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Pathology

ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF PAPILLARY CARCINOMA THYROID

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ABSTRACT

Introduction : Papillary thyroid carcinoma is the most common thyroid neoplasm.
Histopathological diagnosis is the gold standard and the diagnosis is based on the characteristic nuclear features. Focal presence of nuclear features in certain benign lesions and absence of papillary architecture in the variants of papillary carcinoma leads to diagnostic difficulties. The aim of this study is to assess the expression of Immunohistochemical markers in the variants of papillary thyroid carcinoma.

Materials and Method : 30 cases of papillary thyroid carcinoma diagnosed in our institution were selected . Histo-morphological features were analysed and Immunostaining with Cytokeratin 19, HBME 1 and Ki 67 was done in the representative blocks. The results of Cytokeratin 19 and HBME 1were analysed based on the semi-quantitative scoring. Ki 67 results were analysed based on the labeling index.

Results: All the cases (100%) showed positivity for Cytokeratin 19 and 93.3% cases showed positivity for HBME 1. In 31.8% cases, the surrounding non neoplastic thyroid tissue also showed positivity for Cytokeratin 19 but it was focal and weak. Ki 67 labeling index of diffuse sclerosing and columnar cell variants was compared with that of the classical variant. The two variants showed higher labeling index compared to the classical variant.

Conclusion : Immunohistochemistry aids in improving the diagnostic accuracy and assessment of prognosis of the variants.Use of a panel of markers is helpful because the sensitivity and specificity of different markers varies and no single marker is highly sensitive or specific for the diagnosis of papillary carcinoma thyroid.

KEYWORDS : Papillary thyroid carcinoma, Cytokeratin 19, HBME 1, Ki 67, Immunohistochemistry.

INTRODUCTION

Thyroid cancer is the most common malignancy occurring in the endocrine organs.Carcinomas arising from the follicular epithelial cells of thyroid accounts for about more than 95% of cases[1]. The most common well differentiated tumor arising from the follicular epithelial cells is papillary carcinoma thyroid[1, 3]. Papillary carcinoma thyroid constitutes about 80% of all thyroid malignancies[4]. Papillary thyroid carcinoma arises at any age and rarely diagnosed as a congenital tumor. Most common presentation is in the third to fifth decade. The male: female ratio is 1:2 to1:4. They tend to be biologically indolent and have an excellent prognosis. The survival rates are more than 95%. The variants of papillary carcinoma are Conventional, Follicular, Encapsulated, Papillary microcarcinoma, Tall cell, Diffuse sclerosing, Oncocytic, Columnar cell, Solid , Clear cell, Cribriform morular, Macrofollicular, PTC with prominent hobnail features, PTC with fasciitis-like stroma, Combined papillary and medullary carcinoma, PTC with dedifferenatiation to anaplastic carcinoma[6].

Papillary carcinoma thyroid can be diagnosed histopathologically by the presence of distinct architecture and nuclear features such as nuclear clearing, overlapping, nuclear grooves and pseudoinclusions[4,5] . The histopathological diagnosis of papillary carcinoma thyroid is easier in typical cases. Difficulty in diagnosis arises when there is only focal presence of nuclear features or in the absence of typical architecture such as in follicular variant which is the second common variant next to classical type[4].Thyroid lesions such as multinodular goiter with adenomatoid hyperplasia have papillary architecture with focal nuclear features and adenomas such as hyalinising trabecular adenoma present with nuclear features similar to papillary carcinoma thyroid [5].

The approach to these challenging lesions should include ancillary techniques such as immunohistochemistry, which improves the standard of diagnosis. The proposed diagnostic markers for papillary carcinoma thyroid include high-molecular-weight cytokeratins , cytokeratin 7, vimentin, CD57, thyroid transcription factor-1, galectin-3, thyroglobulin, cytokeratin 19, epithelial membrane antigen, CA19-9, S-100, HBME-1, Ki 67, progesterone receptor protein, estrogen receptor protein, cytokeratin 20, chromogranin A and RET [7]. Of these the most commonly used markers are cytokeratin 19, HBME-1, galectin-3, RET, fibronectin and thyroid transcription factor-1.

MATERIALS AND METHODS

Our study was conducted in the Department of Pathology, KAPV Government Medical college, Trichy. A total of 30 cases diagnosed as papillary carcinoma thyroid were included in the study.

The specimens received were fixed in 10% buffered formalin and sections were taken from appropriate sites. After processing and embedding in paraffin wax, sections of about 3-5 µm were cut and stained with hematoxylin and eosin. Cases of papillary carcinoma thyroid diagnosed by histopathological examination were selected and representative blocks were taken.

Age,sex,gross and microscopic features of the selected cases were recorded. Immunostaining of the sections taken from the representative block was done with CK 19, HBME1 and Ki 67.

