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Association of BRAF V600E immunoexpression with clinicopathological variant groups in papillary thyroid carcinoma

Hung Nguyen Van[1, 2*], Thanh Nguyen Tuan[3], Minh Tran Ngoc[2, 3], Tuyen Pham Van[1, 3], Luan Dao Thi[1, 2]

[1]Department of Pathology, Hanoi Medical University
[2]Department of Pathology, Hanoi Medical University Hospital
[3]Centre of Pathology and Cytopathology, Bach Mai Hospital

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Abstract:

The detection of BRAF V600E immunoexpression in papillary thyroid carcinoma (PTC) is extremely important because of its association with poor prognosis. Is poor prognosis really associated with all histological variants of thyroid papillary carcinoma? To contribute to this elucidation, using the BRAF V600E mutation-specific antibody, the present study was performed to determine the association between BRAF V600E immunoexpression and clinicopathological variant groups of PTC. Tumour tissue samples collected from 95 patients with papillary thyroid cancer and tumour size <3.5 cm in diameter were processed by routine microscopic technique and using Ventana Benchmark XT automated stainer. BRAF V600E immunoexpression in the group of the aggressive clinicopathological group is directly proportional to lymph node and distal metastasis (p=0.005) in contrast to the favourable clinicopathological group. Finally, the lymph nodal and distal metastasis (poor prognosis) of the aggressive clinicopathological group of PTC can be better predicted when combining BRAF V600E immunostaining with conventional histopathological technique, conversely, these results could not be achieved in the favourable clinicopathological group. These findings suggest that BRAF V600E immunoexpression is a potential candidate for the prognosis of the aggressive clinicopathological group.

Keywords: **BRAF V600E immunoexpression, favourable and aggressive clinicopathological groups, papillary thyroid carcinoma.**

Classification number: **3.2**

Introduction

PTC is the most common malignant thyroid disease, accounting for 45-80% of all thyroid cancers. The BRAF (B-isoform of Raf kinase) V600E mutation is common in PTC, with a reported frequency of 25-82.3% [1, 2]. The mutant BRAF protein activates serine/threonine BRAF kinase, which can activate mitosis. BRAF V600E also causes metalloprotease overexpression in the matrix, thereby increasing the mobility and spread of tumour cells. BRAF mutations have been detected in 20-80% of thyroid papillary carcinomas with favourable prognoses, and high frequencies of mutant BRAF are more common in papillary than in follicular variants [3-5].

The BRAF V600E mutation has been associated with age, lymph node metastasis, extra-thyroid expansion, tumour, node, metastasis (TNM) stage, lymph node metastasis, recurrence, and reduced survival but not distal metastasis [6-9]. However, several studies on the association between the BRAF V600E mutation and unfavourable clinical features in PTC showed controversial results and therefore, the association between BRAF V600E mutation and the clinical prognosis of PTC needs to be confirmed [10, 11]. Meanwhile, other studies have demonstrated that characteristics of the unfavourable/aggressive histological variant group (columnar cell, tall cell, hobnail, solid/trabecular) include large tumour size, extra-thyroid expansion, early lymph node metastasis, and high recurrence rate. Favourable histological variants (microcarcinoma, encapsulated, follicular, and cribriform) often have opposite characteristics [3, 4, 12].

Patients who have PTCs with the BRAF V600E mutation have a 1.5-2.1-times higher risk of disease recurrence, extrathyroidal expansion, lymph node metastasis, and TNM stage than patients carrying tumours with wild-type BRAF genotype [13]. According to the American Thyroid Association (ATA) guidelines, the BRAF V600E mutation is one of the risk stratification factors for PTC according to ATA guidelines [14]. The mutant-specific BRAF antibody is useful in detecting the BRAF V600 mutation with more than 95% sensitivity and specificity. Indeed, antibodies specific to BRAF mutations may be more sensitive than molecular tests in detecting BRAF V600E mutations [15-19]. Recently, targeted treatments based on the expression of mutant BRAF protein in thyroid cancer, especially the papillary variant, has increased with quite satisfactory results [20-22]. Thus, the precise identification of aggressive histological variants of the PTC and BRAF V600E mutation status is crucial in preventing sub-optimal or inappropriate patient treatment [12].

The presence of aggressive variants significantly impacts the prognosis and treatment of patients with PTC based on the ATA guidelines. The identification of aggressive variants can modify the therapeutic approach, especially in small thyroid tumours [14]. Therefore, this study aims to determine the relationship between BRAF V600E immunoexpression and clinicopathological variant groups (e.g., aggressive and favourable variants, tumour size, lymph node metastasis, and distal metastasis) of PTC.

