Short Term Outcome of Radioactive Iodine Ablation Therapy in Patients of Papillary Thyroid Carcinoma with B-RAF Mutation

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ABSTRACT

most prevalent malignancy among all forms of thyroid cancers is papillary thyroid carcinoma (PTC). This is one of the few cancers that is rapidly increasing. The prognosis is excellent with an overall 10-year survival rate of 90%. However, few patients show poor prognosis and disease recurrence. Recently genetic mutations have been considered to be contributing factors to the clinical and behavioral metastatic risk factors. The most common genetic mutation involved in PTC is BRAF(V600E) mutation. The objective of this study was to see outcome of PTC patients associated with BRAF mutation after radioactive iodine ablation (RAIA).

Methodology: This prospective cohort study was carried out at the National Institute of Nuclear Medicine & Allied Sciences (NINMAS). A total of 63 patients with PTC after thyroidectomy were referred to NINMAS for RAIA were included in this study. All the necessary information was collected. Clinical staging of thyroid cancer was classified according to the Tumor-node-metastasis (TNM) classification of AJCC 8th edition; 2018. All of them were tested for BRAF(V600E) mutation. BRAF mutation-negative groups were selected to match the patients with BRAF positive mutation. Patients underwent a visit three months interval after RAIA evaluating thyroglobulin (Tg) levels, anti-thyroglobulin antibody (Anti Tg Ab) levels, neck ultrasound (US), whole-body 131I scan. Patients were given to repeated radioiodine with a higher dose than the previous dose based on disease stage, recurrence, and metastasis. Tg level <2 ng/dl was considered disease-free (DF) and Tg > 2 ng/dl was considered persistence of disease (PD) based on Tg. Progression of disease was considered in case of rising Tg, local recurrence, positive post-therapy scan (RxWBS) or diagnostic whole-body scan (DxWBS). The outcome based on metastasis, recurrence, or progression of the disease of these patients was observed.

Result: A total of 63 patients, male 25 (39.7%) and female 38(60.3%), were included in this study. Among them, 23 (36.51%) were BRAF (V600E) positive, and 40 (63.49%) were negative. After one year follow up 23(36.51%) were disease free; 4(17.39%) were BRAF positive, and 19(82.61%) were BRAF negative; 38(60.32%) showed persistent disease, 17(44.74%) were BRAF positive and 21(55.26%) were negative; 2(3.17%) BRAF positive patients showed disease progression and; p-value was 0.016 which was statistically significant.

Conclusion: In this study, patients with positive BRAF mutation showed aggressive presentation and poorer outcome. BRAF mutation negative patients showed higher rate of disease-free condition. BRAF analysis in PTC patients provides important prognostic value. These patients might be benefited by receiving more intensive management and frequent follow up.

Keywords: Papillary thyroid carcinoma, BRAF (V600E) mutation, short term outcome.

Bangladesh J. Nucl. Med. Vol. 27 No. 2 July 2024
DOI: https://doi.org/10.3329/bjnm.v27i2.79189

INTRODUCTION

The most prevalent malignancy among all forms of thyroid cancers is papillary thyroid carcinoma (PTC). Among women, it is the most rapidly increasing and the second most common cancer in men. There are risk factors for PTC, like radiation exposure, positive family history, previous history of carcinoma, solitary thyroid nodule in men, rapidly growing nodule, etc. However, the exact cause is still unknown. It was evaluated that carcinogenesis and tumor development consist of multistep accumulations of adverse genetic and epigenetic events (1). Genetic alterations involved in thyroid carcinogenesis are BRAF (b-type Rapidly Accelerated Fibrosarcoma) mutation, RAS (Rat Sarcoma Virus) mutation, and RET (Rearranged During Transformation) rearrangements.

BRAF is the most frequent mutation in PTC, and the transverse point mutation at codon 600 (BRAF V600E) is the most common type of BRAF mutation (2). Though the prognosis is excellent, few patients show poor prognosis and disease recurrence. The prognosis of PTC depends on the

patient's age, gender, tumor size, histological findings, extrathyroidal extension, clinical lymph node metastasis, and remote metastasis. A recent study shows that genetic mutations have contributed to the clinical and behavioral metastatic risk factors (3). The most common genetic mutation involved in PTC is BRAF, and sub-type V600E constitutes 99% of all BRAF mutations (4). BRAF analysis in PTC patients may provide significant prognostic value, and these patients might benefit by receiving more intensive management and frequent follow-up. In this background, the objective of this study was to see the association between BRAF mutation in PTC patients and their outcome after radioactive iodine ablation (RAIA).

