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ORIGINAL RESEARCH

Diagnostic Utility of CD56, CK19 and p63 in Differentiation of Papillary Carcinoma Thyroid from Follicular Neoplasms

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ABSTRACT

Background: Papillary thyroid carcinoma is the commonest malignancy of thyroid. The diagnosis of such lesions can be easily rendered by using standard histological criteria. However, distinguishing the follicular variant of papillary carcinomafrom other follicular neoplasmsis difficult**. Objectives:** In this study we investigated the role of CD56, CK19 and p63 in differentiating papillary carcinoma thyroid from follicular neoplasms.

Materials and Methods: The immunohistochemical expression of CD56, CK19 and p63 was evaluated individually and in combinations in 30 samples of thyroid lesions. Thyroid lesions included: 26 Papillary carcinoma thyroid [classic papillary carcinoma (21) and follicular variant of papillary carcinoma thyroid (5)] and 4 cases of follicular carcinoma**.**

Results: CD56 was found to be 100% sensitive and 84.62% specific in differentiating papillary thyroid carcinoma from follicular neoplasms. p63 was 50% sensitive and 57.69% specific in differentiating papillary thyroid carcinoma from follicular carcinoma.CD56 and CK19 were more specific than p63 in differentiation of Papillary carcinoma thyroid from follicular neoplasms. (p=0.039,0.001 respectively).Combination of all the three markers showed sensitivity and specificity of 100% and 53.85% respectively.

Conclusion: Combined utility of CD56, CK19 and p63 is helpful in diagnosing papillary thyroid carcinoma including follicular variant and it's differentiation from follicular carcinoma**.**

Keywords: Papillary thyroid carcinoma, follicular carcinoma, CD56, CK19 and p63.

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INTRODUCTION

Papillary carcinoma thyroid is the most commonmalignancy arising in thyroid gland comprising approximately 80% ofthyroid epithelial malignancies.[1] The "gold standard" in the diagnosis of thyroid nodule is histologic evaluation using routine hemotoxylinand eosin (H&E) staining. However, morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPTC) causes diagnostic difficulties and significant inter observer variability amongst the pathologists. Diagnostic dilemma may arise when an encapsulated nodule with a follicular growth pattern exhibits clear nuclei with

grooves and so distinguishing follicular adenoma from encapsulated FVPTC becomes difficult.[2] Several immunohistochemical markers have been investigated such as CD56, CK19, p63, galectin-3, HBME-1, CK903, CITED1, CD44, CD57 to aid in the diagnosis of these problematic cases. Up till now there is noconsensus about an immunohistochemical panel that would reliably overcome such diagnostic obstacles.

CD56 is a cell surface glycoprotein, neural cell adhesion molecule (NCAM) constitutionally expressed in normal thyroid follicular cells and regulates cell motility hence its expression may affect the migratory capacity of tumor cells.[3] Decreased expression of CD56 has frequently been found in malignant thyroid tumours, especially in papillary thyroidcarcinoma.[4]

Cytokeratin polypeptide 19 (CK19) is a type 1 intermediate filament protein synthesised in simple and stratified epithelia.[1] It is useful in the diagnosis of papillary carcinoma, with its strong and diffuse cytoplasmic staining patternand more heterogeneous expression in follicular carcinoma.[5,6,7]

p63 is a p53 homologuenuclear transcription factor encode proteins which transactivateson p53 activity and inducts cells into apoptosis. P63 is consistently expressed inmyoepithelial cells, squamous and basal cells such as in myoepithelial cells of the breast, squamous cell carcinoma and basal cells of the prostatic ducts and acini.[8]

In the current study, we evaluated the expression and diagnostic utility of CD56, CK19 and p63 in distinguishing papillary thyroid carcinomas including the follicular variant from other follicular mimics.

MATERIALS & METHODS

This was a cross sectional observational study conducted in a tertiary care institute in NEW DELHI, India between year 2017-2019. Thyroidectomy specimens were subjected to histopathological examination. The clinical data were obtained from medical files and informed consent were taken. Ethics permission was obtained from hospital ethics committee.

Only cases diagnosed as Papillary carcinoma thyroid, follicular variant of papillary thyroid carcinoma and follicular carcinoma were included. Rest of the thyroid lesions were excluded from the study.

