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*BRAF*V600E Immunohistochemistry in Papillary Thyroid Carcinomas: Relationship Between Clinical and Morphological Parameters

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ABSTRACT

Objective: To investigate the association of the *BRAF*V600E mutation with papillary thyroid carcinoma using clinical, morphological and prognostic parameters. We also intend to assess the utility of the *BRAF*V600E immunohistochemistry and compare it with *BRAF* polymerase chain reaction (RT-PCR).

Material and Method: We applied *BRAF*V600E immunohistochemistry in a cohort of 107 papillary carcinomas, 19 adenomas and 13 normal thyroid tissues that was chosen retrospectively between 2011 and 2015. Statistical analysis was based on semiquantitative immunohistochemistry findings. We also applied *BRAF* RT-PCR in a subgroup of 14 papillary carcinomas, 13 metastatic lymph nodes and 4 adenomas that was chosen randomly.

Results: In regard to the comparison of *BRAF*V600E immunohistochemistry and *BRAF* RT-PCR, a 3+ nuclear and cytoplasmic immunoexpression was considered 'positive'. The *BRAF*V600E mutation was most frequently observed in classic variant cases. No mutation was detected in follicular variant cases. The mutational status of the primary tumour and the lymph node metastasis was consistent. A significant relationship of the *BRAF*V600E mutation was found with prognostic factors such as higher pT stage, classic variant, lymphatic invasion, perineural invasion, lower mitotic index, lack of tumour capsule, intrathyroidal spread and extrathyroidal extension.

Conclusion: Immunohistochemistry, using the VE1 clone, is a reliable technique for detection of the *BRAF*V600E mutation. Our results with immunohistochemistry are consistent with a previous effort. In our study, despite the correlation between some pathological prognostic parameters and the *BRAF*V600E mutation; poor prognosis was found to be irrelevant overall. Morphological parameters seem to be keener than the *BRAF*V600E mutation. Nevertheless, different series display different results, possibly due to environmental factors. Considering this and the proven success of targeted therapies against the *BRAF*V600E mutation a thorough assessment would be important.

Key Words: Thyroid, Papillary carcinoma, *BRAF*V600E, Immunohistochemistry, Reverse Transcriptase PCR, Prognosis

INTRODUCTION

Papillary carcinoma of the thyroid (PTC) is one of the most common cancers with an incidence rate of 14.42 per 100 000 person-years in 2010–2013 (1). PTCs have been known to have a better prognosis than other malignant tumours of the human body, although around 10% exhibit a worse clinical course than expected in PTCs. Several immunohistochemical stains have been used, such as cytokeratin 19 (CK19), Hector Battifora mesothelial cell-1 (HBME-1) and Galectin-3, to diagnose and detect this small group. The rapid development in molecular pathology has led to deeper efforts in coming up with the privileges of targeted therapy options.

The most relevant genetic alterations in PTC are generally mutually exclusive and in the vast majority of cases cause activation of the *MAPK* pathway; such as *BRAF*, *RET* and *RAS* mutations with *BRAF* mutations in the centre of attention (2). Mutations affecting the *BRAF* proto-oncogene are point mutations, small in-frame deletions, insertions and chromosomal rearrangements; the most frequent of which is the *BRAF*V600E point mutation. As a group, *BRAF* mutations activate *BRAF* kinase and lead to chronic stimulation of the mitogen-activated protein kinase pathway. In PTCs, *BRAF* mutations have been postulated as a cause of tumour recurrence (3) and worse prognosis (4, 5), along with initial tumour pathogenesis. Polymerase

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chain reaction (RT-PCR) and Sanger sequencing are the gold standard techniques to detect *BRAF*V600E mutation, whereas immunohistochemistry (IHC) needs more scientific evidence of high specificity and sensitivity. Some morphological findings like multicentricity, lymph node metastasis, tumour extension beyond the thyroid parenchyma and Psammoma bodies (6) may also predict the *BRAF*V600E mutation.

CK19 is a low molecular weight cytokeratin found in simple and complex epithelia, as well as in some carcinomas. An increased intensity of CK19 immunostaining is used for the diagnosis of PTC. HBME-1 is a marker of the apical surface of the mesothelium. An apical membranous staining of HBME-1 is also seen in PTCs. Galectin-3 is a β-galactoside binding lectin in charge of cell adhesion. Nuclear and cytoplasmic immunostaining is seen in PTCs. Ki-67 detects the nuclei of cells in late G1, S, G2 and M phases. Proliferation index in PTCs is no more than 5% in general (7).

