




Prognostic Significance of Various Clinicopathologic Parameters and BRAF V600E Mutation in Papillary Thyroid Microcarcinoma—An Observational Study

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Ind J Med Paediatr Oncol 2023;44:345–352.

Abstract

Introduction A specific subset of papillary microcarcinoma of thyroid (PMC) can metastasize regionally and to distant organs, and thus, have a significant effect on the overall survival of the patient cohort.

Objectives We aim to analyze the prognostic significance of various clinicopathologic parameters including BRAF^{V600E} mutation by immunohistochemistry in PMC, in order to identify the subset of cases with aggressive behavior.

Materials and Methods Data regarding the PMC cases was retrieved retrospectively from medical records. The clinicopathologic factors like age, tumor size, focality, capsular invasion, histologic subtype, lymphovascular invasion, perithyroidal fat invasion (PTFI), lymph node (LN) metastasis, and distant metastasis were studied in depth. Tissue microarray was constructed to perform immunohistochemistry with CK19 and BRAF^{V600E}. Information regarding overall survival (OS) and development of metastasis, if any, was noted. Chi-squared test was performed to know the association between various factors. To determine odds ratio, logistic regression was done. Survival analysis was done using Kaplan–Meier and Cox-regression analysis.

Results PMC was diagnosed in 48 patients (M:F = 1:2.4), between 22 and 70 years of age (median = 46.5 years). Chi-squared test showed significant association of fibrosis with tumor size more than or equal to 0.5 cm, infiltrative borders, PTFI, and LN metastasis. Tumor size was also associated with infiltrative borders; and LN metastasis with PTFI. BRAF^{V600E} positivity showed significant association with histologic pattern, PTFI and distant metastasis. On logistic regression, tumor size showed significantly increased odds ratio with presence of fibrosis and infiltrative borders. Presence of fibrosis also showed significant association with infiltrative borders and LN metastasis. BRAF^{V600E} had significantly increased odds ratio with histologic pattern, both on univariate and multivariate logistic regression. Kaplan–Meier analysis revealed

Keywords

- ▶ thyroid
- ▶ papillary microcarcinoma
- ▶ prognosis
- ▶ BRAF^{V600E}
- ▶ lymph node metastasis

Ethics approval obtained from the institutional ethics committee (letter no.EC/NIMS/2809/2021).

DOI <https://doi.org/10.1055/s-0043-1761412>.
ISSN 0971-5851.

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significantly reduced OS with presence of LN metastases (p -value = 0.050, log-rank test). Cox-regression did not yield a significant hazard ratio for the various factors studied.

Conclusion This study shows association of LN metastasis with intratumoral fibrosis, PTFI and reduced OS. Intratumoral fibrosis was also associated with tumor size more than 5mm, infiltrative borders and PTFI. Increasing tumor size and infiltrative borders also showed an association. In addition, $BRAF^{V600E}$ positivity was found to be associated with histologic pattern, PTFI and distant metastasis.

Introduction

Papillary microcarcinoma (PMC) is defined as papillary thyroid carcinoma (PTC) of size ≤ 1 cm.¹ Most of them are diagnosed incidentally in thyroidectomy or autopsy specimens. Advances in imaging modalities (including PET/CT, done both for screening purposes and for surveillance of other known malignancy) have led to increased early detection of small thyroid nodules.^{2,3} Many of these either remain clinically silent for the entire lifetime or have excellent long-term outcome. However, a proportion of cases metastasize to cervical lymph nodes (LNs) and distant sites. Various factors like multifocality, tumor size more than 5mm, LN metastasis and extrathyroid extension (ETE) have been found to predict the risk of recurrence.⁴⁻⁷ Identification of a subset of patients with aggressive behavior is important to stratify patients for implementation of radical therapeutic approach similar to classical PTC.