BRAF is part of a signaling pathway known as the RAS/MAPK (Mitogen Activated Protein Kinase) pathway. BRAF is a serine-threonine kinase. It has been associated with the reduced uptake of ^{131}I, partly due to the protein kinase signaling pathway activated by MAPK (5). The binding of RAS to the cell membrane prompts the activation of BRAF (2). These kinases are intracellular effectors of the MAPK signaling cascade and relay the signals downstream to regulate the expression of several genes. These genes are responsible for cell proliferation, differentiation, apoptosis, and resistance to pro-apoptotic movements (6). Oncogenic activation of MAPK occurs when it is triggered by the binding of growth factor (GF) to a receptor tyrosine kinase (RTK). This activates the RAS, BRAF, MEK, and ERK phosphorylation cascade, which causes cellular proliferation and differentiation (7).

BRAF mutation has a direct relationship with both the dedifferentiation of thyroid tumors and resistance to 131I therapy. This resistance is mediated fundamentally by the BRAF (V600E) mutation. This mutation stimulates the induction of tumor growth factor (TGF-B) secretion, which represses the expression of the sodium/iodide symporter (NIS) that is necessary for the uptake of iodine in the cell in addition to promoting changes required for migration and cellular invasion (8). This loss of expression is the basis of refractoriness to treatment with ^{131}I. Consequently, mutated PTC shows a decrease in avidity for ^{131}I (9). So, it would be wise to think that more aggressive surgery would be the best therapeutic option (10). Almost 90% of the patients may be cancer-free with an aggressive approach to managing the disease (11). MAPK signaling pathways' enzymes, specifically BRAF and RAS genes, have contributed to metastatic risk factors (12). However, in their latest update in 2015, the American Thyroid Association (ATA), while recognizing its role as a marker for the risk of recurrence, still does not recommend the routine study of the mutational state of the tumor.

Several studies expressed a strong association between BRAF mutations and poor clinicopathological outcomes of patients with PTCs (13). This association had not been observed in Bangladeshi PTC patients before. In this background, this study was performed to see the initial result of ^{131}I ablation in PTC patients associated with BRAF mutation.

PATIENTS AND METHODS

Informed written consent was taken from all the participants. A total of 63 patients with thyroidectomy for PTC who came to NINMAS for RAIA were included in this study. All the necessary information was collected. Clinical staging of thyroid cancer was classified according to the Tumor-node-metastasis (TNM) classification of AJCC 8th edition; 2016.

All 63 patients were tested for BRAFV600E mutation. BRAF negative groups were selected to match the patients having the mutation. Patients underwent a visit three months after RAIA. Thyroglobulin (Tg) levels, anti-thyroglobulin antibody (Anti Tg Ab) levels, neck ultrasound (US) were evaluated in subsequent visits. After one-year whole-body ^{131}I scan was done according to protocol of NINAS. Patients were given repeated radioiodine with a higher dose than before based on disease stage, recurrence, and metastasis. Tg level <2 ng/dl was considered disease-free (DF), and Tg > 2 ng/dl was considered persistence of disease (PD) based on Tg. Progression of disease was considered in case of rising Tg, local recurrence, positive post-therapy scan (RxWBS), or diagnostic whole-body scan (DxWBS). We observed the outcome as DF, PD, progression of the disease of these patients.

RESULT

This study included a total of 63 patients, of whom 25 (39.7%) were male, and 38 (60.3%) were female. BRAF (V600E) mutation was evaluated by DNA sequencing of the PCR-amplified exon 15. Overall, the percentage of BRAF(V600E) in PTC was 23 out of 63 (36.51%).