The aim of this study is to evaluate the immunoexpression of *BRAF*V600E, and its correlation with clinicopathologic parameters.

MATERIALS and METHODS

The study involved the use of formalin-fixed paraffin-embedded tissue sections of histopathologically diagnosed cases of PTC (n = 107) and adenoma (n = 19) from the archives of the Department of Pathology. Of the PTC cases, 23 were microcarcinomas: 2 were follicular variants, 1 was oncocytic and 20 were classic. Twenty-three of the PTC group had metastatic lymph nodes available. These lymph nodes were assessed similarly. The slides that had been routinely stained with hematoxylin and eosin, CK19, HBME-1, Galectin-3 and Ki-67 were re-evaluated. The pT stage, necrosis, calcification, lymphatic invasion, vascular invasion, perineural invasion, tumour capsulation and capsule invasion, extrathyroidal extension, multicentricity, intrathyroidal spread and surgical margin status were assessed as prognostic parameters. To avoid controversy, certain criteria were used (8) as elaborated below.

Vascular invasion was defined as a direct tumour extension into the blood vessel lumen or a tumour aggregate within the vessel lumen. The criteria for vascular invasion are as follows:

- The affected vessel must be located within the capsule or immediately beyond the capsule but not within the tumour nodule itself.
- The vessel should have a clearly identifiable wall with endothelial lining.
- If a tumour extends directly into the vessel lumen, it should form a polypoid mass protruding into the lumen or exhibit thrombus formation in association with the tumour and not just bulging into the lumen.
- The cell aggregates within the lumen should be histologically identical to the tumour cells and be composed of epithelial cells and not of reactive endothelial cells.
- The intravascular tumour aggregate should be attached to the wall of the blood vessel and covered by a layer of endothelial cells.

Extrathyroidal extension was defined as tumour penetration through the thyroid pseudocapsule into the adjacent skeletal muscles or other organs.

Intrathyroidal spread was defined as an intraglandular dissemination of a tumour via lymphatic channels, and multiple small or larger satellite foci in the vicinity or remotely from the main tumour mass.

Information regarding the gender and age of the patients was obtained from the automation system of our hospital. Clinical follow-up was provided by the general surgery department. PTC cases were classified as either ‘good prognosis (GP)’ or ‘poor prognosis (PP)’ upon the clinical occurrence of lymph node metastasis, local recurrence and/or distant metastasis. Clinicopathologic features are shown in Table I. Serial sections (4 μm thick) were obtained from the paraffin-embedded blocks of the selected preparations and fixed on positively charged slides to perform IHC. *BRAF*V600E IHC was performed manually using the Novolink® Polymer Detection System (Leica, Australia). Additional information on IHC is summarised in Table II. Positive and negative control slides were also stained. All 31 cases (14 primary tumours (PT), 13 metastatic lymph nodes and 4 adenomas) were selected randomly, and *BRAF* mutation analysis was performed on these cases using the Cobas® 4800 RT-PCR System (Roche Diagnostics, USA).

Assessment of Immunostaining

The assessment of *BRAF*V600E IHC in PTCs, melanomas and colonic adenocarcinomas is still under debate (9). However, in our study, nuclear with or without cytoplasmic staining was considered positive as in most of the literature. A semiquantitative approach was used based on the staining intensity of positively stained cells: negative, 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining) of any proportion of tumour cells (Figures 1-4). Any proportion of tumour cells with membranous and cytoplasmic staining with CK19, apical membranous staining with HBME-1 and nuclear and/or cytoplasmic staining with Galectin-3 was considered positive. The eyeballing technique was used for the Ki-67 labelling index, and 100 tumour cells were counted in hot spot areas. A proportion of nuclear-stained cells with Ki-67 was recorded and divided into groups with a threshold of 5%.

Statistical Analysis

Statistical assessments were performed using the SPSS software (SPSS version 15, SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation together with a range (minimum–maximum). The comparison of categorical variables was performed using the chi-square test. Fisher's exact test was used to compare *BRAF*V600E IHC and RT-PCR. A *P*-value of less than 0.05 was accepted as statistically significant.

