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CYTOKERATIN 19 EXPRESSION IN DIAGNOSIS OF PAPILLARY THYROID CARCINOMAS

Pathology

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ABSTRACT

Papillary thyroid carcinoma (PTC) is the most common form of malignant thyroid neoplasm. Despite the propensity for lymphatic dissemination to cervical nodes, the majority of them have an excellent long term prognosis, hence accurate diagnosis plays a key role.[1] Diagnostic dilemma may arise when an encapsulated nodule with a follicular pattern of growth exhibits clear nuclei with grooves or darkly staining colloid and distinguishing follicular adenoma (FA) from encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) becomes difficult.[2]The distinction between these lesions is important because prognosis and management differ. Multinodular goitre (MNG) with delicate papillary budding and focal nuclear clearing may be confused with PTC.[3]The use of monoclonal antibodies for immunohistochemistry can improve diagnostic accuracy when combined with standard morphologic criteria. PTC have been shown to express CK19 with strong diffuse cytoplasmic reactivity. **SUMMARY:** CK19 expression is helpful in diagnosing PTCs where there are diagnostic dilemmas morphologically.

KEYWORDS

Papillary Thyroid Carcinoma , Cytokeratin 19 , Follicular Adenoma

INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common form of malignant thyroid neoplasm. Despite the propensity for lymphatic dissemination to cervical nodes, the majority of them have an excellent long term prognosis, hence accurate diagnosis plays a key role.[1] However, identification of these features remains, at times, difficult because of its focal presence and thus the distinction of PTC from other thyroid lesions like follicular adenoma may not be possible.

Diagnostic dilemma may arise when an encapsulated nodule with a follicular pattern of growth exhibits clear nuclei with grooves or darkly staining colloid and distinguishing follicular adenoma (FA) from encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) becomes difficult.[2]The distinction between these lesions is important because prognosis and management differ.

There are several other thyroid lesions that may contain papillary processes with nuclear features in a focal manner, which pose diagnostic difficulties with PTC. Multinodular goitre (MNG) with delicate papillary budding and focal nuclear clearing may be confused with PTC.[3]The use of monoclonal antibodies for immunohistochemistry can improve diagnostic accuracy when combined with standard morphologic criteria.

The use of monoclonal antibodies for immunohistochemistry can improve diagnostic accuracy when combined with standard morphologic criteria. Several immunohistochemical stains have been investigated for their possible role as diagnostic markers for PTC.They are cytokeratin19 (CK19), HBME1(anti-mesothelioma antibody), galectin-3, RET and thyroid transcription factor 1(TTF1).[1]PTC have been shown to express CK19 with strong diffuse cytoplasmic reactivity.

MATERIALAND METHODS:

A prospective study on thyroid neoplasms was conducted in the department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences (CAIMS) Karimnagar, over a period of two years . The data necessary for study has been retrieved from the histopathology records at the department. A total of 54 cases of thyroid neoplasms were studied in detail correlating the clinical and histopathological findings. The biopsy material was provided by the department of surgery, ENT and Oncology Institute, CAIMS Hospital. The type of specimens included total thyroidectomy specimens, hemi thyroidectomy specimens and lobectomy specimens. The applied nomenclature is adopted by the latest 2017 WHO classification of Endocrine tumours.

Multiple sections were studied from each tumour by paraffin embedding technique. The tissue has been fixed in 10% formalin and is processed in an automatic tissue processor and later paraffin embedding has been done. Sections of 4 to 5 µ thickness were cut on a Leica microtome and the routine stain which has been used for all the tumours was Harry's haematoxylin and Eosin. A detailed microscopic examination has been carried out.

Immunohistochemistry is done in all the cases. Cytokeratin polypeptide 19 (CK19) is a type I intermediate filament protein that is expressed in stratified and simple-type epithelia.

Primary Antibody
Name: Cytokeratin 19 (CK 19)
Clone: Mouse anti-human Cytokeratin 19 Monoclonal Antibody (Clone BA17)
Supplier: Master Diagnostica
Catalog Number: 2473-1Dilution: 1:100
Incubation Time/Temp: 60 min/room temperature

Antigen Retrieval
Device: IHC-TekTM Epitope Retrieval Microwave
Buffer/pH value: IHC-TekTM Epitope Retrieval Solution
Heat/Cool Temperature: 95 ºC/room temperature
Heat/Cool Time: 20 minutes/20 minutes

Detection Methods
Standard Method: Avidin-biotin complex Method
Enhanced Method: Polymeric Methods

Chromogen Substrate
Reagent: DAB(3,3′-Diaminobenzidine)
Incubation Time/Temperature: 1-3 minutes/room temperature

Counterstain
Reagent: Mayer's Hematoxylin
Staining Time: 30 seconds

RESULTS

Staining Pattern:Granular Cytoplasmic

Positive Control: Colon Carcinoma tissue
Negative Control: Omit primary antibody, isotype control, absorption control Blocking: 2-5% normal serum to reduce unspecific background staining; 0.5-3% H2O2 to block endogenous peroxidase activity; avidin/biotin to block endogenous biotin activity if necessary.

