Original Article

Elucidating the Prognostic Role of *BRAF*^{V600E} and the Activation Status of the Downstream MAPK Pathway in PTC: A Study from a Tertiary Centre in India

Nadeem Kocheri, Priti Chatterjee[1], Shipra Agarwal, Mehar C. Sharma[2], Sanjana Ballal[3], Chandrasekhar Bal[3], Sunil Chumber[4]

Departments of Pathology, [2]Neuropathology, [3]Nuclear Medicine and [4]Surgery, All India Institute of Medical Sciences, New Delhi, [1]Department of Pathology, Lady Hardinge Medical, College, New Delhi, India

Abstract

Introduction: Papillary thyroid carcinoma (PTC) has an excellent prognosis, but few cases are treatment-resistant. To check the applicability of combined *BRAF*^{V600E} and MEK-targeted therapy, the current study correlated *BRAF*^{V600E} with the MAPK pathway activation status in a cohort of PTCs. The prognostic relevance of *BRAF*^{V600E} and the usability of immunohistochemistry (IHC) for detecting the mutation were also assessed. **Methods:** Randomly selected 50 PTC and 15 non-PTC cases were re-classified according to the 2022 WHO classification. The *BRAF* mutation status was compared with the IHC of BRAF^{V600E}, pERK1/2, pMEK1/2, and clinicopathological variables, including response to radioactive iodine and disease-free survival. **Results:** *BRAF*^{V600E} mutation was present in 38%. Most (87.8%) cases were immunopositive for pMEK1/2 and 40% for pMEK1/2. Although *BRAF*^{V600E} mutation did not correlate with the MAPK activation status, it had an adverse impact on tumour sensitivity to radioiodine ($P < 0.05$). Five of the seven radioiodine-resistant tumours were *BRAF*^{V600E}-mutated. An Allred cut-off score of 7 had a sensitivity of 100% and a specificity of 84% for detecting the mutation by IHC. All the non-PTC cases were *BRAF*-wild type, but 20% showed weak immunopositivity for mutated protein and were scored 6. **Conclusions:** *BRAF*^{V600E}-mutated PTCs are more likely to be RAI-resistant. MAPK pathway activation status did not vary significantly with *BRAF* mutation. Immunopositivity for pMEK1/2 in most suggests a scope for MEK1-targeted therapy in recalcitrant PTC cases even in the absence of the *BRAF* mutation. In addition, IHC is a reliable technique for detecting *BRAF*^{V600E} mutation but needs validation by correlation with molecular studies.

Keywords: *BRAF*^{V600E}, IHC, MAPK, papillary thyroid carcinoma, pERK, pMEK

Introduction

Papillary thyroid cancer (PTC) is the most prevalent endocrine malignancy.[1,2] *BRAF*^{V600E} mutation, commonly present in PTC,[3] acts through the MAPK (RAS/RAF/MEK/ERK) pathway.[4] It has variably been associated with higher stage and radioactive iodine (RAI) resistance.[5,6] Tyrosine kinase inhibitors show an excellent response in RAI-resistant PTC. However, resistance to these inhibitors is an emerging problem.[4,7-9] Combined BRAF and MEK inhibition has been approved in *BRAF*^{V600E}-mutated anaplastic thyroid cancer.[10] Its applicability in PTC remains underexplored. We evaluated *BRAF*^{V600E} mutation and the activation status of the MAPK pathway in PTC and compared the results with patient outcomes, and immunohistochemistry (IHC) for the mutated protein.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/indjem/

DOI: 10.4103/ijem.ijem_235_23

Materials and Methods

This was a retrospective study performed after obtaining appropriate ethical approval by the Institute Ethics Committee. Randomly selected 50 cases of PTC were enrolled in the study. Tumours measuring <1 cm were excluded. The cases were retrieved from the archives of the Department of Pathology and re-evaluated for recharacterisation as per the 2022 WHO

