**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
chain reaction (RT-PCR) and Sanger sequencing are the gold standard techniques to detect \( \text{BRAF} \text{V600E} \) 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 \( \text{BRAF} \text{V600E} \) 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 \( \beta \)-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 \( \text{BRAF} \text{V600E} \), 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 \( \text{pT} \) 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:

- 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 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 \( \mu \text{m} \) thick) were obtained from the paraffin-embedded blocks of the selected preparations and fixed on positively charged slides to perform IHC. \( \text{BRAF} \text{V600E} \) 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 \( \text{BRAF} \) mutation analysis was performed on these cases using the Cobas® 4800 RT-PCR System (Roche Diagnostics, USA).

### Assessment of Immunostaining

The assessment of \( \text{BRAF} \text{V600E} \) 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 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 BRAFV600E IHC and RT-PCR. A P-value of less than 0.05 was accepted as statistically significant.

**Table 1: Clinicopathologic features of the cases in the PTC group.**

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

**PTC:** Papillary carcinoma of the thyroid.
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 BRAFV600E IHC, 31 of the PTC cases were negative. Twelve cases showed a Ki-67 proliferation index higher than 5%. For BRAFV600E 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 BRAFV600E is truly positive’. The sensitivity of BRAFV600E 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 BRAFV600E IHC findings are reconsidered and summarised in Table V.
Table III: BRAFV600E IHC.

<table>
<thead>
<tr>
<th>Group</th>
<th>BRAFV600E staining intensity (n)</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>PTC-classic</td>
<td>11</td>
<td>3</td>
<td>7</td>
<td>27</td>
<td>48</td>
</tr>
<tr>
<td>PTC-follicular</td>
<td>13</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>PTC-oncocytic</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Papillary microcarcinoma</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Adenoma</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Normal thyroid tissue</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>39</td>
<td>21</td>
<td>34</td>
<td>139</td>
</tr>
</tbody>
</table>

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

Table IV: BRAF RT-PCR results.

<table>
<thead>
<tr>
<th>RT-PCR (-)</th>
<th>IHC (−)</th>
<th>IHC (1+)</th>
<th>IHC (2+)</th>
<th>IHC (3+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR (+)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>31</td>
</tr>
</tbody>
</table>

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

Table V: BRAFV600E IHC upon RT-PCR results.

<table>
<thead>
<tr>
<th>Group</th>
<th>BRAFV600E staining intensity (n (%))</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/1+/2+</td>
<td>3+</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>PTC-classic</td>
<td>21</td>
<td>27 (59.5)</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>PTC-follicular</td>
<td>25</td>
<td>0 (0)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>PTC-oncocytic</td>
<td>10</td>
<td>1 (9)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>PMC</td>
<td>17</td>
<td>6 (26)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>34 (31.8)</td>
<td>107</td>
<td></td>
</tr>
</tbody>
</table>

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 \textit{BRAFV600E} 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 \textit{BRAFV600E} mutation (Phi = 83.7%, \(p = 0.0001\)).

The \textit{BRAFV600E} 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 (\(p = 0.0001\)). No association was found between the \textit{BRAFV600E} 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 \textit{BRAFV600E}-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.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Case} & \textbf{PT-\textit{BRAF}} & \textbf{PT-\textit{BRAF}-%} & \textbf{MLN-\textit{BRAF}} & \textbf{MLN-\textit{BRAF}-%} \\
\hline
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 & \\
\hline
\end{tabular}
\caption{\textit{BRAFV600E} positive cells (3+ intensity) in PTs and conjugate MLNs.}
\end{table}

\textbf{PT-\textit{BRAF}}: \textit{BRAFV600E} mutation in primary tumour, \textbf{PT-\textit{BRAF}-%}: Percentage of \textit{BRAFV600E} positive cells in primary tumour, \textbf{MLN-\textit{BRAF}}: \textit{BRAFV600E} mutation in conjugate metastatic lymph node, \textbf{MLN-\textit{BRAF}-%}: Percentage of \textit{BRAFV600E} positive cells in conjugate metastatic lymph node, \textbf{PT}: Primary tumour, \textbf{MLN}: Metastatic lymph node.
In cases where the \(\text{BRAF}V600E\) 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, \(\text{BRAF}V600E\) 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. \(\text{BRAF}\) mutations are the major promising development for PTC recently, providing a highly efficient anti-tyrosine kinase therapy.

Several studies have compared \(\text{BRAF}V600E\) IHC with molecular techniques. For instance, Qiu et al. \((11)\) assessed \(\text{BRAF}V600E\) 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 \(\text{BRAF}\) RNA in situ hybridisation. Zagzag et al. \((13)\) accepted 3+ \(\text{BRAF}V600E\) 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 \(\text{BRAF}V600E\) IHC ranges from 89% to 100% and from 61% to 100%, respectively. In our study, we accepted 3+ \(\text{BRAF}V600E\) staining as positive, disregarding the percentage of tumour cells, and compared IHC with RT-PCR. The sensitivity of \(\text{BRAF}V600E\) 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 \(\text{BRAF}V600E\) 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 \(\text{BRAF}V600E\) 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 PTCs \((16)\), which is similar to our results.

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

To start with associations between the \(\text{BRAF}V600E\) mutation and clinicopathologic parameters, we found no association 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, \(\text{BRAF}V600E\) 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 \(\text{BRAF}V600E\) mutation, interestingly having extrathyroidal extension in common \((6,21,22)\).

Surprisingly, the \(\text{BRAF}V600E\) 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 the association between \(\text{BRAF}\) 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 signifi cant association 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 HBME-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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