Cytokeratin19(CK 19) scoring[4]
Cytokeratin-19 shows cytoplasmic positivity.The intensity of staining of immunoreactive cells and their % distribution pattern was evaluated according to the following table.

Table 1- CK 19 Scoring for percentage of cells stained and for intensity of staining

Percentage of cells stained	Reaction	Intensity of Staining	Intensity score
0	No visible reaction	No staining	0
1-5	1+	Weak	1
>5-25	2+	Moderate	2
>25-75	3+	Strong	3
>75	4+	-	-

In terms of percentage of staining, 3+ or 4+ staining is considered positive stain and negative for 1+ or 2+. In terms of intensity of staining, 2+ or 3+ staining is considered as positive and negative for 0 and 1 score.

OBSERVATIONS AND RESULTS

The present study includes all the thyroid neoplasms that are reported in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar over a period of two years . A total of 54 cases were obtained.

Biopsy specimens included total thyroidectomy specimens and hemi thyroidectomy specimens. Total Thyroidectomy specimens constituted maximum in number in this study.

Table 2: Incidence of Variants of Papillary Thyroid Carcinoma

Sl No	Variants of Papillary Thyroid Carcinoma	No. of cases (%)
1	Conventional Papillary Thyroid Carcinoma	23 (70)
2	Follicular variant of Papillary Thyroid Carcinoma(FVPTC)	04 (12)
3	Encapsulated variant of Papillary Thyroid Carcinoma(EVPTC)	04 (12)
4	Papillary Microcarcinoma	02 (06)
5	Columnar variant of Papillary Thyroid Carcinoma	0
6	Oncocytic variant of Papillary Thyroid Carcinoma	0
Total		33 (100)

Table 3: Incidence of Cytokeratin 19 (CK 19) Immunoreactivity in Thyroid Neoplasms

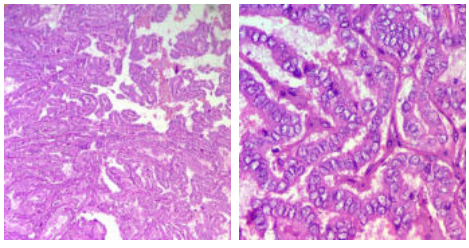
Sl.No	Lesions	CK 19 POSITIVE (%)	CK 19 NEGATIVE (%)	TOTAL (%)
1.	Papillary Thyroid Carcinoma	33 (61)	0 (0)	33 (61)
2.	Follicular Adenoma	01 (02)	09 (16)	10 (18)
3.	Nodular Colloid Goitre	01 (02)	10 (19)	11 (21)
Total	54	35 (65)	19 (35)	54 (100)

In this study, Papillary Thyroid Carcinomas showed 100% expression of Cytokeratin 19 whereas Follicular Adenomas and Nodular Colloid Goitre showed negative immunostaining with Cytokeratin 19 with an exception of one case each showing CK19 expression.

Table 4: Incidence of Thyroid Neoplasms in relation to Cytokeratin 19 scoring

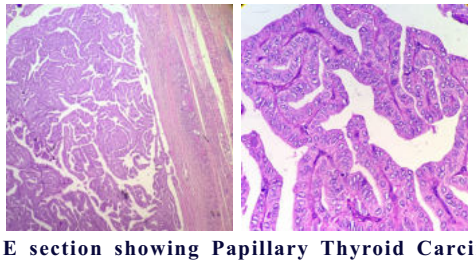
SLNo	Lesions	No. of Cases	Negative/ score 0	1+	2+	3+	4+	1+	2+	3+
1.	Papillary Thyroid Carcinoma	33	-	-	-	11	22	-	03	30
a)	Conventional Papillary Thyroid Carcinoma	23	-	-	-	03	20	-	03	20
b)	Follicular Variant of Papillary Thyroid Carcinoma	04	-	-	-	03	01	-	-	04
c)	Encapsulated Variant of Papillary Thyroid Carcinoma	04	-	-	-	03	01	-	-	04
d)	Papillary Microcarcinoma	02	-	-	-	02	-	-	-	02
2.	Follicular Adenoma	10	01	05	03	01	-	08	01	-
3.	Nodular Colloid Goitre	11	05	04	01	-	01	05	-	01
Total		54 (100 %)	06 (11%)	09 (17 %)	04 (07 %)	12 (22 %)	23 (43 %)	13 (24 %)	04 (07 %)	31 (58 %)

Negative scoring is seen in 11% of the cases which included Nodular Colloid Goitre cases. All the papillary thyroid carcinomas showed 3+ and 4+ scoring in percentage of cells with CK19 expression, whereas follicular adenomas showed only 1+ scoring. The intensity of CK19 expression in Papillary Thyroid Carcinomas is 3+. Few cases show 2+ scoring.



