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Original research article

Expression of Cytokeratin-19 And HBME-1 in Various Thyroid Neoplasm at a Tertiary Care Centre

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Abstract

Background: To distinguish between malignant and benign lesions of the thyroid gland and due to several overlapping histomorphological features and pitfalls in thyroid pathology, there is a need to establish a panel of immunomarkers that could aid in proper diagnosis. This study is intended to investigate the ability of CK-19 & HBME-1 in differentiating between benign and papillary carcinoma thyroid

Methods: This study is being conducted in the Department of Pathology, Kakatiya Medical College and MGM hospital, Warangal, Telangana during a period between 2018 to 2021. A total of 60 cases are studied out of which 32 cases were diagnosed as follicular adenoma, 16were PTC, and 12 were FVPTC. Excision biopsies specimens from the lesions were fixed in formalin, paraffin-embedded, and routinely stained in H and E. IHC staining with markers HBME - 1 and CK-19 was done and their expression is correlated.

Results: The highest incidence of thyroid nodules in the present study was in the 4th decade in males. Papillary Thyroid Carcinoma (PTC) was most common in malignant thyroid nodules, out of 28 malignant lesions selected 16 were classic PTC and 12 were FVPTC. Positive CK19 expression observed in 25 out of 28 malignant cases (89.28%) included 15 out of 16 cases of classic PTC (93.75%). Positive HBME-1 expression observed in 27 out of 28 malignant cases (96.4%) included 16 out of 16 cases of classic PTC (100%) and 11 out of 12 cases of FVPTC (91.6%). Negative HBME-1 expression was observed in 1 case out of 12 FVPTC (3.5%). Negative HBME-1 expression was observed in 26 out of 32cases (81.25%) of benign lesions. HBME-1 showed a high statistically significant difference between papillary carcinoma of the thyroid and other benign thyroid nodules ($P < 0.000001$).

Conclusion: Conventional PTC may not pose a problem for diagnosis but its variants and its benign morphological mimics are challenging. Our study concurs with published literature to suggest strong expression of CK19 and HBME-1 in FVPTC and PTC. CK19 and HBME-1 are impressive positive markers for malignant lesions probably helping in the new category of encapsulated follicular pattern thyroid nodules.
Keywords: -Thyroid Neoplasm, PTC, FVPTC, HBME -1, CK-19, IHC staining.

Introduction

Thyroid nodules are very common (about 5% of the general population) and are usually discovered during routine medical care. With the emergence of ultrasound, impalpable thyroid nodules can be detected in 20-67% of the general population. [1, 2] Thyroid cancer represents about 5-24% of thyroid nodules and 1-2% of all malignancies. [3] Papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid malignancies. [4] Histopathology is the gold standard for diagnosing thyroid lesions. The diagnosis of papillary carcinoma is based on the nuclear morphology of a thyroid neoplasm. Chan et al., [5] and described the grooved nucleus with conventional hematoxylin and eosin (H & E) stained sections and considered it a useful diagnostic criterion for papillary carcinoma. The appreciation of the nuclear features of PTC is crucially dependent on the tissue processing (fixative, duration of fixation, and thickness of sections). Morphologic similarities between benign and malignant lesions are frequent; papillary and follicular architectures and nuclear irregularity may be seen both in benign and malignant lesions. [6-8] Moreover, severe chronic lymphocytic thyroiditis, Hashimoto's thyroiditis, and reactive atypia attributed to inflammation result in nuclear morphology similar to that of papillary carcinoma, with nuclear enlargement, chromatin clearing, and even grooves. [9, 10] If the nuclear features of PTC are insufficiently appreciated, severe problems arise in differentiation from the follicular variant of papillary thyroid carcinoma (FVPTC), and follicular adenoma (FA). [11] Therefore, the diagnosis of non-invasive, encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) versus follicular adenoma is prone to considerable interobserver variability. [12] Immunohistochemical and molecular methods were investigated to aid in the diagnosis of these problematic cases. Several immunohistochemical markers such as Galectin-3, P-63, and CD56 have been recommended to help in the discrimination between these controversial thyroid nodules. Nonetheless, up till now, there is no agreed consensus about an immunohistochemical panel that would reliably overcome such diagnostic obstacles. In this study, we evaluated the expression of HBME-1and CK-19 separately and in combination in benign thyroid nodules and papillary Carcinoma. HBME-1, a mesothelioma marker, is evolving as a promising antibody for identifying thyroid malignancy. HBME-1 stains mostly follicular derived malignant tumors, including both well differentiated and poorly differentiated carcinoma. [13] Cytokeratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Cytokeratin 19 is a type 1 keratin encoded by the KRT19 gene.CK19 was proposed as a possible marker for epithelial tumors by Bjorklund in 1957. [14] It is one of the smallest cytokeratin (40Kda) present in both simple and complex epithelium. Involved in the organization of myofibres; links contractile apparatus to dystrophin at costameres of striated muscles. The use of CK19 expression in cases of thyroid tumors is well established. CK19 has been proposed as an immunohistochemical marker to distinguish papillary thyroid carcinoma from other tumors with follicular patterns and with benign lesions. Several studies have reported the diffuse positive reactivity for CK19

