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# A pilot study on the prevalence of BRAF V600E mutation in papillary thyroid carcinoma in the Pakistani population

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## ABSTRACT

**Background:** Papillary thyroid carcinoma (PTC) is the most common type of malignancy of the endocrine system. B-Type Raf Kinase (BRAF) V600E is the prevalent mutation in PTC and plays an important role in the diagnosis, prognosis, and management of thyroid cancer. Despite global studies, the prevalence of the BRAF V600E mutation in the Pakistani population is still unexplored. This study aimed to determine the frequency of the BRAF V600E mutation in PTC patients of Pakistani origin.

**Method:** DNA was extracted from a total of 26 Formalin-fixed paraffin-embedded tissue specimens of PTC and was amplified by PCR. The point mutation BRAFV600E was detected in exon 15 of the BRAF gene by Sanger sequencing.

**Result:** A total of 10 out of 26 of the classical PTC patients were positive for the BRAF V600E mutation, which is in alignment with the global prevalence of the BRAF V600E mutation.

**Conclusion:** In conclusion, this pilot study gives initial insight into the prevalence of the BRAF V600E mutation in PTC patients of Pakistani origin. This study will contribute to improving diagnosis, management, and risk stratification in PTC. However, a large-scale study is needed to verify the findings of this study.

**Keywords:** Papillary thyroid cancer, BRAFV600E, mutation, prevalence, Pakistani

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## Introduction

Thyroid cancer is the 7th most common endocrine neoplasm with the 24th highest death rate globally [1]. In Pakistan, it ranks 16th most common cancer and 23rd in mortality rate [2]. It is 3 times more prevalent in women than in men [3]. Tumors derived from thyroid epithelial cells display diverse neoplastic phenotypes [4]. Among the histological types of thyroid cancer, papillary thyroid carcinoma (PTC) is the most common, accounting for approximately 79% of cases. In addition, 13% are follicular carcinomas, 5% are Hürthle-cell carcinomas, 4% are medullary thyroid carcinomas, and 2% anaplastic thyroid carcinomas [5]. PTC is characterized by an overall good prognosis and low mortality, while a ~30% risk of recurrence and metastasis was seen [6].

B-Type Raf Kinase (BRAF) mutation is a common genetic mutation in PTC and a potent activator of mitogen-activated protein kinase (MAPK). The MAPK signaling pathway regulates cell growth, proliferation, and division. BRAF mutations result in constitutive activation of the MAPK signaling pathway, leading to uncontrolled

growth and proliferation. Among the BRAF gene mutations, the c.T1779A point mutation in exon 15 of the BRAF gene results in Valine to Glutamic acid substitution at codon position 600, which represents 90% of the gene mutations [7].

BRAF V600E mutation holds significant clinical implications as it often co-occurs with pTERT mutations, linked to adverse outcomes including higher recurrence rates, reduced Radioactive Iodine avidity, and increased mortality. The presence of both mutations needs more effective treatment and management strategies, including decisions on the extent of surgery and long-term surveillance. Furthermore, BRAF mutations serve as critical markers for identifying RAI-refractory disease and selecting targeted therapies, such as kinase inhibitors, to improve progression-free survival rates in aggressive cases [8].

As the BRAF V600E mutation is highly prevalent among the PTC, the molecular testing of the BRAF V600E mutation can augment the fine needle aspiration cytology (FNAC), particularly in indeterminate cytology cases. The BRAF V600E testing can help to avoid

unnecessary surgeries by reducing diagnostic uncertainty and effectively managing thyroid cancer.

The prevalence of the BRAF V600E mutation in PTC shows significant regional variation, ranging from 25% to 84%, with higher rates in the Asian region. In East Asia, a high mutation rate of 84.9% in China [9], 83.1% in Japan [10], 76.8% in Taiwan [11], and 75.5% in Korea [12] was reported. In the Middle East, Saudi Arabia reflects a 46.3% incidence rate, comparable to findings from Western populations, where prevalence generally ranges from 45% to 60% [7,11]. South Asia shows comparatively lower rates, with India reporting 51% [13]. Despite its significance, the prevalence of BRAF V600E mutations in PTC remains unreported in the Pakistani population. This knowledge gap is particularly critical given Pakistan's unique genetic landscape and environmental exposures.

So, this study aimed to investigate and report the presence of the BRAF V600E mutation in PTC within the Pakistani population, which will enhance the diagnostic yield and improve the treatment of thyroid cancer.

