Original Article

Immunohistochemical Expression of HBME-1 in a Spectrum of Thyroid Neoplasms

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Abstract

Introduction: Thyroid neoplasms are the most common malignancy of the endocrine system, representing 3.8% of new cancer cases, and it is the ninth most common cancer overall. The immuno-histochemical marker Hector Battifora Mesotheilial-1 (HBME-1) is a monoclonal antibody that now finds its diagnostic utility as a positive marker for well-differentiated thyroid carcinomas. The aim was to study the expression of HBME-1 and to differentiate between malignant and non-malignant lesions by demonstrating their usefulness in the categorisation of thyroid neoplasms. **Methods:** A prospective study was conducted at a tertiary care centre from August 2022 to May 2023, comprising 25 cases. All thyroidectomy specimens were included. They were subjected to histopathological examination using routine haematoxylin and eosin stain, and further confirmation was made by immuno-histochemical staining for HBME-1. **Results:** There were a total of 25 cases consisting of 23 (92%) females and two (8%) males. The maximum number of cases was seen in the age group of 21–30 years (27%), followed by 31–40 years (24%) and 41–50 years (24%). The most common thyroid neoplasm noted was the follicular variant of papillary thyroid carcinoma (40%) with HBME-1 being strongly positive for papillary thyroid carcinoma and its variants compared to other tumour sub-types, where it shows diffuse and focal expression. **Conclusion:** HBME-1, due to its high sensitivity, can be significantly used as a marker for identification and differentiation, particularly papillary thyroid carcinoma. However, using a panel of markers comprising Galectin-3 and Ck-19 along with HBME-1 increases the accuracy and specificity for the correct diagnosis of thyroid neoplasms.

Keywords: HBME-1, malignancy, thyroid, thyroid neoplasm

Introduction

Thyroid neoplasms are the most common malignancy of the endocrine system, representing 3.8% of new cancer cases and the ninth most common cancer overall. The clinical behaviour of thyroid cancer is highly variable, from indolent, slowly progressing to highly aggressive tumours with malignant lesions comprising only 10%. The immuno-histochemical marker HBME-1 (Hector Battifora Mesotheilial-1) is a monoclonal antibody that now finds its diagnostic utility as a positive marker for well-differentiated thyroid carcinomas as it has high sensitivity, which can be helpful in morphologically unequivocal cases. Among previously studied bio-markers, HBME-1 is potentially helpful in diagnosing thyroid lesions.[1]

To know the immuno-histochemical expression of HBME-1 in diagnosing thyroid neoplasms and to differentiate between malignant and non-malignant lesions by demonstrating their usefulness in the categorisation of malignant lesions of thyroid neoplasms, which helps in better prognosis.

Materials and Methods

A prospective study was conducted at a tertiary care centre from August 2022 to May 2023 comprising 25 cases of thyroid neoplasms. Histopathological specimens were received in 10% formalin after fixation tissue was dehydrated by passing the tissue through a series of ascending grades of alcohol. Then clearing was done by passing it through two changes of

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xylene; wax blocks were made. Thin sections of 3–4 micron thickness were cut, and slides were prepared and stained with haematoxylin and eosin (H and E) stain and examined microscopically. Histopathological diagnosis was considered as a reference standard for categorisation into non-malignant and malignant thyroid lesions. Further confirmation was made with immuno-histochemical staining by HBME-1.

All the thyroid specimens irrespective of age, classified as benign or malignant, that have undergone lobectomy, hemithyroidectomy, sub-total thyroidectomy, near-total thyroidectomy, and total thyroidectomy were included.

All the patients who reported thyroiditis, inadequate thyroid lesion sample, metastasis to the thyroid gland, uncooperative and unwilling to do surgery, or patients suffering from serious illness were excluded from the study.

Immuno-histochemical evaluation

The immuno-histochemical marker HBME-1 shows positivity in both the cytoplasm and cell membrane predominantly. Staining of the follicular colloid in the absence of staining of the follicular epithelium and/or cytoplasm was considered non-specific and negative. The intensity of staining of immuno-reactive cells and their % distribution pattern was evaluated [Table 1].[2]

Ethical aspects

The study was approved by Institutional Ethical Committee EC/NEW/INST/2021/225 vide letter no DHR-EC/2022/SC/07/29 on 28/07/2022. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures in the study follow the guidelines laid down in the Declaration of Helsinki (1964).

Results

In our study, there were a total of 25 cases consisting of 23 females (92%) and 2 males (8%). The maximum number of cases was seen in the age group of 21–30 years (28%), followed by 31–50 years (24%) and the least number of cases in < 20 years (4%). The expression of HBME-1 in 25 cases has been depicted according to the intensity of staining and the percentage of positive cells [Figure 1].

