Comprehensive evaluation of risk factors for lymph node metastasis in patients with papillary thyroid carcinoma

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Abstract. With the increasing incidence of papillary thyroid cancer (PTC), it is important to risk-stratify patients who may have a more aggressive tumor biology. The present study aimed to evaluate the risk factors for lymph node metastasis (LNM) in patients with PTC, which may provide a significant reference for clinical diagnosis and treatment. In total, 1,045 patients with PTC [313 with PT microcarcinoma (PTMC) and 732 with non-PTMC] between August 2016 and August 2019 were investigated. The B-type Raf kinase (BRAF) V600E mutation was tested in all samples. The clinical data (sex, age, tumor location, sample type and pathological features) were retrospectively analyzed. Logistic regression analysis was performed to evaluate independent risk factors for LNM. A total of 181/313 (57.8%) PTMC cases and 145/732 (19.8%) non-PTMC cases had a BRAF V600E mutation. In the PTMC cases, significant differences in sex and sample type were identified (BRAF V600E mutation vs. wild-type). In the non-PTMC cases, significant differences in sex and age were identified (BRAF V600E mutation vs. wild-type). Female sex and tumor diameter ≤1 cm were significant independent predictors of LNM in PTC. In PTMC, female sex was a significant independent predictor of LNM. A bilateral tumor was an independent protective factor for LNM in PTC, PTMC and non-PTMC. The BRAF V600E mutation rate of ultrasound-guided fine-needle aspiration cytology was higher compared with FFPE in PTMC (P=0.018). In contrast to previous studies, the results of the present study suggested that being female and having a tumor of diameter ≤1 cm were risk factors for LNM, and that the BRAF wild-type of PTMC may be more aggressive than other types. Notably, the position of the tumor in the bilateral thyroid was also an independent protective factor for LNM. Therefore, ultrasound-guided fine-needle aspiration should be recommended for gene analysis (BRAF V600E) in PTMC. In addition, clinicians should consider an individualized treatment according to gene mutations, sex, age, tumor size and the location of the tumor, in order to achieve an improved therapeutic efficacy.

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Abbreviations: PTC, papillary thyroid carcinoma; PTMC, papillary thyroid microcarcinoma; US-FNA; ultrasound-guided fine-needle aspiration; FFPE, formalin-fixed paraffin-embedded; FNAC, fine-needle aspiration cytology; H&E, hematoxylin-eosin staining; LNM, lymph node metastasis; OR, odds ratio

Key words: genetic testing, papillary thyroid carcinoma, papillary thyroid microcarcinoma, non-papillary thyroid microcarcinoma, BRAF mutation

Introduction

Papillary thyroid carcinoma (PTC), is the most common histological subtype of thyroid cancer, which accounts for >90% of all thyroid malignancies, and its incidence rate has increased rapidly in recent years (1-4). This recent marked change is primarily attributable to the increased use of fine-needle aspiration (FNA) or ultrasonography-guided biopsy as the early diagnosis methods in patients without palpable thyroid nodes (5,6). Although the mortality rate of PTC is relatively low, 20-50% of patients have a risk of poor clinical outcomes, including distant metastases (7), a high rate of long-term persistence of the disease and the possibility of recurrence (8). Papillary thyroid microcarcinoma (PTMC) with a tumor diameter ≤1 cm occurs in >50% of all new-onset thyroid cancer types, and its incidence has been increasing rapidly over the last several decades worldwide (9,10). Clinically, numerous studies have reported that PTMC had a favorable prognosis in the majority of cases following surgical interventions (11-13). However, PTMC tumor growth is usually slow and certain patients developed clinically problematic tumor growth after multiple years of observation (14,15). In addition, the majority of PTMC cases have an indolent nature and excellent outcomes, and the expert consensus recommended that PTMC should be identified and managed separately (2,12).

The B-type Raf kinase (BRAF) mutation has been the subject of intensive research to investigate its tumorigenic role and clinical implications in thyroid cancer types, particularly PTC. It has been revealed that ~90% of BRAF mutations are T1799A transverse point mutations, resulting in a valine to glutamic acid switch at codon 600 (V600E) (16,17). The kinase activity of BRAF V600E is 460-fold higher compared with the wild-type BRAF, and this active conformation may constitutively activate its downstream effects to transform healthy cells or induce cancer proliferation without the need of RAS for activation (18). These results suggested that the mutation is an early event in PTC development, and there is a complex process that may affect tumorigenesis and tumor aggressiveness.