Table I: Clinicopathologic features of the cases in the PTC group.

Gender	
Male	22% (*n* = 24)
Female	78% (*n* = 83)
Mean age	44 years
Type of resection	
Total thyroidectomy	44% (*n* = 47)
Lobectomy	56% (*n* = 60)
Surgical margins	
Positive	23% (*n* = 25)
Negative	77% (*n* = 82)
Clinical follow-up	
Available	91% (*n* = 97)
N/A	9% (*n* = 10)
Mean follow-up time	25 months
Survival	
Alive	99% (*n* = 106)
Dead	1% (*n* = 1)
Lymph node metastasis	
Synchronous	24% (*n* = 26)
Later	1% (*n* = 1)
Local recurrence	5% (*n* = 5)
Distant metastasis	2% (*n* = 2)
Prognostic group	
Good prognosis	68% (*n* = 73)
Poor prognosis	32% (*n* = 34)

PTC: Papillary carcinoma of the thyroid.

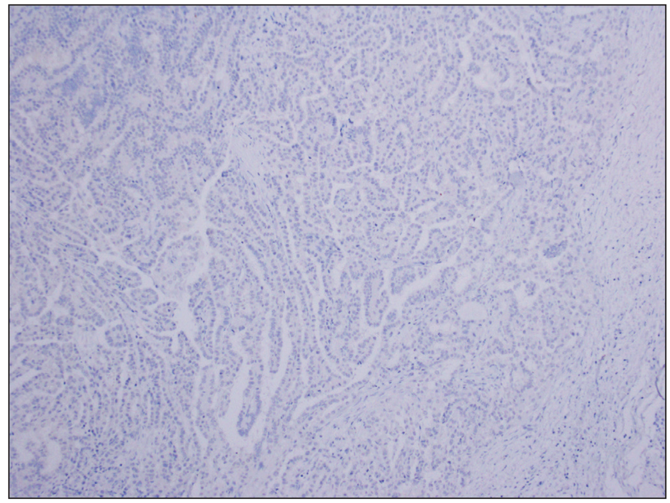

Figure 1: *BRAF*V600E IHC negative (x200).

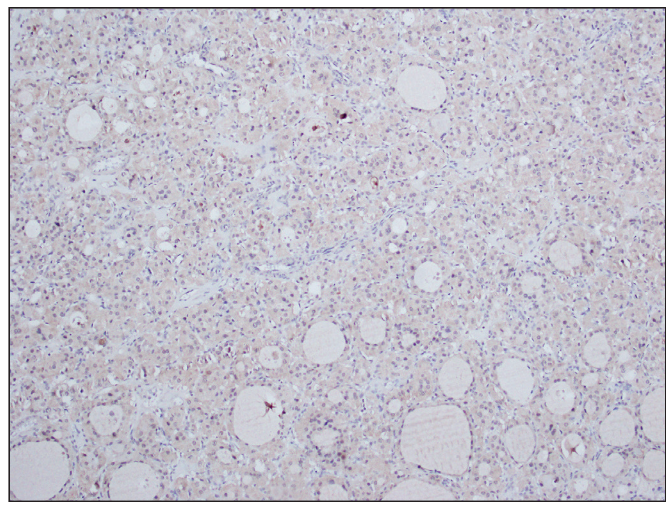

Figure 2: *BRAF*V600E IHC 1+ (x200).

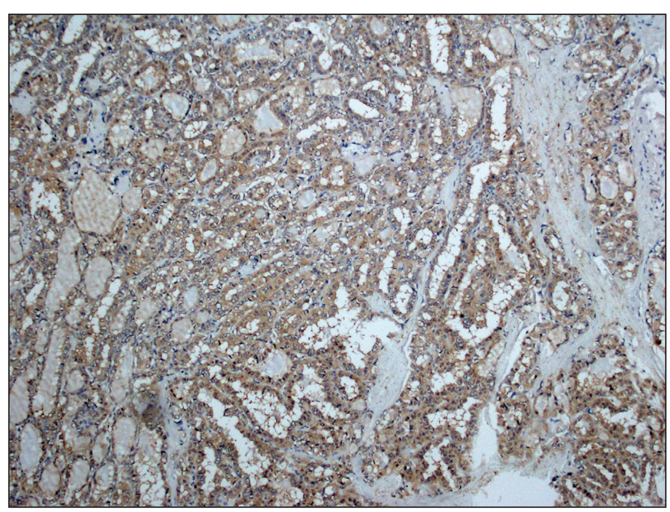

Figure 3: *BRAF*V600E IHC 2+ (x200).

