Accuracy of HBME-1 as an Immunohistochemical Marker Differentiating Benign from Malignant Follicular Thyroid Nodules

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Abstract

One of the common clinical findings among adults is the presence of follicular thyroid nodules especially in females. Difficulties in the diagnosis of follicular patterned thyroid lesions on fine needle aspiration (FNA) cytology examination are well know problems, and histologic evaluation of surgically resected follicular patterned lesions can be challenging as well. Fortunately, only a small percentage is malignant. Accurate diagnosis of these thyroid nodules is critical for the proper clinical management but sometimes it is very difficult to differentiate Clinically, radio-logically and even histologically. Methods: We investigated the accuracy of immunoexpression of HBME-1 in 24 surgically removed benign thyroid nodules including 16 hyperplastic nodules (HN) and 8 follicular cell adenomas (FA), and 17 malignant tumors including 6 follicular carcinoma (FC), 7 classic papillary carcinoma (PTC), and 4 follicular variant papillary carcinoma (FVPC).Results: The staining results showed that malignant tumors express HBME-1significantly more than benign nodules. The sensitivity of this marker for the distinction between benign and malignant lesions ranged from 83.3% to 87%. Immunoexpression was usually diffuse and strong in malignant tumors, and focal and weak in the benign lesions.Conclusion: Our findings indicate that immunomarker HBME-1is significantly more expressed in malignant tumors compared to benign lesions and may be of additional diagnostic value when combined with routine histology.

Introduction

About 40% of the population between 30 and 60 years-old has thyroid nodules, most of which are benign [1]. Difficulties in the diagnosis of follicular patterned thyroid lesions on fine needle aspiration (FNA) cytology examination are well know problems, and histologic evaluation of surgically resected follicular patterned lesions can be challenging as well. Follicular neoplasms are classified as benign or malignant depending on the presence or absence of capsular and or vascular invasion. However, evaluation of these features can be challenging on histologic examination due to the presence of incomplete capsular penetration or equivocal vascular invasion, and for this reason, many end up with a general inconclusive diagnosis of "follicular lesion" [1-2].Also one of the common diagnostic dilemma is encountered when an encapsulated lesion with follicular growth pattern has some but not all the nuclear features diagnostic of papillary thyroid carcinoma [2-4]. It is estimated that only about 10% of the resected lesions are proven to be malignant . Furthermore, the surgical approach of these lesions may cause anxiety and social distress, and may incur high cost for the healthcare system [5].Searching for relatively less invasive technique before surgery of utmost importance not only to the patients by avoiding unnecessary surgery and to insurance system by decreasing the overall cost but also from the view of medical ethics [6]. Currently, the standard diagnosis depends on the histomorphologic features of routine hematoxylin and eosin (H&E) stained slides, but inter-observer or intra-observer disagreements in the diagnosis of follicular thyroid lesions are well known and

documented [2]. For example, in a recent study, review of 200 thyroid tumors by seven Italian pathologists showed good agreement for papillary and anaplastic carcinomas, moderate for medullary and poor for follicular thyroid carcinomas. Recent studies have focused on identifying IHC markers that can help in differentiating benign from malignant lesions, and follicular variant of papillary carcinoma from follicular carcinoma or adenoma [7-9] Several markers have been investigated on aspiration biopsy material and histologic specimens such as CK19 galectin-3, HBME-1, CK 903, CITED1, Ret oncoprotein CD 44, CD 57, cyclin D1 and p27. The findings were generally encouraging and promising although some studies demonstrated inconclusive or conflicting results [2,4,5,10,11]

The aim of this study was to investigate the ability of HBME-1as a promising IHC markers, to distinguish between benign (non-neoplastic and neoplastic) and malignant (follicular and papillary carcinomas) thyroid lesions removed by surgical resection.

