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Original Article

The clinical evaluation of the relationship between papillary thyroid microcarcinoma and Hashimoto's thyroiditis

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Abstract: To evaluate the clinical relationship between papillary thyroid microcarcinoma (PTMC) and Hashimoto's thyroiditis (HT). A total number of 168 patients with histologically confirmed PTMC were enrolled into this study and divided into two groups according to the presence (n=49) or absence (n=119) of concurrent HT. The clinicopathological characteristics between two groups were compared, including age, gender, tumor location, single or multiple lesions, tumor size, invasion of envelope, lymph node metastasis. The correlation between PTMC and HT was analyzed by multivariate logistics analysis. Furthermore, PTMC tissue removed during surgery were collected for immunohistochemistry to evaluate the expression of $BRAF^{V600E}$. Thirty-three percent (38/115) female patients with PTMC were accompanied with HT, which is higher than that of male patients (20.75%, 11/53). In PTMC patients with HT, the average age was younger [(42.18±9.84) *vs.* (46.35±11.23) years] and tumor size was smaller [(0.65±0.12) *vs.* (0.87±0.22) cm] compared with those without HT. Multivariate logistics analysis showed that concurrent HT was an independent risk factor for PTMC (OR 3.012, 95% CI 2.025~3.731, P<.05). The rate of positively stained anti-$BRAF^{V600E}$ was lower in the cases with HT than those without HT. The expression of $BRAF^{V600E}$ was weaker in female than male patients with HT. Additionally, the expression of $BRAF^{V600E}$ was relatively stronger in the patients without HT who suffered from invasion of envelope and lymph node metastasis. The risk of concurrent HT in PTMC patients increases predominantly. The concurrence of HT in PTMC is of higher incidence in female patients, younger age, smaller tumor size and lower incidence of envelope invasion and lymph node metastasis. Additionally, the expression of $BRAF^{V600E}$ is relatively weaker in PTMC patients with concurrent HT. For future work, large-scale perspective follow-up and epidemiological study may help to establish an effective PTMC risk-evaluation system for the patients with HT.

Keywords: Papillary thyroid microcarcinoma, hashimoto's thyroiditis, clinical correlation

Introduction

Primary thyroid carcinoma is the most common endocrine malignancy including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, anaplastic thyroid carcinoma and medullary thyroid carcinoma etc. PTC represents about 85% of thyroid cancers and most are histologically classified as conventional PTCs. Early detection and treatment is crucial for the prognosis of papillary thyroid microcarcinoma (PTMC) smaller than 1 cm [1]. Hashimoto's thyroiditis (HT), first described by a Japanese surgeon Hakaru Hashimoto, is an autoimmune disease of thyroid gland. Its annual incidence is estimated to be 30~150/100000 while the incidence of PTC is approximately 77/100000 [2]. Chronic inflammation may favor the tumor pathogenesis. Some of underlying mechanisms have been elucidated. But the relationship between PTC and HT remains controversial in term that whether PTC is an active response to HT, or HT is a premalignant state of PTC [3]. In this study we enrolled 168 cases of PTMC with or without HT. And we retrospectively analyzed their clinicopathological characteristics and expression of $BRAF^{V600E}$ in order to evaluate the clinical correlation between PTMC and HT.

Materials and methods

Patients

This study was approved by the Research Ethics Committee of Renji Hospital affiliated to

Table 1. The comparison of basic characteristics between the PTMC patients with or without HT

Groups	Cases with HT (n=49)	Cases without HT (n=119)
Gender		
Male	11	42
Female	38	77
Average age (y)	42.18±9.84	46.35±11.23
Tumor size (cm)	0.65±0.12	0.87±0.22

Shanghai Jiao Tong University, School of Medicine. Written informed consent was obtained from all the participants. From December 2012 to June 2015, 168 consecutive patients with PTMC who underwent surgery were enrolled in this study at Renji Hospital (South Campus) affiliated to Shanghai Jiao Tong University, School of Medicine. There were 53 males and 115 females aging from 32 to 77 years (mean age 45.37±10.82 years). During surgery, PTMC was removed and histologically confirmed after operation. There were 49 cases with HT and 119 cases without HT and they were divided into two groups accordingly. The epidemiological data of patients in the two groups is displayed **Table 1**. Of the 168 PTMC cases, multiple lesions were found in 22 cases. And for the 146 cases with single lesion, 66 cases were found in right lobe, 55cases in left lobe and 15 cases in isthmus. The size of PTMC is larger than 0.5 cm in 110 cases and ≤0.5 cm in 58 cases. The lesions invaded envelope of thyroid gland in 9 cases and 41 cases suffered from lymph node metastasis.