Table II: Details of immunohistochemical findings.

Antibody	Clone	Dilution	Incubation time (min)	Temperature (°C)
*BRAF*V600E	VE-1 (mm); Spring Bioscience	1/100	60 (manual)	37
CK19	AB53 (mm); Dako	1/100	30 (Bond-Max)	56
HBME-1	HBME-1 (mm); Dako	1/50	30 (Bond-Max)	56
Galectin-3	9C4 (mm); Dako	1/100	30 (Bond-Max)	56
Ki-67	SP6 (rm); Abcam	1/100	30 (Bond-Max)	56

IHC: Immunohistochemistry, **mm:** Mouse monoclonal, **rm**: Rabbit monoclonal.

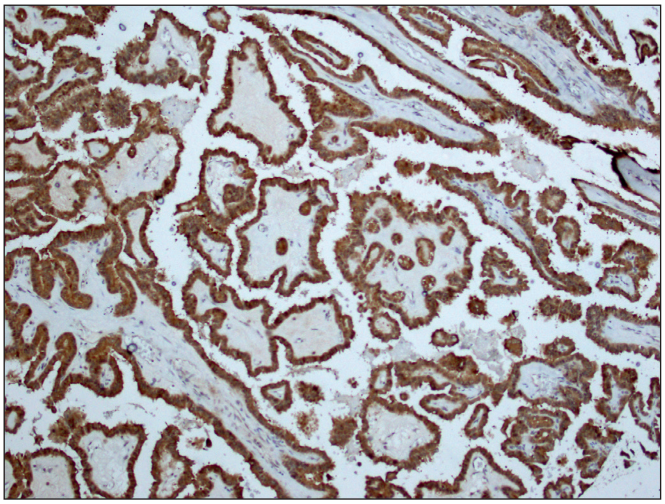

Figure 4: *BRAF*V600E IHC 3+ (x200).

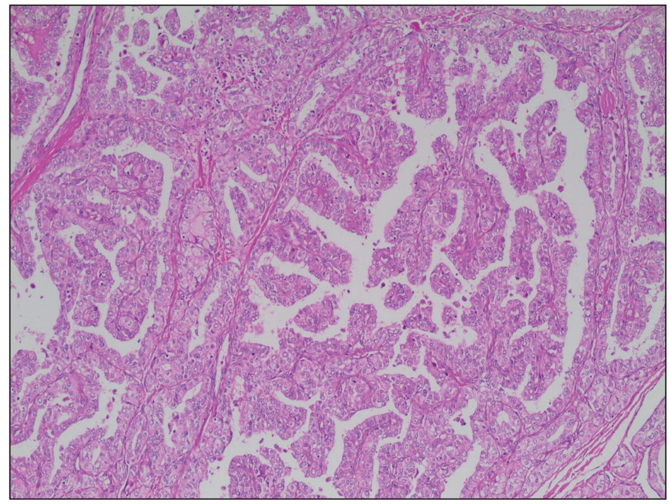

Figure 5: Classic variant papillary carcinoma (H&E; x200).

RESULTS

Of the PTC cases, 43% (*n* = 46) was pT1, 27% (*n* = 29) pT2 and 30% (*n* = 32) pT3. The mean tumour size was 20.98 mm (min. 3 mm and max. 75 mm) in PTCs and 25.79 mm (min. 9 mm and max. 67 mm) in adenomas. Of the PTC cases, 45% (*n* = 48) was classic variant, 23% (*n* = 25) follicular, 10% (*n* = 11) oncocytic and 22% (*n* = 23) microcarcinomas (Figures 5-8). Of the microcarcinomas, 87% (*n* = 20) was classic variant, 9% (*n* = 2) follicular and 4% (*n* = 1) oncocytic. Of the adenomas, 15.8% (*n* = 3) was oncocytic.

Twelve of the PTC cases were negative for HBME-1, one was negative for CK19 and six were negative for Galectin-3. In *BRAF*V600E IHC, 31 of the PTC cases were negative. Twelve cases showed a Ki-67 proliferation index higher than 5%. For *BRAF*V600E IHC, positive cases exhibited varying percentages of staining as shown in Table III.