As with PTC in general, lymph node metastasis (LNM) has been reported to be a risk factor for increased tumor recurrence rates and is also associated with a decreased survival rate (19). Lutz *et al* (20) revealed that an imbalance in DNA repair gene expression was associated with aggressive clinicopathological features in PTC. Therefore, the aim of the present retrospective observational study was to investigate the associations between BRAF V600E mutations and clinicopathological features and to identify the risk factors for LNM in patients with PTC.

Materials and methods

Patient population. Clinical data of 1,045 patients with PTC were collected for analysis between August 2016 and August 2019 from The First Affiliated Hospital of Chongqing Medical University (Yuzhong, China). Based on tumor diameter, patients were diagnosed with PTMC (n=313) or non-PTMC (n=732). All participants in the study were Chinese, without a blood relationship with each other, and all provided written informed consent.

All patients met the inclusion criteria, which were as follows: i) underwent either a resection or a diagnostic procedure (biopsy or cytological specimen); ii) confirmed to have PTC via intraoperative rapid pathology or postoperative pathology detection; and iii) presence of gene mutation. Different locations of thyroid tumor were divided into seven regions: Left lobe, right lobe, bilaterality, isthmus, left lobe and isthmus, right lobe and isthmus, bilateral lobes and isthmus according to ultrasound imaging results. The information of sex, age, diagnosis date and sample type are available for the 1,045 patients in the hospital information system of The First Affiliated Hospital of Chongqing Medical University (Table I).

Pathological examination. PTC tissues were embedded in paraffin and were sectioned into 4-μm thick sections according to standard procedures. The sections were processed for hematoxylin and eosin (H&E) staining. For this purpose the sections were fixed with 95% ethanol for 20 min at room temperature, and washed twice with PBS for 1 min each time. The 60% neutral balsam was used as a blocking reagent for 3-5 sec at room temperature. The hematoxylin staining was performed for 12-15 min and eosin for 5 min at room temperature. Slides were used for observed under a light microscope (magnification, x400). Different types of PTC and the presence of LNM were reviewed independently by two blinded pathologists; any inconsistent diagnostic cases were discussed with a third pathologist. The color ultrasound diagnosis was routinely used before and after the surgical resection of thyroid tumor or used before the fine needle aspiration, the ultrasound-guided fine-needle aspiration (US-FNA) was routinely used before the surgical resection of thyroid tumor or conservative treatment and the H&E staining was used for morphological detection.

Sample collection, DNA extraction and mutation screening. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) or fine-needle aspiration cytology (FNAC) samples using TRIzol® reagent (cat. no. 15596-026; Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. DNA concentrations of all samples were determined using a NanoDrop ND-1000 spectrophotometer at 280 nm (Thermo Fisher Scientific, Inc.). Gene mutations were detected via amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and the sequences used were as follows: BRAF, forward, 5'-GCTTGCTCTGATAGGAAAATGAG-3'; and reverse, 5'-GGGCCAAAAATTTAATCAGTGG-3'. The thermocycling conditions of PCR were as follows 1 cycle of 94˚C for 5 min; followed by 15 cycles of 95˚C for 25 sec, 64˚C for 20 sec, 72˚C for 20 sec and 93˚C for 25 sec; and then finally 31 cycles of 60˚C for 35 sec and 72˚C for 20 sec. The ARMS-PCR reagents were provided by AmoyDx Diagnostics Co., Ltd. (cat. no. 20143401824).

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software (IBM Corp.). Quantitative data are presented as the mean ± standard deviation, while qualitative data are represented as a percentage or frequency. χ^2 or Fisher's exact tests were used to evaluate the difference in clinical features between two different groups. Univariate and multivariate logistic regression analyses were performed to assess independent risk factors for the presence of LNM

Table I. Patient characteristics.