Conventional type of PTC showing papillary fronds with central fibrovascular cores and lined by optically clear nuclei showing nuclear grooves.

(H &E 10X ; 40X)



H & E section showing Papillary Thyroid Carcinoma encapsulated and composed of complex branching papillary fronds.

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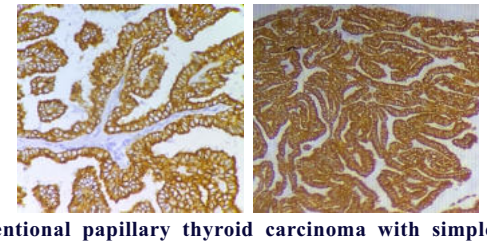
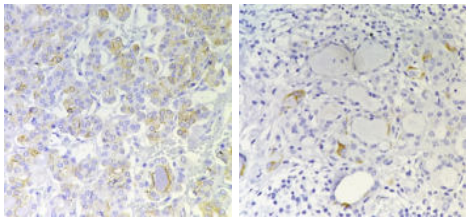


Coventional papillary thyroid carcinoma with simple and complex branching showing intense granular cytoplasmic positivity for Ck19.



Follicular adenoma showing intensity scoring 1+ and negative scoring for CK19 in the above photographs

DISCUSSION

Papillary thyroid cancer is the most frequently observed malignant tumour in the thyroid, accounting almost for 94% of all thyroid carcinoma. In general, the prognosis of PTC is favourable and ten-year survival rate for PTCs is greater than 90%.[5] However, about 20% of the differentiated thyroid cancer will present with metastasis.[6]So accurate biomarkers which can predict the aggressive behaviour of thyroid carcinoma is critical for clinical management.[7]

Thus, the diagnosis of Papillary Thyroid Carcinoma is very important. A common dilemma is encountered with tumours showing follicular growth pattern. The presence or absence of capsular or vascular invasion distinguishes benign from malignant follicular tumours, but identification of this finding can be challenging due to incomplete capsular invasion.[4]

Another situation encountered is when some of the nuclear features of Papillary thyroid carcinoma are present, in the absence of papillary architecture, distinguishing the follicular variants of papillary thyroid carcinoma from cellular adenomatous goitre may be difficult.

The histomorphologic diagnosis of thyroid neoplasm remains the cornerstone in the classification of thyroid follicular lesions. However, for those tumors that are poorly differentiated or undifferentiated, not follicular derived, and exhibit equivocal histomorphologic features, the application of immunohistochemical biomarkers may play an active or complementary role in their accurate classification.[8]

To overcome these difficulties, immunohistochemical markers play an important role for establishing the diagnosis.[9,10]Thyroid transcription factors (TTFs) TTF1, paired box gene 8 (PAX8), and TTF2 (FOXE1) are crucial for thyroid organogenesis and differentiation.[11-13] The transcription factors control the expression of thyroglobulin (TGB), thyroperoxidase (TPO), thyroid-stimulating hormone receptor, and thyroid iodine transporter.[14]Immunohistochemically, they serve as organ-specific immunomarkers.

Among the variety of biomarkers reported in the literature, Hector Battifora mesothelial–1 (HBME-1), galectin-3 (GAL3), cytokeratin 19 (CK19), Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain, 1 (CITED1), and TPO are most promising.

The combination of HBME-1, GAL-3, and CK19 is by far the most common panel evaluated by investigators, and their diffuse expression has not been reported in benign lesions. A distinct membranous staining pattern for trophoblastic cell surface antigen 2 (TROP2), a 35-kDa type 1 transmembranous glycoprotein, in PTCs is noted, in contrast, follicular neoplasms (FAs and FTCs) are nonreactive or showed only rare focal, weak cytoplasmic staining. TROP2 is a potential novel immunomarker for the identification of PTC that can be used in a panel to increase diagnostic accuracy when encountering a difficult follicular cell–derived lesion.[15]

Cytokeratin 19 is a low-molecular-weight cytokeratin found in a variety of simple or glandular epithelia, both normal and their neoplastic counterparts. In the thyroid gland, normal follicular epithelium usually has shown no detectable CK19 expression.[16] Many studies reported a strong and diffuse staining pattern of CK19 in PTC. The overall sensitivity of CK19 is 79.3% for malignancy, 82.2% for PTC, and 44.3% for FTC. The specificity is 63.1%. Overexpression of CK19 is a good indicator for PTC however, the sensitivity for follicular carcinoma is low.

