ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immunohistochemical Evaluation Of BRAF Expression In Papillary Carcinoma Of Thyroid Along With Its Histopathological Variants.

Shajitha Aafrin M[1], K Valarmathi[2], Nalli R Sumithra[3], and N Kiruthika[4]*.

[1]Department of Pathology, Govt. Stanley Medical college, Chennai-1, Tamil Nadu, India.
[2]Professor, Department of Pathology, Govt. Stanley Medical college, Chennai-1, Tamil Nadu, India.
[3]Professor, Department of Pathology, Govt. Stanley Medical college, Chennai-1, Tamil Nadu, India.
[4]Assistant Professor, Department of Pathology, Govt. Stanley Medical college, Chennai-1, Tamil Nadu, India.

ABSTRACT

The most common type of thyroid carcinoma worldwide is Papillary carcinoma accounting 85%. Most of these tumours are occurring from 20 to 50 years of age with female preponderance. Papillary thyroid carcinoma has 20-year survival rate over 90%. Despite its good prognosis, some subtypes of PTC also have aggressive properties, such as early local invasion, lymph node metastasis. Early detection and appropriate treatment will prolong the survival rate of patients. This study aimed to determine the diagnostic role of BRAF mutation in papillary thyroid carcinoma and variants by evaluating the frequency of expression of BRAF in Papillary thyroid carcinoma and its variants. The study was conducted during the period of May 2019 to April 2022 in 60 Paraffin embedded blocks of patient specimens with confirmed histopathological diagnosis of papillary thyroid carcinoma and its variants. Age, sex, type of thyroidectomy, tumor site, size, histological variant, TNM stage and nodal metastasis were observed for all papillary thyroid carcinoma cases. Immunohistochemical evaluation of BRAF was observed in both classical and variants of papillary thyroid carcinomas and the results were correlated with clinicopathological variables. This study shows a significant association between aggressiveness of the tumor and BRAF expression, between age of presentation, nodal involvement and stage of tumor with BRAF expression. Because of this significant expression in high grade tumors, BRAF can be used as a prognostic marker. This study initiates the development of BRAF targeted therapy for papillary thyroid carcinoma.

Keywords: Papillary thyroid carcinoma, BRAF, prognostic marker, Targeted therapy.

https://doi.org/10.33887/rjpbcs/2023.14.5.12

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Thyroid carcinoma accounts only 2-4% while others are benign [1]. Papillary thyroid carcinoma is the most common type of thyroid carcinomas accounting 85% of the tumors. At least 90% of pediatric thyroid carcinomas are papillary carcinomas. Most of these tumors are occurring from 20 to 50 years of age, with female preponderance [2] . Papillary thyroid carcinoma has twenty-year survival rate over 90%. Despite its good prognosis, some subtypes of PTC also have aggressive properties, such as early local invasion or extensive lymph node metastasis. Early detection and appropriate treatment will prolong the survival rate of patients [3].

BRAF (B-Raf proto-oncogene) encodes a cytoplasmic serine/ threonine kinase with a crucial role in regulating the mitogen-activated protein kinase (MAPK) signal transduction pathway. A missense mutation in codon 600 of Exon 15 (v600E) of BRAF gene has been identified in multiple neoplasms including Melanoma, Colorectal carcinoma, papillary thyroid carcinoma, hairy cell leukemia and Langerhan cell histiocytosis [4]. BRAF targeted therapy is a promising strategy for patients with advanced Papillary Thyroid Carcinoma [5]. In this study we aimed to determine the diagnostic and prognostic role of BRAF mutation in papillary thyroid carcinoma and its histopathological variants. BRAF could be an important tool in taking therapeutic decision in patients with Papillary thyroid carcinoma.

A combination therapy of BRAF inhibitors and MAP/Extracellular signal Related Kinase (ERK) Inhibitors hold great hope in treatment of BRAF mutated malignancy and this has a powerful role in changing the course of Papillary Thyroid Carcinoma and Anaplastic Thyroid Carcinoma [6].

Aim

To evaluate the frequency of expression of BRAF in papillary carcinoma thyroid ,its histopathological variants and its usefulness in prognostication.