For diagnosis of these lesions, standard histological criteria proposed by WHO were followed i.e presence of papillae and characteristic nuclear features including nuclear enlargement, nuclear overlapping, ground glass nuclei, nuclear pseudo inclusion, prominent longitudinal groove. Follicular carcinoma was diagnosed by presence of capsular and/or vascular invasion.FVPTC ,now classified as NIFTP (non-invasive follicular thyroid neoplasm with papillary like nuclear features) was diagnosed by the following features,(1)encapsulation or clear demarcation (2) follicular growth pattern with(<1% papillae, nopsammoma bodies,<30% solid/trabecular/insular growth pattern) (3) nuclear features of papillary carcinoma (4)no lymphovascular or capsular invasion (5) no tumor necrosis (6) no high mitotic activity(<3 mitosis/10HPF).

The immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections using standard streptavidin-biotin peroxidase complex methods. The slides prepared were stained using antibodies against CD56 (monoclonal, 123C3. D5, Bio SB), CK19(monoclonal, RCK108, Biogenex) and p63 (monoclonal, 4A4, Biocare). A positive membranous and/or cytoplasmic positivity in >10% of the tumor cells qualified the case as positive for CD56 and CK19.Any nuclear p63 staining was accounted as positive expression of p63.

RESULTS

There was a total of thirty cases included in the study of which maximum number of cases were in the second decade(30%,9/30) with M:F of 1:1.5.The mean age for follicular carcinoma, papillary thyroid carcinoma and follicular variant of papillary carcinoma thyroid was 43.5 years,34.76 years and 24.6 years respectively.70% (21/30) cases had classic papillary thyroid carcinoma,16.67% (5/30) cases had follicular variant of papillary carcinoma thyroid and 13.33% (4/30) cases had follicular carcinoma.

Among the PTC,86% (18/21) cases were CD56 negative,100% (21/21) cases were CK19 positive and 52.38% (11/21) cases were p63 positive. Among the FVPTC,80% (4/5) cases were CD56 negative,100% (5/5) cases were CK19 positive and 100% (5/5) cases were p63 negative.

Among the FC,100% (4/4) cases were positive for CD56 ,75% (3/4) cases were positive for CK 19 and 50% (2/4) cases were positive for p63.

Expression of CK19 was noted in 100% (26/26) cases of PTC,75% (3/4) cases of follicular carcinoma .CK19 was found to be 100 % specific for differentiation of papillary carcinoma thyroid from follicular carcinoma.

84.6% (22/26) cases of PTC including FVPTC did not express CD56.In contrast, 100% cases of follicular thyroid carcinoma (4/4) were positive for CD56.Sensitivity of CD56 in differentiating papillary thyroid carcinoma from follicular neoplasm was found to be100%, while specificity was 84.62%.

Positive nuclear staining of p63 was noted in42.30%cases (11/26) of PTC and 50% (2/4) cases of follicular carcinoma. p63wasfound to be 50% sensitive and 57.69% specific in differentiating papillary thyroid carcinoma from follicular carcinoma.CD56 and CK19 were found to be more specific than p63 in differentiation of PTC from follicular neoplasms. (p=0.039,0.001 respectively).

Application of combined markers CD56+CK19 showed positive expression in all cases of follicular carcinoma. (4/4).Only one case of FVPTC showed positivity.(1/5).Coexpression of CD56+CK19 was significantly higher in follicular carcinoma compared to FVPTC with a significant p value of 0.016.Coexpressionof CK19+ p63 did not show any significant difference in PTC and non PTC lesion(p value 0.073). Coexpression of CD56+ p63 showed significant difference in PTC and non-PTC lesion with p value of 0.044. Combined expression of CD56, CK19 and p63is significantly higher in PTC compared to non-PTC lesion with a p value of 0.044.

100% sensitivity was seen with combined expression of CD56 +CK19, CD56+p 63, CD56+ CK19 +p63 for differentiation of PTC from non-PTC lesion and maximum specificity of 84.62% was seen with combined expression of CD56 +CK19 for differentiating the two lesions. Combination of all the three markers showed sensitivity and specificity of 100% and 53.85% respectively.

