# Assessment of CD56, HBME-1 and cytokeratin 19 expressions in papillary thyroid carcinoma and benign thyroid nodule

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**Abstract**

Neoplasms of thyroid are the commonest type of the endocrine neoplasms across the globe. Papillary thyroid carcinoma (PTC) constitutes about 80% of thyroid neoplasms. Morphological overlapping is quiet common between follicular patterned thyroid neoplasms. This study was a cross-sectional analysis carried out over a period of two years and Immunohistochemical staining was carried out on all 50 cases. Our study showed 96.67% of PTC group had diffuse and strong staining with HBME-1, and the staining was not observed in 86.67% of benign lesions. There is a significant difference between PTC group and benign group for the HBME-1 staining. The loss of CD56 expression was observed in 83.33% of malignant lesions. In benign lesions, staining prevalence of HBME-1, CK19, and CD56 was 13.33%, 20%, and 100% respectively.

## Introduction

Thyroid nodules are significantly common worldwide and are usually detected during routine clinical examination. Among adult populations the prevalence of palpable thyroid nodules constitute 4–7%.[1] Neoplasms of thyroid are the commonest type of the endocrine neoplasms and it constitute about 1% of overall malignancies. Among the malignant neoplasms of thyroid, Papillary thyroid carcinoma (PTC) constitutes about 80%.[2] In the current scenario, histopathologic evaluation of thyroid using hematoxylin and eosin (H&E) staining is the “gold standard” diagnostic tool for detecting thyroid neoplasms. However, morphological overlapping is quiet common between follicular lesions and follicular variant papillary thyroid carcinoma (FVPTC). Consequently, there are cases in which histopathological criteria do not allow the differentiation between benign and malignant follicular-patterned thyroid lesions, making the distinction between these two groups quite subtle and challenging [3]

Immunohistochemical Markers such as Hector Battifora Mesothelial Cell-1 (HBME-1), Galectin-3 (Gal-3) and Cytokeratin-19 (CK19) have been used recently in the diagnosis of thyroid pathology. Although a wide range of sensibility and specificity values of these markers has been reported by different studies along the time.[4-6] none of those studies demonstrated conclusive results.

Similarly, other studies shown that loss of CD56 expression in malignant thyroid lesions.[6,7] CD56 is a newly reported, “promising” marker in thyroid pathology that is expressed in natural killer cells, activated T-lymphocytes, and neural and muscle tissue, but to present date the literature data is few and inconsistent. [8,9]

Our aim was to study the applicability of CD56, HBME-1, and CK19 in discriminating the PTC including the follicular variant from other follicular thyroid lesions and neoplasms. Although there were similar studies on this subject, but many of theses had demonstrated inconclusive or conflicting results. We aimed to support the literature with our results and to make a contribution to the routine practice.

## MATERIALS & METHODS

This was a cross-sectional study included 50 specimens of surgically removed, formalin-fixed, and paraffin-embedded thyroid lesions that were received at the Department of pathology tertiary care hospital, Kerala for over a period of two years from August 2015 to July 2017.

### Immunohistochemistry

All 50 samples were subjected to immunohistochemical (IHC) staining with HBME-1 (Clone: HBME-1, Thermo Scientific®), CK19 (Clone: A53-B/A2.26 Thermo Scientific®), and CD56 (clone: 123C3.D5 Thermo Scientific®) antibodies. The paraffin-embedded tissue sections were deparaffinized by one-night incubation in the oven at 56°C and waiting in xylene. Tissue sections were rehydrated through absolute alcohol. Antigen retrieval in citrate buffer was used after the sections were treated in a microwave 3 times for 5 min, and the sections were then left to cool for 20 min. Respectively, sections were incubated in tris-buffered saline (TBS) 10 min, 3% hydrogen peroxide 20 min, TBS 10 min. Primary antibodies for HBME-1, CK19, and CD56 were applied to sections and incubated for 30 min. This was followed by the secondary biotin-conjugated antibody (Biotinylated Goat antiserum) for 20 min and finally, the peroxidase-conjugated streptavidin for another 20 min. 3-amino-9-ethylcarbazole chromogen was added for 25–35 min and then counterstained with Harris hematoxylin followed by dehydration, clearing, and mounting