Materials and Methods

A total of 41cases of surgically resected thyroid lesions sent by the endocrinologist as cases with clinically palpable thyroid nodules. All the cases are euthyroid, 20 cases were originally hypothyroid but medically controlled by L-Thyroxin , 13 cases were euthyroid from beginning and 8 cases were originally hyperthyroid but medically controlled by theouracil. The patients referred to surgeon after thyroid US. is done by expert radiologist unaware of the patients condition.16 cases showed significantly sizable solitary thyroid nodule and the rest showed classic pattern of multinodular goitre. All the patients showed either none or non specific enlarged cervical LN. Almost all the cases are females except 3 cases were males. The age of those patients range from 24-56 years with mean age was 37.4±11.3.The surgical resections were done from July 2009 till September 2010 in surgical department of Zagazig University hospitals. The resected lesions sent to pathology lap for further managment. The cases included 16 benign non-neoplastic lesions diagnosed as hyperplastic colloid nodules or cellular colloid nodules (HN), 8 cases of benign tumors (FA) and 17 cases of malignant tumors (6 cases of FC, 7cases of PTC and 4 cases of FVPC).(Figure 1 showed distribution of cases in our study)

	(HN)	(FA)		(FC)	(PTC)	(FVPC)
Series1	16	8		7	6	4
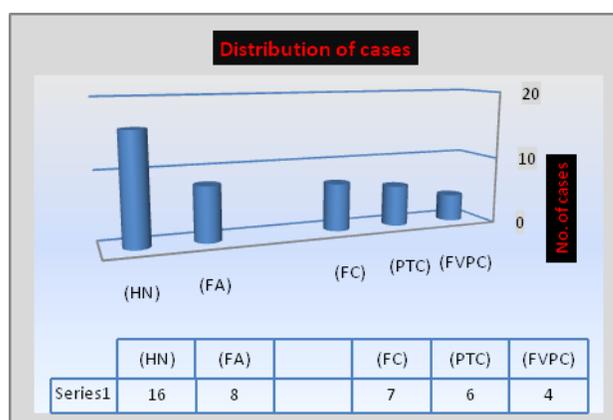
Cases with equivocal features or indefinite diagnosis were excluded from the study. The diagnosis of hyperplastic colloid nodules was based on the presence of follicles containing colloid and lined by bland follicular cells with basal small round nuclei lacking crowding, overlapping or other PTC-type nuclei. The evaluation and classification of the thyroid tumors was based on WHO thyroid tumor classification published in 2004. The diagnosis of follicular

adenoma was made based on the presence of encapsulated mass with homogenous follicular proliferation, lack of PTC nuclear features and absence of vascular and/or capsular invasion. The diagnosis of classic PTC was based on the presence of papillary structures with fibrovascular cores and specific nuclear features widely known as typical of papillary carcinoma. The FVPC was diagnosed based on the presence of follicular growth pattern with classic PTC-type nuclear features in at least several areas of the tumor. Follicular carcinoma was diagnosed based on the presence of follicular proliferation with complete thick capsule and full capsular penetration and/or vascular invasion, and atypical hyperchromatic nuclei that lacked features of PTC nuclei. All specimens were fixed in 10% zinc formalin, embedded in paraffin and 4 micron-thick sections stained with hematoxylin and eosin for routine histological examination. Immunohistochemical staining was performed using HBME-1 immunohistochemical stains were performed on a Ventana Benchmark automated strainer.

Table 1 shows the characteristics of the used antibody

Antibody	Clone	Dilution	Antigen retrieval	Company
HBME	HBME-1	1:50	HIER EDTA Buffer	Daco Corp

After standard protocols for deparaffinization of the 4 micron-thick sections, and microwave antigen retrieval, the tissue sections were incubated with available commercial monoclonal antibodies diluted at 1: 50.The staining was completed using a streptavidin-biotincomplex detection method. Positive controls were mesothelioma for this marker .

The stained slides were examined in pathology lap blindly and independently without knowing the original histologic diagnosis. A case was considered positive when cytoplasmic or nuclear staining of 10% or more of the lesional cells was found reactive with the antibody. The staining results were then correlated with the original histologic diagnoses and data tabulated.

Interpretation and Analysis: HBME1 staining was cytoplasmic with membranous accentuation, only cells with distinct strong membranous staining were counted as positive reaction. A lesion was considered positive when 10% or more of the cells showed reactivity for the specific antibody.

The calculation was done using a known statistical computer softwere program (SPSS 10.0 for Windows;SPSS Inc. Chicago, Illinois, USA).