in papillary thyroid carcinoma. With this background, we in the current study tried to evaluate the expression of CK-19 and HBME-1 immunostaining on all diagnosed thyroid neoplasms by H&E.

Material and Methods

This prospective study was conducted in the Department of Pathology, Kakatiya Medical College and Mahatma Gandhi Memorial Hospital, Warangal during the study period from June 2018 to June 2021. Included n=60 specimens of surgically removed, formalin-fixed, and paraffin-embedded thyroid lesions which were received at our department of pathology included. Relevant demographic data was collected for all 60 cases.

Inclusion criteria

1. All the cases diagnosed as Benign and Malignant thyroid neoplasm of thyroid lesions were included.

Exclusion criteria

1. All other inflammatory thyroid lesions like Granulomatous Thyroiditis, and Hashimoto Thyroiditis were excluded.
2. All benign cases like Multinodular Goitre, Colloid Cyst, etc were excluded.

Out of n=60 cases, n=32 cases were benign thyroid lesions and n=28 cases were malignant. *Benign cases*: Follicular adenoma (32/32) *Malignant Cases*: Classical papillary carcinoma of the thyroid (16/28). Follicular variant of papillary carcinoma (12/28). The specimens received were fixed in 10% buffered formalin, grossed, and sections were taken from representative sites. The sections were then processed in an automated tissue processor and embedded in paraffin wax. Representative areas containing tumor/non-neoplastic areas with suspicious foci were selected and submitted for IHC. Three sections of 4 microns thicknesses were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other two on a poly- L-lysine-coated slide for immunohistochemical staining. Standard procedure for H&E staining was employed using Harris Haematoxylin and aqueous Eosin. Chan and Saw criteria were followed for diagnosis of papillary thyroid carcinoma. [5]

Immunohistochemistry: All n=60 samples including n=32 benign and n=28 malignant lesions were subjected to immunohistochemistry markers CK19 and HBME-1. The kits for CK19 mouse monoclonal antibody immunohistochemical staining were obtained from DAKO (Monoclonal Mouse Anti-Human CD56, 123C3). For HBME-1 mouse monoclonal antibody immunohistochemical staining was obtained from DAKO (Monoclonal Mouse Anti-Human p63, RCK108). Staining was done according to the manufacturer's protocol. Interpretation of IHC markers: Strong cytoplasmic expression without membranous staining of the cells qualified the case as positive for CK19. A positive cytoplasmic expression without cytoplasmic staining in 5% or more of neoplastic cells qualified the case as "positive (+)" Strong and complete membranous immunoreactivity without cytoplasmic staining of the cells qualified the case as positive for HBME-1. Staining in 5% or more of neoplastic cells qualified the case as "positive" (+).

Scoring for immunohistochemistry markers in the semiquantitative Assessment According to Nemeth et al., [15]

Score ‘0’ < 5% cells showing positive expression
Score 1+ 6-25% cells showing positive expression
Score 2+ 26-50% cells showing positive expression
Score 3+ >50% cells showing positive expression

Statistical analysis: The $\chi 2$ test and Fisher's exact test were used for comparing the expressions of the applied immunohistochemical markers in papillary thyroid carcinomas and other thyroid lesions. The statistical analysis was performed using SAS 9.1, and the results with the p-value (<0.05) were significant. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the markers in diagnoses were compared.