MATERIALS AND METHODS

The study was conducted during the period of May 2019 to April 2022. It was carried out in specimens obtained from patients with confirmed histopathological diagnosis of papillary thyroid carcinoma and its histopathological variants. The study was approved by the Ethical committee of Government Stanley Medical College and hospital.

Study Design

Descriptive Cross-sectional study.

Inclusion Criteria

60 cases of papillary thyroid carcinoma and its histopathological variants reported in hemithyroidectomy and total thyroidectomy specimens were included in the study.

Exclusion Criteria

Nodular Colloid Goitre, Adenomatous hyperplasia, Hashimoto's thyroiditis and thyroid carcinoma types other than papillary thyroid carcinoma were all excluded from the current study.

Methodology

With histopathological examination, they were categorized as Papillary thyroid carcinoma and its histopathological variants. Age and sex of the patient, site of Papillary thyroid carcinoma, were all recorded. The variants of Papillary thyroid carcinoma included in the study are Conventional / Classic variant, follicular variant and Diffuse sclerosing. Staging of Papillary thyroid carcinoma was done according to AJCC (8^{th} edition) TNM classification. Presence of nodal metastasis has been taken into account as even a single lymph node with metastasis and absence of nodal metastasis as when the lymph node was free of tumor invasion. The Immunohistochemical expression of BRAF for all the 60 cases were recorded.4 microns thick sections were cut and Slides coated with chrome alum were used. Sections were subjected to antigen

retrieval using pressure cooker technique with TRIS EDTA (pH- 9.2) buffer solution and then treated by HRP (horse radish peroxidase) polymer technique (Path insitu IHC protocol).

IHC Scoring Criteria For BRAF

Immunohistochemically stained sections were examined under a minimum 5 high power (40x) fields. BRAF expression- Cytoplasmic staining of the tumor cells will be considered positive.

The staining intensity was graded on a scale of 0 to 3 were

- 0 - no staining.
- 1+ - weak/ slight staining.
- 2+ - moderate staining.
- 3+ - intense staining.

Quantitative criteria

- 3+->60% of neoplastic cells in cytoplasm / nucleus/ both (cytoplasm and nucleus) /extracellular matrix.
- 2+-30 - 60% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix.
- 1+-<30% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix.
- 0 -No reactivity.

Inferential Statistics

Quantitative outcome: Categorical BRAF expression was compared between each case of papillary thyroid carcinoma using Chi square test. P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis. [Machines IB. IBM SPSS Statistics for windows, Version 22.0. IBM Corp Armonk, NY; 2013].

DISCUSSION

Various researches found a significant correlation between aggressive tumors and BRAF expression. Also, the researches proved its significant variation in expression among the histopathological variants of papillary thyroid carcinoma that correlates with their aggressive properties. As BRAF has a chief role in tumorigenesis of papillary carcinoma thyroid, the therapy targeting BRAF may give a better results in patients.

Papillary Thyroid Carcinoma

The percentage of BRAF expression in papillary thyroid carcinoma cases was 33.33%. 20 out of 60 cases showed BRAF expression in the papillary thyroid carcinoma. Made Priska Rusmana et al in 2018 in his study of BRAF expression in Papillary thyroid carcinoma observed expression in 21 out of 36 (58.3%) cases. Xiaoli Zhu et al reported BRAF expression in 101 out of 150 (75.6%) cases. Our current study observed BRAF expression in 20 out 0f 60 (33.33%) of Papillary thyroid carcinoma cases.

Age distribution of Papillary thyroid carcinoma and their comparison with BRAF expression

Among the Papillary thyroid carcinoma cases, 9 (15%) patients were aged up to 20 years, 13 (21.7%) patients were between the age group between 21 – 30 years, 24 (40%) patients were between 31 – 40 years of age, 5(8.3%) patients were between 41 - 50 years of age and 9 (15%) were aged 51 - 60 years of age.