Compared with the diffuse, strong reactivity in PTC, most studies indicated focal reactivity in benign lesions. Cytokeratin 19 may have added value as part of a panel of immunomarkers in the diagnosis of PTC.

In this study,Cytokeratin 19 expression in Papillary Thyroid Carcinoma, follicular adenomas with papillary like nuclear features and Nodular Colloid Goitre with papillary foci is evaluated.

CK19 belongs to the intermediate filaments it is normally expressed in ductal epithelium such as pancreas, bile ducts and so on, and has been applied to hepatocellular carcinomas, PTCs, squamous carcinomas and colorectal adenocarcinoma[17-20].

In 54 cases, CK19 have shown positivity in 35 cases (65%) which is similar to the study done by Scognamiglio et al.[21]47 cases (82.45%). In 21nonmalignant cases, CK19 is positive in 02 cases (9.5%), which is less when compared to Scognamiglio et al[21] study (79.16%).
CK19 showed positivity in 33 (100%) of 33 papillary carcinoma cases, higher than that of de Matos et al[22] (72.6). While de Matos et al.[22] study showed positivity in 8 (21%) of 38 follicular carcinoma cases.

In nonmalignant lesions, the present study showed lower positivity for FA 1 case (09%) and Nodular Colloid goitre 1 case (10%) when compared to de Matos et al[22] study, which showed 33.3% and 16.7%, respectively. In terms of intensity for malignant lesions, moderate or strong positivity is seen in 33 cases (65%) and weak stain is seen in 19 (35%) cases of non neoplastic lesions.

For 21 nonmalignant lesions, negative or weak staining was seen in 19 cases (90.4%), which is much higher than Scognamiglio et al[21] study (27.9%).

In the majority of PTC, we found strong and diffuse expression of CK19. Our results are in concordance with the previous studies related to CK19 expression in PTC. [23,24] Beesley and McLaren[25] point out (moderate/strong) positive CK19 immunoreactivity in all PTC cases (n = 26) and weak/absent immunostaining in the majority of benign lesions and follicular carcinoma.

Calangiu et al.[26] provided some new information on the usefulness of CK19 in differentiating the classical and follicular papillary carcinomas and the tall-cell variant of PTC. They observed higher CK19 immunostaining in the classical and follicular types, however, they did not analyze the correlation of CK19 immunostaining with the postsurgical course of the patients.

CONCLUSION:

According to our results, positive expression of the CK19 in Papillary Thyroid Carcinoma is related to the progression of disease.

CK19 may be useful predictor for the Papillary Thyroid Carcinoma progression because their expression is associated with the total tumour diameter of PTCs. CK19 seems to be useful in differentiating the follicular variant of PTC from FA and PTC from the papillary aspects in Nodular colloid Goitre. The presence of CK19 immunoreactive cells arise the suspicion of a Papillary Carcinoma, thus requiring a careful examination.

When it is difficult to diagnose PTMC (Papillary Microcarcinoma) by morphology alone, the utilization of CK19 and other markers combined with morphologic evaluation may be helpful to the differential diagnosis of PTMC.

We would conclude that immunohistochemical markers that include HBME1, GAL3, CK19, and CITED1 are helpful in the diagnosis of PTC and its variants. Strong expression of 2 or more markers supports the diagnosis of PTC, particularly if HBME1 is one of the positive antibodies.

Negative staining with 3 or 4 antibodies, in contrast, strongly supports the diagnosis of FA. Using these antibodies can have a confirmatory role in distinguishing FVPTC and FA, being most helpful in preventing overdiagnosis and underdiagnosis of FVPTC.

In conclusion, although no single immunohistochemical marker by itself is completely sensitive and specific for papillary thyroid carcinoma, the combination of HBME1 and CK19 attains high sensitivity and specificity. It is important to remember that HBME1 must stain the basolateral membrane in order to be considered positive.

When this criterion is strictly applied, positive HBME1 staining is highly specific for Papillary thyroid carcinoma. Although CK19 staining is common to both benign thyroids and Papillary thyroid carcinoma, a negative stain is good evidence against Papillary thyroid carcinoma.

CONFLICTS OF INTEREST: NONE

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