Results

A total of n=60 cases were enrolled in the present study, with benign thyroid neoplasm observed in n=32 (53.3%) cases and malignant thyroid neoplasm identified in n=28 (46.6%) cases. n=32 benign thyroid lesions were diagnosed as Follicular adenoma. Out of n=28 cases, n=16(26.6%) cases were diagnosed as classical Papillary thyroid carcinoma and n=12 (20%) cases were the follicular variant of papillary carcinoma. Out of 60 cases, n=12(20%) cases were male and n=48(80%) cases were female patients with a male to female ratio of 1:4 (table-1). In benign lesions ratio is 1:4 and in malignant lesions, 1:6. Incidence of thyroid nodules was higher in females.

Table 1: Age and Sex Distribution of cases in the study

Age Group	*Male*	*Female*	*Total*
20 -29	02	14	16
30 - 39	04	18	22
40 - 49	02	12	14
50 - 59	01	04	05
60 - 69	01	02	03
Total	12	48	60

In the present study youngest patient was 20 years old (female) and the oldest patient was 65 years old (female). The highest incidence of thyroid nodules in the present study was in the 4th decade and there is an increased preponderance in females constituting about 85% of the cases. The incidence of both benign and malignant neoplasm is more in females than in males. The incidence of follicular adenoma is more common than papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma in females (table 2).

Table 2: Sex distribution of Thyroid Neoplasia

Neoplasm	*Female*	*Male*	*Total*
Benign	28	04	32
Follicular Adenoma	28	04	32
Malignant	23	05	28
Classical PTC	13	03	16
FVPTC	10	02	12

Table 3: Age and sex distribution among benign and malignant neoplasms

Age Group	Benign		Malignant		Total
	Female	Male	Female	Male	
20 - 29	10	01	05	00	16
30 - 39	14	03	04	01	22
40 - 49	03	00	10	01	14
50 - 59	01	00	02	02	05
60 - 69	00	00	02	01	03

The higher incidence of benign lesions among females is more in the 4th decade while the incidence of the malignant lesion is more in the 5th decade.

Cytokeratin-19 Expression Benign Thyroid Lesions: Negative CK19 expression was observed in n=28 out of n=32 cases (87.5%) of benign lesions which includes follicular adenomas, remaining n=4 out of n=32 cases (12. 5%) of follicular adenoma showed positive expression for CK19. All of the negative cases displayed CK19 expression in 50% of the Cells (score 3).

Malignant Thyroid Lesions: Positive CK19 expression was observed in n=25 out of n=28 malignant cases (89.28%) including n=15 out of n=16 cases of classic PTC (93.75%) and 10 out of n=12 cases of FVPTC (83.3%). Negative CK19 expression was observed in the n=1 case of classic PTC (6.25%) and n=2 out of n=12 FVPTC (16.6%).

