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DIAGNOSTIC UTILITY OF HBME1 AND CD56 IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PAPILLARY THYROID CARCINOMA FROM NODULAR COLLOID GOITRE WITH HYPERPLASIA

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ABSTRACT
Differentiating papillary thyroid carcinoma (PTC) from nodular colloid goitre with hyperplasia may be difficult when characteristic nuclear features are equivocal. This study evaluated HBME1 and CD56 immunohistochemical expression in PTC and its variants and assessed their diagnostic utility. A retrospective and prospective study was conducted at the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, from August 2018 to August 2020. Fifty thyroidectomy specimens were selected, comprising 33 PTCs and variants and 17 nodular colloid goitres with hyperplasia. HBME1 and CD56 were assessed immunohistochemically using predefined scoring systems. HBME1 was positive in 32/33 PTCs (97.0%) and 1/17 nodular hyperplasia cases (5.9%). Its sensitivity, specificity and diagnostic accuracy were 97.0%, 94.1% and 96.0%, respectively. CD56 was negative in 27/33 PTCs (81.8%) and positive in all 17 nodular hyperplasia cases. Its sensitivity, specificity and diagnostic accuracy were 81.8%, 100% and 88.0%, respectively. HBME1 showed moderate or strong positivity in 87% of PTCs. HBME1 demonstrated greater sensitivity, whereas CD56 demonstrated greater specificity for distinguishing PTC from nodular hyperplasia. These findings support the use of HBME1 and CD56 as ancillary immunohistochemical markers in diagnostically difficult thyroid lesions.

KEYWORDS: Papillary thyroid carcinoma; HBME1; CD56; Immunohistochemistry; Nodular hyperplasia; Thyroid

INTRODUCTION
Thyroid lesions comprise a broad spectrum of non-neoplastic, benign and malignant conditions. In the present dissertation, the majority of thyroid specimens received during the study period were non-neoplastic, while papillary thyroid carcinoma constituted the predominant malignant thyroid neoplasm. The predominance of papillary carcinoma among malignant thyroid lesions has also been reported in previous studies.[1,2]

Papillary thyroid carcinoma is primarily diagnosed on histomorphological grounds, particularly by recognition of characteristic nuclear changes. However, differentiation from nodular colloid goitre with hyperplasia can be difficult when the nuclear features are incomplete or questionable. In such situations, ancillary immunohistochemistry may improve diagnostic confidence.[3,4]

CD56 has been investigated as a diagnostic marker in thyroid lesions. Studies cited in the dissertation have demonstrated reduced or absent CD56 expression in PTC compared with non-neoplastic thyroid lesions, although its reported sensitivity and specificity have varied.[5,6]

HBME1 has also been evaluated extensively in thyroid lesions. It demonstrates predominantly membranous staining with variable cytoplasmic positivity and has been reported to show increased expression in malignant thyroid lesions. Its diagnostic performance, however, has varied among studies.[7–10]

Other studies have examined the utility of HBME1 and CD56 individually or in combination for differentiating PTC from benign or hyperplastic thyroid lesions.[11–13]

The present study was undertaken to evaluate the histomorphological features of PTC and its variants, determine HBME1 and CD56 immunohistochemical expression, and assess the usefulness of these markers in differentiating PTC from nodular colloid goitre with hyperplasia.

MATERIAL AND METHODS
Study design and setting
This was a prospective and retrospective study conducted at the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, from August 2018 to August 2020. During the study period, 559 thyroid specimens were received for histopathological evaluation.

Ethical clearance was obtained from the Ethical Committee of Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. The dissertation does not provide an ethics approval number.

Study population

Of the 559 thyroid specimens, 493 were total thyroidectomies, 51 were hemithyroidectomies, 11 were completion thyroidectomies and 4 were trucut biopsy specimens.

For the immunohistochemical study, 50 thyroidectomy specimens were evaluated, comprising:

- 33 cases of PTC and its variants
- 17 cases of nodular colloid goitre with hyperplasia.