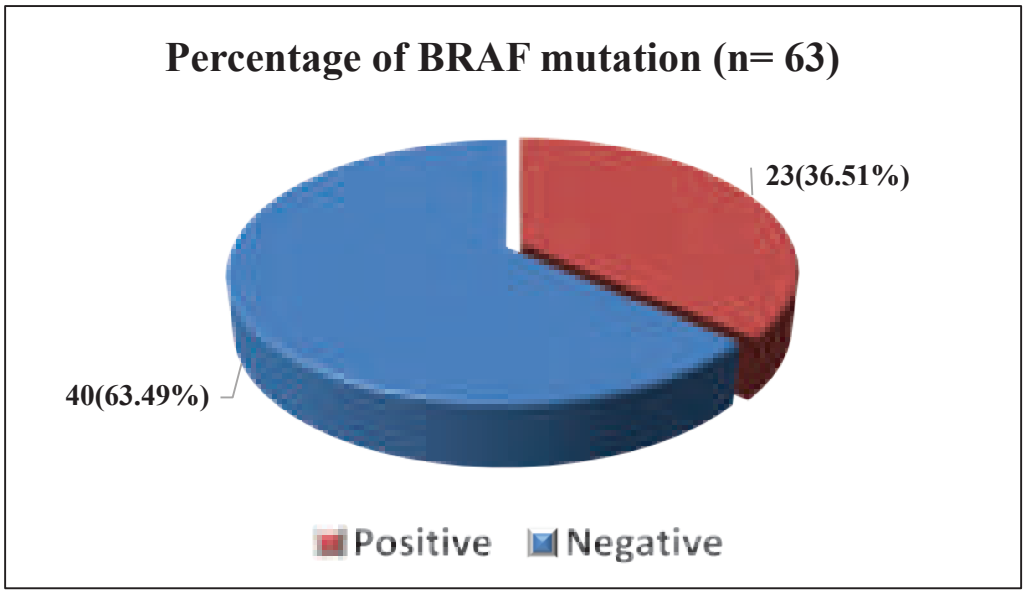

Figure1: Pie-chart showing the percentage of BRAF(V600E) mutation.

Distribution of study population by gender (n=63):

This study included a total of 63 patients, of whom 25 (39.7%) were male, and 38 (60.3%) were female.

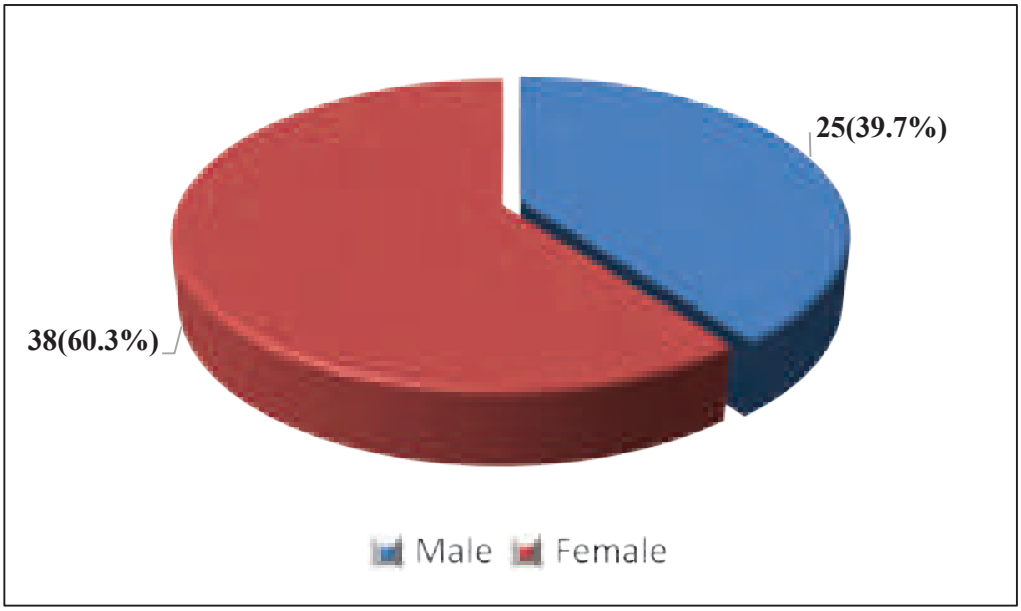

Figure-2: Pie-chart showing the gender distribution of the study patients

Distribution of study population by Tg status:

One year follow up of the study population with Tg showed that persistence of disease (PD) is strongly associated with BRAF (V600E) mutation (p-value 0.008, 0.002, and 0.001 in first, second and third follow up respectively-(Table-1)

Table 1: Study population according to thyroglobulin (n=63)

