Original Article

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BRAFV600E Mutation in Papillary Carcinoma Thyroid

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ABSTRACT

Background: Thyroid malignancies comprise 1 % of total human malignancies, Papillary Thyroid Carcinoma (PTC) is the most common malignant tumour of thyroid cancers. It is estimated that 80 % of thyroid malignancies are comprised of papillary carcinoma thyroid, which shows increased incidence in women. The most important etiology of PTC is mutation of RET/RAS/BRAF which activates MAPK signaling pathway. The BRAF V600E activating point mutation appears to be highly specific for PTC.

Methods: It is cross sectional study of 56 cases studied in the Government Medical college, Thiruvananthapuram, Kerala. Consecutive cases of histo-pathologically proven cases were selected and Immunohistochemical (IHC) studies for BRAFV600E were conducted, and results were recorded.

Result: Our study showed female preponderance and equal distribution of cases above and below 45 years of age. Size of the primary tumour was more than 1cm for majority of cases. In our study out of 56 cases studied 45 patients showed BRAFV600E expression comprising of 80% positivity. A 44% of the total cases studied showed lymph node metastasis.

Conclusion: We conclude in our study that BRAFV600E mutation is most common mutation in primary PTC patients. BRAFV600E expression most seen in elderly patients and shows strong association with lymph node metastasis. IHC assay is a reliable method to detect BRAFV600E mutation in PTC and can be used as alternative to molecular methods on routine practice

Keywords: *Papillary thyroid carcinoma; BRAFV600E mutation; Tumour aggressiveness; RET/RAS/BRAF mutation; Immunohistochemistry.*

Introduction

Thyroid malignancy is most common of all the endocrine malignancies, and it accounts for 90% of all the endocrine neoplasms. PTC is a not life-threatening malignancy however minority of cases may recur and cause significant morbidity. It could be treated with total thyroidectomy and radioiodine ablation.

Objectives: Primary objective: To know the Proportion of BRAFV600E mutation positivity in Papillary carcinoma thyroid specimens in Department of Pathology, Government Medical college, Trivandrum. Secondary objective: Association between the mutation status of BRAFV600E and the tumour aggressiveness in papillary thyroid carcinoma.

The most important etiology of PTC is imposed on activating mutation of RET/RAS/BRAF/MAPK pathways in papillary carcinoma thyroid [1]. RET and RAF being most important mutation in PTC, and in well differentiated tumors it seen that RET/PTC gene mutation playing major role in causation of tumour. TP53 and PTEN gene mutation has been seen in progressive and poorly differentiated tumour with tumour aggressive behavior. Other risk factors involved in PTC are ionizing radiation, nodular diseases of the thyroid. Risk of relapse or persistent disease is observed in 30 % of patients of PTC.

The T1799A single hotspot mutation leading to V600E amino-acid substitution is the only PTC-specific mutation of the BRAF gene detected in PTC [10-11], with no benign or other well differentiated thyroid neoplasm having been found to harbor this mutation, with a prevalence of 29–83 %.

Specific markers determine the type and aggressiveness behavior of the tumour, Molecular markers are essential part of the tumour diagnosis of any malignancy and helps in targeted therapy. The use of molecular markers for thyroid cancer diagnosis, prognosis, targeted therapy, and surveillance has been exciting area of the study and change. BRAF is a serine–threonine kinase abundantly expressed in thyroid follicular cells [1], which activates Mitogen Extracellular Kinase (MEK1) and MEK2. Thyroid cancer progression and dedifferentiation involves several genetic alterations including two distinct molecular mechanisms: point mutation or chromosomal rearrangement. Studies have suggested that BRAF mutation serve as a novel prognostic biomarker that predicts poor clinicopathological outcomes with lymph node metastasis and large tumour

size helps to identify patients who should undergo more aggressive clinical follow up [2,3]. Light Cycler PCR with allele specific fluorescent probe melting curve analysis (LCPCR) has been used successfully to detect BRAF activating point mutations in PTC.[2]