[*]Corresponding author: Email: nguyenvanhung@hmu.edu.vn

Materials and methods

Tissue samples

Ninety-five patients with primary PTC who underwent an initial operation (total thyroid lobectomy with or without lymph node dissection) at the Hanoi Medical University Hospital, Vietnam, between October 2019 and June 2020 were enrolled in this retrospective study. Recurrent PTCs, secondary thyroid carcinomas, and thyroid tumours ≥3.5 cm in size were excluded. PTC tissue samples were obtained from patients residing in the northern regions of Vietnam.

Data were obtained from archived records including age, sex, lymph node metastasis, distal metastasis, histological type, primary tumour location, and tumour size. The corresponding slides and tissue samples embedded in paraffin blocks were also obtained. Formalin-fixed paraffin-embedded tissue blocks were used to prepare hematoxylin and eosin slides to determine areas of tumour cells. The identification of histological variants of PTC was based on the histological classification of thyroid cancer by the World Health Organization in 2017 [4].

Based on clinical behaviour, aggressive (high-risk) and favourable prognosis groups were identified among the PTC variants [12, 23]. The favourable prognosis group consisted of four variants: microcarcinoma, encapsulated, follicular, and cribriform. The clinically high-risk aggressive group (ability to penetrate and metastasize early and extra-thyroid expansion) also consisted of four variants: tall cell, columnar cell, hobnail, and solid/trabecular. The histological characteristics of the aggressive variant group were as follows: tall cell variant, ≥30% tall cells (height twice their width) with oncocytic cytoplasm; columnar cell variant, columnar cells with pseudostratified nuclei, no eosinophilic cytoplasm, and hypercellular neoplasm with thin papillary and glandular structures (the nuclear characteristics of this variant are not as clear as that for classic PTC); solid/trabecular, solid nested variant, lack of tumour necrosis, and absence of marked mitotic activity; and the hobnail variant, ≥30% hobnail cells, micropapillary growth patterns, and high nuclear/cytoplasmic ratio. The clinicopathological characteristics included large tumour size, diffuse tumours, extra-thyroid expansions, patient age (50-60 years) or infiltrative form with extra-thyroid expansion, rapid growth rate, early lymph node metastasis, high rate of recurrences, and higher risk of metastasis. Wild-type BRAF expression was more common than the BRAF V600E point mutation [3-5, 23, 24].

Distal metastases were evaluated via retrospective data analysis of imaging studies (scintigraphy) or histopathologic examination of resected tumours and/or metastases. In the present study, lymph node metastases were usually cervical ones. Distal metastases appeared in the lungs and/or mediastinal lymph nodes. This study has been reviewed and approved by the Ethics Committee of Hanoi Medical University and adheres to the ethical standards laid down in the Declaration of Helsinki amended by the World Medical Association (WMA) General Assembly, Seoul, Korea, October 2008.

Immunohistochemistry

Tissue slices (4-5 µm) were dried, dewaxed, and rehydrated. Immunostaining was performed using a Ventana Benchmark XT automatic stainer. A kit with BRAF V600E mutant-specific antibody (diluted 1:100, VE1, mouse monoclonal, LOT: E12804, RFF 790-4855, VENTANA) was used. An OptiView DAB immunohistochemistry (IHC) detection kit (Roche Diagnostics), which included pre-treatment with cellular conditioner 1 (pH 8.5) for 64 min, was used for visualization of antibody binding. Tissue slices were then incubated at 37°C with the VE1 antibody for 16 min. Incubation of the antibodies was followed by back-staining with hematoxylin and bluing reagent at room temperature for 4 min each. Subsequently, the immunostaining slides were removed, washed with a drop of dishwashing detergent, and mounted.

A Nikon optical microscope (Nikon Microscope ECLIPSE Ci-L, Epi-FL 4) was used to assess immunostaining. Immunostaining for the BRAF V600E antigen was considered positive when the cytoplasm of the tumour cells was reddish-brown. A BRAF V600E-positive colorectal adenocarcinoma was used for the positive control and a section with no primary antibody incubation was the negative control. Interpretation of immunostaining results was performed by two pathologists (HNV, TNT), who were blinded to the clinical evidence.

Scoring of BRAF V600E immunoreactivity

IHC results were reported as a function of tumour cells with cytoplasmic staining as follows: 0, no colouring or weak colouring intensity of 10% or less of tumour cells; 1+, weak colouring in more than 10% of tumour cells; 2+, moderate colouring in over 10% of tumour cells; and 3+, strong colouring in over 10% of tumour cells. The tumour was considered positive at 1+ or higher and negative at 0 [25].