Clinical information including age, sex, clinical history, thyroid function assays, ultrasound/imaging findings and cytological findings was obtained.

Histopathological evaluation

Histopathological diagnosis was based on examination of hematoxylin and eosin-stained sections. PTC was diagnosed based on its characteristic nuclear morphology. The dissertation evaluated conventional PTC and its morphological variants.

Immunohistochemistry

Immunohistochemical analysis for HBME1 and CD56 was performed in the 50 selected cases. Slides were evaluated and scored, and the results were compared with histopathological parameters.

HBME1 scoring

HBME1 staining was evaluated according to the percentage of positively stained tumour cells and staining intensity. Extent was scored from 0 to 4 according to the percentage of positive cells, while staining intensity was categorized as weak, moderate or strong. The final immunohistochemical score was obtained by multiplying the extent and intensity scores. Scores of 0, 1–4, 5–8 and 9–12 were categorized as negative, weakly positive, moderately positive and strongly positive, respectively.

CD56 scoring

CD56 positivity was assessed based on membranous staining, with or without cytoplasmic staining, according to the percentage of positively stained tumour cells. A score of 0 represented staining in <10% of cells, 1 represented 10–25%, 2 represented 26–50% and 3 represented >50% of cells.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 23.0. Descriptive statistics, frequency and percentage analyses were used for categorical variables, while mean and standard deviation were used for continuous variables. ROC curve analysis was used to evaluate the diagnostic efficacy of HBME1 and CD56. Pearson's chi-square test was used to determine statistical significance for categorical data. A P value of 0.05 was considered statistically significant.

RESULTS

Distribution of thyroid lesions

During the 24-month study period, 559 thyroid specimens were received. Of these, 80.0% were non-neoplastic, 3.5% were benign and 16.5% were malignant.

Table 1. Distribution of thyroid specimens according to lesion category

Category	Number	Percentage
Non-neoplastic	448	80.0
Benign	19	3.5
Malignant	92	16.5
Total	559	100

Among malignant thyroid neoplasms, PTC was the predominant tumour, accounting for 80.4% of malignant lesions.

Table 2. Distribution of malignant thyroid neoplasms

Malignant lesion	Number	Percentage
Papillary carcinoma	74	80.4
Follicular carcinoma	11	12.0
Medullary carcinoma	3	3.2
Poorly differentiated carcinoma	2	2.2
Anaplastic carcinoma	2	2.2
Total	92	100

Age and sex distribution
The age of patients with PTC ranged from 17 to 76 years. The highest frequency was observed in the 31–40-year age group. There was a female predominance.

Histological variants
The conventional variant was the most frequent subtype, accounting for 73% of PTCs. Representative histomorphological and immunohistochemical findings of conventional PTC are shown in Figure 1. The follicular variant accounted for approximately 12% of PTCs. Representative histomorphological and immunohistochemical findings of follicular variant papillary thyroid carcinoma are shown in Figure 2.

Table 3. Histological variants of PTC

Variant	Number	Percentage
Conventional	54	73.0
Follicular variant	9	12.1
Papillary microcarcinoma	5	6.7
Oncocytic variant	2	2.7
Tall-cell variant	2	2.7
Warthin-like variant	1	1.4
Columnar-cell variant	1	1.4
Total	74	100

Metastasis
Among the 74 PTC cases, 33 showed cervical lymph-node positivity and two cases had lung metastasis.
Among the 33 PTC cases included in the IHC study, 18 had nodal metastasis. Of these, 10 showed strong HBME1 positivity, 7 showed moderate positivity and 1 showed weak positivity.

HBME1 expression
Among 33 PTC cases, 32 (97.0%) were HBME1-positive and one (3.0%) was negative. Among the 17 nodular hyperplasia cases, 16 (94.1%) were negative and one (5.9%) was positive.