The most common BRAF mutation (BRAF V600E) accounts for over 90 % of all BRAF mutations and consists of a thymine-to-adenine transversion at position 1799 in exon 15 of BRAF, leading to a valine-to-glutamate transversion at residue 600 and thus facilitating ATP binding. [7] Analysis of BRAF exon 15 in large series of thyroid carcinomas indicated a high frequency of BRAF (V600E) in classical and tall cell variants of PTCs. [8,9] BRAF is translocated to the cell membrane after being bound and activated by RAS, which results in the phosphorylation and activation of mitogen activated protein kinase (MAPK) and other downstream targets of MAPK signaling pathway .The T1799A BRAF mutation causes a V600E amino acid change in the BRAF protein, resulting in oncogenic activation of mutated BRAF kinase.[16,17] B–type raf kinase (BRAF) mutation was recently discovered to be an most common aberrant activation of MAPK pathway in Human cancers.[18,19]

The most common genetic alterations in PTCs include BRAF mutation, RAS mutation, and RET/PTC rearrangement which mainly involve in the RAS/BRAF/MAPK signal pathway which mediates cellular responses and growth signals .BRAF mutation was strongly associated with patient age, with a higher prevalence of BRAF V600E among elderly patients.[20] Mayo clinic study Jin etal[21] also reported significant association of of B-raf mutation with Lymph node metastasis and extra thyroidal invasion. The BRAF mutation occurred most in tall cell variant of PTC and second most common in conventional PTC and least commonly in follicular variant of PTC with average prevalence of 77, 60 and 12 % respectively [22-24].

Molecular testing evaluation of BRAF mutation status is not available at many hospitals, since its time consuming, expensive and requires expertise in molecular techniques. Conversely, IHC allows *in-situ* assessment of expression of mutant protein within the tumour cells. Recently mutation specific antibody clone was developed (Clone VE1)18 which allowed immuno-histochemical visualization of BRAF V600E protein with high sensitivity and specificity. The sensitivity and specificity of IHC staining for BRAF V600E is 98.8 % and 100 % respectively.

The comparative analysis of the IHC approach with molecular biology methods for BRAFV600E detection demonstrated that IHC performed very well and at a level equivalent to the pyrosequencing and SNaPshot methods [25] but was more sensitive than the direct sequencing method. IHC approach can be good alternative to the molecular approach to the BRAF V600E detection.

Tumour spread and metastasis

Distant metastasis is uncommon in PTC. It is generally associated with lymph node metastasis. Studies have revealed that the distance metastasis prevalence is approximately 8% in BRAF mutated cases. BRAF V600E mutation is associated with impaired iodine uptake, conferring the poor prognosis and resistance to radioactive iodine adjuvant therapy[26]. BRAF mutation are associated with extrathyroidal extensions into adjacent neck structures like Trachea requiring more aggressive treatment with more chances of morbidity. Extrathyroidal extension is important factor relating to the PTC prognosis, relating in local recurrence/persistence of the disease.[27]

Prognosis and predictive factor

PTC usually has an excellent prognosis. Survival rate at 5 years is 96 %, 10 years is 93 % and at 20 years is > 90 %. [28] Many clinical and pathological factors are associated with prognosis of thyroid carcinoma. The most frequently used factors are age, sex , lymph node metastasis, size of primary tumor, histopathology subtypes and multifocality. [4,5] Of which, Independent risk factors associated with PTC are: 1). Patient age at diagnosis- the age at the time of diagnosis is known to be of great prognostic significance. Mortality increases with age > 40-45 years. PTC in children and adolescents associated with good outcome. 2). Tumor size- increased risk of death with size > 3-4 cm. 3). Staging- extrathyroidal extension result in adverse prognosis. 4). Distant metastasis- metastasis to lungs have an adverse influence on prognosis.[6]

Materials and Methods

This was a cross-sectional study conducted over one year in the Department of Pathology, Government Medical College, Thiruvananthapuram, Kerala after obtaining clearance from the institutional ethical committee. 56 specimens histopathologically (Figure 1) diagnosed as Papillary carcinoma thyroid in the Department of Pathology, were studied.