CK 19 immunostaining was done using monoclonal mouse antibody – DAKO, clone RCK 108, isotype IgG_1 kappa. Normal colonic tissue was taken as positive control .For negative control, one of the sections was treated with buffer instead of primary antibody.

HBME 1 immunostaining was done using monoclonal mouse antibody – DAKO, clone HBME 1, isotype IgM kappa. Normal peritoneal tissue was taken as positive control .One of the sections treated with buffer instead of primary antibody was used as negative control.

Ki 67 immunostaining was done using monoclonal mouse antibody – BIOGENEX, clone BGX-Ki 67, isotype IgG_1 kappa.

Semi –quantitative scoring was done based on the percentage of positively stained cells. The results were tabulated and statistically analysed.

Table 1:Semi - quantitative scoring for CK19 and HBME45[5].

% of positively stained cells %	Score
Nil	0 (absent)
Less than 5	1 +
5 – 25	2 +
25 – 75	3 +
> 75	4 +

RESULTS:

The gross and microscopic features were recorded. Immunohistochemical study with three markers was performed and the results were analysed.

The most common variant observed in this study was the classical variant (19 cases, 63.33%) and the next common variant was follicular variant (6 cases 20%)

Immunohistochemical findings:-

The three IHC markers done in this study were cytokeratin 19, HBME-1 and Ki 67. A panel of markers is usually suggested because no single marker is specific or sensitive for identification of papillary thyroid carcinoma.

Cytokeratin 19 Expression :

CK 19 expression was seen in all cases (30cases, 100%) of papillary carcinoma thyroid and a semiquantitative scoring of CK 19 expression based on the percentage of positively stained cells was done. The staining pattern was predominantly cytoplasmic. The classical variant showed 4+ positivity (Fig.1) in 16 cases (84.21%), 3 + positivity in 2 cases (10.52%) and 2 + positivity in 1 case (5.26%). In the follicular variant 3 cases (50%) showed 4 + positivity, 2 cases (33.33%) showed 3 + positivity and only 1 case (16.6%) showed 2 + positivity. The columnar cell variant (1 case) showed 3 + positivity. All other variants showed 4 + positivity. All the cases showed diffuse cytoplasmic positivity. Cytokeratin expression was also seen in the adjacent benign thyroid lesions which were associated with papillary carcinoma thyroid. But the positivity was only focal, cytoplasmic and number of positively stained cells were less than 25%. None of the lesions showed diffuse positivity.

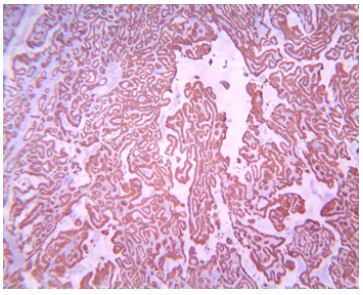

Figure 1: Photomicrograph shows CK 19 Immunostaining – diffuse and 4+ positivity.

HBME 1 EXPRESSION:

HBME-1 expression was analysed in all the cases. Membranous staining was considered positive. The results were analysed according to the semi-quantitative score. The classical variant showed 4+ positivity (Fig.2) in 11 cases(57.89%), 3+ positivity in 3 cases(15.78%) and 2+ positivity in 4 cases (21.055%). The stain was found negative in one case (5.26%). In the follicular variant, 1 case (16.66%) showed 2+ positivity, 1 case (16.66%) showed 3+ positivity and 3 cases (50%) showed 4+ positivity. One case in this variant was negative. The intracystic variant showed 3+ positivity in 1case and 2+ positivity in 1 case. All other variants showed 4+ positivity.

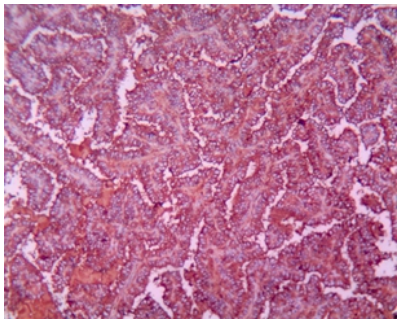

Figure 2: Photomicrograph shows HBME 1 expression- 4+ positivity in the classical variant

KI 67 EXPRESSION :

The expression of Ki 67 in the variants was analysed. Granular nuclear staining was considered positive. The results were analysed according to the labeling index. The labeling index was calculated based on the number of positively stained cells per 100 follicular cells. The labeling index was higher in the diffuse sclerosing variant and columnar cell variant (3.6 and 3.2 respectively) compared to the other variants (Fig.3).