The clinicopathological data between two groups were compared, including age, gender, tumor location, single or multiple lesions, tumor size, invasion of envelope and lymph node metastasis. The correlation between PTMC and HT was analyzed by multivariate logistics analysis. PTMC tissue removed during surgery was collected for immunohistochemistry to evaluate the expression of $BRAF^{V600E}$.

Histology and immunohistochemistry

After surgical resection, thyroid specimens were collected and processed by an experienced pathologist into 3 μm sections for hematoxylin and eosin (H&E)-stain and immunohistochemistry. Dako Envision Detection System (USA) and mouse-derived anti-human $BRAF^{V600E}$ monoclonal antibody was used in immunohistochemistry according to the manufacturer's instructions.

Firstly all the H&E-stained sections were reviewed to confirm the diagnosis based on the WHO Classification of Tumor, Pathology and Genetics of Tumors of the Endocrine Organs. Immunohischemical evaluation was performed by two experienced oncologists blindly according to established criteria. The presence of brown-yellow granules in cytoplasm is recognized to be positive and semi-quantitative scoring system was used to evaluate the expression of $BRAF^{V600E}$ by immunohistomorphometry. Transparency is recorded as 0 point, light-yellow as 1 point, brown-yellow as 2 points and brown as 3 points. The average point from 5 sections was recorded to be the final score.

Statistical analysis

Data were expressed as mean ± standard deviation and SPSS 17.0 was applied for all the analysis of this study. Student t-test between two groups and chi-square test was used to compare the difference between the two groups. Multivariate logistics analysis was applied to analyze the correlation between PTMC and HT. A p values <0.05 were considered to be statistically significant.

Results

The clinicopathological characteristics between two groups of PTMC with and without concurrent HT

Of all the 168 cases, 53 males and 115 females were included (male: female ratio is 1:2.2). All of them were histologically confirmed to be PTMC. Among them, 49 cases were with concurrent HT (29.17%) while 119 cases without HT (70.83%). The ratio of females PTMC patients with concurrent HT was significantly higher than those of male patients (female 33.04%, 38/115 *vs.* male 20.75%, 11/53, P<0.05). PTMC patients with concurrent HT were younger [with HT (42.18±9.84) years *vs.* without HT (46.35±11.23) years] and with smaller tumor size [with HT (0.65±0.12) cm *vs.* without HT (0.87±0.22) cm] compared with PTMC patients without HT. Among the 49 PTMC cases with HT, there were 28 cases with single

Table 2. The comparison of the clinicopathological characteristics between the PTMC patients with or without HT

Groups	Cases with HT (n=49)	Cases without HT (n=119)
Gender		
Male	11 (20.75%)	42 (79.25%)
Female	38 (33.04%)#	77 (66.96%)
Age (y)		
<45	32 (65.31%)*	56 (47.06%)
≥45	17 (34.69%)*	63 (52.94%)
Single or multiple lesions		
Single	28 (57.14%)	63 (52.94%)
Multiple	21 (42.86%)	56 (47.06%)
Tumor size (cm)		
≤0.5	20 (40.82%)*	38 (31.93%)
>0.5	29 (59.18%)	81 (68.07%)
Invasion of envelope		
Yes	2 (4.08%)	7 (5.88%)
No	47 (95.92%)	112 (94.12%)
Lymph node metastasis		
Yes	11 (22.45%)	30 (25.21%)
No	38 (77.55%)	89 (74.79%)

Note: #compared with male patients with HT; *compared with the PTMC cases without HT, P<0.05.

lesion and 21 cases with multiple lesions, and only 2 cases with invasion to the envelope and 11 cases with lymph node metastasis (**Table 2**).

Logistic multivariate regression analysis

Multivariate logistics analysis showed that concurrent HT was an independent risk factor of PTMC (OR 3.012, 95% CI 2.025~3.731, P<0.05).