Sample size: Sample size is calculated by using formula $n=4pq/d^2$. p is the proportion of BRAFV600E mutation in papillary thyroid carcinoma. P=83% q=100-p=17. Relative precision=10% Confidence interval=95% D is the maximum variability that can be afforded, taken as 10% of p Thus the sample size is 56. This was the minimum number of cases required for the study. All consecutive specimens received during the study period were included in the study.

Study Instruments: 1. Formalin-fixed paraffin-embedded tissue blocks. 2. Hematoxylin and eosin-stained sections. 3. IHC Marker- BRAFV600E. 4. Hospital records.

Methods of Data Collection: Clinical details of the patients were recorded from case sheets. H&E sections were studied for a histological subtype, and stage of the tumour. Formalin-fixed paraffin-embedded blocks were used for IHC staining using primary antibody – BRAFV600E. Interpretation of BRAFV600E were done and result entered as positive or negative. The procedures and evaluation were done by single observer.

Immunohistochemical evaluation: The IHC results were scored based on tumor cells with cytoplasmic staining as follows: 0: no staining or faint staining intensity in 10% or fewer cells. 1+: faint staining in more than 10% of cells; (Figure 4). 2+: moderate staining in more than 10% of cells (Figure 3). 3+: strong staining in more than 10% of cells (Figure 2). The tumor was recorded as positive when the score was 2+ or greater, negative when the score was 0 and 1+.

Data analysis: All collected data was entered in Microsoft excel sheet with all relevant details and analyzed using statistical software SPSS version 23.0. The qualitative variables were expressed in percentage. The association was tested using appropriate statistical test.

Statistical tests: Categorical and quantitative variables were expressed as frequency (percentage) and mean ± SD respectively. Prevalence of BRAFV600E expression in papillary thyroid carcinoma was expressed using 95 % CI. Chi-square test was used to find association of BRAF expression with selected categorical variables. For all statistical interpretations, $p<0.05$ was considered the threshold for statistical significance. Statistical analyses was performed by using a statistical software package SPSS, version 20.0.

Results

Clinical data: A total of 56 cases were studied out of which female patients showed the predominance below and above the age of 45years (Table 1). Majority of tumour were measuring more than 1 cm which accounted for 73% of cases and showed strong association with BRAFV600E mutation

Prevalence: Out of 56 cases studied, 45 cases showed BRAFV600E positivity comprising of 80%(Table 2). Lymph node metastasis was recorded in 25 cases out of 56 cases which accounts for 44.6%.(Table 3)

Discussion

Papillary carcinoma is one of the most common thyroid malignancies comprising more than 80 % of all thyroid cancers. Papillary thyroid carcinomas is well known

Table 1: Distribution of age and gender.

Age	Female		Male		Total	
	Count	Percent	Count	Percent	Count	Percent
<=45 yrs	21	47.7	7	58.3	28	50.0
>45 yrs	23	52.3	5	41.7	28	50.0
Total	**44**	**100.0**	**12**	**100.0**	**56**	**100.0**

Table 2: Percentage distribution of the sample according to BRAF expression.

BRAF expression	Count	Percent	95% CI
Positive	45	80.4	70 – 54.82
Negative	11	19.6	

Table 3: Association between the mutation status of BRAFV600E and Lymph node metastasis.

LN mets	BRAF expression				χ^2	p
	Positive		Negative			
	Count	Percent	Count	Percent		
Present	25	100.0	0	0.0	11.04	p<0.01
Absent	20	64.5	11	35.5		

Table 4: Correlation of BRAF mutation with selected clinico-pathological prognostic factors[14].

Authors	No of BRAF (+) (%)	Age	Sex	Extrathyroidal extension	LN mets
Rosenbaum et al(2005)	(54) 65%	0.0001	-	-	-
Kim et al (2006)	(140) 73%	NS	0.0060	0.06	0.02
Lupi et al(2007)	(217) 44%	NS	NS	0.0001	0.0009
Ito et al(2009)	(242) 38%	0.05	NS	NS	0.0050
Wang et al(2008)	(54) 50%	0.02	NS	0.02	-
Present study	(46) 80%	0.019	0.770	0.06	<0.01