The frequency of $BRAF^{V600E}$ mutation in PTC ranges from 60 to 80% and is associated with high risk clinicopathological features. Similar incidences have also been noted in PMC accounting for 37.5% to 74% of cases and are associated with aggressive features despite their small size.⁸⁻¹⁰

There are not many studies on PMC in Indian literature till date.¹¹⁻¹⁵ In this article, we have analyzed the prognostic significance of various clinicopathologic parameters including $BRAF^{V600E}$ mutation by immunohistochemistry (IHC) in PMC.

Materials and Methods

This is a retrospective study of cases diagnosed as PMC in our institute, from January 2014 to May 2020 ($n = 48$). The demographic and clinical data were retrieved from histopathology requisition forms and medical records. The inclusion criterion was thyroidectomy specimens diagnosed as PMC on routine histopathology. And exclusion criteria were tumor size exceeding 1 cm and concurrent presence of other malignancy (e.g., follicular/oncocytic carcinoma).

The hematoxylin and eosin (H&E)-stained histopathology sections of these cases were reviewed by three pathologists to confirm the original diagnosis and to also document various histopathological parameters like tumor size, focality, capsular invasion, histologic subtype, lymphovascular invasion, perithyroidal fat invasion (PTFI), and stage of the tumor. The pathological changes in the adjacent thyroid

(including both neoplastic and non-neoplastic entities) were also analyzed.

Immunohistochemistry

For performing IHC, a tissue array block was constructed by extracting tumor tissue from formalin fixed paraffin embedded blocks of the cases using manual Quick-Ray needle. Of the total 48 cases, paraffin blocks of only 37 patients were available for constructing the tissue microarray and performing subsequent IHC. After initial review of H&E-stained slides, the tumor areas were marked and tissues were extracted from corresponding area on the paraffin block using 5mm tip of Quick Ray needle. The extracted tissues were then transferred to a recipient block. One or two cores were extracted per case depending on the availability of tumor tissue and each array block held 14 tumor tissue cores. Four micron sections were cut from these tissue array blocks and IHC with CK19 (Clone EP72, Pleasanton, California, USA) and $BRAF^{V600E}$ (Clone VE1, Ventana Hoffmann La -Roche Ltd., Switzerland) antibodies was performed on fully automated immunostainer ('Ventana GX' with 'Ventana Benchmark GX, Hoffmann La - Roche Ltd, Switzerland'). The IHC results for these two antibodies performed on tissue microarray slides were analyzed. In addition, results of CK19 IHC wherever performed at time of initial diagnosis were also reviewed.

Follow-up details were noted where ever available. Information regarding overall survival, persistence of disease, and development of recurrence/metastasis if any was also noted.

Statistical Analysis

The nominal data was compared using ratios. Continuous numerical data was studied using median. Percentages were used for both nominal and ordinal data. Chi-squared test was performed to find out the association between factors like tumor size, focality, histopathological variant, infiltrative margins, fibrosis, PTFI, LN metastasis, distant metastasis, and $BRAF^{V600E}$ positivity. To determine odds ratio, univariate and multivariate logistic regression was done. Kaplan-Meier survival analysis was done to compare time to death between various prognostically significant groups. Cox-regression analysis (univariate and multivariate) was done to know the association of survival time with covariates and to calculate the hazard ratios for each variable. These were done using Statistical Package for the Social Sciences (SPSS) software version 29.

Ethics

Ethics approval was obtained from the NIMS institutional ethics committee (approval letter no.EC/NIMS/2809/2021, dated 28.08.2021). Waiver of informed consent was obtained due to the retrospective nature of the study. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki declaration of 1964, as reviewed in 2013.

Results

Of the total 1,088 thyroidectomy specimens received during study period, 32.4% (353) had malignant tumors. Of these, 58.6% (207) were PTCs and 14.4% (51) PMCs. Among the PMC cases, three cases were excluded as they had a history of concurrent malignancy like follicular carcinoma, oncocytic (Hurthle cell) carcinoma and mucoepidermoid carcinoma.