The results of *BRAF* RT-PCR of randomly selected cases are shown in Table IV. For comparison of IHC and RT-PCR, the highest likelihood ratio (8.18) was obtained with the hypothesis 'Only 3+ IHC of *BRAF*V600E is truly positive'. The sensitivity of *BRAF*V600E IHC was calculated at 90.9%, whereas the specificity was 88.8%. In addition, the positive predictive value was 95.2%, and the negative predictive value was 80%. Given the likelihood ratio at 8.18, 1 of every 10 tests was meant to be wrong. In this respect, the *BRAF*V600E IHC findings are reconsidered and summarised in Table V.

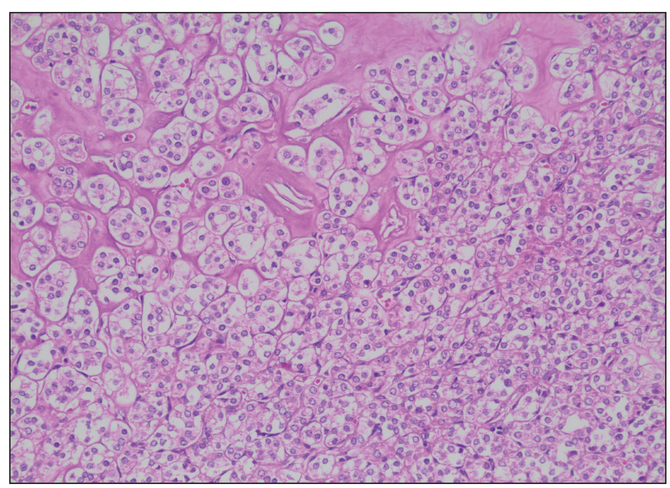

Figure 6: Follicular variant papillary carcinoma (H&E; x200).

Table III: *BRAF*V600E IHC.

Group	*BRAF*V600E staining intensity (*n*)				
	0	**1+**	**2+**	**3+**	**Total**
PTC-classic	11	3	7	27	48
PTC-follicular	13	11	1	0	25
PTC-oncocytic	2	5	3	1	11
Papillary microcarcinoma	5	3	9	6	23
Adenoma	12	7	0	0	19
Normal thyroid tissue	2	10	1	0	13
Total	45	39	21	34	139

IHC: Immunohistochemistry, **PTC:** Papillary carcinoma of the thyroid.

Table IV: *BRAF* RT-PCR results.

	IHC (–)	**IHC (1+)**	**IHC (2+)**	**IHC (3+)**	**Total**
RT-PCR (–)	10	5	5	1	21
RT-PCR (+)	1	0	1	8	10
Total	11	5	6	9	31

RT-PCR: Polymerase chain reaction, **IHC:** Immunohistochemistry.

Table V: *BRAF*V600E IHC upon RT-PCR results.

Group	*BRAF*V600E staining intensity (*n* (%))		
	0/1+/2+	**3+**	**Total**
PTC-classic	21	27 (59.5)	48
PTC-follicular	25	0 (0)	25
PTC-oncocytic	10	1 (9)	11
PMC	17	6 (26)	23
Total	73	34 (31.8)	107

IHC: Immunohistochemistry, **RT-PCR:** Polymerase chain reaction, **PTC**: Papillary carcinoma of the thyroid, **PMC:** Papillary microcarcinoma.

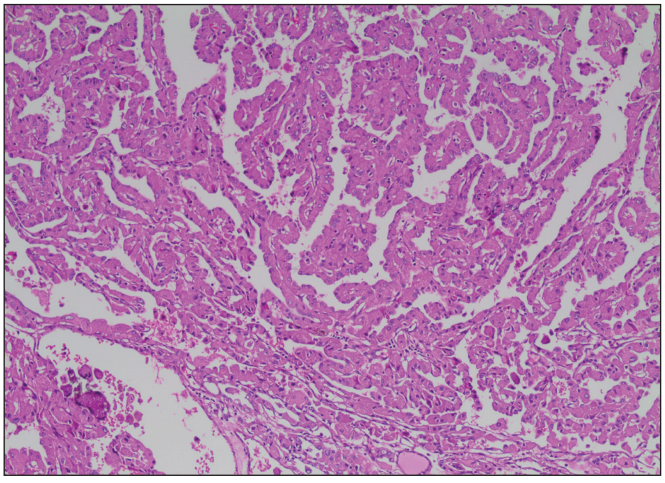

Figure 7: Oncocytic variant papillary carcinoma (H&E; x200).

