Analysis of the Association Between Basic and Clinicopathological Characteristics of Papillary Thyroid Carcinoma Patients with BRAF Mutation

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ABSTRACT

Introduction: The prognosis of papillary thyroid carcinoma (PTC) depends on the patient's age, gender, tumor size, histological findings, extrathyroidal extension, lymph node metastasis, and distant metastasis. Genetic mutations, particularly BRAF (V600E), contribute to metastatic risk factors.

Methodology: This prospective cohort study was carried out at the National Institute of Nuclear Medicine and Allied Sciences (NINMAS). A total of 63 patients with PTC after thyroidectomy who were referred to NINMAS for RAIA were included in this study. All the necessary information was collected regarding patients (age, sex) and tumors (size, focality, lymph node involvement, extra-thyroid invasion, distant metastases, staging, and grading). Clinical staging of thyroid cancer was classified according to the tumor-node-metastasis (TNM) classification of the AJCC 8th edition, 2018. All of them were tested for the BRAF (V600E) mutation. Any association between aggressive presentation and BRAF-positive PTC patients was observed.

Results: 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. Extrathyroidal extension, lymphovascular invasion, capsular margin involvement, and larger tumor size were significantly associated with the BRAF mutation. No significant association was found with age, histological type, lymph node involvement, multifocality, tumor staging, or grading.

Conclusion: The study suggests that patients with a positive BRAF mutation may benefit from more intensive management and frequent follow-up for aggressive presentation and larger tumor size.

Key words: Papillary thyroid carcinoma, BRAF (V600E) mutation.

Bangladesh J. Nucl. Med. Vol. 26 No. 2 July 2023
DOI: https://doi.org/10.3329/bjnm.v26i2.71471

INTRODUCTION

Thyroid carcinoma is the most common endocrine-related cancer, and its incidence has continuously increased in the last three decades worldwide (1). In the United States, the incidence increased at an annual rate of 5.4% in men and 6.5% in women from 2006 to 2010 (2). The most prevalent malignancy among all forms of thyroid cancer is papillary thyroid carcinoma (PTC). Different factors are associated with thyroid carcinoma. Genetic alterations involved in thyroid carcinogenesis are BRAF (b-type Rapidly Accelerated Fibrosarcoma) mutations, RAS (Rat Sarcoma Virus) mutations, and RET (Rearranged During Transformation) rearrangements. BRAF is the most frequent mutation in PTC. The transverse point mutation at codon 600 (BRAF V600E) is the most common type of BRAF mutation (3). PTC is treated by surgical excision, radioiodine therapy, and levothyroxine suppression. The prognosis is excellent. The overall 10-year survival rate is 90% (4). However, few patients show poor prognosis and disease recurrence. Recently, genetic mutations have been postulated to contribute to clinical and behavioral metastatic risk factors (5). Several studies have delineated an association between the BRAF mutational status and many PTC clinicopathologic parameters (6). Several studies have expressed a strong association between BRAF mutations and poor clinicopathological outcomes in patients with PTCs (7). This association had not been observed in Bangladeshi PTC patients before. In this background, this study was performed to see the association between the basic and clinicopathological characteristics of PTC patients with BRAF mutations.

PATIENTS AND METHODS

Informed written consent was obtained from all the participants. We selected 63 patients with thyroidectomy for PTC who came to NINMAS for RAIA as the subject of this study. All the necessary information was collected regarding the patient's age, sex, tumor size, multifocality, lymph node involvement, extra-thyroid invasion, distant metastases, and grading. Clinical staging of thyroid cancer was classified according to the tumor-node-metastasis (TNM) classification of the AJCC 8th edition, 2016. All 63 patients with total thyroidectomy for PTC were tested for the BRAFV600E mutation. BRAF negative groups were selected to match the patients having the mutation concerning gender, age, TNM staging, and histological type. Any association between the aggressive presentation of PTC patients and BRAF mutation positivity was observed. Statistical analyses were carried out using the Statistical Package for Social Sciences version 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for continuous variables. Frequencies and percentages indicated the quantitative observations. A chi-square test was used to analyze the categorical variables, shown with cross-tabulation, and the quantitative variables by an unpaired t-test. A P-value of <0.05 was considered statistically significant.