Address for correspondence: Dr. Shipra Agarwal, Department of Pathology, All India Institute of Medical Sciences, New Delhi, India.
E-mail: drshipra0902@gmail.com

Submitted: 04-Jun-2023 **Accepted:** 16-Oct-2024
Published: 30-Dec-2024

How to cite this article: Kocheri N, Chatterjee P, Agarwal S, Sharma MC, Ballal S, Bal C, *et al*. Elucidating the prognostic role of *BRAF*^{V600E} and the activation status of the downstream MAPK pathway in PTC: A study from a tertiary centre in India. Indian J Endocr Metab 2024;28:617-21.

classification.[2] In each case, the patient demographics, size of the tumour, focality, metastatic profile, and clinical outcome, including response to RAI, were documented. The response to RAI was considered complete when a tumour lacked evidence of RAI uptake and radiological disease, along with undetectable Thyroglobulin and anti-thyroid antibodies. Radioiodine-resistant cancer refers to the following scenarios: negative RAI whole body scan, metastases progressing despite RAI uptake, persistent disease despite cumulative I-131 of >22.2 GBq (600 mCi), one or more lesions not demonstrating RAI uptake, the de-novo radioiodine refractory disease, or a combination of FDG-positive, elevated thyroglobulin, and I-131 negative lesions (TENIS syndrome).[11] Fifteen non-PTC thyroid lesions, including follicular adenoma (FA; n = 6), follicular thyroid carcinoma (FC; n = 8), and hyperplastic thyroid nodule (HTN; n = 1), were used as a negative control for Sanger sequencing and BRAF^{V600E} IHC.

DNA extraction and BRAF mutation assessment

Tumour DNA was extracted from representative paraffin-embedded tissue blocks by using a commercially available kit as per the manufacturer's protocol (Promega). The extracted DNA was evaluated for *BRAF* mutation analysis by PCR followed by Sanger Sequencing. Forward and reverse primers used were 5'-CTAGTAACTCAGCAGCATCTCAG-3' and 5'-CTCTTCATAATGCTTGCTCTGATAG-3', respectively, and the PCR product derived was 250 base pairs long. Sequencing was performed on ABI 3500 Genetic Analyser, and the data were analysed using Chromas software.

Immunohistochemical analysis

Five-micron-thick formalin-fixed paraffin-embedded representative sections were stained for BRAF^{V600E} (clone VE1, 1:50; Spring Bioscience) by using the Ventana automated immunohistochemical stainer according to the manufacturer's specifications (Ventana Medical Systems, Tucson, AZ). The VE1 antibody exhibits a cytoplasmic staining pattern with variable staining intensity ranging from weak to strong. Allred score, which is a semi-quantitative system considering the proportion and intensity of positive cells, was applied as follows: Proportion score: 0: 0%, 1: 1%, 2: 2%–10%, 3: 11%–33%, 4: 34%–66%, and 5: 67%–100%; Intensity score: 0: negative, 1: weak, 2: moderate, and 3: strong. The proportion and intensity scores were added to get the total score ranging from 0 to 8.[12] When staining intensity was heterogeneous within a tumour, the intensity was evaluated based on the highest staining intensity. Tissue sections from a case of malignant melanoma were used as positive control.

To evaluate the activation status of the MAPK pathway, immunostaining using antibodies for the activated (phosphorylated) forms of the downstream molecules, pERK1/2 (4376, Cell Signalling Technology, USA; 1:400 dilution), and pMEK1/2 (2338, Cell Signalling Technology, USA; 1:50 dilution), was performed manually. Nuclear and/or cytoplasmic staining was considered positive.[13] The quality of immunohistochemical staining was evaluated using endothelial cells as an internal positive control. The intensity was recorded on a scale from 0 to 3 and multiplied by the percentage of cells showing positivity at a given intensity. Hence, the expression was quantified on a scale of 0–300. Thus-obtained IHC score was considered positive if it was >30 and was given a final score of 0: <30 (negative); I: 30–99 (low expression); II: 100–199 (medium expression); and III: >200 (high expression).[14]