Characteristics	Total (n=1,045)	PTMC (n=313)	Non-PTMC (n=732)	P-value
Sex				0.524
Male	298	85	213	
Female	747	228	519	
Age (7-85), years	41.97±12.94	42.57±11.40	41.60±13.78	0.009[a]
<55	889	280 (89.5%)	609 (83.2%)	
≥55	156	33 (10.5%)	123 (16.8%)	
Lymph node metastasis	326	181 (57.8%)	145 (19.8%)	0.000[a]
BRAF V600E mutation	839	273 (87.2%)	566 (77.3%)	0.000[a]
Different locations of thyroid tumor				0.254[b]
Left lobe	382	104 (33.2%)	278 (38.0%)	
Right lobe	480	147 (47.0%)	333 (45.5%)	
Isthmus	6	3 (1.0%)	3 (0.4%)	
Bilateral lobes	150	52 (16.6%)	98 (13.4%)	
Left lobe and isthmus	7	1 (0.3%)	6 (0.8%)	
Right lobe and isthmus	15	6 (1.9%)	9 (1.2%)	
Bilateral lobes and isthmus	5	0 (0.0%)	5 (0.7%)	
Sample type				0.058
FNAC	303	78 (24.9%)	225 (30.7%)	
FFPE	742	235 (75.1%)	507 (69.3%)	

Quantitative data are presented as the mean ± standard deviation or n (%). [a]P<0.05; [b]Fisher exact test. PTMC, papillary thyroid microcarcinoma; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

in PTC, and the results are reported as odds ratios (OR) with a 95% CI. P<0.05 was considered to indicate a statistically significant difference.

SPSS univariate analysis is helpful for selecting variables that will be in the final logistic regression model. Following testing for all possible interactions among independent variables, the best fitted logistic regression model was established to determine the possible risk factors for LNM in PTC. Next, adjusted ORs and 95% CIs were calculated for all significant variables.

The two stepwise multiple logistic regression was used in the multivariate analysis to compare parameters with LNM or without in PTC as the dependent variable, and sex, age, mutation, tumor type, sample type and different location of thyroid tumor as the independent variables.

Results

Clinicopathological characteristics of 1,045 patients with or without PTMC. A retrospective study of 732 patients with non-PTMC and 313 patients with PTMC between August 2016 and August 2019 was performed to assess the clinicopathological characteristics at diagnosis, including sex, age, sample type (FFPE tissues or FNAC), LNM, different locations of thyroid tumor (left lobe, right lobe, bilaterality, isthmus, left lobe and isthmus, right lobe and isthmus, or bilateral lobes and isthmus) and BRAF V600E mutation status (Table I). In total, 298 male and 747 female patients have been analyzed in the present study. The mean age was 41.97±12.94 years. The patients were divided into two subgroups according to age: Young subgroup (<55 years; n=889) and old subgroup (≥55 years; n=156). The sample type consisted of FFPE (n=742) and FNAC (n=303). LNM was present in 181 cases (57.8%) of PTMC and in 145 cases (19.8%) of non-PTMC. Collectively, LNM was present in 31.2% of patients.

With regards to the location of the thyroid tumor, it was present in 382 patients with PTC in the left lobe, in 480 cases in the right lobe, in six cases in the isthmus, in 150 cases in the left and right lobes, in seven cases in the left lobe and isthmus, in 15 patients in the right lobe and isthmus, and in five cases in the bilateral lobes and isthmus. The BRAF V600E mutation occurred in 273 (87.2%) of PTMC cases and 566 (77.3%) of non-PTMC cases. The total mutation rate of BRAF V600E was 80.3%.

The clinicopathological characteristics and sample types were compared between PTMC and non-PTMC in the present study (Table I). The BRAF V600E mutation rate in the PTMC group was significantly higher than that in the non-PTMC group (P=0.00). The frequency of LNM in the PTMC group was also significantly increased compared with that in the non-PTMC group (P=0.00). The age cut-off value of 55 years between the PTMC group and the non-PTMC group was significantly different (P=0.009). Other clinical parameters exhibited no significant differences between the two groups.

BRAF V600E mutational status and clinical characteristics in patients with PTMC or non-PTMC. The association between BRAF mutation status and clinical characteristics of 313 patients with PTMC was analyzed. The BRAF V600E

Table II. Association between clinicopathological characteristics and BRAF V600E mutation.

	PTMC			Non-PTMC		
Characteristics	BRAF V600E mutation	BRAF wild-type	P-value	BRAF V600E mutation	BRAF wild-type	P-value
Sex			0.026[a]			0.003[a]
Male	80	5		180	33	
Female	193	35		386	133	
Age, years	42.02±11.31	43.07±11.48	0.505	43.32±14.06	36.83±11.74	0.017[a]
<55	242	34		464	149	
≥55	31	6		102	17	
Lymph node metastasis			0.964			0.280
Yes (+)	158	23		449	138	
No (-)	115	17		117	28	
Different locations of thyroid tumor			0.475[b]			0.448[b]
Left lobe	88	16		211	67	
Right lobe	127	20		259	74	
Isthmus	3	0		3	0	
Bilateral lobes	49	3		79	19	
Left lobe and isthmus	1	0		3	3	
Right lobe and isthmus	5	1		8	1	
Bilateral lobes and isthmus	0	0		3	2	
Sample type			0.018[a]			0.699
FNAC	75	4		176	49	
FFPE	198	36		390	117	