RESULT

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

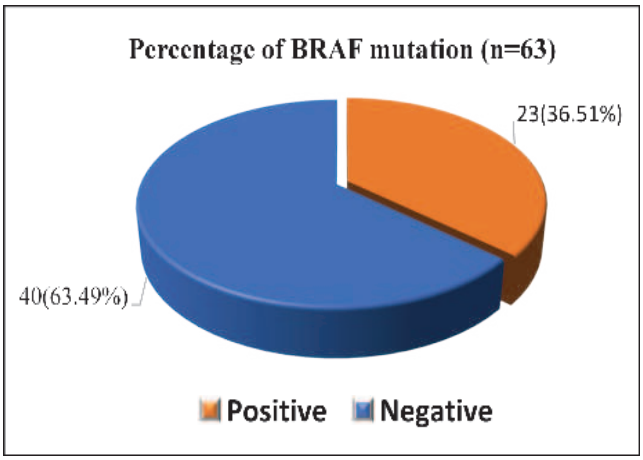

Figure 1: The percentage of BRAF(V600E) mutation. This chart demonstrates that out of 63 patients 23 (36.51 %) is BRAF positive and 40 is BRAF negative (63.49%).

The demographic characteristics of the study show that BRAF mutation is not associated with patients age or gender (p-value 0.456 and 0.547, respectively) (Table 1).

Table-1: Demographic Distribution of the study patients (N=63)

Variables	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
Age group (years)			
18-19	0(0.0%)	4(10.0%)	
20-29	8(34.8%)	13(32.5%)	
30-39	6(26.1%)	12(30.0%)	
40-49	3(13.0%)	6(15.0%)	
50-59	5(21.7%)	5(12.5%)	
60-69	1(4.3%)	0(0.0%)	
Mean±SD	37.9±12.9	33.4±11.5	0.456
Range (min-max)	(20- 63)	(18-58)	
Sex			
Male	8(34.8%)	17(42.5%)	
Female	15(65.2%)	23(57.5%)	0.547
Male : Female ratio	1:1.9	1:1.4	

Data were expressed as frequency, percentage, and mean±SD
Unpaired student t-test and Chi-square test was performed to compare between two groups
p-value ≤ 0.05 considered as a level of significant.

In this study, the relationship between BRAF (V600E) positivity and tumor size and capsular margin involvement was evaluated. Table 2 shows that the BRAF mutation is strongly associated with greater tumor size and capsular margin in PTC patients. The relationship between BRAF (V600E) positivity and extrathyroidal extension and lymphovascular invasion was analyzed by univariate analysis. Among the 63 patients, five showed extrathyroidal extension (four with BRAF mutations and one without BRAF mutations), and there was no extrathyroidal extension in the rest of the 58 patients. Lymphovascular invasion was seen in 16 patients (10 with BRAF mutation and six without BRAF mutation) among 63 patients, and the rest of the 47 patients showed no lymphovascular invasion. Table 2 shows that the BRAF mutation is strongly associated with extrathyroidal invasion (p-value 0.053) and lymphovascular involvement (p-value 0.012). No association was observed with stages and grades of the tumor with BRAF mutation (Table-3).

Table-2: Relationship of tumor size, capsular margin involvement, extrathyroidal extension, lymphovascular invasion with BRAF mutation (N=63)

Tumor size (cm)	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
<2 cm	3(13.0%)	7(17.5%)	
2-4 cm	17(73.9%)	26(65.0%)	
>4 cm	3(13.0%)	7(17.5%)	
Total	23(100.0%)	40(100.0%)	
Mean±SD	3.43±1.18	2.64±0.89	0.003
Capsular margin			
Involved	8(34.8%)	4(10.0%)	0.016*
Uninvolved	15(65.2%)	36(90.0%)	
Total	23(100.0%)	40(100.0%)	
Extra thyroidal extension	4(17.4%)	1(2.5%)	0.035*
Lymphvascular invasion	10(43.5%)	6(15.0%)	0.012*

Data were expressed as frequency, percentage, and mean±SD

Unpaired student t-test and Chi-square test was performed to compare between two groups

p-value ≤ 0.05 considered as a level of significant

Table-3: Relationship of histopathological grade & stage with BRAF mutation (N=63)

Histopathological grade and stage	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
Grade			
Grade 1	22(95.7%)	38(95.0%)	
Grade 2	1(4.3%)	1(2.5%)	0.693
Grade 3	0(0.0%)	1(2.5%)	
Total	23(100.0%)	40(100.0%)	
Staging			
Stage I & II for age <55 years			
Stage I, II, III & IV for age >55 years			
Stage I	14(60.9%)	34(85.0%)	
Stage II	3(13.0%)	2(5.0%)	0.141
Stage III	5(21.7%)	4(10.0%)	
Stage IV	1(4.3%)	0(0.0%)	
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 a level of significant.

No relationship was found with lymph node involvement or focality with BRAF mutation (P-value 0.110 and 0.159 respectively).