Table 4. HBME1 expression in PTC and nodular hyperplasia

HBME1 expression	PTC n (%)	Nodular hyperplasia n (%)
Positive	32 (97.0)	1 (5.9)
Negative	1 (3.0)	16 (94.1)
Total	33 (100)	17 (100)

Moderate or strong HBME1 positivity was observed in 87% of PTC cases.
Among the PTC variants, all conventional PTC, follicular variant PTC, papillary microcarcinoma, oncocytic, tall-cell and Warthin-like variant cases evaluated were positive. The single columnar-cell variant was negative.
ROC analysis for HBME1 demonstrated an area under the curve of 0.955 with P = 0.0005.
The sensitivity was 97.0% and specificity 94.1%.

CD56 expression
CD56 was negative in 27/33 PTC cases (81.8%) and positive in 6/33 cases (18.2%). All 17 nodular hyperplasia cases were CD56-positive.

Table 5. CD56 expression in PTC and nodular hyperplasia

CD56 expression	PTC n (%)	Nodular hyperplasia n (%)
Positive	6 (18.2)	17 (100)
Negative	27 (81.8)	0
Total	33 (100)	17 (100)

CD56 demonstrated a sensitivity of 81.8% and specificity of 100% for differentiating PTC from nodular colloid goitre with hyperplasia.

Table 6. Diagnostic performance of HBME1 and CD56

Parameter	HBME1	CD56
Sensitivity	97.0%	81.8%
Specificity	94.1%	100%
Diagnostic accuracy	96.0%	88.0%
AUC	0.955	[NOT AVAILABLE IN DISSERTATION RESULT TABLE USED HERE]

DISCUSSION

The present study evaluated the diagnostic utility of HBME1 and CD56 immunohistochemistry in differentiating papillary thyroid carcinoma (PTC) from nodular colloid goitre with hyperplasia. The study included 33 PTC cases and 17 cases of nodular hyperplasia for immunohistochemical evaluation. HBME1 demonstrated high sensitivity, whereas CD56 demonstrated high specificity in distinguishing PTC from nodular hyperplasia.

In the present study, 80.0% of the 559 thyroid specimens were non-neoplastic, 3.5% were benign and 16.5% were malignant. The predominance of non-neoplastic thyroid lesions was comparable with findings reported by Tsegaye et al. and Rahman et al.[1,2]

Papillary thyroid carcinoma constituted 80.4% of the malignant thyroid neoplasms in the present study. This predominance is comparable with observations reported by Hussain et al. and Gorva et al.[13,14] The conventional variant was the most frequent subtype, accounting for 73% of PTCs, followed by the follicular variant. Variations in the distribution of PTC subtypes have been reported in different series, including those of Girardi et al. and Muzaffar et al.[15,16]

HBME1 expression

HBME1 was positive in 32 of 33 PTC cases (97.0%), while only one of the 17 nodular hyperplasia cases (5.9%) showed positivity. Moderate or strong HBME1 expression was observed in 87% of PTC cases. The high frequency of HBME1 expression in PTC observed in the present study is consistent with findings reported by de Matos et al. and Erdogan-Durmus et al.[7,17]

The low frequency of HBME1 positivity in nodular hyperplasia is also in keeping with observations from previous studies. Liu et al. reported significant HBME1 expression in PTC compared with benign thyroid lesions.[8] Prasad et al. demonstrated HBME1 expression in 85% of PTCs, with only limited expression in non-neoplastic thyroid lesions.[18]

Park et al. evaluated HBME1 together with other immunohistochemical markers in thyroid nodules and demonstrated its diagnostic usefulness in differentiating malignant from benign thyroid lesions.[19] The high sensitivity of HBME1 in the present study further supports its usefulness as an ancillary marker in diagnostically challenging thyroid lesions.

The present study demonstrated a sensitivity of 97.0% and specificity of 94.1% for HBME1. Nasr et al. reported HBME1 positivity in 49 of 51 PTCs, with positivity in only a small proportion of benign thyroid lesions.[21] These findings are comparable with the high diagnostic performance observed in the present study.