## Methodology

### Study population

The study was conducted on 26 PTC-diagnosed patients from 2023 to 2025. Formalin-fixed paraffin-embedded (FFPE) tissue samples were processed at Cancer Genomic Laboratory, PINUM Cancer Hospital, for the detection of BRAF V600E mutation. The BRAF testing was conducted before radioactive therapy to refine risk stratification regarding disease recurrence. FFPE blocks were evaluated by the Department of Pathology, Faisalabad Medical University, to estimate the tumor content and mark the tumor area on the slides.

### DNA extraction

Genomic DNA was extracted from FFPE tissue samples using the QIAamp DNA FFPE Tissue Kit. Marked tissue sections were trimmed, homogenized in xylene using a tissue lyser (Qiagen/TissueLyser LT), and washed twice with 1 ml of absolute ethanol to completely remove residual xylene, followed by centrifugation at 15,000 rpm for 2 minutes. The tissue pellet was air-dried at room temperature and resuspended in 180 µl of ATL buffer and 20 µl of proteinase K, followed by incubation at 56°C on Dry Bath (WiseTherm/HB-48) overnight. 200 µl of each AL buffer and absolute ethanol were added, and the lysate was transferred to the QIAamp MinElute column. The column was washed with 500 µl of each AW1 buffer and AW2 buffer. The DNA was eluted in 25 µl of ATE buffer. The extracted DNA was stored at -20°C for further analysis.

### PCR amplification

Exon 15 of the BRAF gene was amplified using primers (FW: TCATAATGCTTGCTCTGATAGGA, Rev: GGCCAAAATTTAATCAGTGGA). 20 µl PCR

reaction mixture contained: 10 µl 2× PCR Master Mix (abm), 1 µl of 2.5 pM of each primer, and 8 µl of DNA. Then, PCR was performed using SuperCycler Thermal Cycler (Kyratec/SC300G) under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, and final extension at 72°C for 5 minutes. The presence of the PCR product was confirmed by agarose gel electrophoresis.

### Capillary electrophoresis of BRAF EXON 15

PCR product was purified using ExoSAP-IT™ Express PCR Product Cleanup (Applied Biosystems) to inactivate primers and dNTPs as per standard protocol. The purified amplicons were processed for sequencing using BigDye™ Terminator v3.1 Cycle Sequencing kit. The reaction mixture contained 0.5 µl BigDye Terminator v.3.1 reaction mix, 1.75 µl sequencing buffer, 1 µl of each sequencing primer, 2 µl purified PCR product, and 4.75 µl nuclease-free water. The sequencing reaction was run at 96°C for 1 minute, followed by 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. Sequencing reaction was purified using the ethanol precipitation method and processed on the SeqStudio™ Genetic Analyzer (Applied Biosystems/A33770). Sequencing data were analyzed with Variant Reporter 3.0 to identify the BRAF V600E mutation.