[a]P<0.05; [b]Fisher exact test. PTMC, papillary thyroid microcarcinoma; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

mutation demonstrated a significant association with the male sex (P=0.026) and sample type from FFPE tissues (P=0.018) compared with the BRAF wild-type in patients with PTMC. However, there was no difference in LNM, age and location of thyroid tumor between the BRAF V600E mutation and the BRAF wild-type (Table II).

The BRAF V600E mutation had a significant association with the male sex (P=0.003) compared with the BRAF wild-type in patients without PTMC. In addition, significant differences were identified in the age cut-off value of 55 years between BRAF V600E mutation and BRAF wild-type in patients without PTMC (P=0.017). However, there was no difference in other clinical features between the BRAF V600E mutation and the BRAF wild-type (Table II).

The clinicopathological characteristics and sample type in all patients with the BRAF V600E mutation were compared (Table III). A lower rate of LNM (P=0.00; χ^2=42.369) was present in PTMC cases compared with that in non-PTMC cases. The age cut-off value of 55 years was significantly different (P=0.013) between PTMC and non-PTMC groups. However, there was no difference in other clinical features between PTMC and non-PTMC groups.

Univariate and multivariate analysis of risk factors for LNM in PTC, PTMC and non-PTMC. In PTC (Table IV), the female sex (OR=1.834; 95% CI=1.297-2.592; P=0.001) and PTMC (OR=3.267; 95% CI=2.418-4.413; P<0.001) were characterized as independent risk factors for LNM. Furthermore, a bilateral tumor (OR=0.29; 95% CI=0.1676-0.497; P=0.000) and sample type FFPE (OR=0.643; 95% CI=0.470-0.879; P=0.006) were characterized as protective factors for LNM. However, there was no difference between BRAF V600E mutation and LNM (P>0.05).

In PTMC (Table V), the female sex (OR=2.66; 95% CI=1.490-4.760; P=0.001) was characterized as an independent risk factor, while a bilateral tumor (OR=0.18; 95% CI=0.075-0.418; P=0.00) was characterized as a protective factor. Age, BRAF V600E mutation and sample type did not demonstrate any statistical differences with LNM (P>0.05).

In non-PTMC cases (Table VI), the sample type FFPE (OR=0.568; 95% CI=0.389-0.8291; P=0.003) was characterized as a protective factor for LNM. The sex, age, BRAF V600E mutation and different locations of thyroid tumor did not show statistical differences with LNM (P>0.05).

Discussion

With the increasing incidences of non-PTMC and PTMC, it is important to risk-stratify patients who may have a more aggressive tumor biology in what was traditionally considered to be

Table III. Comparison of progression between PTMC and non-PTMC with BRAF V600E mutation.

	BRAF V600E mutation			
Characteristics	PTMC	non-PTMC	χ^2	P-value
Sex			0.537	0.464
Male	80	180		
Female	193	386		
Age, years	42.02±11.31	43.32±14.06	6.135	0.013[a]
<55	242	464		
≥55	31	102		
Lymph node metastasis			42.369	0.000[a]
Yes (+)	158	449		
No (-)	115	117		
Different locations of thyroid tumor			5.644	0.445[b]
Left lobe	88	211		
Right lobe	127	259		
Isthmus	3	3		
Bilateral lobes	49	79		
Left lobe and isthmus	1	3		
Right lobe and isthmus	5	8		
Bilateral lobes and isthmus	0	3		
Sample type			1.153	0.283
FNAC	75	176		
FFPE	198	390		

[a]P<0.05; [b]Fisher exact test. PTMC, papillary thyroid microcarcinoma; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

Table IV. Univariate and multivariate analysis of risk factors for lymph node metastasis in papillary thyroid carcinoma.