### Interpretation of immunohistochemical staining of CD56, HBME-1, and cytokeratin 19

The results of immunohistochemical staining were evaluated by two independent observers semiquantitatively. For all three antibodies, percentage and severity of staining were assessed. HBME-1 and CK19 expressions were scored as follows: 0, staining in <5% of the cells; 1, staining in 5–30% of the cells; 2, staining in 31–69% of the cells; 3, staining in >70%

of the cells. CD56 expression was scored as follows: 0, staining in <10% of the cells; 1, staining in 11–25% of the cells; 2, staining in 26–50% of the cells; 3, staining in >50% of the cells.

**Statistical analysis**

Data were analyzed using the SPSS program version 21 (IBM®). Descriptive statistics for the evaluation of results have been shown in the form of mean; the nominal variables have been shown as the number of cases and percentage (%). Comparison of qualitative variables between groups was carried out using the Chi-square test. A $P = 0.05$ was chosen as the level of significance.

**Results**

The study consisted of 30 cases (60%) of PTC, which included 26 cases (86.67%) of CPTC and four cases (13.33%) of FVPTC, and 15 cases (30%) of follicular adenomas ([Table 1]. The ages of the patients were between 24 and 76 years, and the arithmetic mean of age for neoplastic lesions 43.38 (SD 12.97), arithmetic mean of age for benign and malignant cases were found be 38.8 (SD±7.57) and 45.34 (SD±14.34) respectively.

About 84% of the patients ($n = 42$) were female and 16% of the patients ($n = 8$) were male. Among both benign and malignant groups, female gender showed higher proportion, but there was no significant difference between the two groups in terms of gender.

About 30.77% (n=8) and 19.23% (n=5) of CPTC cases showed multifocality and extrathyroidal extension respectively. [table 2]

**Immunohistochemical results**

The percentage and severity of staining for HBME-1, CK19, and CD56 was shown in Table 1&2. In PTC cases, +3 staining percentages for HBME-1, CK19, and CD56 were 76.67 %, 86.66 %, and 3.33%, respectively.

Assessment of CD56 staining in the 30 PTC cases showed negative CD56 expression in 25 cases (83.34%). All of the benign cases showed positive staining with CD56. About 86.66% (n=13) of these cases had +3 staining [Table 1].

Between benign and malignant groups, there found a significant difference for percentages of HBME-1, CK19, and CD56 staining with Chi-square test ($P < 0.001$). The percentages of HBME-1, CK19, and CD56 staining for subtypes of lesions are shown in Table 2.

**Table 1: Expression of HBME-1, CK 19 and CD56 in papillary thyroid carcinoma and benign lesion**

| | 0 (%) | +1 (%) | +2 (%) | +3 (%) | Total (%) | |
|---|---|---|---|---|---|---|
| **HBME-1** | | | | | | |
| PTC | **3.33** | **10** | **10** | **76.67** | 100 | <0.001 |
| Benign | **86.66** | **6.67** | **6.67** | **0** | 100 | |
| **CK19** | | | | | | |
| PTC | **0** | **6.67** | **6.67** | **86.66** | 100 | <0.001 |
| Benign | **80** | **13.33** | **6.67** | **0** | 100 | |
| **CD56** | | | | | | |
| PTC | **83.34** | **10** | **3.33** | **3.33** | 100 | <0.001 |
| Benign | **0** | **6.67** | **6.67** | **86.66** | 100 | |