Figure 1: Gross Picture Follicular Adenoma

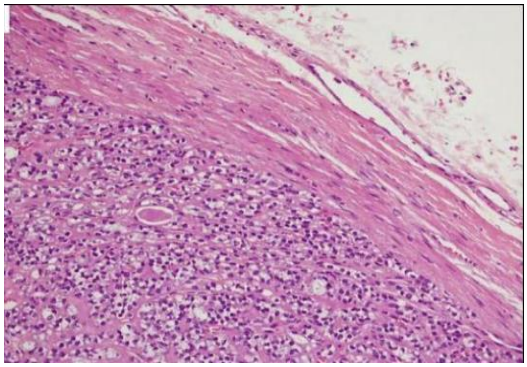

Figure 2: H&E 100x Follicular Adenoma

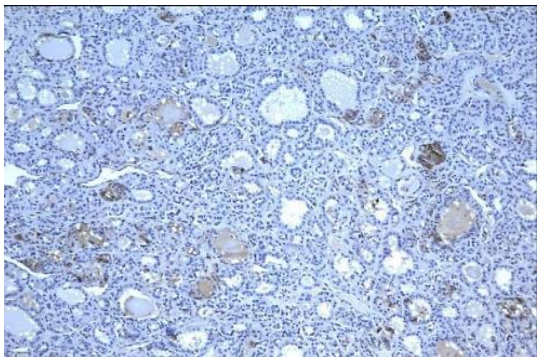

Figure 3: HBME-1 expression in Follicular adenoma

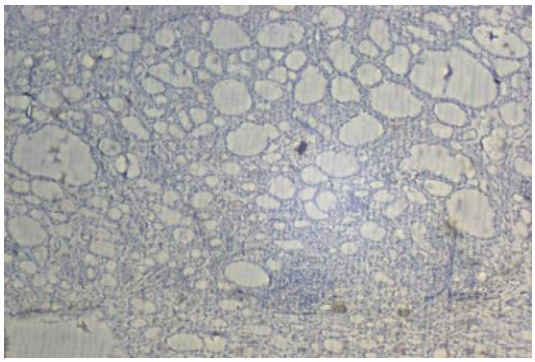

Figure 4: CK-19 expression in Follicular adenoma

Figure 5: Gross Picture Classic PTC

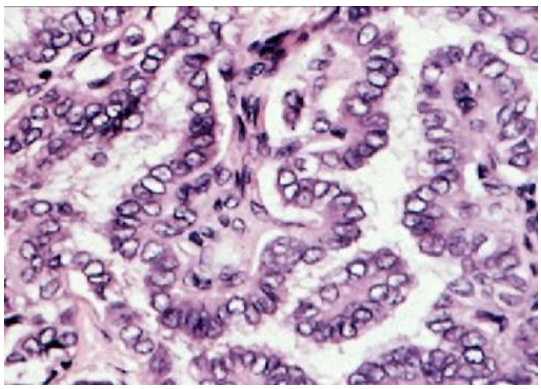

Figure 6: H&E 400X Classic PTC

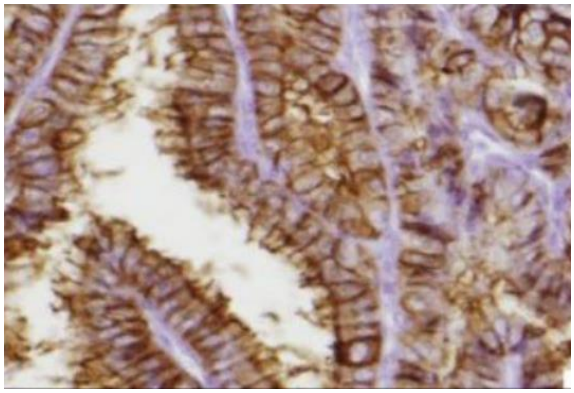

Figure 7: 40X HBME-1 Expression in PTC

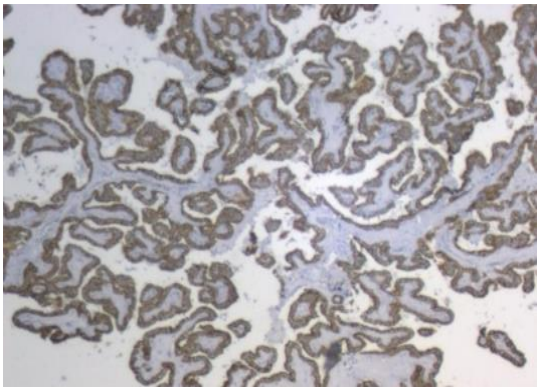

Figure 8:100X CK 19 Expression In PTC

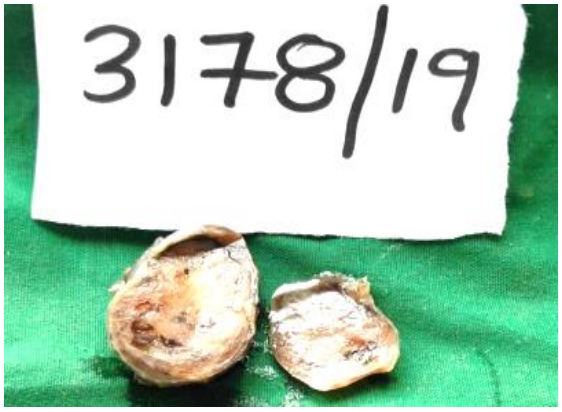

Figure 9: Gross Picture FVPTC

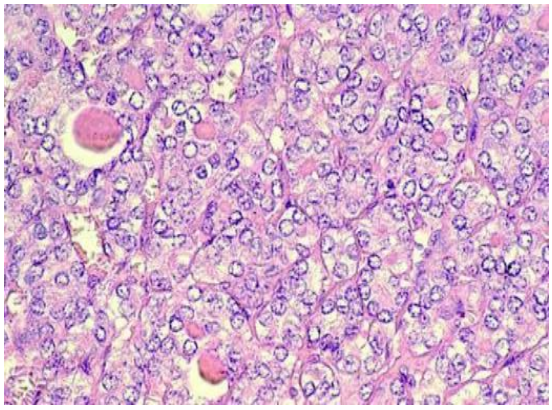

Figure 10: (40X) H&E FVPTC

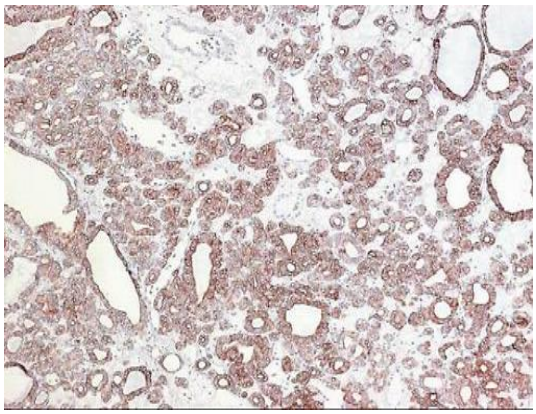

Figure 11: 10X HBME-1 expression in FVPTC

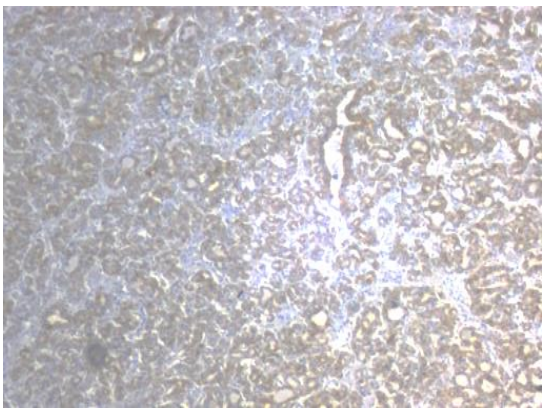

Figure 12:10X CK-19 expression in FVPTC

Table 4: CK19 expression in Benign & Malignant lesions

HPE Diagnosis	*CK19 Expression*				Total
	Positive (%)	*Diffuse*	*Focal*	*Negative (%)*	
Follicular Adenoma	04 (12.5%)	2/28	00	28 (87.5%)	32
PTC	15 (93.75%)	14	01	01 (6.25%)	16
FVPTC	10 (83.3%)	08	02	02 (16.6%)	12

28/32 (87.5%) benign lesions showed less than 5% of cell stains from CK19 and are considered to be negative for CK19 expression. 93.75% of classic PTC and 83.3% of FVPTC showed >50% cells stain with CK19 and are considered to be diffusively positive for CK -19, while<5% cell stain is to be negative