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex (male as reference)				
Female	1.868 (1.340-2.604)	0.000[a]	1.834 (1.297-2.592)	0.001[a]
Age (<55 as reference)				
≥55	1.106 (0.757-1.616)	0.603		
BRAF V600E (yes as reference)				
No (-)	0.72 (0.505-1.043)	0.084	-	-
Tumor type (non-PTMC as reference)				
PTMC	2.93 (2.198-3.910)	0.000[a]	3.267 (2.418-4.413)	0.000[a]
Sample type (FNAC as reference)				
FFPE	0.712 (0.530-0.955)	0.024[a]	0.643 (0.470-0.879)	0.006[a]
Different locations of thyroid tumor (left lobe as reference)				
Right lobe	0.82 (0.611-1.103)	0.191	0.76 (0.556-1.034)	0.080
Bilateral lobes	0.32 (0.189-0.543)	0.000[a]	0.29 (0.1676-0.497)	0.000[a]
Isthmus	1.11 (0.200-6.117)	0.909	0.89 (0.158-5.089)	0.901
Bilateral lobes and isthmus	0.55 (0.061-4.996)	0.597	0.43 (0.044-4.138)	0.461
Right lobe and isthmus	1.11 (0.370-3.304)	0.858	0.93 (0.292-2.926)	0.894
Left lobe and isthmus	0.37 (0.044-3.094)	0.358	0.441 (0.052-3.743)	0.453

[a]P<0.05. OR, odds ratio; CI, confidence interval; PTMC, papillary thyroid microcarcinoma; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

Table V. Univariate and multivariate analysis of risk factors for lymph node biopsy in papillary thyroid microcarcinoma.

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex (male as reference)				
Female	2.91 (1.671-5.077)	0.000[a]	2.66 (1.490-4.760)	0.001[a]
Age (<55 as reference)				
≥55	1.54 (0.773-3.056)	0.220		
BRAF V600E (yes as reference)				
No (-)	0.93 (0.481-1.805)	0.835		
Sample type (FNAC as reference)				
FFPE	0.80 (0.476-1.333)	0.387[a]		
Different locations of thyroid tumor (left lobe as reference)				
Right lobe	0.70 (0.427-1.169)	0.176	0.71 (0.423-1.184)	0.188
Bilateral lobes	0.15 (0.068-0.369)	0.000[a]	0.18 (0.075-0.418)	0.000[a]
Isthmus	1.78 (0.157-20.263)	0.641	2.87 (0.236-35.051)	0.408
Bilateral lobes and isthmus	0.44 (0.039-5.066)	0.514	0.48 (0.040-5.732)	0.561
Right lobe and isthmus	0.44 (0.078-2.539)	0.362	0.48 (0.081-2.833)	0.417
Left lobe and isthmus	0.00	1.000	0.00	1.00

[a]P<0.05. OR, odds ratio; CI, confidence interval; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

Table VI. Univariate and multivariate analysis of risk factors for lymph node metastasis in non-papillary thyroid microcarcinoma.

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex (male as reference)				
Female	1.42 (0.929-2.165)	0.105	1.44 (0.940-2.202)	0.094
Age (<55 as reference)				
≥55	1.095 (0.675-1.778)	0.713		
BRAF V600E (yes as reference)				
No (-)	0.79 (0.500-1.241)	0.304		
Sample type (FNAC as reference)				
FFPE	0.572 (0.392-0.835)	0.004[a]	0.568 (0.389-0.8291)	0.003[a]
Different locations of thyroid tumor (left lobe as reference)				
Right lobe	0.81 (0.550-1.197)	0.291		
Bilateral lobes	0.43 (0.215-0.850)	0.015[a]		
Isthmus	0.0	0.999		
Bilateral lobes and isthmus	0.0	0.999		
Right lobe and isthmus	1.67 (0.407-6.874)	0.476		
Left lobe and isthmus	0.67 (0.077-5.829)	0.716		

[*]P<0.05. OR, odds ratio; CI, confidence interval; FNAC, fine-needle aspiration cytology; FFPE, formalin-fixed, paraffin-embedded.

an indolent disease. This stratification will have management implications, including whether or not to observe the patient outcomes, the extent of surgical resection, the use of radio-iodine ablation therapy (the β rays emitted during 131 iodine decay could selectively destroy thyroid acinar epithelium without affecting the adjacent tissues) and the frequency of follow-up (21). Li *et al* (22) reported that PTMC had an indolent course and excellent prognosis, while the results of the present study demonstrated that the incidence of LNM was more frequent in PTMC than in non-PTMC. The correlation analysis

indicated that the incidence of the BRAF V600E mutation in male patients with PTMC was significantly higher than that in female patients. Lee *et al* (23) recommended that the male sex may be an independent prognostic factor for recurrence in non-PTMC, but it was not a prognostic factor in PTMC. In the present study, the incidence of the BRAF V600E mutation in FNAC was significantly increased compared with that in FFPE, which was consistent with previous studies (24,25). However, LNM was associated with BRAF wild-type in PTMC cases, which was inconsistent with a previous study (26). Therefore, the results of the present study demonstrated that the BRAF V600E mutation may be more prevalent among male patients and is more easily detected in FNAC of PTMC.