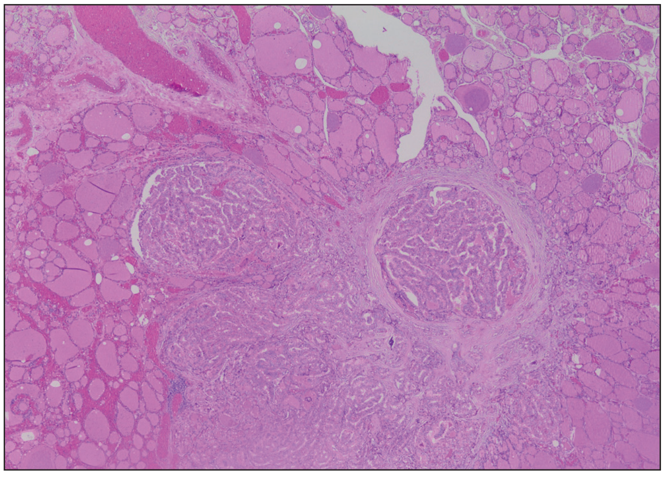

Figure 8: Papillary microcarcinoma (H&E; x40).

In cases where a metastatic lymph node (MLN) is present, the *BRAF*V600E positivity was 11% in PT. Two of those showed no staining in MLN, whereas PT was positive. Another two cases showed a lower percentage of positive tumour cells in MLN (Table VI). However, PTs and conjugate MLNs were statistically correlated upon the *BRAF*V600E mutation (Phi = 83.7%, $p = 0.0001$).

The *BRAF*V600E mutation was found to be statistically correlated with a higher pT stage ($p = 0.003$), classic morphology ($p = 0.003$), lower mitotic index ($p = 0.020$), lymphatic invasion ($p = 0.013$), perineural invasion ($p = 0.006$), a lack of tumour capsule ($p = 0.016$), extrathyroidal extension ($p = 0.0001$) and intrathyroidal spread ($p = 0.0001$). Noassociation was found between the *BRAF*V600E mutation and the patient's age, sex, synchronous lymph node metastasis, necrosis, calcification, vascular invasion, tumour capsule invasion, multicentricity, expressions of CK19, HBME-1, and Galectin-3 and the Ki-67 proliferation index. Of the *BRAF*V600E-mutated cases, 52.9% exhibited nodular hyperplasia in the non-tumoural parenchyma, whereas 23.5% showed lymphocytic thyroiditis. Neither had a significant association.

The clinical prognosis was assessed in two separate groups as described earlier. The poor prognostic group was found to be statistically correlated with a higher pT stage ($p = 0.005$), classic morphology ($p = 0.011$), calcification ($p = 0.017$), lymphatic invasion ($p = 0.008$), vascular invasion ($p = 0.0001$), lack of tumour capsule ($p = 0.004$), extrathyroidal extension ($p = 0.0001$), intrathyroidal spread ($p = 0.001$) and positive surgical margin ($p = 0.002$). No association was found with the patient's age, sex, necrosis, mitotic index, perineural invasion, tumour capsule invasion and multicentricity. HBME-1 positive cases were found to be correlated with PP ($p = 0.049$), whereas CK19 expression, Galectin-3 expression and Ki-67 proliferation index were irrelevant.

Table VI: *BRAF*V600E positive cells (3+ intensity) in PTs and conjugate MLNs.

Case	PT-*BRAF*	PT-*BRAF*-%	MLN-*BRAF*	MLN-*BRAF*-%
1	Negative		Negative	
2	Positive	90	Positive	90
3	Positive	80	Positive	70
4	Negative		Negative	
5	Positive	100	Negative	
6	Negative		Negative	
7	Negative		Negative	
8	Positive	100	Positive	100
9	Negative		Negative	
10	Positive	90	Positive	40
11	Negative		Negative	
12	Negative		Negative	
13	Negative		Negative	
14	Negative		Negative	
15	Positive	100	Positive	100
16	Negative		Negative	
17	Positive	90	Positive	90
18	Positive	100	Positive	100
19	Negative		Negative	
20	Positive	100	Positive	100
21	Positive	100	Positive	100
22	Positive	100	Negative	
23	Negative		Negative	

PT-*BRAF*: *BRAF*V600E mutation in primary tumour, **PT-*BRAF*-%:** Percentage of *BRAF*V600E positive cells in primary tumour, **MLN-*BRAF*:** *BRAF*V600E mutation in conjugate metastatic lymph node, **MLN-*BRAF*-%:** Percentage of *BRAF*V600E positive cells in conjugate metastatic lymph node, **PT:** Primary tumour, **MLN:** Metastatic lymph node.