ISSN: 0975-8585

Table 1: Descriptive Analysis of age group in years in Papillary carcinoma (N=60)

	Frequency	Percent
Upto 20 yrs	9	15.0
21 - 30 yrs	13	21.7
31 - 40 yrs	24	40.0
41 - 50 yrs	5	8.3
51 - 60 yrs	9	15.0
Total	60	100.0
Mean ± SD = 35 ± 12 yrs		

Table 2: Comparison between Age distribution with BRAF Expression

			BRAF Expression		Total	χ2 - value	p-value
			Positive	Negative			
Age distribution	Upto 20 yrs	Count	2	7	9	18.215	0.001 **
		%	10.0%	17.5%	15.0%		
	21 - 30 yrs	Count	3	10	13		
		%	15.0%	25.0%	21.7%		
	31 - 40 yrs	Count	4	20	24		
		%	20.0%	50.0%	40.0%		
	41 - 50 yrs	Count	3	2	5		
		%	15.0%	5.0%	8.3%		
	51 - 60 yrs	Count	8	1	9		
		%	40.0%	2.5%	15.0%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level							

.

The difference in BRAF expression between age groups was statistically significant. (P value 0.001). (Table-2)

Made Priska Rusmana et al in his study observed that patients of age group between 55-65 were suffered more in aggressive papillary thyroid carcinoma with BRAF expression and the current study proves statistical significance having higher expression among the age group of 40 to 60 years.

Gender distribution of Papillary thyroid carcinoma and their comparison with BRAF expression

Table 3: Descriptive analysis of Gender distribution in Papillary thyroid carcinoma

Gender distribution		
	Frequency	Percent
Male	1	1.7
Female	59	98.3
Total	60	100.0

Among the BRAF expressed cases in Papillary thyroid carcinoma, 19(95%) patients were females and remaining 1(5%) was male.

ISSN: 0975-8585

Table 4: Comparison between Age distribution with BRAF Expression

			BRAF Expression		Total	χ2 - value	p-value
			Positive	Negative			
Gender	Male	Count	1	0	1	2.034	0.333 #
		%	5.0%	0.0%	1.7%		
	Female	Count	19	40	59		
		%	95.0%	100.0%	98.3%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
# No Statistical Significance at p > 0.05 level							

The difference in BRAF expression between gender was statistically not significant. (P value 0.333). (Table-4)

Made Priska Rusmana et al in his study observed that there was no statistical significance between BRAF expression and gender in Papillary thyroid carcinoma which was consistent with our study.

Tumor Focality of Papillary thyroid carcinoma and their comparison with BRAF expression

Among the cases in Papillary thyroid carcinoma group, 16 (80%) patients had unifocal tumor, 4 (20%) patients had multifocal tumor.

Table 5: Comparison between Tumor focality with BRAF Expression

			BRAF Expression		Total	χ2 - value	p-value
			Positive	Negative			
Tumor site	Unifocal	Count	16	40	56	8.571	0.009 **
		%	80.0%	100.0%	93.3%		
	Multifocal	Count	4	0	4		
		%	20.0%	0.0%	6.7%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level							

The difference in BRAF expression among unifocal and multifocal tumors of papillary thyroid carcinoma was statistically significant. (P value 0.009). (Table-5)

Wei X Y et al in his study found that there was statistical significance between BRAF expression and focality of tumor in Papillary thyroid carcinoma which was consistent with our study.

Size of tumor and comparison with BRAF expression

Among the cases in Papillary thyroid carcinoma expressed BRAF, no(0%) patients belong to T1a (0.1-1.0 cm) or T1b (1.01 -2.0cm) category, 8 (40%) patients belong to T2 (2.01-4.0 cm) category and12 (55%) patients belong to T3a (> 4.0 cm) category.

Table 6: Comparison between T Stage with BRAF Expression

ISSN: 0975-8585

			BRAF Expression		Total	$\chi 2$ - value	p-value
			Positive	Negative			
T Stage	T1a	Count	0	9	9	37.714	0.0005 **
		%	0.0%	22.5%	15.0%		
	T1b	Count	0	18	18		
		%	0.0%	45.0%	30.0%		
	T2	Count	8	13	21		
		%	40.0%	32.5%	35.0%		
	T3	Count	9	0	9		
		%	45.0%	0.0%	15.0%		
	T3a	Count	3	0	3		
		%	15.0%	0.0%	5.0%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level							

The difference in BRAF expression across tumor size of papillary thyroid carcinoma was statistically significant. (P value 0.0005). (Table-6)

Koperek Osker et al in his study observed that there were statistically significant differences in BRAF expression among groups T1, T2, and T3, which indicates that BRAF is associated with the size of intrathyroidal PTC.