P value significant - <0.05, NS- Non significant

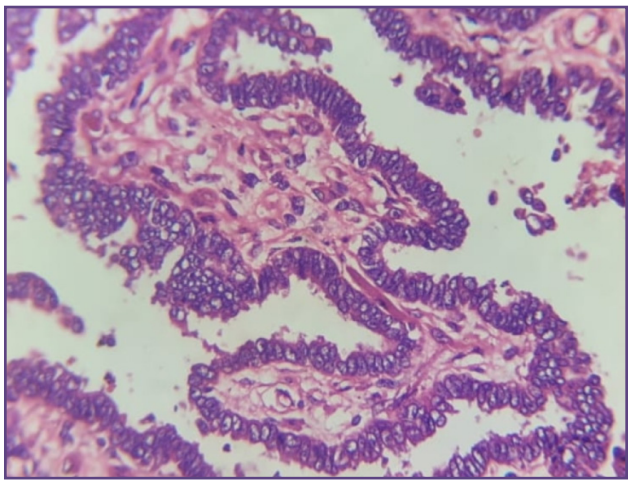

Fig. 1: 40X H and E image showing classical papillary thyroid carcinoma with papillae showing oval elongated, crowded ground glass appearing (orphan Annie) nuclei with true fibrovascular core.

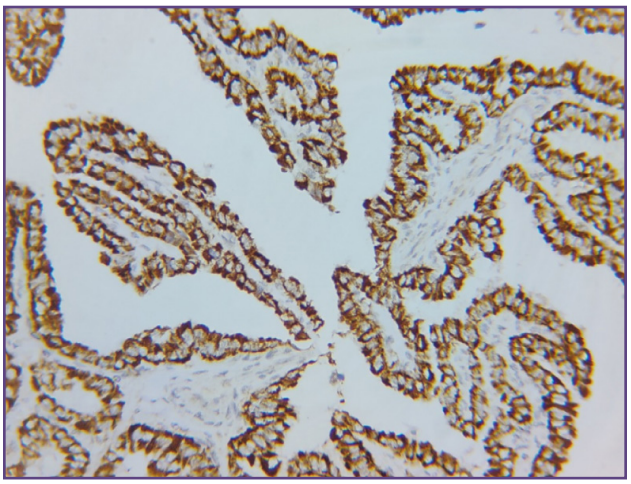

Fig. 2: 40x Immunohistochemical stain of BRAFV600E antibody showing strong cytoplasmic positivity graded as 3+ in the papillary thyroid carcinoma.

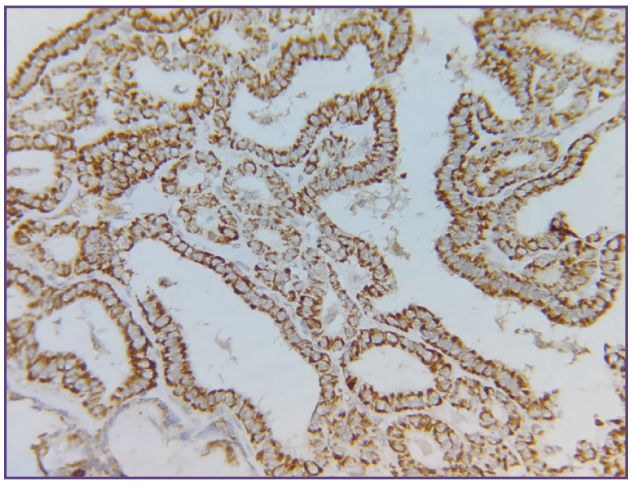

Fig. 3: 40x Immunohistochemical stain of BRAFV600E antibody showing moderate cytoplasmic positivity graded as 2+ in the papillary thyroid carcinoma.