Statistical analysis

Statistical analysis was performed on STATA 16.0. Fischer's exact test was used to compare *BRAF*^{V600E} mutation status with clinicopathological variables, namely age at presentation (55 years as cut-off), tumour size (4 cm as cut-off), lymph nodal metastasis, multifocality, gross extrathyroidal extension, distant metastasis, and RAI-responsiveness. The disease-free survival (DFS) was analysed by plotting the Kaplan-Meier survival curves. The cut-off BRAF^{V600E} IHC score, predictive of the mutation, was calculated using logistic regression and receiver operating characteristic (ROC) analyses. The area under the ROC curve (AUC) was used to summarise the discrimination power of the test. A P value of < 0.05 was considered statistically significant.

Ethical aspects

The study was performed in compliance with Declaration of Helsinki 1964 and its later amendments. It was also approved by the Institute review board, All India Institute of Medical Sciences, New Delhi, India (Ref. No. IECPG-617/22.12.2016). Informed consent was waived in view of retrospective nature of the study.

Results

The mean age of the PTC patients was 37 years (+14 years; range: 10–68 years). There was a female preponderance (female:male = 1.8:1). The mean tumour size was 4.63 cm (+2.5 cm; range: 1–10 cm). Multifocality was present in 36% (18/50), gross extrathyroidal extension in 9.1% (3/33), and regional lymph node metastasis in 38% (19/50). Three (7.8%) of the 38 patients with available follow-up had hematogenous metastasis. The mean follow-up, available in 33 patients, was 10.2 months (+5.1 months; range: 5–28 months). Of these 33 patients, 29 were given RAI. Most (82.8%, 24/29) patients went into remission post-RAI (complete response), and five (17.2%) showed an incomplete response to RAI (RAI-resistant). The four who were not given RAI included two who were surgically ablated and two who had TENIS syndrome. Thus, a total of seven patients were RAI-resistant. The two with TENIS syndrome were treated with radiotherapy and sorafenib, an oral multikinase inhibitor, respectively. While the former patient became disease-free, the latter had persistent but stable disease and was subsequently lost to follow-up.

On histopathological analysis, most (27, 54%) of the cases were classical PTC. Seventeen (34%) had a follicular pattern, of

which seven had an infiltrative morphology, lacking a capsule (infiltrative follicular subtype of PTC; IFVPTC), and ten were encapsulated. Of the latter, five showed capsular and/or vascular invasion and were categorised as invasive encapsulated follicular variants of PTC (EFVPTC); the remaining five lacked true papillae, psammomatous calcification, and evidence of capsular and vascular invasion, leading to their reclassification as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).[2,15,16] The rest of the cases included two tall cell PTC (4%), a case of columnar cell PTC (2%), a case of solid PTC (2%), a case of Warthin-like PTC (2%), and a case of PTC with nodular fasciitis-like stroma (2%).

Mutation analysis for *BRAF*^{V600E}

The prevalence of *BRAF*^{V600E} mutation was 38% (19/50) cases. Thirteen of the 27 classical PTC (48%) [Figure 1a] harboured the mutation. Two of the IFVPTC (2/7), both tall cell PTC (2/2), the Warthin-like PTC (1/1), and PTC with nodular fasciitis-like stroma (1/1), were also *BRAF*-mutated. The alteration was not found in any of the EFVPTC (0/5), NIFTP (0/5), columnar cell PTC (0/1), solid PTC (0/1) cases, in the follicular neoplasms, or the HTN.