In cases where the *BRAF*V600E mutation was present, poor prognostic incidents such as lymph node metastasis, local recurrence and distant metastasis were more frequent. However, we could not reveal any statistical significance ($p = 0.255$). On the other hand, *BRAF*V600E positive cases constituted a minority of 40% in the poor prognostic group.

DISCUSSION

PTCs are malignant tumours with an increasing incidence rate (1). The widespread use of radiological techniques and fine-needle aspiration biopsy has been partly responsible for this increasing incidence, although the vast majority of new cases are microcarcinomas (10). Lobectomy or total thyroidectomy with subsequent radioactive iodine ablation in selected patients is usually more effective; however, approximately 10% of PTC patients suffers from recurrence, lymph node or distant metastasis and hence require further intervention. *BRAF* mutations are the major promising development for PTC recently, providing a highly efficient anti-tyrosine kinase therapy.

Several studies have compared *BRAF*V600E IHC with molecular techniques. For instance, Qiu et al. (11) assessed *BRAF*V600E IHC by distinguishing samples as positive and negative without considering staining intensity and percentage of stained tumour cells. The study also compared IHC with RT-PCR and Sanger sequencing. Jung et al. (12) also compared IHC with RT-PCR and *BRAF* RNA *in situ* hybridisation. Zagzag et al. (13) accepted 3+ *BRAF*V600E staining as positive and compared IHC with direct sequencing. Ilie et al. (14) accepted the results as positive if 100% of the tumour cells stained 3+ and compared IHC with direct sequencing. To sum up, the sensitivity and specificity of *BRAF*V600E IHC ranges from 89% to 100% and from 61% to 100%, respectively. In our study, we accepted 3+ *BRAF*V600E staining as positive, disregarding the percentage of tumour cells, and compared IHC with RT-PCR. The sensitivity of *BRAF*V600E IHC was 90.9%, whereas the specificity was 88.8%. It is important to note that the RT-PCR system we used detects V600D and V600K mutations along with V600E, thus giving a non-discriminatory result.

Using the criteria 3+ nuclear and cytoplasmic staining in the PTC group, the *BRAF*V600E mutation rate was 31.8%. This rate increased up to 59.5% in classic variant cases, but it decreased to 26% in papillary microcarcinomas (PMCs) and 9% in oncocytic variant cases. In follicular variant and adenoma cases, no mutation was detected. In the recent literature, the *BRAF*V600E mutation rate has been reported between 35% and 70%, and the mutations were more often associated with a classic variant, tall cell variant and poorly differentiated/anaplastic carcinomas that arise from well-differentiated PTCs (15). The mutation rate is much lower in follicular carcinomas (16), which is similar to our results.

Intratumoural heterogeneity is a substantial phenomenon for understanding pathogenesis and its clinicopathologic role. As in other *BRAF*-harbouring tumours such as malignant melanomas and colorectal and pulmonary adenocarcinomas, PTCs have been shown to exhibit heterogeneously mutated tumour cells. Guerra et al. (17) showed *BRAF*-mutated tumour cells in MLNs of cases with *BRAF* negative primary, prompting that *BRAF* mutations constitute a subclonal alteration and may arise *de novo* in *BRAF* negative tumours later on. On the other hand, de Biase et al. (16) revealed a direct proportion between tumour size and percentage of *BRAF*-mutated tumour cells, suggesting that *BRAF* mutation is an early period alteration. Walts et al. (18) stated 100% concordance of *BRAF* mutation between PT and MLN and 92.3% concordance between different areas of PTs. In their experience, two *BRAF*-mutated PT cases exhibited *BRAF*-negative MLNs and recurrent tumours afterward. We observed a range of 80–90% *BRAF*-mutated tumour cells in PTs, two of which exhibited *BRAF*-negative MLNs and the other two showed a lower percentage of *BRAF*-mutated tumour cells in conjugated MLNs. The existence of such subclones disturbs the efficacy of targeted therapies. In this regard, quantitative *BRAF* mutation analysis may be suggested in PT, MLN, distant metastasis or recurrent tumour samples.