Demographic and Clinical Findings

The remaining 48 patients diagnosed with PMC included 29.2% (14) men and 70.8% (34) women (M:F = 1:2.4) aged between 22 and 70 years (median, 46.5 years). Of these, only 22.9% (11) presented with palpable solitary nodule in thyroid. In 43.7% (21) patients, tumors were incidentally detected either at ultrasonography of neck (14.6%, 7 cases) or thyroidectomies for other causes (multinodular goiter—27%, 13 cases; Grave's with compressive symptoms—2%, 1 case). The remaining 33.3% (16) patients presented with metastasis in cervical LN (20.8%, 10 cases) and distant sites (12.5%, 6 cases). In the later cases, PMCs were detected in subsequent thyroidectomies.

Surgical Management

The surgical management in these patients included hemithyroidectomy in 29.2% (14), subtotal thyroidectomy in 20.8% (10), near total thyroidectomy in 6.25% (3), and total thyroidectomy in 43.75% (21). Concurrent neck LN dissection was done in 39.6% (19) of these patients. Three patients who had initial hemithyroidectomy subsequently underwent completion thyroidectomy.

Pathological Findings (► Fig. 1, ► Table 1)

Though majority were unifocal/solitary tumors, a significant number were multifocal tumors (2–6 in number). Hence, a total of 81 tumor foci were detected in 48 patients. The size of the tumors ranged from less than 1 mm to 10 mm with median size of 5 mm. Most of the tumors were right sided, unencapsulated with near equal distribution of tumors having circumscribed and infiltrative borders. On histology, follicular variant was the most common type followed by classical variant. Variation in architectural pattern between different foci of multicentric tumors was noted in some cases. Though none of our cases showed any evidence of either lymphovascular invasion or ETE, PTFI by the tumor was noted in 8.3% (4) cases.

LN metastases were detected in 62.5% (15/24) patients with LN dissection. In 26% (4/15) of these patients, there was

discordance between architectural pattern of tumor in thyroid and LN metastases. In these four cases, the tumor showed follicular pattern in the thyroid, whereas the LN metastases showed classic papillary pattern. There was another case where the primary tumor in thyroid showed predominant classic pattern with about 30% tall cell area, whereas the LN metastasis had completely tall cell pattern.

The common findings in adjacent thyroid included lymphocytic thyroiditis, Hashimoto's thyroiditis, and adenomatous goiter. Concurrent oncocytic (Hurthle cell) adenoma was detected in 4.2% (2) cases. Details of selected gross and microscopic findings of the cases are provided in ► Table 1.

Normal parathyroid glands were found in thyroidectomy specimen in 20.8% (10) cases (14.6%, [7] cases showed one and 6.25%, [3] cases showed two glands) and ectopic thymic tissue in neck node dissection of 2% (1) cases.

The distant metastases noted in 12.5% (6) patients affected scalp, gluteal region, mandible, sternum, sacroiliac bone, and mediastinum.

IHC Findings (► Fig. 2)

Of the 77% (37) cases included in tissue array block, IHC findings of only 73% (35) cases could be evaluated due to tissue loss in two cases. In addition, there were four cases in which IHC results of CK19 performed at the time of original diagnosis were available for review. Thus, final results of IHC analysis are derived from 81.25% (39) cases for CK19 and 73% (35) cases for *BRAF*^{V600E}. CK 19 was positive in all (100%, 39/39) and *BRAF*^{V600E} in 54.3% (19/35) cases tested.

Follow-Up

Follow-up details were available in 68.75% (33) cases and the follow-up period ranged from 2 to 54 months (mean, 23.3 months). Five (10.4%) patients received radioactive iodine therapy following surgery. During follow-up, 9% (3/33) patients developed metastases to brain (3%, 1), lung (3%, 1), and LN (3%, 1) at 5 months, 6 months, and 39 months after initial surgery, respectively. During the follow-up period, 9% (3/33) patients died at 2, 18, and 54 months after initial surgery. One of the deceased was a patient who had brain metastasis at follow-up and died at 18 months after initial surgery.