Statistical analysis

Statistical analyses were accomplished using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, U.S.A.). The link between the BRAF mutation and the clinicopathologic characteristics was analysed using an r-Pearson correlation, p-value. Differences were considered to be statistically significant at $p<0.05$.

Results

Patient characteristics

The clinicopathological features of PTC are shown in Table 1. Ninety-five patients with PTC were included in the study; 90.6% of

Table 1. Clinicopathological features of PTC.

Variables		n (%)
Gender		
	Male	9 (9.4)
	Female	86 (90.6)
Age (y)		
	10-19	1 (1.1)
	20-29	9 (9.5)
	30-39	23 (24.2)
	40-49	34 (35.8)
	50-59	17 (17.9)
	≥60	11 (11.6)
Location		
	Right lobe	47 (49.5)
	Left lobe	41 (43.1)
	Isthmus	7 (7.4)
Size		
	<1 cm	22 (23.2%)
	1-<2 cm	67 (70.5%)
	2-3.5 cm	6 (6.3)

patients were female (female:male ratio of 9.5:1). Patient ages ranged from 18-68 years (mean: 43.2±11.2 years). The highest number of cases were in the 40-49 years age group (35.8%, 34 cases), followed by 30-39 years (24.2%, 23 cases). Patients aged 10-19 years accounted for the lowest number of cases (1.1%, 1 case).

Tumours appeared in the right lobe in 47 patients (49.5%) and the left lobe in 41 patients. Seven patients had a tumour in the isthmus. No patients had tumours in both lobes. The minimum tumour size was 0.3 cm and the largest tumour size was 3.2 cm The average tumour size was 2±0.4 cm. Tumours were ≤1 cm in size in 22 cases (23.2%), 1-2 cm in 67 cases (70.5%), 2-3 cm in 5 cases, and 3.2 cm in 1 case.

Histopathological and immunohistochemistry characteristics

Table 2 shows that eleven variants of PTC were identified. The most common variants were conventional (26 tumours, 27.4%), microcarcinoma (22 tumours, 23.2%), and fasciitis-like stroma (16 tumours, 16.8%). The remaining 31 tumours consisted of eight PTC variants, including the clear cell variant. Four variants of PTC were not identified in the study population including diffuse sclerosing, oncocytic, spindle cell, and Warthin-like (Fig. 1).

Table 2. Correlation of histological variants with BRAF V600E in thyroid papillary carcinoma.

Histology		**BRAF V600E**	
Histological variants	***n** (%)*	***BRAF+** (n, %)*	***BRAF-** (n, %)*
Conventional	26 (27.4)	22 (84.6)	4 (15.4)
Follicular	6 (6.3)	2 (33.3)	4 (66.7)
Encapsulated	4 (4.2)	3 (75)	1 (25)
Microcarcinoma	22 (23.2)	17 (77.3)	5 (22.7)
Tall cells	8 (8.4)	5 (62.5)	3 (37.5)
Columnar cells	3 (3.2)	1 (33.3)	2 (66.7)
Cribriform	3 (3.2)	1 (33.3)	2 (66.7)
Hobnail	2 (2.1)	2 (100)	0
With fibromatosis/ fasciitis-like stroma	16 (16.8)	10 (62.5)	6 (37.5)
Solid/trabecular	4 (4.2)	2 (50)	2 (50)
Clear cells	1 (1.1)	1 (100)	0
Total	95 (100)	**66 (69.5)**	29 (30.5)

r-Pearson correlation = -0.325; p=0.01.

Positive BRAF V600E immunostaining was detected in 66 tumours (69.5%) while 28.4, 29.5, and 11.6% had immunostaining scores of 3+, 2+, and 1+, respectively. The remaining 29 tumours (30.5%) were negative for BRAF expression. BRAF V600E immunoexpression is associated with histological variant and was more common in the following variants: conventional (84.6%), encapsulated (75%), microcarcinoma (77.3%), tall cell (62.5%), hobnail (100%), fasciitis-like (62.5%), clear cell (100%), and less in variants: follicular, columnar, and cribriform (same at 33.3%) (r-Pearson correlation=-0.325; p=0.01) (Table 2). Thus, the high frequency of BRAF V600E immunoexpression is in both aggressive and non-aggressive variants.