The expression of BRAF^{V600E} in PTMC cases with and without HT

The rate of positively stained anti-BRAF^{V600E} was lower in the cases with HT (**Figure 1**) than those without HT (with HT 65.31%, 32/49 cases vs. 70.59%, 84/119 cases, 70.59%, P>0.05). The correlation between BRAF^{V600E} and the clinicopathological characteristics of the PTMC patients with or without HT was displayed in **Table 3**. Among the positively stained cases with HT, 19 female patients (76.00%) were recorded as 0~1 and 1~2 points for immunohistomorphometry while it was 62.50% in male patients. Therefore, the expression of BRAF^{V600E} was weaker in female patients with HT. The difference of expression of BRAF^{V600E} between the cases with and without HT was not significant in terms of age, number of lesions and size of tumors. Additionally, significant stronger expression (2~3 points) of BRAF^{V600E} was found in PTMC patients (**Figure 2**) with invasion of envelope and lymph node metastasis in group without HT (P<0.05). The finding means that the expression of BRAF^{V600E} was relatively stronger in the patients without HT but with invasion of envelope and lymph node metastasis.

Discussion

PTC is the most common malignant tumor of the thyroid, and HT is the most common autoimmune thyroid disease. With the growing popularity of Doppler ultrasound, fine needle aspiration biopsy and thyroid function test, increasing number of these two diseases in recent years has been diagnosed. Most PTMC are less than 1 cm. Previous studies have demonstrated that chronic inflammation can lead to tumor formation, but the relationship between PTC and HT is still controversial. Dailey [4] first proposed the hypothesis that there might be a link between these two diseases in 1955. Several studies show that the PTC with HT is more common in women, often with multiple lesions, less invasion of envelope or lymph node metastasis and low postoperative recurrence rate. HT has a higher coincidence rate with PTC, but the prognosis may be relatively good. Thus whether there is a causal link between PTC and HT is still not clear. According to the literature, most studies show that there is a correlation between these two diseases and PTC is a risk factor of HT [5, 6], but the existence of selection bias and/or confounding bias in the research results should be taken into account. From an epidemiological point of view it is necessary to study the prevalence of PTC in patients with HT on large sample size.

The pathological features of HT are diffused lymphocytic infiltration in the thyroid gland, and atrophy of the glandular parenchyma in the late stage of fibrosis. And the level of anti-thyroid peroxidase antibody (Tpo-Ab) and anti-thyroid globulin antibody (Tg-Ab) was elevated.

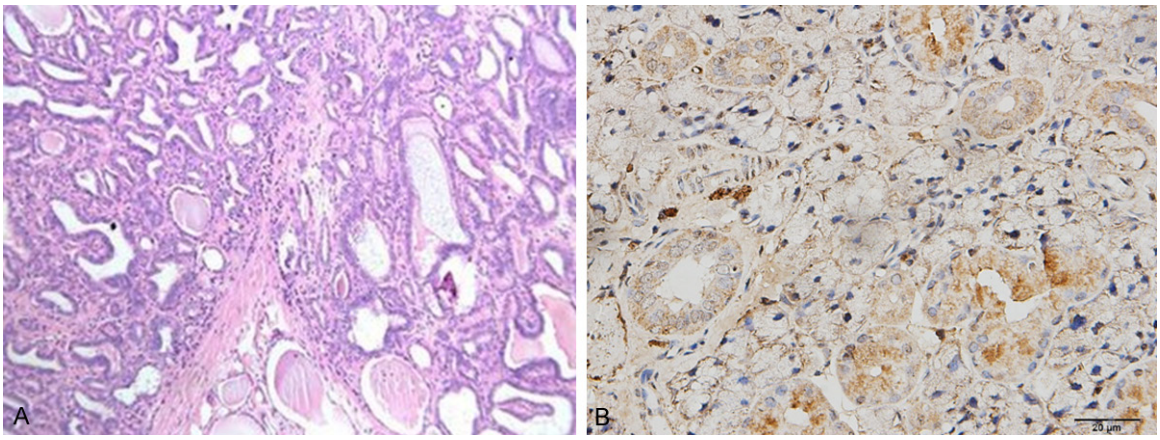

Figure 1. The positive rate of $BRAF^{V600E}$ was lower in the cases with HT (A: H&E staining, and B: IHC staining).