HBME-1 Expression Benign Thyroid Lesions: Negative HBME-1 expression was observed in 26 out of 32cases (81.25%) of benign lesions which includes follicular adenomas, the remaining 6 out of 32 cases (18.7 5%) of follicular adenoma showed positive expression for HBME-1. All of the negative cases displayed HBME-1 expression in 50% of the Cells (score3).
Malignant Thyroid Lesions: Positive HBME-1 expression observed in 27 out of 28 malignant cases (96.4%) included 16 out of 16 cases of classic PTC (100%) and 11 out of 12 cases of FVPTC (91.6%). Negative HBME-1 expression was observed in 1 case out of 12 FVPTC (3.5%).

Table 6: HBME-1 Expression in Benign & Malignant Lesions

HPE Diagnosis	HBME-1 Expression				Total
	Positive (%)	Diffuse	Focal	Negative (%)	
Follicular adenoma	6/32 (18.75%)	01	05	26/32 (81.25%)	32
PTC	16/16(100%)	16	00	00	16
FVPTC	11	11	00	01	12

In the present study, 81.25% of follicular adenoma cases showed<5% cell stain with HBME-1 and they are considered negative for HBME-1 expression. 100% of classic PTC and 91.6% of FVPTC cases expressed HBME-1 in >50% cells and they are considered to be positive for HBME-1.
CK-19 shows a high statistically significant difference between papillary carcinoma of the thyroid and other benign thyroid nodules ($P < 0.00001$). There was also a high statistically significant difference between the Follicular variant of papillary carcinoma and follicular adenoma ($P < 0.00001$).