To further analyze the difference between PTMC and non-PTMC with regard to the biological features, the clinicopathological characteristics and sample type of patients with BRAF V600E mutations were compared. While several studies have reported that BRAF V600E mutation in PTC is associated with aggressive pathological features, including a negative influence on ^{131}I-avidity, decreased thyroperoxidase, an increased risk of lymph node metastasis and recurrence following treatment (27-29), the clinical implications and precise mechanisms in PTMC and non-PTMC are contradictory. For example, Zheng *et al* (30) reported that tumor diameter (>0.5 cm) was an independent risk factor correlated with LNM in PTC. Notably, the results of the present study demonstrated that the LNM rate was significantly increased and was correlated with BRAF wild-type in PTMC, which indicated that the BRAF V600E mutation in PTMC was less aggressive; this result differs to that from a previous meta-analysis (22). A previous study revealed that the majority of PTMC cases with a BRAF V600E mutation do not express BRAF V600E protein (31).

Correct preoperative diagnosis is highly important, and it is generally agreed that improved knowledge regarding predictive risk factors for LNM may guide clinical decisions, but the greater risk of LNM remains debatable. Following adjusting for other significant preoperative clinical factors, univariate and multivariate analysis was performed to identify the risk factors for LNM in the present study. Sex was a prominent patient background parameter for PTC. In recent years, the association between sex and recurrence or survival of PTC has been debated. A previous study reported that the male sex was an independent clinical prognostic factor of poor outcome in PTC (32), but not in PTMC (23). Recently, Roh *et al* (33) reported that there was no association between sex and LNM. In the present study, although the female sex had a lower BRAF V600E mutation presence than the male sex, multivariate analysis demonstrated that the female sex was a risk factor for LNM in PTMC and non-PTMC, which was different from the results of the aforementioned studies. A previous study revealed that the female sex was an independent predictive risk factor of central lymph node metastasis in PTC (34). Controversies in the results of these studies may be associated with different sample types, sample sizes and detection techniques. It has been reported that the disease in the female sex has an earlier age of onset, while male patients have a higher rate of mortality (35,36). Therefore, it is recommended that the molecular mechanisms between LNM and sex in patients with PTC are investigated in future studies.

The results of the present study have suggested that the location of the tumor in the bilateral thyroid was a protective factor for LNM in PTMC and non-PTMC, which was inconsistent with a retrospective cohort study (37).

In conclusion, the results of the present study have demonstrated that the female sex and PTMC were independently associated with LNM in PTC, while the tumor in the bilateral thyroid was a protective factor in LNM. Furthermore, a negative association was identified between the BRAF V600E mutation and LNM. FNAC from tumor samples had a higher rate of BRAF V600E mutation compared with FFPE in PTMC, which suggested that FNAC may be a reliable intervention to detect the BRAF V600E mutation. Therefore, the results of the present study indicated that clinicians should comprehensively consider the following clinical features: Sample type, BRAF mutation, tumor size, patient sex and location of the thyroid tumor. Furthermore, multilevel gene sequencing technologies and therapeutic schedule should be utilized in order to achieve a relatively favorable prognosis.

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Availability of data and materials

All data generated and/or analyzed during the present study are included in this published article.

Authors' contributions

YD, DW and XL conceived and designed the study. HB, YS, YLZ, XC, YJZ developed the methodology. XS, JZ, HL, JL, ZY, CH and YJZ acquired the data (acquired and managed patients and provided facilities). LC, DW and YL analyzed and interpreted the data (statistical analysis, biostatistics and computational analysis). YD, DW and XL wrote, reviewed and revised the manuscript. LC, DW and XL provided administrative, technical or material support (i.e., reporting or organizing data and constructing databases). XL supervised the study. CH performed English language editing All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University Ethics Review Board (approval no. 2020-173). All patients provided written informed consent.

Patient consent for publication

All patients provided written patient consent for publication.

Competing interests

The authors declare that they have no competing interests.

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