Table 3. The correlation between $BRAF^{V600E}$ and the clinicopathological characteristics of the PTMC patients with or without HT

Groups	Cases with HT (n=49)			Cases without HT (n=119)		
Scores	0~1	1~2	2~3	0~1	1~2	2~3
Gender						
Male	1	2	4	10	7	11
Female	9#	10#	6	17	18	21
Age (y)						
<45	8	10	7	12	17	10
≥45	3	2	2	15	16	14
Single or multiple lesions						
Single	8	5	4	15	11	12
Multiple	6	3	6	14	17	15
Tumor size (cm)						
≤0.5	6	4	5	9	10	11
>0.5	7	5	5	17	19	18
Invasion of envelope						
Yes	1	1	0*	1	0	4
No	1	8	7	24	29	26
Lymph node metastasis						
Yes	3	3	2*	4	6	15
No	10	9	5	20	22	17

Note: #compared with male patients with HT; *compared with the PTMC cases without HT, $P<0.05$.

Ultrasonography showed that the echo of glands was not uniform or decreased, with more blood vessels and low echo nodules. The diagnosis of the disease is mainly based on strict standard of histology. In order to guarantee the reliability of the results in the study, we made the conclusion according to the histological characteristics of the specimens after operation. There are many hypotheses about the correlation between PTC and HT. The hypothesis of inflammatory reaction is the first to be put forward and the discussion is also the most [7]. This hypothesis suggests that the presence of diffuse lymphocytic infiltration in HT suggesting an inflammatory response which provides a suitable environment for the activation and development of tumors. Inflammatory reaction of oxygen-free radicals could cause DNA damage and mutation accompanied by the trend factors and cell factors, resulting in the changes of matrix and malignant transformation of epithelial cells. However, from another aspect, it may be a kind of immune response to tumor to block the further growth of malignant cells and leading to a better prognosis. Other hypotheses include TSH hypothesis and molecular pathways hypothesis etc [8-10], however they lack convincing evidence and thus are not widely recognized.

In this study, we compared the clinical and pathological characteristics of PTMC patients with or without HT and found that among the PTMC patients with HT there were more female patients, with younger age, smaller lesions and lower incidence of peri-thyroid invasion. In female PTMC patients 38 cases were found to have concurrent HT accounting for 33.04% of all female patients.

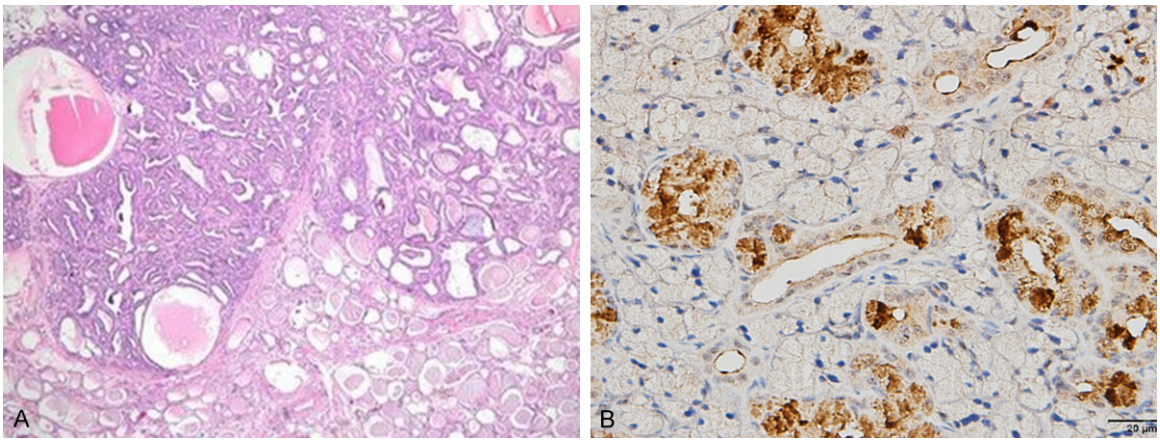

Figure 2. The positive rate of $BRAF^{V600E}$ was higher in the cases without HT (A: H&E staining, and B: IHC staining).

Meanwhile 11 male PTMC patients found to have concurrent HT accounting for 20.75% of all male patients. The difference was statistically significant ($P<0.05$). Therefore the ratio of concurrent HT in female patients with PTMC was higher. Besides, PTMC patients with HT were younger and tumor size was smaller ($P<0.05$). Additionally, the incidence of tumor invasion to the outside of envelope and lymph node metastasis with HT was lower than those without HT, but the difference was not significant.