Table 7: Analysis of CK-19 in benign and malignant lesions

HPE	CK19		X^2	P
	Positive	Negative		
Benign	4	28	32.25	<0.00001*
Malignant	25	3		

* Significant
HBME-1 showed a high statistically significant difference between papillary carcinoma of the thyroid and other benign thyroid nodules ($P<0.000001$). There was also a high statistically significant difference between the Follicular variant of papillary carcinoma and follicular adenoma ($P<0.002$)

Table 8: Analysis of HBME-1 in benign and malignant lesions

HPE	HBME-1		X^2	P
	Positive	Negative		
BENIGN	6	26	36.4	<0.000001*
MALIGNANT	27	1		

* Significant

Discussion
Thyroid nodules are a very frequent finding, and their prevalence steadily increases with age. Incidence among the population is increased with the emergence of modern ultrasonographic (USG) techniques which can detect thyroid nodules of a few millimeters. There is a high female preponderance observed in the population. The microscopic distinction by conventional histology between benign and malignant lesions may be difficult. The "gold standard" in the

diagnosis of thyroid nodules is pathologic evaluation using routine hematoxylin and eosin (H&E) staining. [16] However, the morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPC) is common and is characterized by an almost exclusive follicular growth pattern and a set of nuclear features identical to those of the classic type of PTC. A diagnostic dilemma may arise when an encapsulated nodule with a follicular pattern of growth exhibits clear nuclei with grooves and so distinguishing follicular adenoma from encapsulated FVPTC becomes difficult. There are several other thyroid lesions that may contain papillary processes with nuclear features, which pose diagnostic difficulties with PTC. Multinodular goiter (MNG) with delicate papillary budding and focal nuclear clearing may be confused with PTC. Also, a new category emerged that was named Follicular Neoplasm/atypical cells of undetermined significance (AUS). [17] This category accounts for 10–25% of all cases and represents a therapeutic problem because of the low risk of malignancy. A growing number of promising immunohistochemical (IHC) markers for the differential diagnosis of thyroid neoplasms have emerged, including CD56 and p63, Hector Battifora mesothelial (HBME-1), and galectin-3 (Gal-3), CK19 however, till now none of them are conclusive. [18, 19] In the present study, we have evaluated the usefulness of CK19 and HBME-1 markers in differentiating benign and malignant thyroid lesions especially lesions with follicular patterned and papillary patterns with nuclear clearing and grooves. [20] The expression of CK-19 and HBME-1 is well documented in the published literature. In the present study, there is a female preponderance of 48 (80%) while males 20% (12%) with an F: M ratio of 4:1 which corresponds to the study conducted by Liu Z et al., [21] and Sanuvada R et al., [22] found the F: M ratio of 3:1 and 5:1 respectively. In the present study benign thyroid lesions i.e., follicular adenoma accounts for 32(53.3%) of cases, and malignant lesions account for 28(46.6%) of cases, of these 12(20%) belong to the follicular variant of PTC. No statistically significant difference was noted in age distribution between patients with PTC and those with benign thyroid neoplasm. This is consistent with the study done by Liu Z et al., [21] reported in their study 63.3% of PTC and its variants cases and 36.7% of cases of non-malignant lesions showed no significant difference in age between PTC and benign thyroid neoplasms.

CK-19 Expression in Thyroid Lesions: In our present study CK-19 showed strong and diffuse expression in malignant lesions with papillary and follicular features, and low or absent CK-19 expression was observed in benign neoplasm. Positive CK-19 expression was observed in 89.28% (25/28) of malignant neoplasms of which classic papillary thyroid neoplasm showed 95.8% (15/16) cases of positive expression, while a follicular variant of papillary thyroid carcinoma (FVPTC) showed 83.3% (10/12) positive expression. Among benign neoplasms, only 12.5% (4/32) cases showed positive expression. All positive cases showed strong CK-19 expression in >50% of the cells (score 3). Negative CK-19 expression was observed in 87.5% (28/32) cases of benign thyroid neoplasm i.e follicular adenoma, while 6.25% (1/16) cases of PTC and 16.6% (2/12) cases of FVPTC showed negative expression.