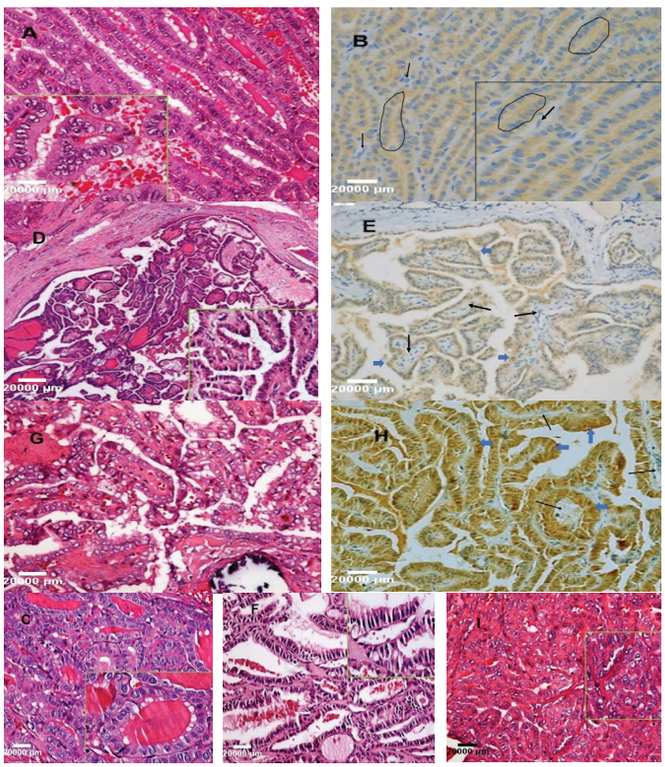

Fig. 1. Variants of PTC. (A) Columnar cell variant, **(B)** BRAF (++), columnar cell variant, **(C)** Follicular variant, **(D)** Encapsulated variant, **(E)** BRAF (+), encapsulated variant, **(F)** Tall cell variant, **(G)** Conventional variant, **(H)** BRAF (+++), conventional variant, **(I)** Solid variant. [H&E (A,C,D,F,G,I) and immunoperoxidase (B,E,H) stain; original magnification: 20x (large box) and 40x (inserts)].
(⇑): internal negative control; *circle and* (➡): positive for yellow brown.

The associations between positive BRAF expression and clinicopathologic factors are shown in Table 3. Tumours were positive for BRAF V600E in 66.7% of men and 69.8% of women (p>0.05). BRAF V600E-positive tumours tended to decrease with age from 88.9% (20-29 years) to 58.8% (50-59 years), then increase in the >60 years age group (90.9%). The differences according to age were significant (p<0.05). The highest incidence of BRAF V600E expression was found in tumours of the right lobe (74.5%), left lobe (65.9%), and isthmus (57.1%). Differences in positive BRAF immunostaining rate by tumour location were not statistically significant (p=0.332).

In the favourable histological group, 3/78 tumours (3.8%) with node metastasis were positive for BRAF V600E (p=0.636). In the aggressive histological group, 10/17 tumours (58.8%) with nodes and distant metastasis (3 lung metastases and 2 mediastinal metastasis) were positive for BRAF V600E (p=0.005). BRAF V600E immunoexpression was associated with patient age (p=0.039), histological variants (r-Pearson correlation=-0.325; p=0.01), and metastasis in the aggressive histological group (p=0.005), but not with gender, tumour location, or tumour size (Table 3).

Table 3. The association between positive BRAF expression and clinicopathologic factors.

Clinicopathologic factors	BRAF V600E (−) (n)	BRAF V600E (−) (n)	Total n (%)
Gende			
Male	3	6	9
Female	26	60	86
p	0.457		
Age			
10-19	1	0	1
20-29	1	8	9
30-39	6	17	23
40-49	13	21	34
50-59	7	10	17
≥60	1	10	11
p	0.039		
Location			
Right lobe	12	35	47
Left lobe	14	27	41
Isthmus	3	4	7
p	0.332		
Size			
<1 cm	18	47	65
1-<2 cm	9	14	23
2–3.5 cm	2	5	7
p	0.476		
Metastasis (lymph node, distal) of favourable group			
Yes	1	2	3
No	21	54	76
p	0.636		
Metastasis (lymph node, distal) of aggressive group			
Yes	0	10	10
No	7	0	7
p	0.005		

Association between BRAF V600E expression and histological variants according to clinical behaviour

As shown in Table 4, only 17/95 tumours could be classified into aggressive prognostic groups by histological type. BRAF V600E immunoexpression was associated with histological type by high expression rate in tall cell (62.5%), solid (50%), hobnail (100%), and lower in columnar variants (33.3%), (r-Pearson correlation=0.870; p=0.000). So, immunostaining with VE1 antibodies should be preferred for aggressive variants: tall cell, solid, and hobnail.

Table 4. Relationship between the BRAF V600E expression and clinicopathologic variant groups.