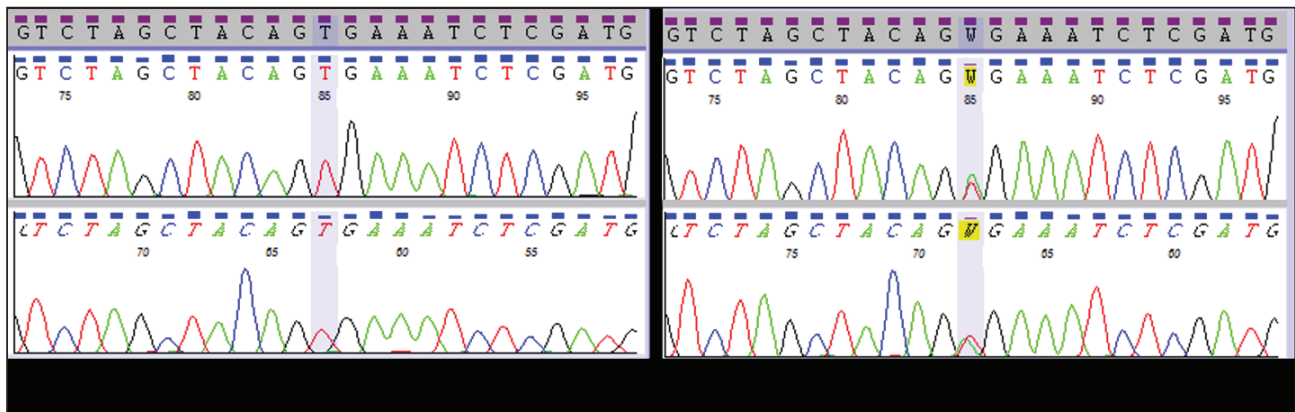
## Results

A total of 26 PTC-diagnosed patients (N = 26) included in this prospective study were of the classical type. The mean age of the patients with PTC was 38 ± 12 years. Of the 26 PTC patients, 35% (9/26) were male, and 65% (17/26) were female. BRAF V600E missense mutations were detected in 10 patients (38%). The electropherogram of the mutated and wild-type BRAF substitution at position c.1779T>A is shown in Figure 1. The mean age of patients with the BRAF V600E mutation was 40 ± 13 years, compared to 37 ± 12 years in those with the BRAF wild type. The results of the study are summarized in Table 1, given below.

## Discussion

Thyroid cancer is one of the most common endocrine tumors with an increasing incidence year by year. Among the four primary histological types, PTC is the most common thyroid malignancy worldwide, with increasing incidence over recent decades. Missense mutations in codon 600 of exon 15 (V600E) account for most of the activating mutations in the BRAF gene [14]. Although PTC is a low-malignant thyroid carcinoma with a good prognosis, the specific genetic variation significantly impacts its behavior and therapeutic outcomes [12].

In the present study, the mean age of PTC patients was 38 ± 12 years. The mean age of BRAF V600E



**Figure 1.** Sanger sequencing detection (a) BRAF V600E wild. (b) BRAF V600E mutated.

**Table 1.** Comparison of histological characteristics of V600E-mutated and wild-type PTC patients.

Clinical and histological factors		V600E mutation detection		Percentage of BRAFV600E mutated patients (%)
		Mutated (n = 10)	Wild (n = 16)	
Cancer type	Classical PTC	10	16	38%
Gender	Male	4	5	40%
	Female	6	11	60%
Mean Age (years)	-	40 ± 13	37 ± 12	-

mutation-positive patients (40 ± 13 years) was slightly higher than that of BRAF wild-type patients (37 ± 12 years). A retrospective study conducted on 80 PTC patients reported a mean age of 57.2 ± 16.8 years for PTC patients, 58.5 ± 15.8 years for BRAF V600E mutation-positive patients, and 51.8 ± 20.0 years for BRAF wild-type patients [15]. The mean age of PTC patients in the present study was relatively lower than previous study. In addition, Patients with a BRAF V600E mutation are older than those with a wild-type, consistent with a previous study. The differences in age distribution may reflect the population-specific factors or study design; however, further investigation is needed to better understand the clinical significance of age in relation to BRAF mutation status in PTC.

The current study identified 38% of PTC patients positive for the BRAF V600E mutation in Pakistan. A study conducted on the Han Chinese population identified 86% BRAF V600E mutation [9]. A contemporary review on the prevalence of BRAF V600E mutation identified 45.6% in Indian, 46.3% in Saudi Arabia, 75.5% in South Korea, and 70.6% in Japan [12]. The significant discrepancies observed among studies may be attributed to differences in sample sizes, tissue sources, sequencing methodologies, and geographic factors, including underlying genetic and environmental variation.

This study is the first to report the frequency of BRAF V600E mutation in PTC within the Pakistani population, highlighting the regional genetic landscape. This information provides a foundation for future research and may

inform clinical management strategies in this population. The 38% BRAF V600E prevalence in the Pakistani population highlights its significance regarding the diagnosis of PTC in thyroid cancer patients. Molecular testing of BRAF V600E can augment FNAC, which may alleviate financial and mental stress related to two-stage surgery. The integration of BRAF V600E testing followed by FNAC can be an effective approach to diagnose PTC in indeterminate cases.

In conclusion, our findings underscore the importance of integrating BRAF mutation testing into routine diagnostics to enhance personalized management of PTC in Pakistan. The study could be expanded with larger cohorts and clinical correlations to further elucidate the genetic and prognostic landscape of thyroid cancer in Pakistan.

**List of Abbreviations**

- BRAF B-Type Raf Kinase
- FFPE Formalin fixed paraffin embedded
- FNAC Fine needle aspiration cytology
- MAPK Mitogen-activated protein kinase
- PTC Papillary thyroid carcinoma

**Conflict of interest**

The authors declare no conflict of interest.

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