The most common thyroid neoplasm noted was the follicular variant of papillary thyroid carcinoma (FV-PTC) (40%) with HBME-1 being strongly positive for papillary thyroid carcinoma (PTC) and its variants compared to other tumour sub-types, where it shows diffuse and focal expression [Table 2].

The reliability immuno-histochemistry (IHC) offers an advantage in cases where histomorphological details are insufficient to establish a definitive diagnosis;[3] there is no correlation between both diagnoses [Figure 2].

IHC is the definitive and confirmatory diagnostic tool; nine cases that did not correlate with the H and E stain diagnosis were excluded based on IHC findings [Supplementary Table 1].

Table 1: Scoring for percentage of cells stained and for intensity of staining

Expression of HBME- 1	Percentage of positive cells
No Immunoreactivity/Negative	No Staining
Weakly positive (+)	Staining <25% of the cells
Focal positive (++)	Staining of 25%-50% of the cells
Strong positive (+++)	Staining of >50% of the cells

Table 2: Final diagnosis after IHC for HBME-1

Neoplasms	Female	Male	Total	Percentage
Classic Papillary Thyroid Carcinoma	3	-	3	12%
Follicular Variant of Papillary Thyroid Carcinoma	9	1	10	40%
Non-invasive follicular Thyroid Carcinoma with Papillary nuclear features	6	-	6	24%
Follicular Neoplasm	4	-	4	16%
Follicular Variant of Medullary Thyroid Carcinoma	1	-	1	4%
Follicular Adenoma	-	1	1	4%
Total	23	2	25	100%

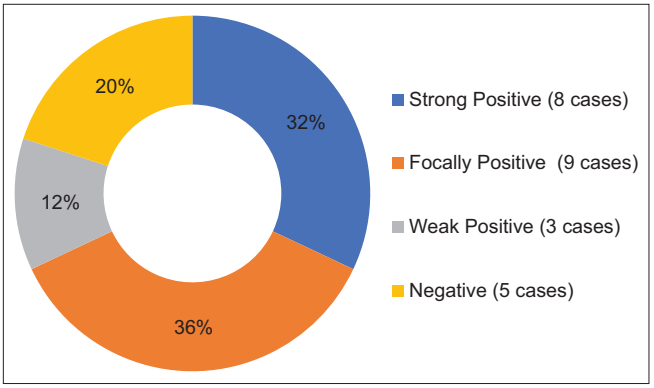

Figure 1: Expression of HBME-1 in 25 cases of thyroid neoplasms

Discussion

The diagnosis of the majority of thyroid neoplasms is straightforward.[4] Papillary thyroid carcinoma is usually a morphologic diagnosis with characteristic nuclear features such as large, overlapping, ground glass nuclei, nuclear grooves, and pseudo inclusions and rarely requires IHC to confirm the diagnosis. Histologically, classic PTC [Figure 3a] and follicular variant [Figure 3b] are the two major low-risk sub-types of PTC with other high-risk variants like tall cell, diffuse sclerosing, and hobnail variants reported in the literature.[5,6] Follicular patterned lesions of the thyroid have high levels of inter-observer and intra-observer disagreement,[7,8] and the main differentials of that include follicular adenoma (FA) [Supplementary Figure 1a], follicular carcinoma (FC), and FVPTC.[9,10] The presence or absence of capsular and/or vascular invasion helps in differentiating benign from malignant follicular tumours, but identification

of this finding can be challenging due to incomplete capsular invasion. Invasive follicular thyroid with papillary-like nuclear features (NIFTP) [Supplementary Figure 1b] is an evolving diagnosis and is usually categorised only if the criteria for it are fulfilled.

HBME-1 targets an unknown antigen of mesothelial microvilli and is abnormally expressed in thyroid malignancies, showing cytoplasmic location with membrane accentuation.[11] HBME-1 is highly expressed in PTC including both classical and follicular variants and follicular thyroid carcinoma with little or no expression in medullary carcinoma of thyroid (MTC) and anaplastic thyroid carcinoma.[12,13] It has gained attention in the past few years, and its expression in benign and malignant thyroid lesions has been investigated. In normal thyroid tissue, there is no expression of HBME-1; however, it is over-expressed in malignant tumours.[14]

In the present study, when assessing the diagnostic performance of HBME-1 as a single protein marker, the sensitivity was 93.75% and the specificity was 33.33%. Similar findings have been reported by Mr. Nasr *et al*.,[15] Y.J. Park *et al*.,[16] Scognamiglio *et al*.,[17] Alshenawy,[18] and Zargari and Mokhtari [Table 3].[19]

Liu and Lin[7] published a review article in 2015, in which they analysed various studies evaluating the role of IHC in diagnosing thyroid lesions. The authors concluded that there is no single biomarker sufficient to differentiate between benign and malignant thyroid lesions.