However, HBME1 expression is not completely restricted to malignant thyroid lesions. Muthusamy and Shah and Palo and Biligi reported HBME1 expression in benign as well as malignant thyroid lesions.[20,22] Therefore, HBME1 staining should be interpreted in conjunction with histomorphological features rather than being used as an isolated diagnostic criterion.

Zargari and Mokhtari also evaluated HBME1 as an immunohistochemical marker in thyroid carcinoma and demonstrated its diagnostic utility.[23] Mataraci et al. reported differences in HBME1 expression between neoplastic and non-neoplastic thyroid lesions.[24] Saleh et al. similarly demonstrated HBME1 positivity in a proportion of hyperplastic and benign thyroid lesions.[25] These findings emphasize that the staining pattern and morphological context are important when interpreting HBME1 immunoreactivity.

In the present study, all conventional PTCs, follicular variant PTCs, papillary microcarcinomas, oncocytic variants, tall-cell variants and the Warthin-like variant evaluated were HBME1-positive. The single columnar-cell variant included in the study was negative. Since only one columnar-cell variant was evaluated, this finding cannot be generalized to this tumour variant.

CD56 expression

CD56 demonstrated a contrasting immunohistochemical pattern. It was negative in 27 of 33 PTC cases (81.8%), whereas all 17 cases of nodular hyperplasia were positive. The reduced or absent CD56 expression observed in PTC is consistent with previous studies evaluating CD56 as a diagnostic marker in thyroid lesions.[3,5]

Shin et al. demonstrated the diagnostic usefulness of CD56 in differentiating PTC from non-neoplastic thyroid lesions.[3] Similarly, El Demellawy et al. evaluated CD56 along with other immunohistochemical markers and reported its usefulness in the diagnosis of PTC.[5]

Park et al. reported decreased CD56 expression in PTC, supporting its potential role in distinguishing PTC from benign thyroid lesions.[11] Golu et al. also reported that absence of CD56 expression may help differentiate PTC from other thyroid lesions.[12]

In the present study, CD56 demonstrated a sensitivity of 81.8% and specificity of 100%. Thus, CD56 positivity strongly favoured nodular hyperplasia in the present series, while CD56 negativity was observed in the majority of PTC cases. The sensitivity was lower than that observed for HBME1, but the specificity was higher.

The variation in CD56 expression reported across different studies indicates that its diagnostic performance may vary according to the case selection and morphological spectrum of the lesions studied. Muthusamy and Shah also evaluated CD56 expression in benign and malignant thyroid lesions and demonstrated its potential diagnostic value.[20]

Diagnostic significance of HBME1 and CD56

The two markers demonstrated complementary immunohistochemical patterns in the present study. HBME1 showed greater sensitivity, while CD56 showed greater specificity for distinguishing PTC from nodular hyperplasia.

HBME1 was positive in 97.0% of PTC cases compared with 5.9% of nodular hyperplasia cases. In contrast, CD56 was negative in 81.8% of PTC cases and positive in all nodular hyperplasia cases. These findings indicate that HBME1 and CD56 provide complementary diagnostic information.

The combined assessment of HBME1 and CD56 may therefore be useful in diagnostically difficult thyroid lesions, particularly when the histomorphological features are equivocal. However, a separate combined-marker contingency analysis was not performed in the present study; therefore, combined sensitivity, specificity or diagnostic accuracy cannot be calculated from the available data.

HBME1 expression and nodal metastasis

Among the 33 PTC cases included in the immunohistochemical study, 18 had nodal metastasis. Of these, 10 cases showed strong HBME1 positivity, seven showed moderate positivity and one showed weak positivity. This distribution suggests that HBME1 expression was frequently observed among PTC cases with nodal metastasis. However, the available analysis does not establish an independent statistical association between HBME1 staining intensity and nodal metastasis. Therefore, the present findings should be regarded as descriptive rather than prognostic.