Statistical Analysis

The chi-squared test showed significant association between presence of fibrosis and tumor size more than or equal to 0.5 cm, infiltrative borders, PTFI and LN metastasis. Apart from presence of fibrosis, tumor size more than or equal to 0.5 cm was found to be associated with infiltrative borders. LN metastasis also showed association with PTFI. *BRAF*^{V600E} positivity showed significant association with histologic pattern, PTFI and distant metastasis. (► Table 2)

The logistic regression analysis was done with those clinicopathological parameters that were found to have significant association on chi-squared test (shown in ► Supplementary Table S1). The odds ratio for tumor size more than or equal to 0.5 cm was significantly increased with

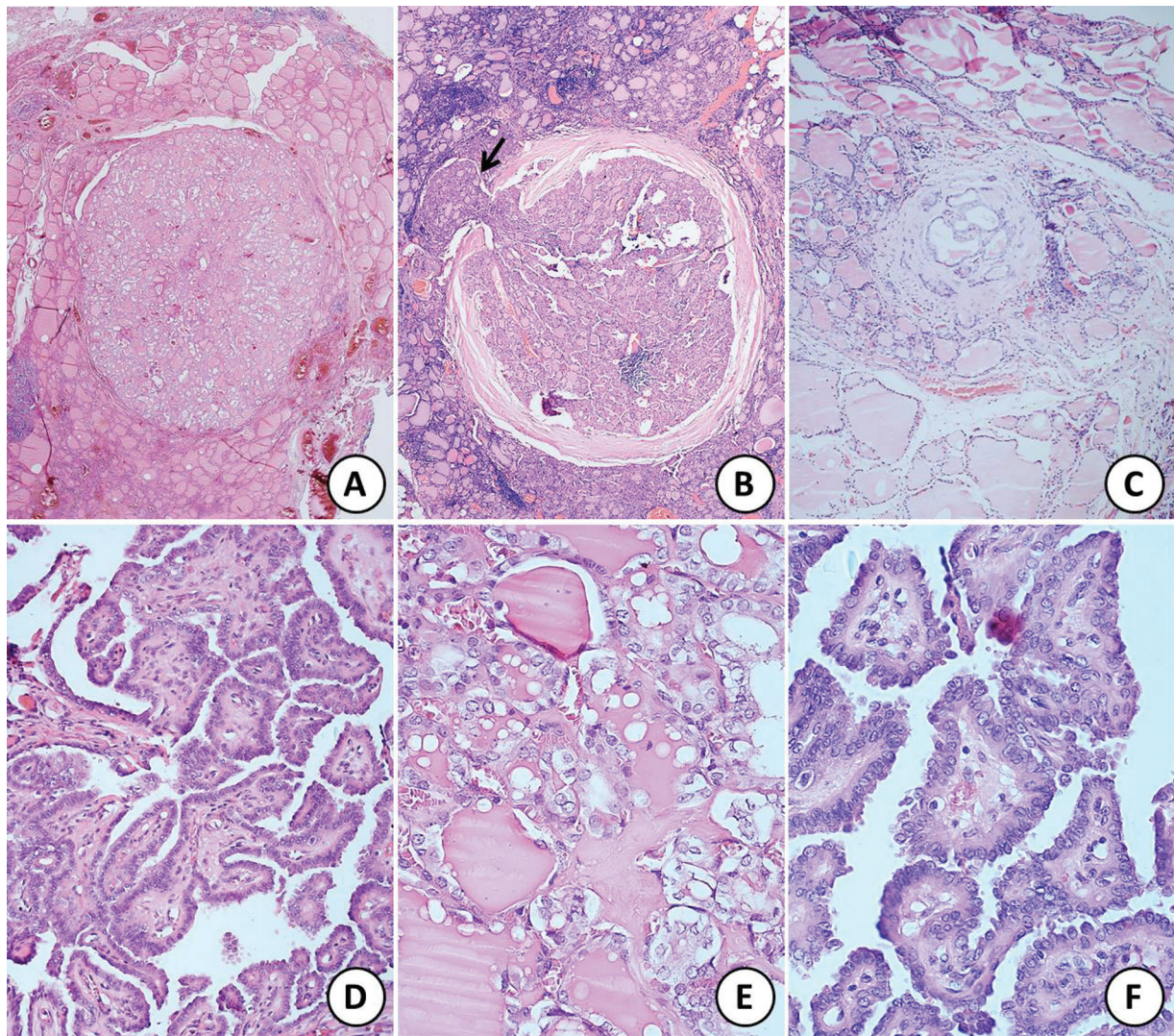


Fig. 1 Sections of papillary microcarcinoma of thyroid showing (A) unencapsulated tumor, (B) encapsulated tumor with focal capsular breach and extracapsular extension (black arrow), and (C) very tiny focus of tumor comprising only a few follicles. All the three above tumors show follicular pattern of growth (hematoxylin and eosin [H&E]; Ax20, Bx40, Cx100). (D) Papillary microcarcinoma with classical papillary pattern of growth (H&E; x200). (E) and (F) Higher magnification to highlight the nuclear features of clearing, grooves, intranuclear inclusions, and overlapping (H&E; Dx400, Ex400).

presence of fibrosis and infiltrative borders (odds ratio = 4.333 and 5.320; p -value = 0.045 and 0.010, respectively). However, multivariate regression did not show statistically significant association between these factors.

Presence of fibrosis had significantly increased odds ratio (positive association) with infiltrative borders, presence of LN metastasis and tumor size more than or equal to 0.5 cm on univariate logistic regression. However, on multivariate analysis only infiltrative borders showed significant association with presence of fibrosis. PTFI did not show any significant association on logistic regression.