Histology			BRAF V600E		
Clinicopathologic variant groups		*n (%)*	*BRAF+ (n, %)*	*BRAF- (n, %)*	p
Favourable histological variant group	Conventional	26 (27.4)	22 (84.6)	4 (15.4)	r-Pearson correlation=-0.239; p=0.035
	Follicular	6 (6.3)	2 (33.3)	4 (66.7)	
	Encapsulated	4 (4.2)	3 (75)	1 (25)	
	Microcarcinoma	22 (23.2)	17 (77.3)	5 (22.7)	
	Clear cells	1 (1.1)	1 (100)	0	
	With fibromatosis/ fasciitis-like stroma	16 (16.8)	10 (62.5)	6 (37.5)	
	Cribriform	3 (3.2)	1 (33.3)	2 (66.7)	
Aggressive histological variant group	Tall cell	8 (8.4)	5 (62.5)	3 (37.5)	r-Pearson correlation=0.870; p=0.000
	Columnar cell	3 (3.2)	1 (33.3)	2 (66.7)	
	Solid/trabecular	4 (4.2)	2 (50)	2 (50)	
	Hobnail	2 (2.1)	2 (100)	0	

Association between histological variants according to clinical behaviour and clinicopathologic features

As shown in Table 5, only 52/95 tumours could be classified into prognostic groups by histological type. In the favourable variant group (35 tumours, 67.3%), 28 tumours were <1 cm (80.0%) and 7 were 2-3 cm (20.0%). Of the aggressive variants (17 tumours, 32.7%), 10 tumours (58.8%) were <1 cm. The differences in tumour size according to clinicopathological groups were statistically significant (p=0.04). Most tumours in the left lobe (90%) and the isthmus (83.3%) belonged to the group of favourable histological variants (p=0.004).

Table 5. Relationship between histological variant groups and other variables.

Variables	n	Group of favourable histological variants	Group of aggressive histological variants	p
Size				
<1 cm	38	28	10	0.04
1–<2 cm	11	7	4	
2–3.5 cm	3	0	3	
Location				
Right lobe	26	12	14	
Left lobe	20	18	2	0.004
Isthmus	6	5	1	
Lymph node metastasis				
Yes	7	1	6	0.003
No	45	34	11	
Distal metastasis				
Yes	4	0	4	0.009
No	48	35	13	

In the lymph node metastasis group, the majority of tumours (6/7, 85.7%) were aggressive variants (Table 5) and in the group without lymph node metastases, the majority of tumours (34/45, 75.6%) were favourable variants (p=0.003). In the group with distal metastases, all tumours (4/4, 100%) were aggressive variants and in the group with no distal metastases, the majority of tumours (35/48, 72.9%) were favourable variants (p=0.009). Therefore, BRAF V600E immunoexpression was related to patient age and histological variant and not to gender, tumour location, tumour size, lymph node metastasis, and distal metastasis. The aggressive variant group had a worse prognosis: larger tumour size (p=0.004), more lymph node metastasis (p=0.003), and more distal metastasis (p=0.009) than the favourable variant group; Therefore, it is necessary to closely manage the aggressive variant group with the BRAF V600E mutation to limit the poor prognosis as much as possible.

Discussion

The frequency at which the BRAF V600E mutation was detected in PTC varies worldwide, for example, in the Philippines (70.59%) [26], Korea (75.8-82.3%), China (62.7%), Hawaii (83.8%), Poland (72.4%), the United States (61.0-77.0%), and the present study, Vietnam (69.5%). The differences between these rates are not significant. These findings are also consistent with the increasing PTC mutation rates detected in recent years [27, 28]. Increasing mutation rates may be occurring because of improved detection methods with better sensitivities and specificities. High rates of BRAF V600E immunoexpression occur with the tall cell variant. Several studies showed that the tall cell variant has the highest BRAF V600E positive

rate (68-100%) [12, 24, 29]. H.Z. Kimbrell, et al. (2015) [1] proved that all cases of tall cell variants were positive. Therefore, in clinical settings, the determination of BRAF V600E mutations in tall cell variants in PTC should be prioritized. The frequency of BRAF V600E mutation in the follicular variant ranges from 21 to 54% [23, 30, 31]. This frequency is consistent with our results for the tall cell variant (62.5%).

Several other factors can influence the measurement of BRAF V600E expression including the research material (frozen or paraffin-embedded tissue), antibody purity, the epitope for the antibody, the amplification system for signal detection, incubation time, and fixation solution for tissue samples [30, 31]. Another possible cause for the varying expression is the gene pair mismatch self-repair that results in the BRAF V600E gene mutation. Excessive oxidation during thyroid hormone synthesis may cause genomic mismatches, which suggests a correlation between iodine concentration (a component in thyroid hormone synthesis) and BRAF mutations [32].