Wei X, Y et al observed in his study that there was significant difference in BRAF expression among tumor size of papillary thyroid carcinoma which was consistent with our study.

Histologic variant of Papillary carcinoma thyroid and comparison with BRAF expression

Among the cases in Papillary thyroid carcinoma expressed BRAF, 18(90%) cases belong to classic variant, 1 (5%) case belong to follicular variant and the remaining 1 (5%) case belong to diffuse sclerosing variant.

Table 7: Comparison between T Stage with BRAF Expression

			BRAF Expression		Total	$\chi 2$ - value	p-value
			Positive	Negative			
Variant	Classic	Count	18	37	55	2.134	0.344 #
		%	90.0%	92.5%	91.7%		
	Follicular	Count	1	3	4		
		%	5.0%	7.5%	6.7%		
	Diffuse sclerosing	Count	1	0	1		
		%	5.0%	0.0%	1.7%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
# No Statistical Significance at p > 0.05 level							

The difference in BRAF expression across histologic variant of papillary thyroid carcinoma was statistically not significant. (P value 0.344). (Table-7)

Made Priska Rusmana et al in his study reported that there was statistical significance between BRAF expression and variants of Papillary thyroid carcinoma which was more expressed in tall cell variant, columnar and diffuse sclerosing variant. This was statistically not significant because of the minimal representation of variants.

Nodal metastasis and comparison with BRAF expression

Among the cases in Papillary thyroid carcinoma expressed BRAF, nodal metastasis was present in 11 (55%) patients and absent in 1 case (2.5%).

ISSN: 0975-8585

Table 8: Comparison between N Stage with BRAF Expression

			BRAF Expression		Total	χ2 - value	p-value
			Positive	Negative			
N Stage	N0	Count	9	39	48	23.494	0.0005 **
		%	45.0%	97.5%	80.0%		
	N1	Count	4	1	5		
		%	20.0%	2.5%	8.3%		
	N1a	Count	7	0	7		
		%	35.0%	0.0%	11.7%		
Total		Count	20	40	60		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level							

The difference in BRAF expression in cases with and without nodal metastasis was statistically significant. (P value 0.005) (Table-8) implying that higher BRAF expression was seen in cases with nodal metastasis.

Chenlei Shi et al reported that primary PTC tumors involving Lymph node Metastasis contained significantly higher concentrations of BRAF expression than tumors without metastases. Kam-Tsin Tang et al in his study observed that BRAF expression in PTC with Lymph node Metastasis was significantly higher than that in the cases without Lymph Node Metastasis.

TNM Stage and comparison with BRAF expression

Among the cases in Papillary thyroid carcinoma expressed BRAF, 16 (80%) patients belong to Stage I and 4 (20%) patients belong to Stage II.

Table 9: Comparison of BRAF expression with TNM Stage in Papillary thyroid carcinoma in the study group (N=60)

TNM Stage		BRAF Expression		Total	Pearson Chi-Square Test	P value
		Positive	Negative			
Stage I	Count	16	38	54	3.3	0.89
	%	80%	95%	90%		
Stage II	Count	4	2	6		
	%	20%	5%	10%		
Total	Count	20	40	60		
	%	100.0%	100.0%	100.0%		

The difference in BRAF expression between the TNM Stage was not statistically significant. (P value 0.89) (Table-9)

Chenlei Shi et al in his study observed that there was no significant difference in BRAF expression among the TNM stage of papillary thyroid carcinoma which was consistent with our study.

Figure 1: Gross picture of Papillary thyroid carcinoma

ISSN: 0975-8585

Figure 2: Histopathology of Papillary thyroid carcinoma (10x)

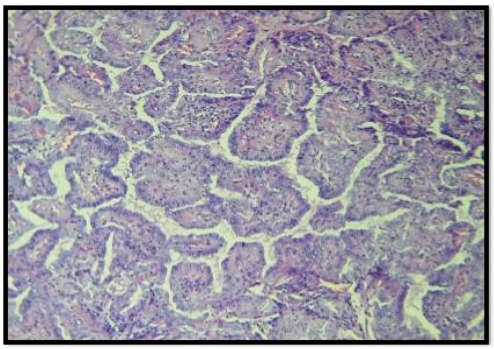


Figure 3: Histopathology of Papillary thyroid carcinoma (40x)