Limitation of the study; The study's representation of certain sub-types was limited, along with low specificity, underscoring the need for a larger sample size to validate HBME-1 expression across various thyroid lesions. Furthermore, a standardised protocol for HBME-1 staining in differentiating benign from malignant thyroid nodules is currently lacking. Establishing a standardised staining protocol and a consistent scoring method is essential to facilitate its adoption in diagnostic laboratories.

Conclusion

Due to overlapping histological features, diagnostic problems in thyroid pathology remain; hence, HBME-1, due to its high sensitivity, can be significantly used as a marker for the identification and differentiation of thyroid neoplasms, particularly papillary thyroid carcinoma. However, using a panel of immuno-histochemical markers comprising Galectin -3 and Ck-19 will be more helpful along with HBME-1 to increase the diagnostic accuracy and specificity for the correct diagnosis of thyroid neoplasms.

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None.

Authors' contribution

Dr. Chawla Archi Arun – Study Concept, Data Analysis and Acquisition, Statistical Analysis, Literature Search, Manuscript Preparation and Editing. Dr. Dhar Reeta – Study Concepts, Literature search, Definition of Intellectual Content, Manuscript Preparation, Editing and Review. Dr. Sahu Shilpi - Study Design, Manuscript Editing and Review.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Data pertaining to this manuscript will be available on request.

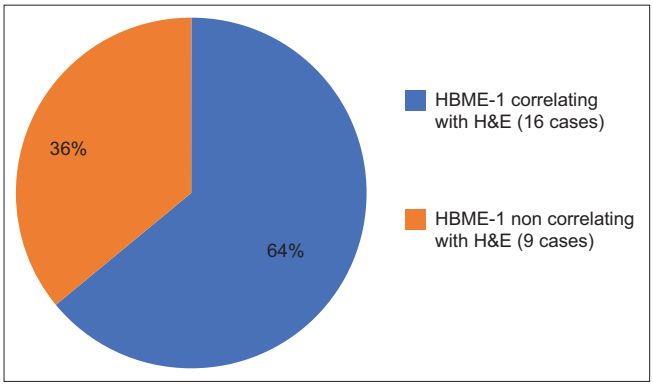

Figure 2: HBME-1 v/s H and E stain correlation

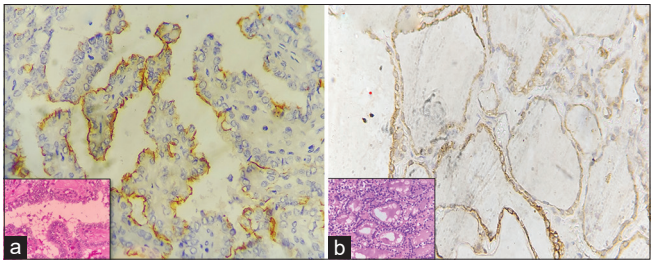

Figure 3: (a) PTC: Expression of HBME-1 strong positive (+++), i.e., more than 50% of the cells stained (40x) with inset classic PTC (H and E 40x). (b) FV-PTC: expression of HBME-1 focal positive (++), i.e., 25%–50% of the cells showing membrane accentuation (40x) with inset FV-PTC (H and E 40x)

Table 3: Comparison of sensitivity and specificity of immuno-histochemical marker HBME-1 with various authors

	Present study (2023) *n*=25	Mr. Nasr *et al*.[15] (2006) *n*=51	Y.J. Park *et al*.[16] (2007) *n*=50	Scognamiglio *et al*.[17] (2006) *n*=127	Alshenawy *et al*.[18] (2014) *n*=70	Zargari & Mokhtari[19] (2019) *n*=102
Sensitivity	93.37%	96%	91.3%	87%	80%	84%
Specificity	33.33%	93%	68.5%	96%	84%	98%

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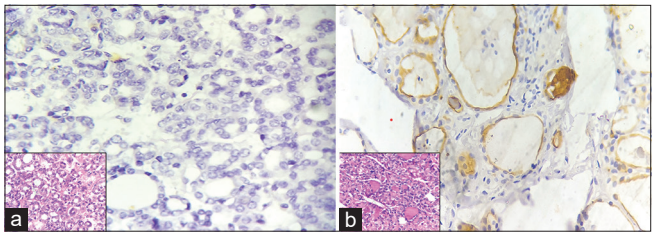
Supplementary Table 1: Re-classification of non-correlating H and E diagnosis cases by final IHC diagnosis

Non-Correlating Initial H and E Diagnosis	Number of Cases	Re-classification after Final IHC Diagnosis
NIFTP	1	Follicular Adenoma
NIFTP	4	FVPTC
FVPTC	3	NIFTP
Follicular Adenoma	1	Follicular variant of Medullary Carcinoma



Supplementary Figure 1: (a) FA- Expression of HMBE-1 -Negative, i.e., no immunohistochemical staining – 0% cells (40×) with inset FA (H and E 40x) (b) NIFTP. Expression of HBME-1-Weak/diffuse positive (+), i.e., less than 25% of the cells stained with inset NIFTP