Immunohistochemistry for *BRAF*^{V600E}

Of the 50 PTC cases, 48 showed variable immunopositivity with antibodies for BRAF^{V600E} protein. While one case was scored as 3, 23 cases as 6, 11 as 7, and 13 as 8. None had a score of 0, 1, 2, 4, or 5. On comparison with the mutation status, none of the cases with a score less than 7 were mutated. Of the 11 cases scored 7, 54.5% (6/11) were mutated. Significantly, all 13 cases scored as 8 were *BRAF*-mutated [Figure 1b]. Of the NIFTP cases, three had a score of 6 and two had a score of 7. While most (80%; 12/15) of the cases of FC, FA, and HTN were negative (score 0), three (20%) were weakly but diffusely immunopositive, yielding a score of 6 [Figure 1b inset].

On ROC analysis, an IHC score of 7 had a sensitivity of 100% (95% CI: 79%–100%), a specificity of 83.87% (95% CI: 66%–94%), a positive predictive value of 79.2% (95% CI: 57%–92%), and a negative predictive value of 100% (95% CI: 84%–100%) for detecting *BRAF*^{V600E} mutation (AUC: 0.974) [Figure 1c].

Immunohistochemistry for pMEK1/2 and pERK1/2

IHC for p-MEK1/2 and p-ERK1/2 was successful in 49 and 50 PTCs, respectively. The staining was patchy and of varying intensity [Figure 1d and e]. While 43 of the 49 cases (87.8%) were immunopositive for pMEK1/2, 20 of the 50 (40%) were scored I and above for pERK1/2. These immunohistochemical results did not correlate with the BRAF mutation status (P = 0.38 and 0.25, respectively). All five NIFTP cases were pMEK1/2-positive, and two for pERK1/2.

Correlation of clinicopathological parameters with *BRAF*^{V600E} mutation

Five of the seven RAI-resistant cases, including both with TENIS syndrome, were mutated for *BRAF*^{V600E}. Thus, *BRAF*^{V600E}-mutated cases were more likely to be non-responsive to RAI. This was statistically significant (P = 0.036). The *BRAF* mutation status, however, did not correlate significantly with