Results

Table 2 shows summary of the immunohistochemical expression of HBME1 marker in each group of the thyroid lesions. In general, high percentages of the malignant tumors (FC, PTC and FVPC) demonstrated strong and diffuse reactivity, while benign lesions (both neoplastic and non-neoplastic) showed lower expression with mainly focal and weaker staining. Benign non-neoplastic lesions (HN) were often negative but some showed focal weak reactivity.

Table 2 Correlation of immunohistochemical staining results with histological diagnosis of thyroid lesions

Benign (24)			Malignant(17)			
Non neoplastic(HN) (16)	Neoplastic(FA) (8)	Total (24)	FC (6)	PTC (7)	FVPC (4)	Total (17)
3(18.7%)	3(37.5%)	6(25%)	5(83.3%)	6(85.7%)	4(100%)	15(88.2%)

Table 3 Sensitivity, specificity, Positive predictive value and Negative predictive value of HBME1 for all Benign vs. Malignant Thyroid Lesions

Senstivity	Speceficity	Positive predictive	Negative predictive
88.2%	75%	71.4%	90%

Non-neoplastic lesions (HN) Of the 16 cases, only 3 (18.7%) were positive for
HBME-1 (Figures 2). Adenomas Of the 8 cases, 3 (37.5%) were positive for
HBME-1.Generally the adenomas appeared less often positive than carcinomas (Figures 3). The data in Table 4 shows that immunoexpression of HBME1
is much lower in the adenomas than that in carcinomas
(mid 40s compared to mid 80s). The sensitivity of the immunoexpression between adenomas and carcinomas is high (88.2%) . However, the specificity is only
moderate for the distinction of adenomas from carcinoma (62.5%)

Table 4 Sensitivity, specificity, Positive predictivevalue and Negative predictivevalue of HBME1 for Neoplastic (adenomas) vs. Malignant Thyroid Lesions

Senstivity	Speceficity	Positive predictive	Negative predictive
88.2%	62.5%	83.3%	71.4%

Carcinomas of all 17 malignant tumors, 15 (88.2.1%) were positive for
HBME-1. FC had high rate of reactivity for HBME-1 (5/6, 83.3.8%) (Figures 4). Classic PTC showed 85.7% (6/7) expression for HBME-1 (Figures4). FVPC was also highly reactive for HBME-1 (4/4, 100%) but this dats could be of limited value due to the few number of cases (Figures5).The data shows high sensitivity for these
subtypes of malignant thyroid tumors and therefore they do not reliably distinguish between them.
Table 5 shows that, generally, there are significant differences in expression between non-neoplastic and malignant tumors with a sensitivity of 88.2%. The differences in percentage reactivity between HN and malignancy was 18.7% vs. 88.2% for HBME-1 (Table 2).

Table 5 Sensitivity, specificity, Positive predictivevalue and Negative predictivevalue of HBME1 for Non-neoplastic (HN) vs. Malignant Thyroid Lesions

Senstivity	Speceficity	Positive predictive	Negative predictive
88.2%	81.2%	83.3%	86.6%

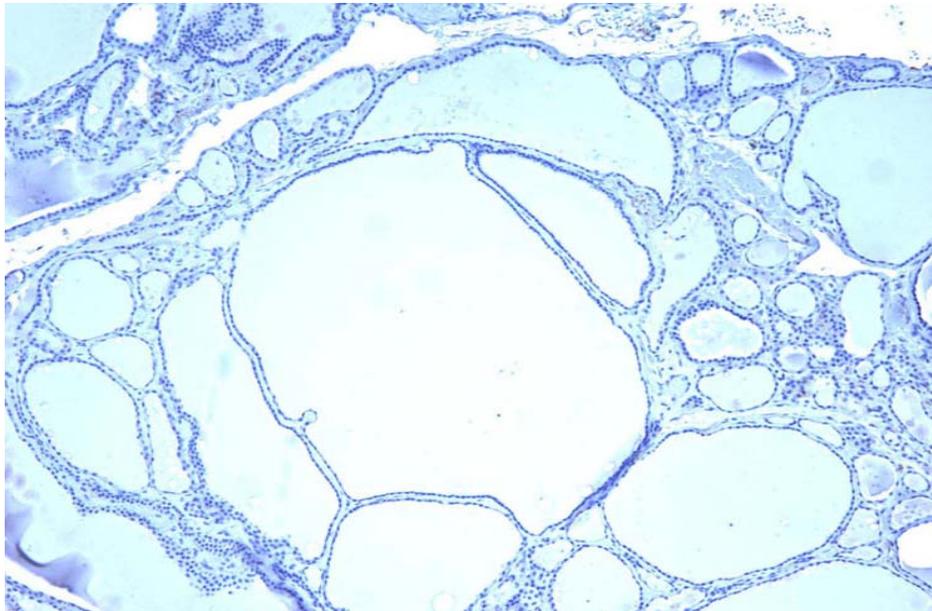*Figure 2 Immunohistochemical stain of hyperplastic colloid nodule shows the cells are negative, for HBME-1 (100×)*