Logistic regression of BRAF^{V600E} versus other factors was also not statistically significant, except with histological patterns. The classic variant showed significant positive association with BRAF positivity, as compared to the follicular variant, on both univariate and multivariate regression (odds ratio = 5.042 and 5.405, p -value = 0.037 and 0.050, respectively). In addition, on multinomial logistic regression the follicular and classic variants showed significantly increased odds ratio with

BRAF positivity, as compared to the diffuse sclerosing variant (as depicted in ► **Supplementary Table S1**).

Kaplan–Meier survival analysis revealed significantly reduced overall survival (OS) with the presence of LN metastases with a p -value of 0.050 (log-rank test) (► **Supplementary Fig. S1**). However, the comparison between the other parameters was not statistically significant. The 5-year follow-up period was not reached for LN metastasis; hence an estimate of survival at 5-years is not possible. One-year and two-year survival rates were 100 and 50% for cases with presence of LN metastasis.

The univariate and multivariate Cox-regression analysis did not yield a significant hazard ratio for the various factors studied.

Discussion

PTC accounts for 70 to 90% of well-differentiated thyroid malignancies and PMC comprises nearly 30% of all diagnosed

Table 1 Gross and microscopic features of PMC cases

Feature		No of cases
Focality	Unifocal (1)	30
	Multifocal (2-6)	18
Location	Right lobe	23
	Left lobe	14
	Isthmus	5
	Bilateral lobes	5
	Isthmus+ right lobe	1
Capsule (no. of foci = 81)	Encapsulated:	12
	With capsular invasion	3/12
	Without capsular invasion	9/12
	Unencapsulated:	69
	Circumscribed	36/69
	Infiltrative	33/69
Histologic variant (no. of foci = 81)	Follicular variant	50
	Classic variant	28
	Tall cell variant	2
	Diffuse sclerosing variant	1
Adjacent thyroid	Calcification	7
	Psammoma bodies	1
	Fibrosis/ sclerosis	6/10
	Stromal hyaline	1
	Lymphocytic thyroiditis	12
	Hashimoto thyroiditis	10
	Adenomatous goiter	9
	Oncocytic (Hurthle cell) adenoma	2
Lymph node dissection (n = 24)	Involved	15/24
	Size of largest metastatic deposit	15-60 mm
	Extranodal extension	3/24
TNM stage	T1a	48
	N1a	1
	N1b	14
	M1	6