Limitations

The study was conducted in a tertiary care centre and the cases were selected from thyroid specimens received during the study period. Therefore, the findings may not represent the prevalence or distribution of thyroid lesions in the general population. The relatively small number of cases included in the immunohistochemical analysis, particularly the individual morphological variants of PTC, also limits the generalizability of subtype-specific observations. Interpretation of HBME1 and CD56 should be performed together with conventional histomorphological assessment, as neither marker demonstrates absolute sensitivity or specificity. The present findings support their role as ancillary immunohistochemical markers rather than substitutes for morphological diagnosis.

CONCLUSION

Histopathology remains the principal diagnostic method for papillary thyroid carcinoma, but diagnostically difficult cases may require ancillary immunohistochemistry. In the present study, HBME1 demonstrated high sensitivity and specificity, while CD56 demonstrated high specificity with lower sensitivity. HBME1 was positive in 97.0% of PTCs and 5.9% of nodular hyperplasia cases, whereas CD56 was negative in 81.8% of PTCs and positive in all nodular hyperplasia cases. Thus, HBME1 was the more sensitive marker and CD56 the more specific marker in this study. The findings support the use of HBME1 and CD56 as complementary ancillary immunohistochemical markers in the differential diagnosis of PTC and nodular colloid goitre with hyperplasia.

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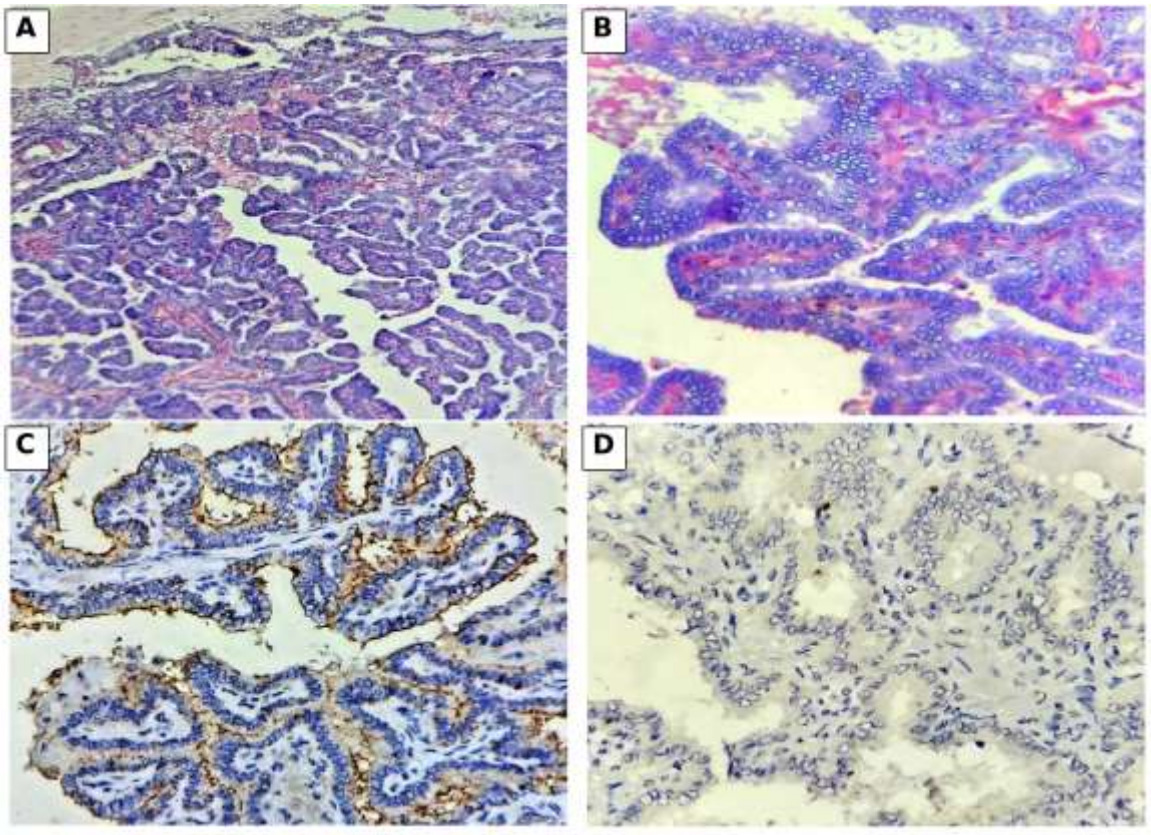