Figure 3Immunohistochemical stain of follicular adenoma shows the tumor cells focally reactive for HBME-1 (100×).
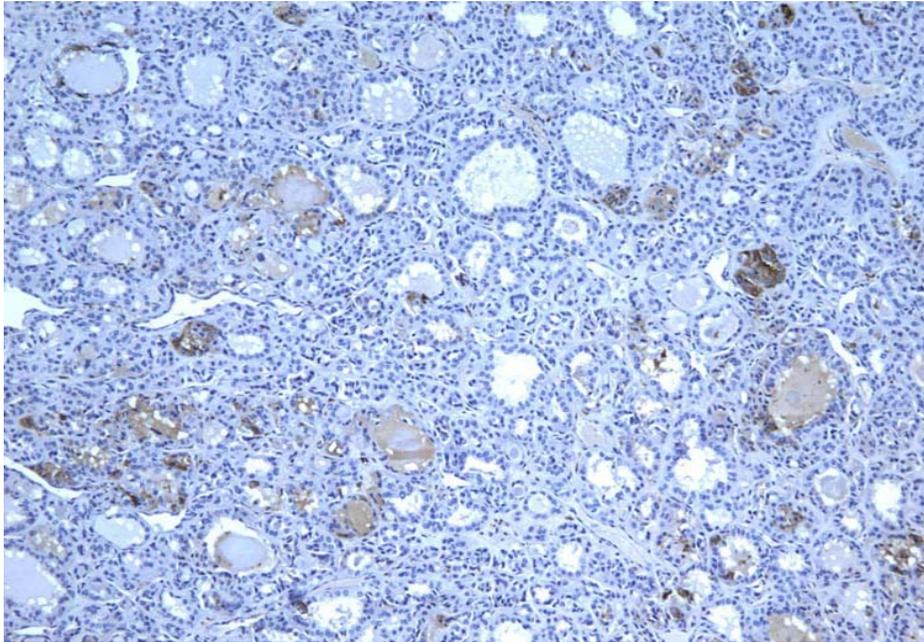