PTC.¹⁶ In the present study, majority of the thyroid malignancies were PTC of which 19.76% were categorized as PMC. The clinical significance of PMC is not clear. Though majority of PMCs have an indolent course and excellent prognosis, some are associated with recurrence and distant metastasis with fatal outcome.^{9,17,18} This study was done with the goal of identifying association of various clinicopathologic factors including the *BRAF*^{V600E} IHC with the risk of aggressive disease in PMC. The various clinicopathological factors including the rate of recurrence are comparable to other studies as shown in ►Table 3.

Though the median age and gender distribution of the cases in the present study were similar to other studies, the median diameter of the tumor varied considerably.^{4,11,19} Multifocality was seen in 37.5% cases similar to Park et al and John et al.^{11,19} Various studies have reported the incidence of ETE ranging from 3.9 to 52.2%.^{4,11,19} The American Joint Committee on Cancer (AJCC) 8th edition does not consider microscopic ETE that is not grossly evident.²⁰ PTFI is no longer considered as ETE. As the definition of ETE has

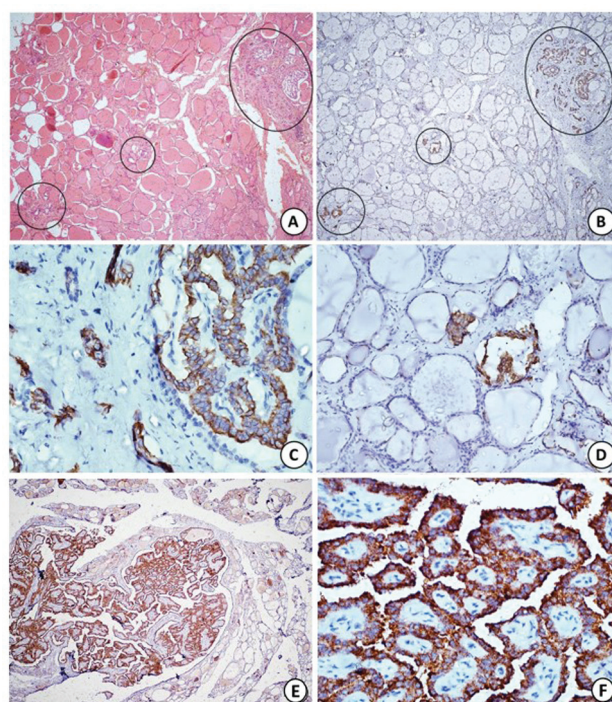


Fig. 2 (A) Section shows multicentric papillary microcarcinoma involving three distinct foci (black circles), two of which are very tiny comprising of only few follicles (hematoxylin and eosin [H&E]; x40). **(B)** Immunohistochemistry (IHC) with CK19 highlights all the foci of papillary microcarcinoma (black circles) including the tiny ones. **(C, D)** Higher magnification shows strong cytoplasmic staining in tumor cells with CK19 (HRP-Polymer; Bx40, Cx400, Dx200). **(E, F)** Diffuse strong cytoplasmic staining with *BRAF*^{V600E} on IHC (Ex40; FX400).

changed recently, only 8.3% cases showed minimal ETE/PTFI that cannot be classified as T3b tumors. Similar to other studies LN metastasis was noted in one-third of the cases.^{4,19} The rate of recurrence occurring in tumor bed, cervical LNs, or at distant sites in the present study was slightly higher than other studies (9 vs. 2.4-6%).^{4,11,19} However the reported range of recurrence rate in PMC is lower compared to conventional PTC.¹

