (73.3%) | 23 (69.7%) | 0.698 | 64 (71.9%) | 22 (73.3%) | 0.880 |
| III/IV | 23 (26.7%) | 10 (30.3%) | | 25 (28.1%) | 8 (26.7%) | |