Outcome	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
1st follow up			
Disease free (Tg<2ng/dl)	7(30.4%)	26(65.0%)	0.008
Persistent of disease (Tg>2ng/dl)	16(69.6%)	14(35.0%)	
2nd follow up			
Disease free (Tg<2ng/dl)	6(26.1%)	27(67.5%)	0.002
Persistent of disease (Tg>2ng/dl)	17(73.9%)	13(32.5%)	
3rd follow up			
Disease free (Tg<2ng/dl)	7(30.4%)	30(75.0%)	
Persistent of disease (Tg>2ng/dl)	14(60.9%)	10(25.0%)	
Progression of disease (rising Tg)	02(8.7%)	00(0.0%)	0.001

Figures in the parentheses indicate the corresponding **percentage;**
Chi-squared Test (χ^2) was done to analyze the data**.**
p-value ≤ 0.05 considered as significant.

Outcome according to risk stratification:

PTC patients are categorized into low, intermediate, and high-risk group according to risk stratification. In this study among 63 patients no one was in high-risk group. Positive BRAF mutation patients of intermediate-risk group showed poorer outcome compared to mutation negative group. Patients of low risk group with BRAF mutation positivity showed statistically significant poorer outcome.

Table 2: Outcome according to risk stratification

Risk group		BRAF mutation Positive (n=14).	Negative (n=27)	p-value
Low	Persistent	11(78.6%)	12(44.4%)	
	Progression of disease	0(0.00%)	0(0.00%)	0.036
	Disease free	3(21.4%)	14(55.6%)	
Total		14(100.0%)	27(100.0%)	
Intermediate	Persistent	6(66.67%)	8(61.5%)	
	Progression of disease	2(22.23%)	0(0.00%)	0.157
	Disease free	1(11.1%)	5(38.5%)	
Total		9(100.0%)	13(100.0%)	

Figures in the parentheses indicate corresponding **percentage;**
Chi-squared Test (χ^2) was done to analyze the data**.**
p value ≤ 0.05 was considered as significant

The study outcome:

DxWBS was performed in all 63 patients and two patients with positive BRAF mutation showed positive DxWBS (one showed metastasis to lung and another metastasis to lateral compartment lymph node). These two patients showed progression of disease. No metastasis was seen in rest of 61 patients. Among 23 BRAF mutation positive patients four were disease free; 17 showed persistent disease and two patients showed progression of disease. In case of 40 BRAF negative patients; 19 were disease free; 21 showed persistent disease and no one showed progression of disease. These results denote that BRAF(V600E) mutation in PTC patients getting RAIA showed a worse prognosis. On the other hand, BRAF negative patients in our study had a better outcome.

Table-3: Outcome of PTC patients (N=63)

Outcome	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
Persistent	17(73.9%)	21(52.5%)	
Progression	2(8.7%)	0(0.0%)	0.016*
Disease free	4(17.4%)	19(47.5%)	
Total	23(100.0%).	40(100.0%)	

Figures in the parentheses indicate the corresponding **percentage.**
Chi-squared Test (χ^2) was done to analyze the data**.**
p-value ≤ 0.05 considered as significant

DISCUSSION

Thyroid cancer is three times more common in women than men, according to European statistics ("Thyroid Cancer." MedicineNet.com). In this study, the ratio of male to female is 1:1.5, which does not correspond to the mentioned finding. In NINMAS, total thyroid carcinoma patients who got RAIA were 585 (in the year when the study was conducted); 499 were female, and 86 were male, and the ratio is 1:5.8. The discrepancy of the balance in this study was because male patients were more sincere than female patients in this study. BRAFV600E mutation in thyroid carcinomas was reported by Kimura et al. (14). Additional research has been done to understand the tumorigenic role and clinical importance after that. The reported incidence of BRAF V600E mutation has ranged from 36% to 83% (15).

Some researchers reported an incidence of 45% BRAF (V600E) mutation (16). Recent studies have reported the differences in the incidence of the BRAFV600E mutation according to the region; for example, in comparison to other countries (range 36% to 65%), the incidence of the BRAFV600E mutation was higher in Korea, ranging from 52% to 87% (17). A higher prevalence in Asian countries, including Japan and Korea, compared with western countries, is reported by other researchers (18). The reason for this dissimilarity in frequency remains unclear but may be due to geographic factors.