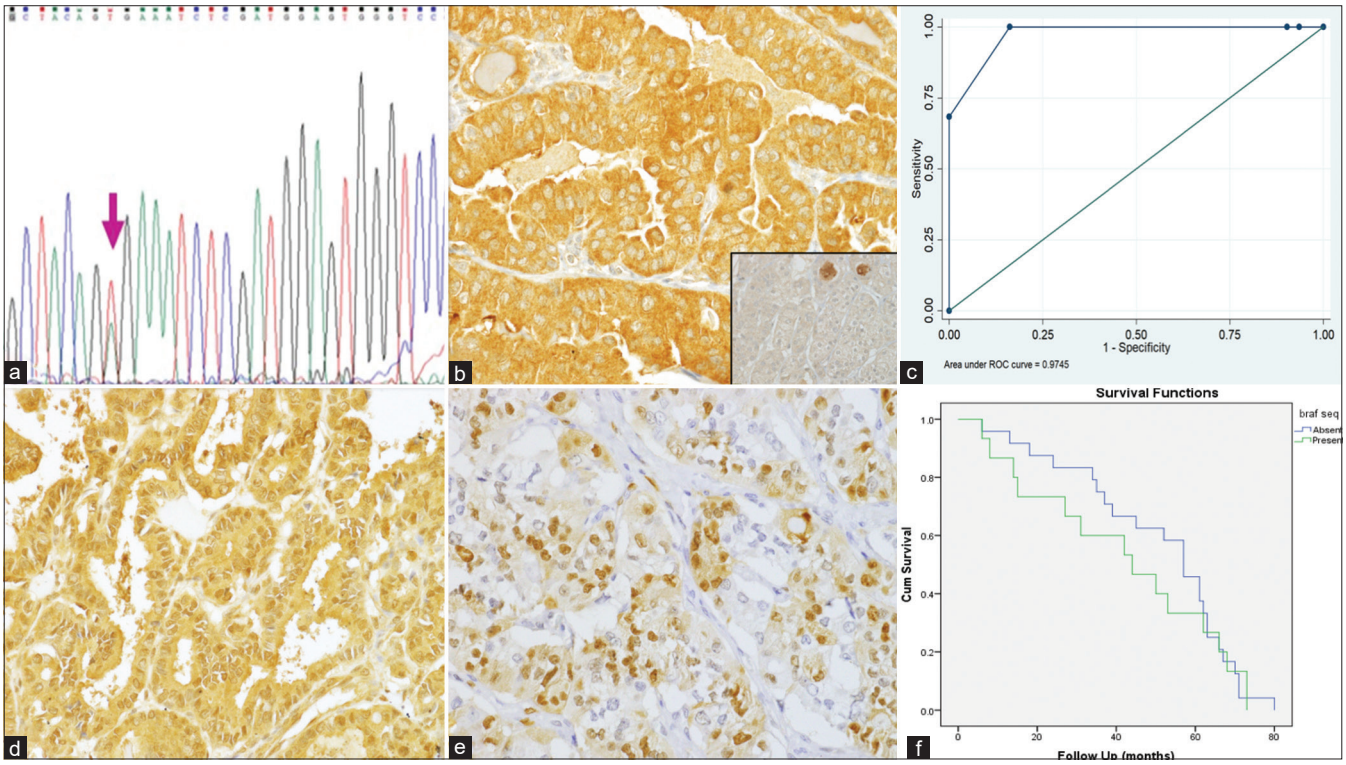

Figure 1: (a) Chromatogram: *BRAF*^{V600E}-mutated PTC. (b) PTC scored 8 for VE1; FA (inset) scored 6 (400×). (c) ROC curve: BRAF^{V600E} IHC. (d) PTC: pMEK1/2 IHC score III (400×). (e) NIFTP: patchy pERK1/2 immunopositivity (400×). (f) DFS curves: *BRAF*^{V600E}-mutated and *BRAF*^{V600E}-wt cases

any other clinicopathological variable, including the DFS [Figure 1f].

Discussion

BRAF mutation involving thymidine to adenine transversion at nucleotide 1799 (T1799A), leading to a change of amino acid Valine to Glutamate in codon 600 (V600E), is the most frequent genetic alteration in PTC. The frequency of this mutation ranges 30%–60% as per Western data and 25%–53% in Indian studies.[17-19] A recent systematic review documented an overall prevalence of this mutation to be 46% in India, with higher rates reported from East Asia.[20] The wide range can be ascribed to different molecular techniques used and the spectrum of the histological subtypes of PTC assessed. In the current study, using Sanger sequencing, the prevalence of *BRAF*^{V600E} mutation was 36.5%, concordant with the published literature. After excluding the NIFTP and EFVPTC cases that, as per the recent WHO definition,[2] lack this molecular alteration, the prevalence of *BRAF*^{V600E} among the PTC cases increased to 47.5% (19/40).