Molecular techniques have been used to detect the BRAF V600E point mutation. These methods are often very expensive, require a lot of time and effort, and are difficult to verify [33]. Immunohistochemical staining is a technique widely used in pathology laboratories. The IHC technique is faster and cheaper than molecular techniques. Importantly, IHC has the added advantage of recognizing individual antigen-carrying tumour cells and evaluating their uniformity [34]. Recently, two mutant-specific antibodies against the BRAF V600E protein have been developed and commercialized: the antibody clone, VE1, developed by Spring Bioscience (Pleasanton, California) and a monoclonal anti-mouse BRAF antibody developed by New East Bioscience (Malvern, Pennsylvania). The VE1 clone has been used in most studies [10, 16, 23]. Currently, available BRAF mutant-specific antibodies have sensitivities and specificities of more than 95% [10, 17-19], and they are more sensitive than molecular tests for detecting the BRAF V600E mutation [17-19, 34]. Molecular techniques are highly dependent on DNA quality and require a sufficient number of tumour cells in the sample [17, 34]. Unlike molecular testing, IHC is less dependent on DNA quality or the proportion of tumour cells present in the sample. Furthermore, the level of mutant protein expression in tumour cells at the individual cell level can be assessed. Thus, each tumour cell in the sample is evaluated for mutations more accurately than molecular analyses [19, 35].

M. Bullock, et al. (2012) [19] suggested that the mutant-specific VE1 antibody should be the gold standard for detecting the BRAF V600E point mutation in PTC. P. McKelvie, et al. (2013) [10] also showed that detection of the BRAFV600E mutation by IHC was more sensitive than standard real-time PCR using a commercial kit (Seegene) to detect the BRAFV600E mutation and recommended using the mutant-specific VE1 antibody (BRAF V600E) in pathology laboratories where conditions for routine sequencing of PTC specimens are not favourable. Several studies have shown that the number of positive cells detected using IHC was higher than the mutant allele frequency, which was perhaps due to the presence of heterogeneous clones for BRAF V600E within the same tumour. The BRAF V600E mutation was found only in PTC cells and not in the stroma. Given that the study samples contain numerous different stromal tissues without the BRAF V600E mutation (stroma and vascular), the allele frequency in PTC may be significantly reduced in molecular tests [17-19, 36].

The detection of the BRAF V600E mutation by IHC upon diagnosis can immediately provide individual prognostic information to guide treatment and monitoring in patients with PTC. The presence of the BRAF V600E mutation significantly increases the risk of lymph node metastasis in PTC. Considerably high frequencies of microscopic lymph node metastases were detected among lymph nodes evaluated as negative by preoperative ultrasound, which ranged from 42% for primary thyroid tumours <2 cm to 86% for tumours >3 cm [10, 17, 33, 34]. P. McKelvie, et al. (2013) [10] demonstrated a significant association between the BRAF V600E mutation and gender, but not with other factors such as age, primary tumour size, extra-thyroid expansion, lymph node metastasis, distal metastasis, or the AJCC stage at the time of diagnosis.

The follicular variant usually has a higher frequency of BRAF V600E mutations and has characteristics more similar to the conventional papillary variant than the encapsulated variant [16, 37, 38]. Synthetic analysis by H. Lui and F. Lin (2015) [33] of 20,764 patients showed that the BRAF V600E mutation was associated with several high-risk clinical variables used in the prognostic system, including extra-thyroid invasion, high TNM stage, lymph node metastasis, recurrence, and total survival but not with distal metastasis. The diagnosis of distal metastasis varies widely among countries and even among medical centres within the same country, which may cause discrepancies when comparing results across studies.

Although several authors have found an association between the BRAF mutation and tumour invasiveness, others have been unable to confirm this association. Accordingly, some studies have found that the presence of a BRAF V60E mutation was not correlated with clinical signs of aggression [10, 35] including age, extra-thyroid expansion, TNM stage, lymph node metastases, recurrence, reduced survival, or distal metastasis [39]. However, PTC histology has been significantly associated with the presence of the BRAF V600E mutation [40] whereas follicular variant histology was not associated with the BRAF V600E mutation.

Classifying the two clinicopathologic variants was useful in determining the association between BRAF V600 immunoexpression and patient age and histological variants in the favourable and aggressive histological groups. In the favourable variant group, the tumour size was often small <1cm (p=0.04) and more preferred in the left lobe (p=0.004). The tumour was more prone to lymph node metastases (p=0.003) in the aggressive variant group and distant metastases (p=0.009).