Our result shows that the prognosis of the PTMC patients with HT is relatively good which is consistent with previous reports. The underlying reason may be due to the following factors: these patients took frequent monitoring so they were detected earlier, and got timely and effective treatment. Gender difference may be another important reason because of the higher ratio of female patients whose recurrence rate is relatively low and the prognosis is relatively good [11]. From the perspective of cytology and molecular biology some scholars put forward the view that killer cells and cytotoxic T cell infiltration and activation of apoptosis pathway may be an important mechanism for relatively good prognosis of HT tumors [12]. Logistic multiple regression analysis showed that the concurrence of HT (OR 3.012, 95% CI 2.025, $P<0.05$) was an independent risk factor for PTMC, and no significant correlation with other factors was found. HT itself is not necessary to take thyroidectomy only if nodules gradually enlarge and result in compression symptoms or in case of malignancy. When analyzing the results of PTC with HT-related studies, the possibility of selection bias or confounding bias and the sampling method being fine-needle aspiration (FNA) or surgical resection should be taken into account [13]. Nevertheless, The PTMC patients with HT still need to check thyroid function regularly with ultrasound, CT scan, or FNA biopsy if necessary in order to accomplish early detection and treatment. Thus we can effectively improve the therapeutic effect of thyroid malignant tumor including PTMC.

Some scholars have found that the proportion of $BRAF^{V600E}$ mutation in PTC cases with HT is lower while $BRAF^{V600E}$ mutation is related to the strong invasive ability of PTC [14, 15]. Our study found that in female PTMC patients, the week immunohistochemical stain of $BRAF^{V600E}$ (score 0 to 1 and 1 to 2) were observed in 19 cases and the ratio was 62.50%, which was 76.00% in male patients. Therefore, the expression of $BRAF^{V600E}$ in female PTMC cases with HT was relatively weaker than male. For the tumor invasion of envelope and lymph node metastasis in the patients with HT, the ratio of strong immunohistochemical staining (score 2 to 3) of $BRAF^{V600E}$ was significantly lower than those without HT ($P<0.05$). This finding means that the expression of $BRAF^{V600E}$ was relatively stronger in PTMC patients without HT who suffered from invasion of envelope and lymph node metastasis.

The above results showed that the weak expression of $BRAF^{V600E}$ might be the reason for better prognosis of PTMC with HT, and the effect of $BRAF^{V600E}$ on the invasion and metastasis of

PTC was further confirmed. PTC is a continuous process associated with multiple genes and processes. $BRAF^{V600E}$ is the key molecule of the classical MAPK pathway and the most common type of gene mutation in PTC which is very important for = diagnosis and prognosis evaluation [16]. $BRAF^{V600E}$ could be involved in tumor development in several aspects: activation of MAPK pathway to promote tumor cell growth; the expression of iodine metabolism-related gene causing radioactive iodine treatment failure and recurrence of disease etc [17, 18]. The mutation of this gene is considered to be a risk factor for poor prognosis, but its existence in HT patients or initiation progression to cancer has not yet been reported. It has been reported that the existence of $BRAF^{V600E}$ could suggest surgical plans for prophylatic central lymph node dissection, and predict lymph node metastasis [19, 20]. In a meta-analysis of the correlation between $BRAF^{V600E}$ gene mutation and the clinicopathological characteristics of PTC, the authors suggested that $BRAF^{V600E}$ gene was closely associated with pathological staging of tumor, lymph node metastasis, single or multiple foci and recurrence of tumor. Based on the above findings, some scholars proposed that the combined evaluation of envelope invasion and the status of $BRAF^{V600E}$ could predict the prognosis of patients with unknown lymph node metastasis and guide the plan of treatment [21, 22]. Based on the results of this study, we consider that it is helpful to establish effective risk evaluation criteria for the patients with HT. Unfortunately the sample size in this study is not large enough to put forward the evaluation criteria. More case and data is needed to expand the sample size for future work.

In conclusion, the concurrence risk of HT in PTMC patients increases significantly. For these patients follow-up should be taken regularly in order to achieve early detection of tumor lesions. Besides, the $BRAF^{V600E}$ expression of PTMC is relatively weak, with higher incidence in female patients, younger age, smaller tumor size and lower incidence of envelope invasion and lymph node metastasis. Previous studies are retrospective analysis and lack detailed clinical data so it is necessary to carry out large sample prospective long-term follow-up and epidemiological study to further explore the correlation between these two diseases so as to establish an effective PTC risk-evaluation system for the patients with HT.

Disclosure of conflict of interest

None.

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