The tumours were also evaluated immunohistochemically for BRAF^{V600E} protein expression. The anti-human monoclonal antibody VE1 detects the BRAF^{V600E}-mutated amino acids sequence from amino acids 596 to 606 (GLATEKSRWSG). This antibody differentiates the BRAF^{V600E}-mutated protein from wild-type BRAF protein and *BRAF*-mutated proteins at codons other than 600.[21,22] Na *et al*. found IHC using the VE1 antibody to have an overall sensitivity and specificity of 100 and 94%, respectively. They scored the staining intensity from 0 to 3, using the follicular colloid as a comparator [Figure 1b inset].[23] Kim recommended an evaluation system for BRAF IHC, similar to the evaluation systems for HER2 status in breast and gastric cancer. They recommended a semi-quantitative scoring system, whereby the scores of the proportion of positive cells (scored 0–5) and staining intensity (scored 0–3) were added. Using their scoring system, they found the cut-off score of BRAF IHC for predicting the mutation to be 5.5. They, hence, suggested that using their scoring system, scores <4 or >6 do not require confirmation by additional tests, unlike the scores of 4 or 5 that require molecular testing.[12] Two Indian studies have evaluated the role of BRAF^{V600E} immunostaining in PTC. Fonseca used the VE1 clone on Ventana and reported 44.44% positivity in their study. A molecularly confirmed case of PTC was used as the positive control. However, no grading or scoring of immunopositivity or correlation with molecular evaluation of the cases was done.[24] Krishnamurthy *et al*.[25] used the RM8 clone (Rabbit monoclonal, Biorbyt Inc) and compared it with qPCR and reported a positivity rate of 26.5%. They graded the intensity of immunopositivity (1 + to 3+). The cases with staining of 2+ and 3+ were considered positive. In the current study, a cut-off Allred score of 7 had a high sensitivity (100%) and specificity (83.87%). All cases scored 8 harboured the mutation as confirmed by Sanger sequencing. Although the antibody clone used by Kim *et al*.[12] and by us is the same, the difference in the results may be due to the different dilutions (1:300 vs 1:50) and the autostainers used. While a LEICA BOND-III Autostainer (Leica Biosystems, Newcastle Upon Tyne, UK) was used by Kim *et al*.,[12] we used Ventana automated immunohistochemical stainer (Ventana Medical Systems, Tucson, AZ). As the method and the antibody used for IHC have a bearing on the interpretation of results, there is a need to standardise the IHC protocol in each laboratory. This is especially important as the results not only aid in diagnosis but also act as an indicator for BRAF^{V600E}-targeted therapy. Our study is limited by the relatively small number of cases. Moreover, Sanger sequencing has relatively low sensitivity, and there is a need to re-analyse the negative cases with more sensitive techniques such as allele-specific PCR. However, unlike the previous studies, we used non-PTC samples for validating BRAF^{V600E} IHC. The weak positivity in some of the latter cases leading to a score of 6 supports the cut-off obtained in the current study. Moreover, for scoring the cases, we preferred using the Allred score as it is one of the most widely used IHC scoring methods. Our results reinforce the need for determining a cut-off score by correlation with molecular studies before using the technique for patient care.

As MEK1-targeted therapy has been approved for treating RAI-resistant anaplastic thyroid carcinoma cases,[26] we further assessed our patient cohort for MEK1/2 immunoexpression. The majority (88%) were positive, but the results did not correlate with the *BRAF* mutation profile. This can be explained as MEK1/2 is the downstream molecule activated by other signals too, including the *RAS* mutations, commonly found in PTC. Moreover, activating mutations of the *MEK1* gene also lead to overexpression of the protein.[26] An important limitation of our study is that molecular alterations other than *BRAF*^{V600E} were not evaluated. However, MEK1/2-immunopositivity in most of our cases suggests the amenability of the tumours to MEK1-targeted therapy, irrespective of the *BRAF* status. The immunohistochemical expression status of pERK1/2 also did not correlate with the *BRAF*-mutation status. Similar results were reported by Mitsiades *et al*., who immunohistochemically evaluated expression levels of phosphorylated and total forms of MEK and ERK. The authors did not find differences in these levels between wild-type and *BRAF*^{V600E}-mutated patient specimens or cell lines.[27] However, some other authors have shown stronger activation of the MAPK pathway in PTCs with *BRAF*^{V600E} mutation.[28]

The prognostic significance of *BRAF* mutation is contentious.[5,17,21,22,29,30] While some reports have demonstrated it to be associated with poor outcomes,[5,17,21,22,29] others have found confounding results.[30] There are no valid explanations for these conflicting observations other than possible heterogeneity of the sample regarding tumour variants as well as the methodology for assessing molecular alterations. In the present study, *BRAF*^{V600E} mutation correlated significantly with RAI resistance but not with the other clinicopathological variables.