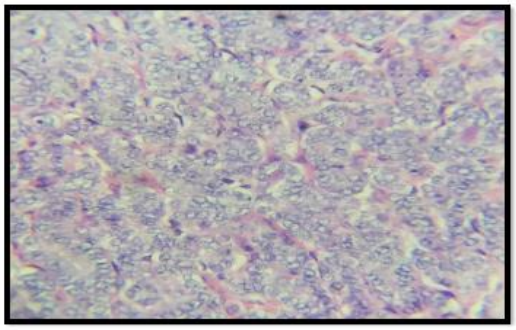


Figure 4: BRAF expression in Papillary thyroid carcinoma-classical-staining intensity -2,staining score-3 (10x)

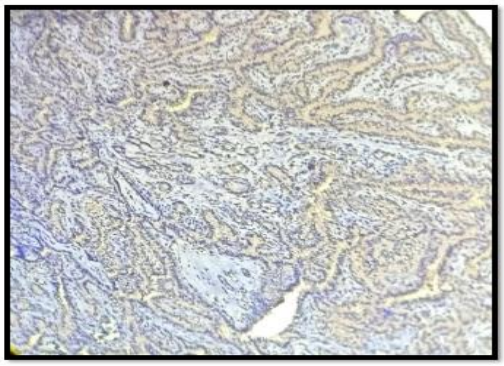
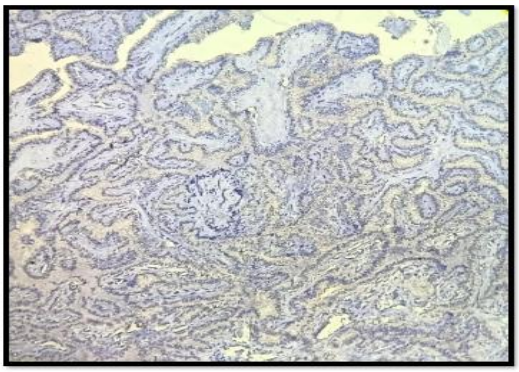


Figure 5: BRAF expression in Papillary thyroid carcinoma-classical-staining intensity -1,staining score-3 (10x)



CONCLUSION

The expression of BRAF in papillary thyroid carcinoma and its variants was correlated with various clinico-pathological variables.

The following are the conclusions of the present study

- There was a significant difference in the expression of BRAF in papillary thyroid carcinoma based on its clinico-pathological features. The stainingintensity was also higher in papillary thyroid carcinoma that has aggressive features in comparison with other papillary carcinoma with less.
- Significant increase in BRAF expression was observed in cases of papillary thyroid carcinoma with lymph node metastasis, showed significant correlation with age and the expression was found to be more among 21 to 40 years of age.
- Among the variants of papillary thyroid carcinoma, though BRAF showed diffuse and moderate positivity in classic variant compared to follicular variant, it was not statistically significant. Only one case of diffuse sclerosing variant had been included in our study and BRAF expression was negative in that case. This points to the limitation of the study and more cases of other variants should be included for statistical significance.

REFERENCES

[1] RV Lloyd, D Buehler, E Khanafshar et al. Papillary thyroid carcinoma. Head And Neck Pathology 2011; 5(1):51-56.
[2] Leslie J Degroot, Edwin L Kaplan, et al. Natural history, treatment, and course of papillary thyroid carcinoma. The journal of Clinical Endocrinology & Metabolism 1990;71(2):414-424.
[3] Virginia A LiVolsi. Papillary thyroid carcinoma: an update. Modern Pathology 2011;24(2):S1-S9.
[4] Y Cohen, M Xing, E Mambo, et al. BRAF mutation in papillary thyroid carcinoma. Journal of the National Cancer Institute 2003;95(8):625-627.
[5] O Koperek, Christoph Kornauth, et al. Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. The American Journal Of Surgical Pathology 2012;36(6):844-850.
[6] Singer G, Oldt R 3rd, Cohen Y, Wang BG, Sidransky D, Kurman RJ, et al. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst 2003;95: 484–6.