Conclusions

Lymph nodal and distal metastases (poor prognosis) in the aggressive (or high-risk) clinicopathological group of PTC can be better predicted when the molecular feature of BRAF V600E is integrated with histopathological characteristics; conversely, these results could not be achieved in the favourable histological group. These features suggest that BRAF V600E immunoexpression may be considered as a potential candidate for the prognosis of the high-risk histological group. Therefore, the BRAF V600E test should be added to the diagnosis in the high-risk clinicopathological group.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] H.Z. Kimbrell, et al. (2015), "BRAF testing in multifocal papillary thyroid carcinoma", *Biomed Res. Int.*, **2015**, pp.1-7.

[2] R. Ciampi, Y.E. Nikiforov (2007), "Minireview: RET/PTC rearrangements and braf mutations in thyroid tumorigenesis", *Endocrinology*, **148(3)**, pp.936-941.

[3] M. Nath, L.A. Erickson (2018), "Aggressive variants of papillary thyroid carcinoma: Hobnail, tall cell, columnar, and solid", *Adv. Anat. Pathol.*, **25(3)**, pp.172-179.

[4] R.V. Lloyd, et al. (2017), *WHO Classification of Tumours of Endocrine Organs, 4th ed.*, International Agency for Research on Cancer (IARC) Publisher, **10**, 355pp.

[5] S. Asioli, et al. (2013), "Papillary thyroid carcinoma with hobnail features: Histopathologic criteria to predict aggressive behaviour", *Hum. Pathol.*, **44(3)**, pp.320-328.

[6] N. Fakhruddin, et al. (2017), "BRAF and NRAS mutations in papillary thyroid carcinoma and concordance in BRAF mutations between primary and corresponding lymph node metastases", *Sci. Rep.*, **7(1)**, pp.1-11.

[7] C. Liu, T. Chen, Z. Liu (2016), "Associations between BRAF V600E and prognostic factors and poor outcomes in papillary thyroid carcinoma: A meta-analysis", *World J. Surg. Oncol.*, **14(1)**, pp.1-12.

[8] G. He, et al. (2014), "Prognostic value of the BRAF V600E mutation in papillary thyroid carcinoma", *Oncol. Lett.*, **7(2)**, pp.439-443.

[9] S. Choden, et al. (2020), "VE1 immunohistochemistry improves the limit of genotyping for detecting BRAFV600E mutation in papillary thyroid cancer", *Cancers (Basel).*, **12(3)**, pp.1-15.

[10] P. McKelvie, et al. (2013), "The prognostic significance of the BRAFV600E mutation in papillary thyroid carcinoma detected by mutation-specific immunohistochemistry", *Pathology*, **45(7)**, pp.637-644.

[11] D. Chen, et al. (2017), "Investigation of BRAF V600E detection approaches in papillary thyroid carcinoma", *Pathol. Res. Pract.*, **214(2)**, pp.303-307.

[12] A. Coca-Pelaz, et al. (2020), "Papillary thyroid cancer-aggressive variants and impact on management: A narrative review", *Adv. Ther.*, **37(7)**, pp.3112-3128.

[13] T.H. Kim, et al. (2012), "The association of the BRAFV600E mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: A meta-analysis", *Cancer*, **118(7)**, pp.1764-1773.

[14] B.R. Haugen, et al. (2016), "2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer", *Thyroid*, **26(1)**, pp.1-133.

[15] K. Dorak, et al. (2014), "Immunohistochemistry with the anti-BRAF V600E (VE1) antibody: Impact of pre-analytical conditions and concordance with DNA sequencing in colorectal and papillary thyroid carcinoma", *Pathology*, **46**, pp.509-517.

[16] J. Zagzag, et al. (2013), "Clinical utility of immunohistochemistry for the detection of the BRAF V600E mutation in papillary thyroid carcinoma", *Surg. (United States)*, **154(6)**, pp.1199-1205.

[17] D. Capper, et al. (2011), "Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody", *Acta Neuropathol.*, **122(1)**, pp.11-19.

[18] O. Koperek, et al. (2012), "Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma", *Am. J. Surg. Pathol.*, **36(6)**, pp.844-850.

[19] M. Bullock, et al. (2012), "Utilization of a MAB for BRAF V600E detection in papillary thyroid carcinoma", *Endocr. Relat. Cancer*, **19(6)**, pp.779-784.

[20] M. Al-Jundi, et al. (2020), "Novel targeted therapies for metastatic thyroid cancer - A comprehensive review", *Cancers (Basel).*, **12(8)**, pp.1-37.