The present study revealed association of LN metastasis with fibrosis and PTFI similar to Neimeier et al.²¹ Unlike the latter, there was no significant association of *BRAF*^{V600E} mutation with LN metastasis in this study. Similar observations were noted in few other studies as well.^{22,23} Studies have shown contradictory results regarding the association of LN metastasis with tumor size more than 5mm.^{16,24,25} The present study did not show any correlation. We also found presence of LN metastasis to be associated with significantly reduced OS on Kaplan–Meier analysis (►Supplementary Fig. S1). The minimal ETE/PTFI was found to be associated with presence of fibrosis, LN metastasis and *BRAF*^{V600E} positivity on chi-squared analysis. However, the odds ratio was not statistically significant with any of the clinicopathologic parameter. This highlights the importance of the change made in AJCC 8th edition, wherein PTFI is no longer considered a criterion in T staging.

A meta-analysis has shown association of *BRAF*^{V600E} mutation with the histological subtype, ETE, and advanced

Table 2 Association of clinicopathological factors with size, presence of fibrosis, LN metastasis, and BRAFV600E IHC status

Characteristic	Size (cm)			Fibrosis			LN metastasis			BRAF		
	<0.5	≥0.5	p-Value	Pre	abs	p-Value	pre	abs	p-Value	pos	neg	p-Value
<i>n</i>	19	29		16	32		15	33		19	16	
Age ≥ 45 y	9	20	0.135	11	18	0.404	10	19	0.551	12	10	0.968
Size ≥ 0.5 cm	–	–	–	13	16	0.037	11	18	0.217	12	12	0.452
Multifocality	7	11	0.939	5	13	0.527	8	10	0.127	8	6	0.782
Inflit borders	5	19	0.007	14	10	<0.001	9	15	0.350	11	7	0.404
Fibrosis	3	13	0.037	–	–	–	9	7	0.009	8	6	0.782
PTFI	1	3	0.533	4	0	0.002	4	0	0.001	4	0	0.021
LN mets	4	11	0.217	9	6	0.009	–	–	–	8	4	0.288
Distant mets	2	4	0.738	1	5	0.355	2	4	0.907	1	5	0.037
Death	1	2	0.822	1	2	0.909	2	1	0.133	1	1	0.900
BRAF positive	7	12	0.452	8	11	0.782	8	11	0.288	–	–	–
Pattern:			0.269			0.465			0.364			0.032
Follicular	12	13		8	17		10	15		6	11	
Classic	7	13		6	14		4	16		11	4	
Tall cell	0	2		1	1		1	1		2	0	
Diff scl	0	1		1	0		0	1		0	1	

Abbreviations: pos, positive; neg, negative; pre, present; abs, absent; IHC, immunohistochemistry; LN, lymph node; mets, metastasis; Inflit, infiltrative; PTFI, perithyroidal fat invasion; scl, sclerosing.

clinical stage suggesting a poor outcome in PMC similar to PTC.²⁶ The present study showed association of BRAF^{V600E} with histologic pattern, PTFI and distant metastasis (as shown in **Table 2** and **Supplementary Table S1**). Choi et al and Fakhruddin et al did not find any statistically significant association between BRAF^{V600E} mutation and other clinicopathologic factors.^{22,23} On the contrary, Virk et al found BRAF^{V600E} mutation associated with LN metastasis, infiltrative borders, fibrosis, ETE, and classic nuclear features of papillary carcinoma.²⁷ Another study suggested that tumors greater than 5mm and a subset of patients with BRAF^{V600E} mutation may develop highly aggressive PMCs. The authors also noted increased frequency of recurrence with ETE, LN metastases, and type of surgical procedure. There was neither any associa-

tion with BRAF^{V600E} mutation on multivariate analysis nor any change in outcome of BRAF^{V600E} mutated patients on aggressive treatment.²⁸ Mercante et al found aggressive tumor behavior in cases showing capsular invasion without ETE and advised radical treatment in them.²⁹