Figure 4 Immunohistochemical stain reveals that tumor cells in follicular carcinoma are strongly and diffusely positive for HBME-1 (400×).
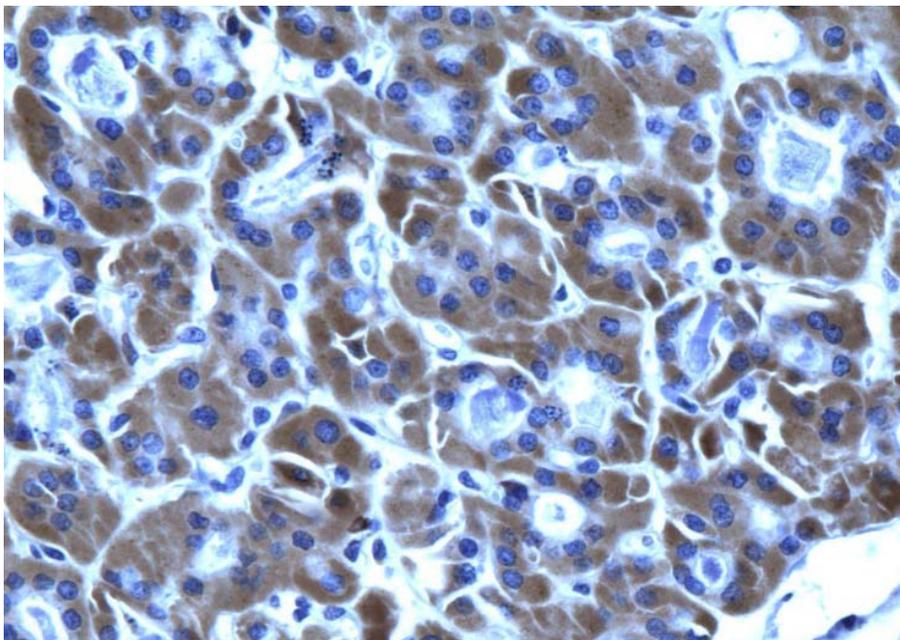
Figure 5 Immunohistochemical stain reveals that the tumor cells of classic papillary thyroid carcinoma are strongly and diffusely positive for HBME-1 (100×)
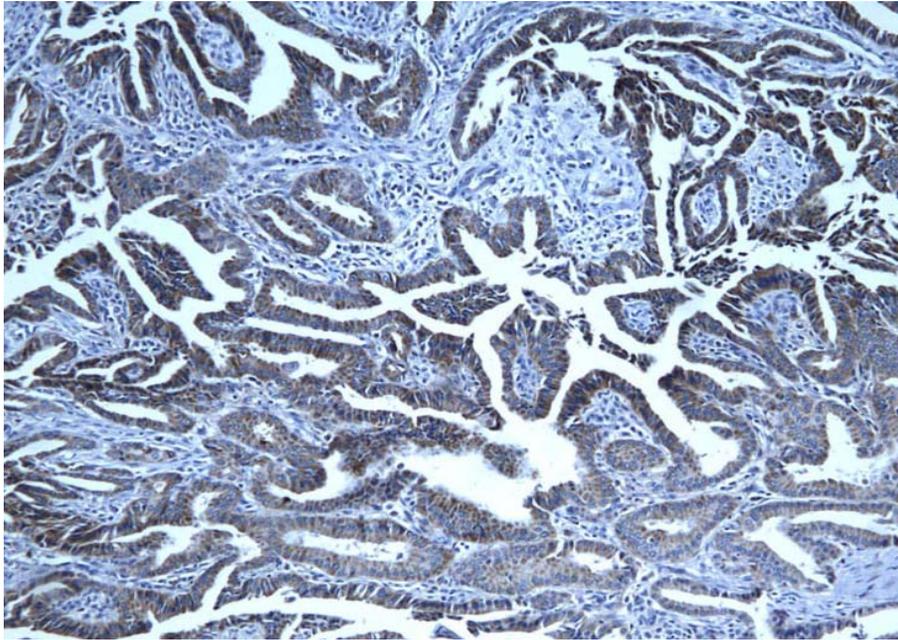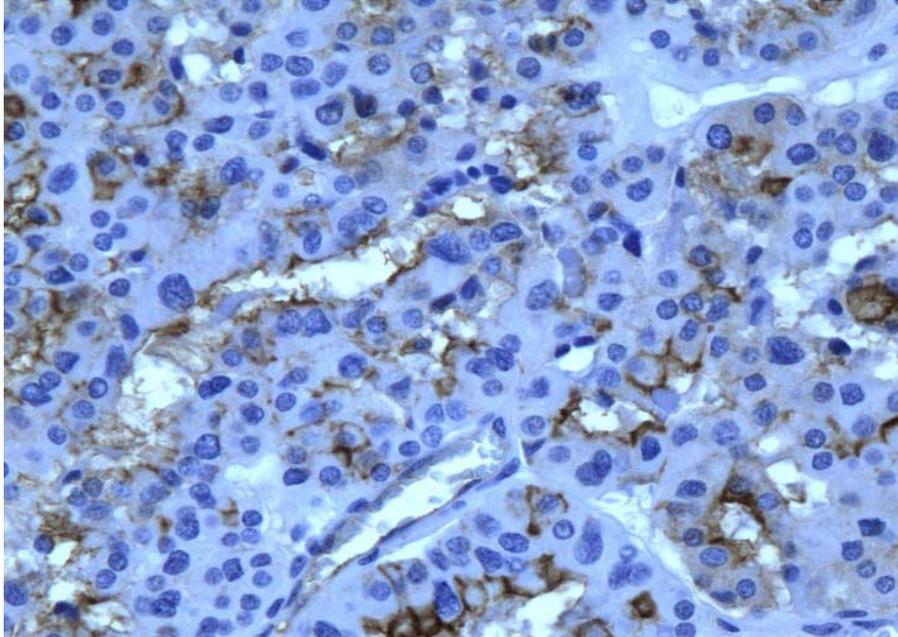