[21] Y. Han, et al. (2021), "Identification of potential BRAF inhibitor joint therapy targets in PTC based on WGCAN and DCGA", *J. Cancer*, **12(6)**, pp.1779-1791.

[22] M. Cabanillas, M. Ryder, C. Jimenez (2019), "Targeted therapy for advanced thyroid cancer: Kinase inhibitors and beyond", *Endocr. Rev.*, **40(6)**, pp.1573-1604.

[23] H.S. Min, C. Lee, K.C. Jung (2013), "Correlation of immunohistochemical markers and BRAF mutation status with histological variants of papillary thyroid carcinoma in the korean population", *J. Korean Med. Sci.*, **28(4)**, pp.534-541.

[24] X. Wang, et al. (2016), "Tall cell variant of papillary thyroid carcinoma: Current evidence on clinicopathologic features and molecular biology", *Oncotarget*, **7(26)**, pp.40792-40799.

[25] X. Zhu, et al. (2016), "Specific immunohistochemical detection of the BRAF V600E mutation in primary and metastatic papillary thyroid carcinoma", *Exp. Mol. Pathol.*, **100(1)**, pp.236-241.

[26] G. Espiritu, et al. (2019), "High preponderance of BRAF V600E mutation in papillary thyroid carcinoma among filipinos: A clinicopathologic study abstract", *J. Glob. Oncol.*, **2019(5)**, pp.1-6.

[27] F.A. Rashid, et al. (2020), "Prevalence of BRAF V600E mutation in Asian series of papillary thyroid carcinoma - A contemporary systematic review", *Gland Surg.*, **9(5)**, pp.1878-1900.

[28] H.G. Vuong, et al. (2017), "The changing characteristics and molecular profiles of papillary thyroid carcinoma over time: A systematic review", *Oncotarget*, **8(6)**, pp.10637-10649.

[29] R. Villar-Taibo, et al. (2017), "Aggressiveness of the tall cell variant of papillary thyroid carcinoma is independent of the tumour size and patient age", *Oncol. Lett.*, **13(5)**, pp.3501-3507.

[30] A.E. Walts, J.M. Mirocha, S. Bose (2015), "Follicular variant of papillary thyroid carcinoma (FVPTC): Histological features, BRAF V600E mutation, and lymph node status", *J. Cancer Res. Clin. Oncol.*, **141(10)**, pp.1749-1756.

[31] Y.Y. Jung, et al. (2015), "Clinicopathologic correlations of the BRAFV600E mutation, BRAF V600E immunohistochemistry, and BRAF RNA in situ hybridization in papillary thyroid carcinoma", *Pathol. Res. Pract.*, **211(2)**, pp.162-170.

[32] H.R. Chung (2014), "Iodine and thyroid function", *Ann. Pediatr. Endocrinol. Metab.*, **1012(19)**, pp.8-12.

[33] H. Lui, F. Lin (2015), "Application of immunohistochemistry in thyroid pathology", *Arch. Pathol. Lab. Med.*, **139(1)**, pp.67-82.

[34] G.V. Long, et al. (2013), "Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma", *Am. J. Surg. Pathol. ?*, **37(1)**, pp.61-65.

[35] A. Mathur, W. Moses, R. Rahbari, (2011), "Higher rate of BRAF mutation in papillary thyroid cancer over time: A single-institution study", *Cancer*, **117(19)**, pp.4390-4395.

[36] C.A. Routhier, et al. (2013), "Comparison of 2 monoclonal antibodies for immunohistochemical detection of BRAF V600E mutation in malignant melanoma, pulmonary carcinoma, gastrointestinal carcinoma, thyroid carcinoma, and gliomas", *Hum. Pathol.*, **44(11)**, pp.2563-2570.

[37] W. Smith, A. Alajegheh, M. Nassiri (2011), "Correlation between BRAF mutation and the clinicopathological parameters in papillary thyroid carcinoma with particular reference to a follicular variant", *Hum. Pathol.*, **42(4)**, pp.500-506.

[38] M. Rivera, et al. (2009), "Encapsulated papillary thyroid carcinoma: A clinicopathologic study of 106 cases with emphasis on its morphologic subtypes (histologic growth pattern)", *Thyroid*, **19(2)**, pp.119-127.

[39] S.Y. Dong, et al. (2017), "BRAFV600E mutation is not associated with central lymph node metastasis in all patients with papillary thyroid cancer: Different histological subtypes and preoperative lymph node status should be taken into account", *Oncol. Lett.*, **14(4)**, pp.4122-4134.

[40] D.J. Givens, et al. (2014), "BRAF V600E does not predict aggressive features of pediatric papillary thyroid carcinoma", *Laryngoscope*, **124(9)**, pp.389-393.