There was an association of increasing size with presence of fibrosis and infiltrative borders in our study. A similar observation was made by Miccoli et al.²⁵ Presence of fibrosis also showed significantly increased odd's ratio with infiltrative borders on univariate and multivariate logistic regression. Tumor-associated stromal reaction, like desmoplasia, fibrosis, or sclerosis were more frequent in BRAF^{V600E} -mutated tumors, but stromal calcification and psammoma bodies were not, according to Virk et al.²⁷ Karkuzhali et al

Table 3 Comparison of the various clinicopathological parameters with other studies

	Current study	Park et al ¹⁹	Shi et al ⁴	John et al ¹¹
Male: Female	14:34 (1:2.4)	42: 237 (1:5.6)	21:230 (1:10.9)	18:59 (1:3)
Median age, years	46.5 ± 15	46.5 ± 11.6	42.9 ± 10.1 ^b	44.54 ± 10.5 ^b
Median diameter, cm	0.5 ± 0.6	0.81 ± 0.67	0.11 ± 0.07 ^b	0.41 ^b
Multifocality (%)	18/48 (37.5)	50/122 (41.0)	12/251 (4.8)	34/77 (43.4)
Extrathyroidal invasion (%)	4/48(8.3) ^a	145/278 (52.2)	25/251 (10)	3/77 (3.9)
LN metastasis (%)	15/48 (31.3)	97/278 (34.9)	84/251 (33.5)	11/77 (14.2)
Thyroiditis (%)	22/48 (45.8)	10/87 (11.5)	40/251 (15.9) ^c	8/77 (10.4)
Recurrent or persistent (%)	3/33 (9) ^d	6/98 (6.1)	6/251 (2.4)	2/77 (2.6)
Mean follow-up duration, months	23.3 ± 17.85	53.4	45.4 ± 3.5	20

Abbreviations: IQR, interquartile range; LN, lymph node; SD, standard deviation.

Median values are accompanied by the IQR and means by the SD.

^a, Perithyroidal fat invasion; ^bthese are mean values; ^c, includes only Hashimoto's thyroiditis; ^d follow-up details were available in 33 cases only.

also found increased stromal changes like calcification with increasing tumor size.¹⁵

Certain histomorphologic patterns like tall cell variant of PMC have been found to be associated with poor prognosis.⁸ However, none showed statistically significant outcome on survival analysis in this study.

We found that CK19 is a highly sensitive marker for PTC including PMC and can help detect even minute tumor foci that can be easily overlooked on routine H&E sections.

The limitations of current study include relatively small number of cases studied, lack of molecular analysis for *BRAF*^{V600E} mutation, and short follow-up time. Larger studies with longer follow-up period and adjunct molecular testing would be helpful.

Conclusion

Despite these limitations, significant conclusions that can be drawn from present study are association of LN metastasis with intratumoral fibrosis, PTFI and reduced OS. Intratumoral fibrosis was also associated with tumor size more than 5mm, infiltrative borders, and PTFI. Increasing tumor size and infiltrative borders also showed an association. In addition, *BRAF*^{V600E} positivity was found to be associated with histologic pattern, PTFI and distant metastasis.

Authors' Contributions

Sobiya Mahnaz Ayesha contributed to designing, definition of intellectual content; literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, editing and review; Monalisa Hui helped in designing, definition of intellectual content; literature search, experimental studies, data analysis, statistical analysis, manuscript preparation, editing and review; Shantveer G Uppin helped in conceptualization, designing, definition of intellectual content, literature search, experimental studies, data analysis, statistical analysis, manuscript preparation, editing and review; Megha Shantveer Uppin and Tara Roshni Paul helped in definition of intellectual content, manuscript preparation, editing, and review; Shubhranshu Jena, Rajsekhar Shanthappa Patil, and Ranganath Ratnagiri contributed to definition of intellectual content, clinical studies, manuscript preparation, editing, and review. Shantveer G Uppin has provided guarantee.

Conflict of Interest

None declared.

Acknowledgements

None.

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