Table-4: Relationship of Lymph node involvement and focality with BRAF mutation (N=63)

Lymph node involvement	BRAF mutation		p-value
	Positive (n=23)	Negative (n=40)	
Yes	14(60.9%)	16(40.0%)	0.110
No	9(39.1%)	24(60.0%)	
Total	23(100.0%)	40(100.0%)	
Focality			
Uni focal	14(60.9%)	31(77.5%)	0.159
Multi focal	9(39.1%)	9(22.5%)	
Total	23(100.0%)	40(100.0%)	

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

DISCUSSION

According to European statistics, thyroid cancer is three times more common in women than men (8). In this study, the ratio of males to females is 1:1.5, which does not correspond to the mentioned finding. Kimura et al. (2003) initially reported the BRAFV600E mutation in thyroid carcinomas (9). Kure et al. (2019) reported an incidence of 45% BRAF (V600E) mutation (10). 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% (11). The reason for this dissimilarity in frequency remains unclear but may be due to geographic factors. This study evaluated the 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.

This study first calculated the correlation between the BRAF (V600E) mutation and patient characteristics, including age and sex, by univariate analysis (Table 1). The presence of BRAF (V600E) was not associated with patient age or gender (p-value 0.456 and 0.547, respectively), in agreement with some (12) but not all previous reports (13). We have examined the percentage of BRAF (V600E) in patients with PTC, came to NINMAS for RAIA, and found that BRAF (V600E) is a common genetic alteration in PTC. In the current study, we assessed the association between BRAF (V600E) positivity and histopathological parameters of tumor aggressiveness.

By univariate analysis, the presence of BRAF (V600E) in the 23 PTCs was strongly associated with greater tumor size (p-value 0.0030; Table 2), capsular margin involvement (p-value 0.016), extra-thyroid invasion (p-value 0.035; Table 2), and lymphovascular involvement (p-value 0.012; Table 2). Some studies have shown a positive correlation between BRAF (V600E) and PTC aggressiveness, but not all studies. Different study populations, heterogeneity of PTCs, histological subtypes, and genetic or environmental differences might be the results' contributing factors.

In this study, the relationship between the BRAF (V600E) mutation and focality and lymph node metastasis was evaluated, but no positive correlation was found. Fakhruddin et al. reported that the BRAF (V600E) mutation is conserved in the primary and paired metastatic lymph node deposits in PTC (14). Oler et al. (2005) and Vasko et al. (2005) observed that the BRAFV600E mutation in lymph node metastasis was occasionally not found in their primary lesion, suggesting that tumor cells that acquire the mutation de novo are probably prompted to metastasis (15, 16). Rodolico et al. (2007) further demonstrated that metastatic lymph nodes harboring the BRAFV600E mutation were larger and had a higher prevalence of extracapsular invasion than those without the mutation (17). However, other studies on paired primary and lymph node metastatic lesions did not find a discordant mutation in most lesion pairs, indicating that the acquisition of BRAFV600E mutations is not a requirement in the progression from localized to metastatic PTC (18).

Two meta-analyses also reported an association of the BRAFV600E mutation with extrathyroidal invasion, aggressive histological type, and advanced disease stages, but not with age, sex, or tumor size, and the association of the BRAFV600E mutation with lymph node metastasis is not a consistent finding (19). Clinicopathological characteristics and staging systems are designed to predict tumor recurrence and disease prognosis. A multicenter study of 219 patients, an American study of 245 conventional PTC cases, and an Italian study of 102 patients demonstrated that the BRAFV600E mutation was associated with aggressive clinicopathological features. They have shown that BRAF was also an independent predictor of tumor recurrence after a median of 15 months, six years, and 15 years of follow-up, respectively (20). In this study, the follow-up period was one year.

Kim et al. (2013 stated that lymph node metastasis, TNM stage, and multifocality were not significantly associated with the BRAFV600E mutation (21). These findings can be correlated with the findings of this study. In Japan, a large cohort study of 631 patients with PTC was conducted recently. They followed up on these patients for an average of 83 months; neither clinicopathological characteristics nor tumor recurrence were associated with the BRAFV600E mutation. (22).

CONCLUSION

This study shows that the BRAF mutation has a positive correlation with papillary thyroid carcinoma. Patients with the BRAF mutation showed aggressive presentations such as extrathyroidal extension, lymphovascular invasion, capsular margin involvement, and larger tumor size. The BRAF (V600E) mutation should be kept in mind during risk stratification. These patients might benefit from receiving more intensive management and frequent follow-up.

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