To start with associations between the *BRAF*V600E mutation and clinicopathologic parameters, we found noassociation with the patient's age and sex, as in the large-scale meta-analysis of Wang et al. (19) and series of Shin et al. (20). In our experience, *BRAF*V600 mutation was found to be correlated with a higher pT stage, lymphatic invasion, perineural invasion, lack of tumour capsule, extrathyroidal extension and intrathyroidal spread. Several studies have stated various morphological findings, and their combinations are correlated with the *BRAF*V600E mutation, interestingly having extrathyroidal extension in common (6,21,22).

Surprisingly, the *BRAF*V600E mutation rate was higher in tumours with a lower mitotic index, as in tumours with a lower Ki-67 proliferation index, despite its incoherency. No effort has been found in the English literature that addresses theassociation between *BRAF* mutations and mitotic index or the Ki-67 proliferation index. Nevertheless, well-differentiated PTCs are known to have a lower proliferation index than other malignancies. We observed that the Ki-67 proliferation index is higher than 5% in 19.6% of the PTC cases, reaching up to 15%. In addition, we did not find any significant association between mitotic/Ki-67 index and worse clinical and/or pathologic prognostic parameters. Guerra et al. (23) showed a higher rate of CK19 expression in *BRAF*-mutated tumours, whereas Galectin-3 was not associated with *BRAF*. In terms of HBME-1 expression and *BRAF*, our effort needs to be published first. However, in our series, no significantassociation was found between the *BRAF*V600E mutation and expression of CK19, Galectin-3 and HBME-1.

In cases where follow-up data are available, a survival analysis could not be made because there was no death by disease. The cases were assessed in two separate groups: GP and PP, as described earlier. The patient's age and sex were not found to be correlated with the prognosis. This is despite the fact that Howell et al. (24) stated that the *BRAF*V600E mutation and older age (≥ 65 years) predict recurrence and Suman et al. (25) associated younger age (≤ 45 years) with central lymph node metastasis.

In our series, PP was found to be associated with a higher pT stage, classic morphology, calcification, lymphatic invasion, vascular invasion, lack of tumour capsule, intrathyroidal spread, extrathyroidal extension, positive surgical margin and loss of HMBE-1 expression. Likewise, Rossi et al. (26) have associated PP in poorly differentiated and anaplastic thyroid carcinomas with loss of HBME-1 expression.

The association between the *BRAF*V600E mutation and PP can be properly summarised by the meta-analysis of Wang et al. (19). In contrast to what has been reported recently. Pelttari et al. (27), with their lengthy follow-up duration, have shown that the *BRAF*V600E mutation is not correlated with lymph node metastasis and/or recurrence. Zheng et al. (28) have revealed that the *BRAF*V600E mutation and the recurrence within PMCs are not related. Nam et al. (21) have also shown that the *BRAF*V600E mutation is not significantly associated with lymph node metastasis. In these series, despite the overall concern, some morphological findings such as extrathyroidal extension are interestingly correlated with the *BRAF*V600E mutation. For instance, Shin et al. (20) revealed that the *BRAF*V600E mutation does not seem to be associated with the overall prognosis but morphological parameters are associated solely and together with aggressive behaviour. We also did not find any association between the *BRAF*V600E mutation and the overall prognosis but with such morphologic parameters.

In conclusion, *BRAF*V600E IHC with VE1 clone can be accepted as a reliable technique for detecting the *BRAF*V600E mutation. Our series of well-differentiated PTCs has exhibited a rate of *BRAF*V600E mutation similar to recent literature. With our effort, morphological findings may be considered keener than the *BRAF*V600E mutation in predicting aggressive behaviour. However, demographic, clinical and morphological findings and genetic alterations should be assessed together to estimate a more precise prognosis. Although further therapeutic interventions are needed, it is better to look for the *BRAF*V600E mutation in PT, lymph node metastasis, recurrent tumour and distant metastasis, if available.

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CONFLICT of INTEREST

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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