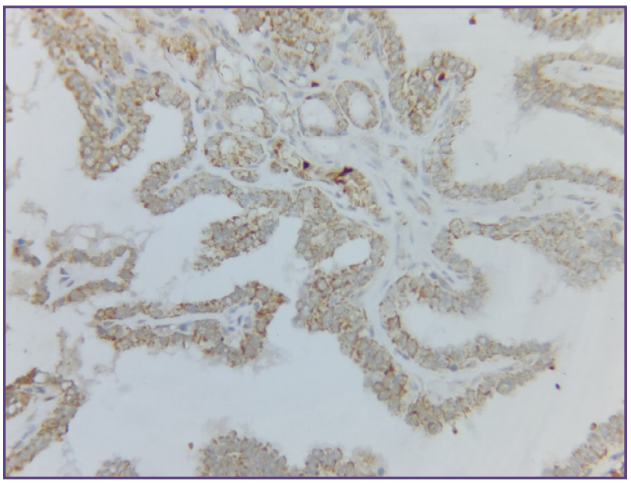

Fig. 4: 40x Immunohistochemical staining of BRAFV600E antibody showing weaker cytoplasmic positivity graded as 1+ in the papillary carcinoma thyroid.

for its excellent prognosis, exceptionally some show aggressive behavior like cervical lymph node metastasis, extra thyroidal extension and increase in the size of the tumour. Ancillary test has become helpful in assessing the aggressive behavior of the tumour and recently the molecular studies have become the essential part of tumour diagnosis and helps in targeted therapy.

Hence in the present study, BRAFV600E mutation is studied to know the association of PTC with increase in tumour size, Lymph node metastasis, Extrathyroidal extension and multifocality. Efforts have been made to study the association of BRAF mutation with age and sex of the patient.

In comparison with the approaches utilized by prior studies [12-13] and our examinations, staining intensities were recorded as weak, moderate, or strong (3+, 2+, 1+ and 0). Faint diffuse staining, any type of isolated nuclear staining, weak staining of single interspersed cells, and staining of monocytes/macrophages were scored as negative. Moderate or strong staining was scored as positive.

No significant relationships were observed between BRAF V600E mutation and either gender (P=0.770) or tumor size (P= 0.119), multifocality (P=0.224) or Extra thyroidal Extension (P=0.06).

The high sensitivity and specificity of the VE1 antibody for the BRAF V600E mutation demonstrated that IHC would be a useful tool for screening for this mutation among PTC patients. A recent meta-analysis [15] reported that the prevalence of the BRAF V600E mutation among PTC patients ranged from 27 % to 90 %, with an average prevalence of 56.3 % and our study showed a prevalence of 80 % among all papillary carcinoma Thyroid.

Several studies have shown that BRAF mutations are associated with patient age and lymph node Metastasis. In accordance with these findings, we found BRAF V600E mutation was strongly associated with patient age, with a higher prevalence of BRAF V600E strong expression among elderly patients and significant number of patients showing lymph node metastasis.

Conclusion

In our study we conclude BRAFV600E mutation is common among primary PTC patients, and it is strongly correlated with elderly age and Lymph node metastasis. BRAF IHC in daily practice can be considered as an alternative or essential tool for the BRAFV600E mutation analysis. It was observed that BRAFV600E mutation was associated with age, tumour stage and prognosis .

The present study was conducted in the Department of Pathology, Government Medical College, and Thiruvananthapuram. Many different methods for BRAF mutation analyses with different sensitivities have been developed. A major drawback of other molecular methods like single-strand conformation polymorphism, gene sequencing and mutation specific PCR is that they are expensive, labor and time-intensive, not always available, and may be difficult to implement in clinical settings, In which our study we included BRAFV600E mutation study by IHC which is Rapid, cost effective and easily applied procedure.

The patients included in the study was grouped into less than or equal to 45 years and more than 45 years, where the study population showed equal proportion of <45 and >45 years of patients. There is a female predominance. Size of the primary tumour was also grouped into less than or equal to 1 cm and more than 1cm, where majority of cases had a size more than 1 cm. A 44 % of the total cases studied showed lymph node metastasis. On evaluating BRAFV600E expression in papillary thyroid carcinoma, 80 % showed a positive expression. The secondary objective was to study the expression of BRAFV600E, and aggressiveness associated with BRAFV600E mutation in papillary carcinoma thyroid. In the present study, we concluded the association between BRAFV600E expression in elderly age and lymph node metastasis

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Ethical Considerations: Institutional ethical committee clearance was obtained. Confidentiality was ensured and maintained throughout the study.

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Competing Interests

Nil

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