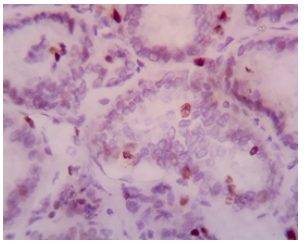

Figure 29: Photomicrograph shows Ki 67 expression- granular nuclear positivity in the diffuse sclerosing cell variant

CK19 showed positive immunostaining in all cases (100%) of papillary carcinoma thyroid including its variants.CK 19 was found positive in 2 cases of colloid goiter,one case of follicular adenoma and 3 cases of follicular hyperplasia. The positivity was only focal with a 1+ positivity in all the cases. HBME 1 was found negative in all the associated benign lesions.

The sensitivity and specificity of the two immunomarkers were calculated based on their expression in papillary carcinoma thyroid and the associated benign lesions. CK 19 showed a 100% sensitivity and 68.18% specificity. The sensitivity and specificity of HBME 1 was 93.33% and 100% respectively.

DISCUSSION

Immunohistochemistry aids in the differentiation between benign and malignant lesions and identification of the variants with different architectural features. CK 19 and HBME1 expression in the cases of papillary carcinoma thyroid was analysed. Ki 67 expression was analysed in certain variants such as diffuse sclerosing and columnar cell, which are found to have an aggressive behavior.[8]

CK 19 EXPRESSION :

CK19 shows a strong diffuse cytoplasmic reactivity in papillary thyroid carcinomas [3]. CK 19 expression is seen in paraffin embedded blocks, but it can also be demonstrated in cytological smears and cell block preparations[9, 10]. 100% of papillary carcinomas show uniform reactivity for CK 19.Eventhough it is expressed in follicular carcinomas and other benign conditions, the extent and intensity of CK19 staining is greater in papillary carcinomas[7].

CK19 is also useful for the diagnosis of papillary carcinoma in FNAC, especially in doubtful cases.The sensitivity and specificity of CK19 ,if used singly is as high as 92% and 97%, respectively[1]. CK19 is of limited value as a marker for routine histopathologic diagnosis but positivity for this marker should raise the suspicion of papillary carcinoma[7].CK 19 negativity is a good evidence against papillary thyroid carcinoma[5].

The present study showed strong and diffuse positivity in all the cases of papillary thyroid carcinoma.

MR Nasr et al (2006)[4] studied the expression of CK 19 immunostain in 51 cases of papillary thyroid carcinoma and observed diffuse positivity in 100% cases of the classical variant and 90% cases of the follicular variant. The study concluded that strong and diffuse expression of CK19 raises the suspicion of papillary thyroid carcinoma and negative staining is a strong evidence against papillary thyroid carcinoma.

Bose et al (2012)[5] did a study in 22 cases of papillary thyroid carcinoma including classical variant, follicular variant and diffuse sclerosing variant. 4 + positivity was seen in 84.62% cases of classical variant and 62.50% of cases of follicular variant. The present study showed 4 + positivity in 84.21% of cases of classical variant , which correlated with the above study.

Scognamiglio et al (2006)[11] studied the expression of CK19 in 49 cases of papillary thyroid carcinoma and diffuse positivity was seen in 100% cases of classical variant and 90% cases of follicular variant. The study concluded that CK 19 was the most sensitive marker among the four markers (CK19, HBME1, Galectin 3 and CITED 1) studied.

The expression of CK 19 in the associated benign lesions were also studied. MR Nasr et al (2006)[4] showed CK19 positivity in 68% of benign lesions including adenomatous nodule and Hashimoto's thyroiditis, but the positivity was weak. In the study by Y.J. Park et al (2007)[12], 9.3% cases of adenomatous nodule showed positivity. Haltas et al (2013)[13] showed focal staining of CK 19 in 29.41% cases. In all the studies the staining pattern was focal and weak. The present study showed focal and weak immunostaining in 31.8% cases.

The sensitivity and specificity of CK 19 was calculated based on the expression in papillary carcinoma and adjacent benign lesions. MR Nasr et al (2006)[4] showed a sensitivity of 100% and specificity of 32%. In the study by Y.J. Park et al (2007)[12], the sensitivity and specificity was 90.3% and 83.1% respectively. Scognamiglio et al (2006)[11] showed a sensitivity of 96% and specificity of 86%. In all the above studies, the sensitivity was higher than specificity. Our study showed a sensitivity of 100% which correlated with that of MR Nasr et al (2006)[4].

Even though non- neoplastic lesions show positive immunostaining for CK 19 , they can be differentiated from papillary thyroid carcinoma by the presence of strong and diffuse positivity in the latter.

HBME1 EXPRESSION:

Hector Battifora Mesothelial (Cell) 1 is a monoclonal antibody. It recognizes an unidentified antigen in the microvilli of mesothelioma cells. The antigen is also expressed in normal tracheal epithelium, and adenocarcinoma of the lung, pancreas, and breast [1]. HBME-1 is a marker of mesothelial cells. It is also an important marker of follicular origin in thyroid tissues and shows greater affinity for malignant lesions[14]. HBME-1 shows positivity in follicle derived malignant thyroid tumors ,both well differentiated and poorly differentiated tumors[1].