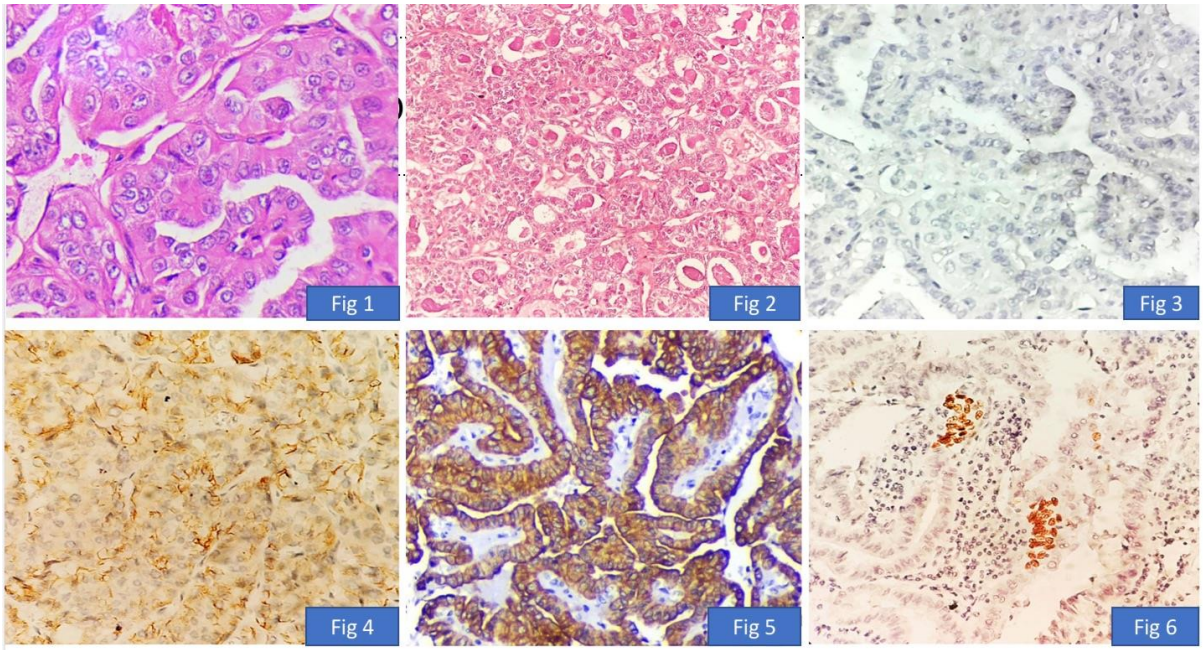

Figure 1: H&E(400X), PTC showing nuclear grooves and inclusions.
Figure 2: H&E (200X), Follicular carcinoma, tumor cells are arranged in follicles.
Figure 3: Negative expression of CD 56 in papillary carcinoma thyroid.
Figure 4: Membranous staining of CD 56 in follicular carcinoma.
Figure 5: Diffuse cytoplasmic positivity of CK 19 in PTC.
Figure 6: Nuclear positivity of p63 in PTC.

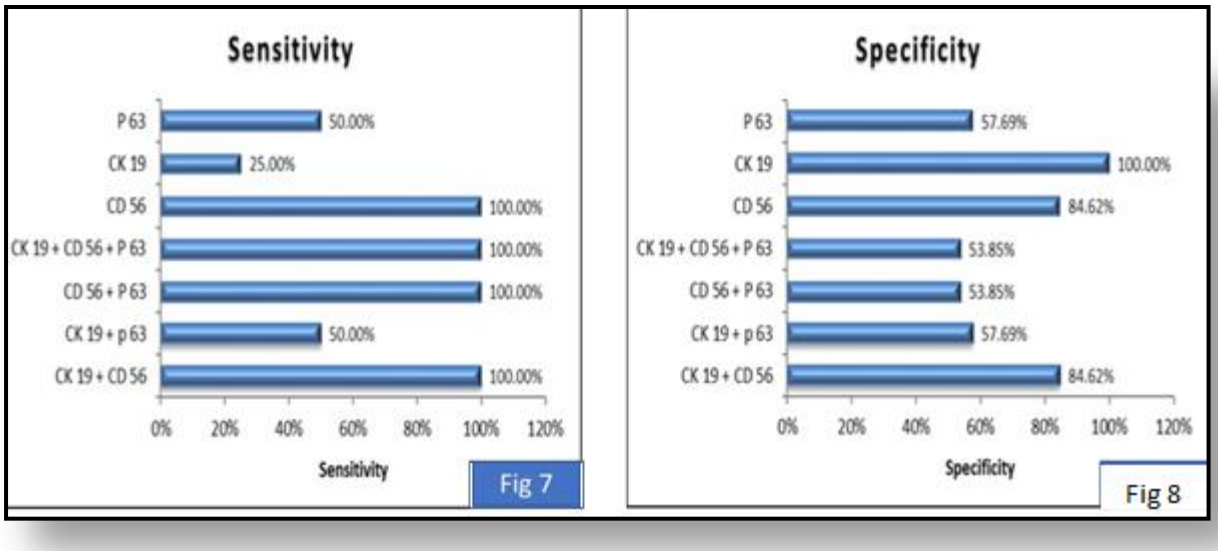

Figure 7: Sensitivity of the individual and combination of markers in papillary carcinoma thyroid vs Non-PTC.
Figure 8: Specificity of the individual and combination of markers in papillary carcinoma thyroid vs Non-PTC.