In terms of CK-19 expression in papillary thyroid carcinoma, the present study showed similar results to that of Liu Z et al., [21] found 93.7% (240 PTC cases) positivity while Sanuvada R et al., [22] showed positivity of 92.75% (48 PTC cases). The present study showed 89.28% positivity (28 PTC cases). In terms of CK-19 expression among FVPTC cases, the present study is in concordance with Noroozinia F et al., [23] study which showed positivity of 85% (46 FVPTC cases) while the present study showed positivity of 83.3% (12 FVPTC cases). Expression of CK-19 among follicular adenoma in the present study showed concordance with Sanuvada R et al., [22] study. The study showed positivity of 79.5% (65 FA cases) while the present study showed positivity of 87.5% (32 FA cases) CK-19 showing a high statistically

significant difference between papillary carcinoma of the thyroid and other benign thyroid nodules (P<0.00001). There was also a high statistically significant difference between the Follicular variant of papillary carcinoma and follicular adenoma (P<0.00001). The sensitivity of CK-19 in discrimination of malignant from benign thyroid lesions was 89.28% and specificity was 87.5% with a positive predictive value of 86.21%, Negative predictive value was found to be 90.32%. which is in concordance with the published literature. Showing closed resemblance with Sanuvada R et al., [22] which showed sensitivity and specificity of 84% and 95.7% respectively.

HBME-1 Expression in Thyroid Lesions: In our present study HBME-1 showed strong and diffuse expression in malignant lesions with papillary and follicular features, and low or absent CK-19 expression was observed in benign neoplasm. Positive HBME-1 expression was observed in 96.4% (27/28) of malignant neoplasms of which classic papillary thyroid neoplasm showed 100% (16/16) cases of positive expression, while a follicular variant of papillary thyroid carcinoma (FVPTC) showed 91.6% (11/12) positive expression. Among benign neoplasms, only 18.75% (6/32) cases showed positive expression. All positive cases showed strong CK-19 expression in >50% of the cells (score 3). Negative HBME-1 expression was observed in 81.25% (26/32) cases of benign thyroid neoplasm i.e follicular adenoma, while 3.5%% (1/12) cases of FVPTC showed negative expression. In terms of HBME-1 expression among PTC cases the present study showed concordance with Nasr MR et al., [24] and Zargari et al., [25] study. Nasr MR et al., [24] showed positivity of 96.5% (51 PTC cases) while Zargari et al., [25] showed a positivity of 96% (48 PTC cases). In the present study, the positivity is 96.4% (28 PTC cases). In terms of HBME-1 expression among FA cases, the present study is in concordance with Nasr MR et al., [24] study which showed negativity of 92.9% (57 FVPTC cases) while the present study showed negativity of 87.5% (32 FVPTC cases) HBME-1 showing high statistically significant difference between papillary carcinoma of the thyroid and other benign thyroid nodules (P < 0.000001). There was also a high statistically significant difference between the Follicular variant of papillary carcinoma and follicular adenoma (P< 0.002). The sensitivity of HBME-1 in discrimination of malignant from benign thyroid lesions was 91.6% and specificity was 87.5% with a positive predictive value of 81.2%, Negative predictive value was found to be 96.03% which is in concordance with the published literature. Showing closed resemblance with Nasr MR et al., [24] and Arcolia et al., [26] studies.

Conclusion

Conventional PTC may not pose a problem for diagnosis but its variants and its benign morphological mimics are challenging. Our study concurs with published literature to suggest strong expression of CK19 and HBME-1 in FVPTC and PTC. CK19 and HBME-1 are impressive positive markers for malignant lesions probably helping in the new category of encapsulated follicular pattern thyroid nodules. In our study, HBME-1 and CK-19 showed high positivity in papillary thyroid carcinoma and its follicular variant. However, strong positivity is seen in 2 cases of follicular adenoma cases for CK-19 and HBME-1. Reflecting on published literature, HBME, CK19, and GALECTIN-3 are sensitive and specific markers for papillary thyroid carcinomas. Therefore, a panel of HBME-1, CK-19 along with GALECTIN-3 could probably help to delineate lesions better. Extensive studies are required to validate the same.

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