Conclusion

BRAF^{V600E} mutation predicts a higher risk of radioactive iodine resistance in papillary thyroid carcinoma (PTC).

Immunohistochemistry provides a reliable technique for detecting *BRAF*^{V600E} mutation. Most tumours express an activated form of MEK1/2, implying the feasibility of MEK1-targeted therapy in recalcitrant PTC cases even in the absence of *BRAF* mutation.

Acknowledgement

None.

Authors' contribution

Conceptualization: S.A., N.K.; Data collection and experimentation: N.K., S.A., P.C., M.C.S., S.B., C.S.B., S.C.; Manuscript preparation (First draft): N.K., P.C.; Manuscript editing and review: N.K., P.C., M.C.S., S.B., C.S.B, S.C., S.A.

Financial support and sponsorship

Indian Council of Medical Research (ICMR), Project ID: 2015-2664; AIIMS, New Delhi Project ID: A320.

Conflicts of interest

There are no conflicts of interest.

Data availability

The authors confirm that the data supporting the findings of this study are available within this article. Raw data are available from the corresponding author, upon reasonable request.

References

1. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today. [Last accessed on May 06].
2. Baloch ZW, Asa SL, Barletta JA, Ghossein RA, Juhlin CC, Jung CK, *et al*. Overview of the 2022 WHO classification of thyroid neoplasms. Endocr Pathol 2022;33:27-63.
3. The Cancer Genome Atlas Research Network 2014 Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-90.
4. Lee S, Rauch J, Kolch W. Targeting MAPK signaling in cancer: Mechanisms of drug resistance and sensitivity. Int J Mol Sci 2020;21:1102.
5. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, *et al*. The association of the BRAF (V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: A meta-analysis. Cancer 2012;118:1764-73.
6. Li C, Lee KC, Schneider EB, Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: A meta-analysis. J Clin Endocrinol Metab 2012;97:4559-70.
7. Dadu R, Shah K, Busaidy NL, Waguespack SG, Habra MA, Ying AK, *et al*. Efficacy and tolerability of vemurafenib in patients with BRAF (V600E)-positive papillary thyroid cancer: MD Anderson cancer center off label experience. J Clin Endocr Metab 2015;100:E77–81.
8. Kim KB, Cabanillas ME, Lazar AJ, Williams MD, Sanders DL, Ilagan JL, *et al*. Clinical responses to vemurafenib in patients with metastatic papillary thyroid cancer harboring BRAF (V600E) mutation. Thyroid 2013;23:1277–83.
9. Karapanou O, Simeakis G, Vlassopoulou B, Alevizaki M, Saltiki K. Advanced RAI-refractory thyroid cancer: An update on treatment perspectives. Endocr Relat Cancer 2022;29:R57-66.
10. U.S. Food and Drug Administration. FDA News Release. FDA approves new uses for two drugs administered together for the treatment of BRAF-positive anaplastic thyroid cancer. Available from: https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm606686.htm.
11. Chakraborty D, Shakya S, Ballal S, Agarwal S, Bal C. BRAF V600E and TERT promoter mutations in paediatric and young adult papillary thyroid cancer and clinicopathological correlation. J Pediatr Endocrinol Metab 2020;26:1465-74.
12. Kim YH, Choi SE, Yoon SO, Hong SW. A testing algorithm for detection of the B-type Raf kinase V600E mutation in papillary thyroid carcinoma. Hum Pathol 2014;45:1483-8.
13. Nakayama N, Nakayama K, Yeasmin S, Ishibashi M, Katagiri A, Iida K, *et al*. KRAS or BRAF mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancer. Br J Cancer 2008;99:2020-8.
14. Ventura AP, Radhakrishnan S, Green A, Rajaram SK, Allen AN, O'Briant, *et al*. Activation of the MEK-S6 pathway in high-grade ovarian cancers. Appl Immunohistochem Mol Morphol 2010;18:499-508.
15. Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LD, *et al*. Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: A paradigm shift to reduce overtreatment of indolent tumors. JAMA Oncol 2016;2:1023-9.
16. Alves VAF, Kakudo K, LiVolsi V, Lloyd RV, Nikiforov YE, Nosé V, *et al*. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): Achieving better agreement by refining diagnostic criteria. Clinics (Sao Paulo) 2018;21:73:e576.
17. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG. BRAFV600E mutation in papillary thyroid carcinoma: Significant association with node metastases and extra thyroidal invasion. Endocr Pathol 2012;23:83-93.
18. Khan MS, Pandith AA, Azad N, Hussain MU, Masoodi SR, Wani KA, *et al*. Impact of molecular alterations of BRAF in the pathogenesis of thyroid cancer. Mutagenesis 2014;29:131-7.
19. Nair CG, Babu M, Biswas L, Jacob P, Menon R, Revathy AK, *et al*. Lack of Association of B-type Raf Kinase V600E mutation with high-risk tumor features and adverse outcome in conventional and follicular variants of papillary thyroid carcinoma. Indian J Endocrinol Metab 2017;21:329-33.
20. Rashid FA, Munkhdelger J, Fukuoka J, Bychkov A. Prevalence of BRAFV600E mutation in Asian series of papillary thyroid carcinoma-A contemporary systematic review. Gland Surg 2020;9:1878-900.
21. Choden S, Keelawat S, Jung CK, Bychkov A. VE1 Immunohistochemistry improves the limit of genotyping for Detecting BRAFV600E mutation in papillary thyroid cancer. Cancers 2020;12:596.
22. Choden S, Keelawat S, Jung CK, Bychkov A. An affordable immunohistochemical approach to estimate the prevalence of BRAFV600E in large cohort studies—establishing the baseline rate of BRAF mutation in an institutional series of papillary thyroid carcinoma from Thailand. Gland Surg 2020;9:1867-1.
23. Na JI, Kim JH, Kim HJ, Kim HK, Moon KS, Lee JS, *et al*. VE1 immunohistochemical detection of the BRAF V600E mutation in thyroid carcinoma: A review of its usefulness and limitations. Virchows Arch 2015;467:155-68.
24. Fonseca D, Murthy SS, Tagore R, Rao V, Rao CS, N Raju KV, *et al*. BRAF status in the variants of papillary thyroid carcinoma. Int J Head Neck Pathol 2018;1:41-7.
25. Krishnamurthy A, Ramshankar V, Murhekar K, Shyamsundar V, Desai PB, Parikh P. Clinical utility of immunohistochemistry using the novel antiBRAF V600E antibody (clone RM8) for detection of the BRAF V600E mutant protein in papillary thyroid cancers. Int J Mol ImmunoOncol 2018;3:28-33.
26. Cabanillas ME, Ryder M, Jimenez C. Targeted therapy for advanced thyroid cancer: Kinase inhibitors and beyond. Endocr Rev 2019;40:1573-604.
27. Mitsiades CS, Negri J, McMullan C, McMillin DW, Sozopoulos E, Fanourakis G, *et al*. Targeting BRAFV600E in thyroid carcinoma: Therapeutic implications. Mol Cancer Ther 2007;6:1070-8.
28. Buffet C, Hecale-Perlemoine K, Bricaire L, Dumont F, Baudry C, Tissier F, *et al*. DUSP5 and DUSP6, two ERK specific phosphatases, are markers of a higher MAPK signaling activation in BRAF mutated thyroid cancers. PLoS One 2017;12:e0184861.
29. Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: A systematic review and meta-analysis. Medicine (Baltimore) 2012;91:274-86.
30. Gouveia C, Can NT, Bostrom A, Grenert JP, van Zante A, Orloff LA. Lack of association of BRAF mutation with negative prognostic indicators in papillary thyroid carcinoma: The University of California, San Francisco, experience. JAMA Otolaryngol Head Neck Surg 2013;139:1164-70.