DISCUSSION

Differentiating follicular variants of PTC from follicular neoplasm is challenging due to morphologic overlap. Although immunohistochemistry is generally accepted as a useful ancillary technique in the diagnosis of papillary thyroid carcinoma, controversy exists

regarding the best stain or combination of stains to distinguish papillary thyroid carcinoma from its mimics. Our study confirms the usefulness of CD56, CK19, p63and suggests that the combination of CD56 and CK19 attains a high sensitivity and specificity for the diagnosis of papillary thyroid carcinoma.CK19, a low molecular weight cytokeratin has diffuse and strong expression in Papillary Carcinoma but it is also focally expressed in normal thyroid epithelium , follicular adenoma and follicular carcinoma.[9,10,11,12,13]

All cases of papillary carcinoma thyroid showed positivity for CK19. Similar results were found by Nasr MR et al and Alshenawy et al.[1,14] 75% cases of follicular carcinoma thyroid were positive for CK19. Similar results were elucidated by Saleh et al.[15] However, None of the cases of follicular carcinoma thyroid showed positive expression in the study conducted by Cheung et al.[6]

In the present study CK19was found to be more specific (100%) but less sensitive (25%) in differentiating papillary carcinoma thyroid from follicular carcinoma whereas it was more sensitive (100%) and less specific (25%) in differentiating FVPTC from follicular carcinoma. Similar results were described by Alshenawy et al.[1] In contrast Sahoo et alreportedthat CK19 expression patterns are not reliable for the distinction between papillary carcinomas and follicular neoplasms.[16]

CD56 is an antigen involved in follicular epithelium differentiation.[17] Loss of CD56 expression was correlated with metastatic potential and poor prognosis in some malignant lesions.[18] 86% cases of classic PTC and 80% cases of FVPTC did not express CD56.In contrast 100% follicular carcinoma were positive for CD56. El Demellawy et al showed 90.3% cases of papillary carcinoma and 9.1% cases of follicular carcinoma with negative expression of CD56.[2] Park et al reported negative CD56 expression in all or most of their studied papillary carcinoma cases and also observed that CD56 expression was more reduced in Papillary Carcinomas with respect to Follicular Carcinomas and Follicular Adenomas.[19] Result of the present study are in concordance with these observations. However, Scarpino et al found significant higher expression of CD56 in63.15% cases of classic papillary thyroid carcinoma and 90% cases of FVPTC.[20]

El Demellawy et al suggested that CD56 is of great value in selecting papillary carcinoma from other follicular neoplasms with 100% sensitivity and 100% specificity.[4] Adela et al suggested, CD56 as sensitive marker (81.1%) and less specific (63.6%).[9] In present study it was found to be 100%sensitive and 84% specific.

P63 isoforms are consistently expressed in basal/stem cells of several types of ectodermally derived multi-layered epithelia and appear to be an effective way to overcome p53 driven cell cycle arrest and apoptosis.[21] Very few studies of p63 expression in papillary carcinoma thyroid have been reported in literature till date which showed less than 50% positivity of p 63 in PTC as was also seen in the present study.

Dina El Demellaey et al showed maximum expression of p63 in cases of papillary thyroid carcinoma (70%).[2] Ji Yung et al showed specificity of 99.2% and sensitivity of only14.7%.[22] In the present study specificity was 57.69% and sensitivity was 50% in differentiation of PTC from follicular neoplasm.

By using various combinations of markers, we achieved higher sensitivity and specificity for differentiation of PTC from follicular neoplasms. Specificity of combined expression of markers CD56 + CK19 was 84.62% which was comparable with the study of Alshenawy et al.[1] In the present study specificity of combined expression of markers CD56 + CK19 for differentiation of FVPTC from follicular neoplasms was 100% .Similar results have been demonstrated by Alshenawy et al.[1] Sensitivity and specificity of combined expression of markers p63+ CD56 and CD56+ p63+ CK19 in differentiating papillary carcinoma thyroid from follicular neoplasms was 100%,80%,100% and 53.85% respectively. No such combination has been studied previously.

CONCLUSION

Lack of CD56 expression in PTC is consistent, sensitive and specific. p63 is less sensitive and specific for diagnosis of PTC. Using an immunohistochemical panel of CD56, CK19 and p63 in diagnosis of PTC, particularly follicular variant is extremely useful.

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Conflict of Interest

The authors declare no conflict of interest.

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