HBME-1 is mainly useful in well differentiated thyroid follicular epithelial tumors such as papillary carcinoma, follicular carcinoma and hurthle cell carcinoma. It shows a membranous staining pattern and cytoplasmic positivity may be variable[3]. Positivity to HBME-1 is indicative of malignancy, but it does not necessarily indicate papillary differentiation [3].

Hector Battifora mesothelial 1 expression was seen in 93.3% cases of papillary thyroid carcinoma in the present study. Among the variants, 94.7% cases of classical variant and 83.3% cases of follicular variant showed membranous positivity with variable cytoplasmic positivity.

MR Nasr et al (2006)[4] showed a positivity in 96% cases of papillary thyroid carcinoma. In a study by Scognamiglio et al (2006) [11] , 88% cases of classical variant and 86% cases of follicular variant showed membranous positivity. The study concluded that the most specific marker among the four markers (CK19, HBME1, Galectin 3 and CITED 1) was HBME 1. The present study had a similar positivity rate compared to MR Nasr et al (2006)[4] . The percentage of positivity in follicular variant was comparable with that of Scognamiglio et al (2006)[11] .

In a study by Y.J. Park et al (2007)[12], HBME 1 positivity was seen in 91.7% cases of classical variant and 94.1% cases of follicular variant. ML Prasad et al (2005)[15] studied 44 cases of classical variant and 13 cases of follicular variant for HBME1 expression and found 85% and 93% positivity respectively.

The sensitivity and specificity of HBME1 was calculated based on the expression in papillary carcinoma and associated benign lesions. MR Nasr et al (2006)[4] showed a sensitivity of 96% and specificity of 93%. In the study by Y.J. Park et al (2007)[12], the sensitivity and specificity was 91.3% and 68.5% respectively. Scognamiglio et al (2006)[11] showed a sensitivity of 87% and specificity of 96%. Our study showed a specificity of 100%.

Ki 67 Expression :

Ki 67 expression showed higher labeling index in diffuse sclerosing variant and columnar cell variant in our study. Lloyd et al [16] showed a marked increase in Ki 67 labeling index in the columnar cell variant compared to other variants and this study correlated with our study. Thompson et al[17] showed the presence of higher Ki 67 labeling index in 2 of the cases of diffuse sclerosing variant in their study. Our study also showed similar results.

The present study showed positivity for both CK19 and HBME1 in all the variants but the intensity of positivity was varied. The positivity was 100% for CK19, but diffuse 4+ positivity was seen in 84.21% cases of classical variant, 50% cases of follicular variant, 100% cases of intracystic, diffuse sclerosing and encapsulated variants. The columnar cell variant showed only 3+ positivity for CK 19. Membranous positivity was seen for HBME1. About 57.89% cases of classical variant, 50% cases of follicular variant, 50% cases of intracystic variant, 100% cases of diffuse sclerosing , encapsulated and columnar cell variants showed 4+ membranous positivity. Ki 67 expression showed higher labeling index in diffuse sclerosing variant and columnar cell variant than the other variants.

CONCLUSION:

Papillary thyroid carcinoma is usually diagnosed by the presence of the typical nuclear features. It has a number of variants with different morphological features and there is inter and intraobserver variation in the diagnosis of these variants. Immunohistochemistry is useful when there is a difficulty in the diagnosis of tumors with questionable nuclear features. No single marker is 100% sensitive or specific for papillary thyroid carcinoma. A panel of markers is usually very useful. CK19 expression confirms the presence of papillary differentiation and it is the most sensitive marker according to many studies. It is not specific as it is positive in certain benign lesions. The diffuse, strong staining pattern confirms the diagnosis of papillary thyroid carcinoma whereas benign lesions show focal and weak staining pattern. HBME1 is another immunomarker which selectively stains malignant lesions and it is negative in benign lesions. HBME 1 is the most specific marker for papillary thyroid carcinoma confirmed by many studies. Therefore, a combined immunostaining with CK19 and HBME1 increases the diagnostic accuracy in papillary thyroid carcinoma. Ki 67 expression confirms the aggressive behavior of the variants.

Papillary carcinoma thyroid has an excellent prognosis compared to other thyroid malignancies. Eventhough histopathology remains the gold standard for the diagnosis of papillary carcinoma thyroid, controversy arises in cases which have questionable nuclear features and absence of papillary architecture. Immunohistochemistry acts as an ancillary tool and improves the diagnostic accuracy, thereby helping the clinicians to render an appropriate treatment.

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