PTC = Papillary thyroid carcinoma; CK19 = Cytokeratin 19

**Table 2: Expression of HBME-1, CK19 and CD56 in subtypes of Papillary Thyroid Carcinoma**

| | 0 (%) | +1 (%) | +2 (%) | +3 (%) | Total (%) |
|---|---|---|---|---|---|
| **HBME-1** | | | | | |
| CPTC (n=26) | **3.85** | 7.69 | 7.69 | **80.77** | 100 |
| FVPTC (n=4) | **0** | 25 | **25** | **50** | 100 |
| **CK19** | | | | | |
| CPTC (n=26) | **0** | **3.85** | **3.85** | **92.3** | 100 |
| FVPTC (n=4) | **0** | **25** | **25** | **50** | 100 |
| **CD56** | | | | | |
| CPTC (n=26) | 88.46 | **7.69** | **3.85** | **0** | 100 |
| FVPTC (n=4) | 50 | **25** | **0** | **25** | 100 |

CPTC= Classical Papillary Thyroid Carcinoma, FVPTC= Follicular Variant of Papillary Thyroid Carcinoma

## DISCUSSION

The most important tool which determines the biological behaviour of thyroid nodules is routine pathological examination. However, follicular pattern can be seen in both benign and malignant lesions. Furthermore, few nuclear features of papillary carcinoma can be seen in benign lesions. All these observations lead to serious differences in the evaluation of the same lesion between different pathologists.[3] The diagnostic concordance rates were found high in

papillary and anaplastic carcinomas but low in FVPTC and FC. These facts indicate the need for further diagnostic immunohistochemical markers in the differential diagnosis of thyroid tumors (TTs) and many studies have been made.[3,10-13]

Nasr *et al.* in their study on immunohistochemical markers on thyroid lesions, had found that HBME-1 staining was observed 96% of the malignant cases and staining was not observed in 93% of benign lesions.[13] similarly, our study showed 96.67% (n=29) of PTC group had diffuse and strong staining with HBME-1, and the staining was not observed in 86.67% (n=13) of benign lesions. There is a significant difference between PTC group and benign group for the HBME-1 staining ($P < 0.01$).[12]

In the current study, in PTC group, the percentage of positive staining for HBME-1 and CK19 was 96.67% (n=29) and 100% (n=30) respectively. The loss of CD56 expression was observed in 83.33% (n=25) of malignant lesions. In benign lesions, staining prevalence of HBME-1, CK19, and CD56 was 13.33% (n=2), 20% (n=3), and 100% (n=15), respectively. According to these findings, the most specific marker was CD56 and the least specific marker was CK19 for distinguishing benign and malignant lesions. HBME-1 was found more specific than CK19 for PTC.

Study done by Scarpino S *et al*, to determine the expression of CD56 in PTC had shown that CD56 staining was not seen in 18 out of 61 PTC cases and focal weak staining was observed in 43 cases. In all PTC cases, absence or decrease in expression of CD56 was observed with PCR. CD56 expression was seen in FA and normal thyroid tissue.[17] Similarly, in our study, CD56 staining was not seen in 83.33% (n=25) out of 30 PTC cases, focal weak staining in 10% (n=3) cases, and strong staining only in 6.67% (n=2) cases. In all benign lesions, there was positive CD56 staining with different rates 86.66%, 6.67% and 6.67% corresponding to +3, +2 and +1 respectively.[14]

Park *et al*. in his study on CD56, galectin-3, and CK19 immunohistochemically in thyroid carcinomas showed that there was no staining with CD56 in 92.5% of PTC cases. They found strong staining (3+) only in one case. Staining percentages of CD56 for FA and NH cases were 93.3% and 90.5%, respectively. For this study, the most specific marker was CD56 in comparison with CK19 and galectin-3.[15] Our study findings were in concordance with these findings.

## Conclusion

In our study, we found that positive staining of HBME-1, CK19, and loosing expression of CD56 support malignancy. However, in Follicular Adenoma we observed strong CD56 staining by immunohistochemically. In the majority of PTC cases, CD56 was negative or there was a loss of expression in various degrees. Eventually, we suggested that CD56 is a helpful antibody for the differential diagnosis of benign and malignant thyroid lesions and may increase the diagnostic accuracy when used with HBME-1 and CK19.

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