Figure 6 Immunohistochemical stain shows that the tumor cells of follicular variant of papillary carcinoma are strongly and diffusely positive for HBME-1 (400×)

Discussion

Thyroid nodules are fairly common clinical findings affecting approximately 40% of the population between 30 and 60 years old in the U.S.A., and thyroid cancer is the most common endocrine malignancy [12-14] Fortunately, most of these nodules are benign tumors or

hyperplastic lesions; however, it is important to identify these benign lesions for proper management and to realize maximum benefit for the patients [15]. Accurate diagnosis then is very critical for post-operative management of patients with thyroid nodules, and incorrect interpretation can lead to significant psychological and social problems, and unnecessary increase in healthcare cost [10,16]. Additionally, since FNA cytology in itself is not a reliable method to differentiate between benign and malignant follicular tumors or lesions, these patients usually undergo surgical resection, although only about 10% will actually have malignant tumors.The current standard in the diagnosis of thyroid lesions is by histologic examination of routine H&E stained sections.However, it is widely known that the interpretation of follicular patterned lesions can be quite difficult [2,4,11]. A somewhat common dilemma is encountered with encapsulated tumors showing follicular growth pattern. Presence or absence of capsular and/ or vascular invasion distinguishes benign from malignant follicular tumors, but identification of this finding can be challenging due to incomplete capsular penetration, equivocal vascular invasion or technical difficulties due to processing or sectioning artifacts. Another challenging situation is encountered when some but not all of the diagnostic nuclear features of papillary carcinoma are present. Recent study by Elsheikh et al [17] and editorial review by J. Rosai [3] pointed clearly to this issue.For all of the aforementioned reasons, investigators have focused during the last several years on finding molecular or IHC markers that can help in the distinction between benign and malignant lesions of the thyroid [7,8,18,19]. Identifying markers that can separate hyperplastic/adenomatous nodules from follicular tumors can be of tremendous benefits to the patients and the healthcare system [20]. As a result, many surgeries for benign lesions can be avoided and patients can be managed medically as needed [19,20]. HBME-1 is a monoclonal antibody directed against an antigen on the mesothelial cell membrane. Several studies have demonstrated its preferential reactivity in malignant thyroid tumors [2,9,11,22]. It has been found to be reactive mostly in papillary thyroid carcinoma and some follicular carcinomas, but usually negative in follicular adenomas. Papotti et al in a study of well-differentiated thyroid tumors of uncertain malignant potential found that a diffuse and strong expression of HBME-1is preferentially observed in the tumors with nuclear changes suggestive of papillary carcinoma [1]. However,they concluded that the diagnosis of these tumors should also depend on previously defined morphologic criteria. In our study, HBME-1 was expressed in 15 of total 17 thyroid carcinomas with a diagnostic sensitivity of 88.2%. However, it was also expressed in 3/16 (18.7%) of benign non-neoplastic lesions and in 3/8 (37.5%) of adenomas. Thus our study shows that HBME-1 is not a very good marker to distinguish adenomas from thyroid carcinomas with over one third of the adenomas expressing this marker.

In summary, immunoexpression of HBME-1 is an important supplementary test in the diagnosis of thyroid neoplasms, albeit it does not replace the conventional histomorphological examination. We found that these markers have somewhat similar sensitivity and specificity of immunoexpression in thyroid malignancy. Therefore, we recommend using immunoexpression of HBME-1 as useful means to increases the likelihood of detecting malignant tumors. This practical low cost IHC test of commercially available markers can help to optimize the management of patients with thyroid nodules and reduce unnecessary surgical resection of benign nodules. Nonetheless, there are still questions to answer and additional studies are needed toward the quest of identifying useful markers for differentiating benign from malignant thyroid nodules.

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