This study evaluated BRAF (V600E) mutation in 63 papillary carcinomas (PTCs) by direct DNA sequencing of the PCR-amplified exon 15. Overall, the percentage of BRAF (V600E) in PTC was 23 out of 63 (36.51%) (Pie diagram-1). The high percentage of BRAF (V600E) mutations in this study corresponds to the reported incidence rate.

Correlation between BRAF (V600E) mutation and clinical outcome:

After radioiodine ablation, patients were followed up for one year at an interval of 3 months. They were considered as free of disease (DF) when patients had no complaints, Tg level was <2 ng/dl with no abnormal findings at neck USG or any distant metastasis at DxWBS. Biochemically persistent

disease (PD) was defined when Tg levels were > 2 ng/mL or anti-Tg were measurable (with undetectable basal Tg levels). Pathological LN detection at neck USG with histological confirmation was considered as morphological PD.

At first follow-up, PD was confirmed in 30 patients; 16 BRAF positive and 14 BRAF negative. DF was established in 33 patients: 7 BRAF positive and 26 BRAF negative (Table 1).

At the second follow-up, PD was confirmed in 30 patients: 17 BRAF positive and 13 BRAF negative. Thirty-three patients were DF; 6 BRAF positive and 27 BRAF negatives. 04 patients showed morphological PD; 3 LN metastases, 01 lung metastases. Two patients with unexplained high Tg (>300 ng/dl) and 04 morphological PD patients were given second therapy. Among the 30 patients showing PD at the first follow-up, 28 showed PD, while 2 were DF, mainly due to the normalization of previously elevated Tg levels. At the third follow-up, PD was confirmed in 26 patients: 16 BRAF positive and 10 BRAF negative. Thirty-seven were FD; 07 were BRAF positive and 30 were BRAF negatives.

The outcome of these 63 patients (based on Tg, anti-Tg Ab, DxWBS, and USG of the neck): PD was 17 (73.9%) with BRAF positive while 21 (52.5%) with BRAF negative; 2 (8.7%) patients with BRAF positive showed progression of disease (rising Tg, metastatic lymph node, positive DxWBS) while no BRAF negative patients showed disease progression; 04 (17.4%) with BRAF positive showed disease-free, and 19 (47.5%) with BRAF negative showed disease-free condition (p-value 0.016; table-3).

Recently, BRAFV600E has taken the focus of the stage due to its possibility of being associated with tumorigenesis and aggressiveness. To explore the clinicopathologic characteristics and the potential utility of BRAFV600E mutation on the diagnostic, prognostic, and therapeutic aspects of PTC, many studies have been conducted. The association of the BRAFV600E mutation with the worse clinicopathological outcome and BRAF mutation independently predicts recurrence was also stated by some researchers (12). They concluded that the BRAFV600E mutation might be a valuable marker for risk stratification in PTC. It is found in many studies that extrathyroidal extensions, lymph node metastases, and advanced stage are the three most common risk factors that are associated with the BRAFV600E mutation (19, 20). The findings of these authors correspond with this study.

At the beginning of this study, all the study subjects were categorized as low, intermediate, and high-risk groups as per the risk stratification described in the ATA guideline 2015. The low-risk group who are BRAF (V600E) positive showed poor prognosis compared to the BRAF (V600E) negative group (Table 2). Researchers from the Mayo Clinic followed patients for up to 15 years and concluded that low-risk patients had a recurrence rate of 3%-5%. However, they noted that thyroid cancer was being diagnosed much earlier, and with the appropriate surgery, the cure was much more likely, and the survival rate after surgery was very high. This study failed to show the recurrence rate and survival rate due to a brief study period.

CONCLUSION

The outcome of PTC patients depends on many factors including BRAF(V600E) mutation which is the most common genetic alterations in these patients. It is shown in recent studies that BRAFV600E mutation is associated with poor clinicopathological characteristics, including lymph node metastasis, extrathyroidal extension, and advanced stage. There are some studies that have failed to establish an association between the BRAFV600E mutation and clinicopathological features. In this study patients with BRAF mutation showed aggressive presentation and poorer outcomes. Patients of the low and intermediate-risk group with BRAF(V600E) mutation showed poor prognosis. BRAF analysis in PTC patients provides important prognostic value. BRAF(V600E) mutation should be keep in mind during risk stratification. These patients might be benefited by receiving more intensive management and frequently follow up.

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