Figure 1. Papillary thyroid carcinoma (PTC)

Representative case of conventional papillary thyroid carcinoma. (A) H&E, 10× showing papillary architecture; (B) H&E, 40× showing the papillary tumour morphology; (C) HBME1 immunohistochemistry, 40× showing positive staining; (D) CD56 immunohistochemistry, 40× showing negative staining.

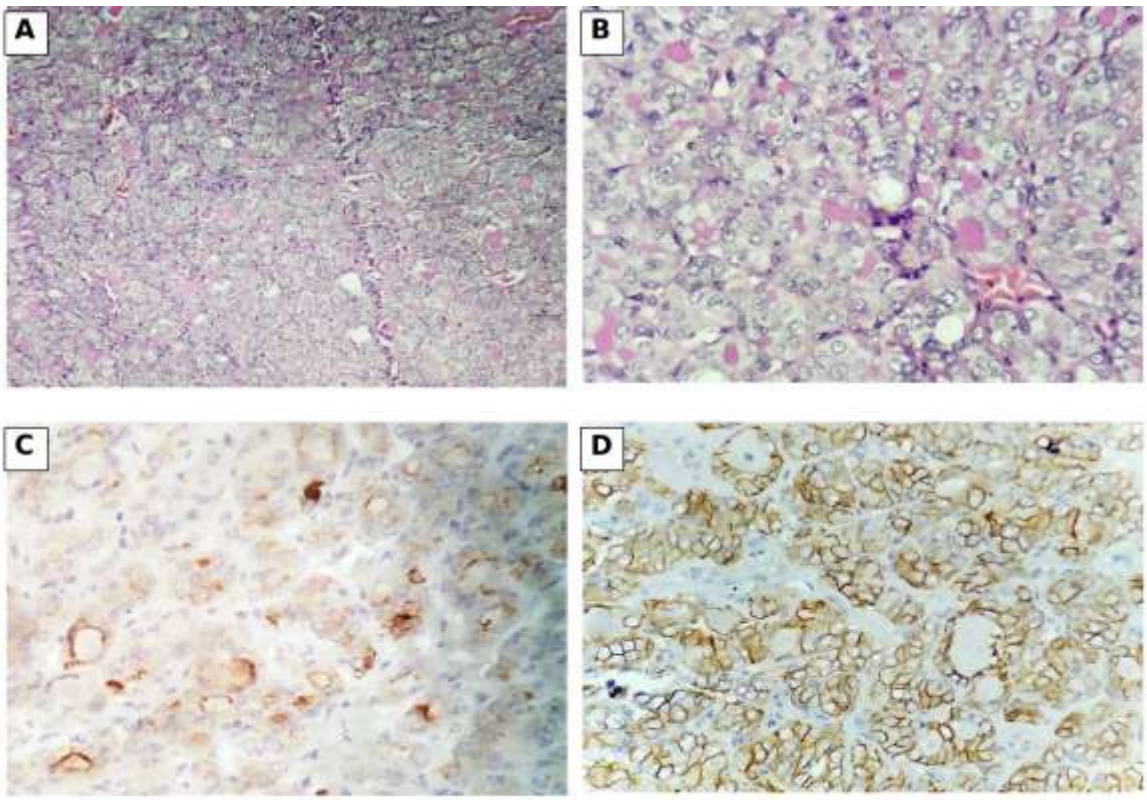

Figure 2. Follicular variant of papillary thyroid carcinoma (FVPTC)
Representative case of follicular variant of papillary thyroid carcinoma. (A) H&E, 10×; (B) H&E, 40× showing the follicular-patterned tumour; (C) HBME1 immunohistochemistry, 40× showing positive staining; (D) CD56 immunohistochemistry, 40× showing positive staining.