

# MONOCLONAL ANTIBODY HBME-1 USEFULNESS IN DIFFERENTIATION OF BENIGN NEOPLASM AND DIFFERENTIATED THYROID CARCINOMA

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**Abstract-** HBME-1 is an antimesothelial monoclonal antibody that recognizes an unknown antigen on microvilli of mesothelial cells. The antibody is only relatively specific for mesothelium and is used in the differential diagnosis of mesothelioma and adenocarcinoma within the context of an appropriate immuno-histochemical panel. HBME-1 has also been reported to strongly and uniformly stain papillary and follicular carcinoma of the thyroid while benign disorders have been usually negative. We studied the immunoreactivity of HBME-1 in 90 cases of benign and malignant thyroid lesions. We found strong positive staining in the majority of papillary carcinomas (28/31), in some of follicular carcinomas (4/6), and in a few follicular adenomas (2/17). Negative staining was found in oxyphilic cell adenoma (0/4), nodular goiter (0/13) and undifferentiated carcinoma. The results suggest that monoclonal antibody HBME-1 is useful in differentiating papillary and follicular carcinoma of the thyroid from benign lesions, especially in more differentiated lesions. Strong and generalized immunoreactivity for HBME-1 in a follicular lesion should raise the suspicion of malignancy, but negative staining specially in poorly differentiated lesion does not rule out malignancy.

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**Key words:** Thyroid lesion, HBME-1, immunostaining, papillary carcinoma, follicular carcinoma

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## INTRODUCTION

The histologic diagnosis and classification of thyroid lesions rests on their microscopic appearance and can be difficult, particularly with well differentiated follicular tumors (1-3). Currently it is recommended that the diagnosis of follicular carcinoma be restricted to tumors with unequivocal full thickness capsular invasion, vascular invasion or metastasis (4). Many immunohistochemical approaches aimed to solve some of these problems have been tried. Measurement of thyroglobulin has been widely used in diagnosis of thyroid tumors,

and it has been found the majority of thyroid carcinomas express this marker with high sensitivity and specificity (5-7). Several other antibodies have been used for diagnosis of thyroid neoplasms, including lactoferrin (8), ceruloplasmin (9) tissue peptide antigen (10), carcinoembryonic antigen (11, 12), thyroid peroxidase (13), epithelial membrane antigen (14) and S-100 protein (15). Unfortunately, many of these antibodies have shown limited potential in assisting distinction between benign and malignant thyroid tumors (15, 16, 8, 10).

The mouse monoclonal antibody HBME-1 was raised using a suspension of cells from an epithelial mesothelioma as immunogen. The antibody recognizes an undetermined antigen abundant on the surface of normal and neoplastic mesothelial cells which also is present in other epithelial cells. Staining often shows a characteristic "thick brush border", a pattern which correlates with the presence of long

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and abundant microvilli (17). Using HBME-1, Miettinen *et al.* reported that thyroid papillary and follicular carcinomas have uniform and strong immunoreactivity whereas normal thyroid, nodular goiter and follicular adenoma do not or show only weak and focal staining. They suggested that HBME-1 could be useful in the evaluation of difficult thyroid lesions (18, 2). We performed this study on 90 cases of benign and malignant thyroid lesions to evaluate usefulness of HBME-1 staining in differential diagnosis of thyroid lesions.

## MATERIALS AND METHODS

We retrieved 90 formalin-fixed paraffin embedded thyroid specimens from the surgical files of the Al-Zahra Hospital in Isfahan. Histopathologic diagnosis based on H&E staining was done. Specimens included papillary carcinoma (31 cases), follicular carcinoma (6 cases), follicular adenoma (17 cases), nodular goiter (13 cases), oxyphilic cell adenoma (4 cases), other benign lesions (11 cases) and other malignant lesions (8 cases). Other benign lesions included thyroiditis (Hashimoto, granulomatous) and other malignant lesions included few anaplastic carcinomas and insular carcinoma of thyroid. The diagnosis of follicular carcinoma was confirmed by the presence of unequivocal full thickness capsular invasion, vascular invasion or distant metastasis.

We used the standard avidin-biotin peroxidase complex (ABC) method. We first tested two different epitope retrieval methods: trypsin pretreatment and heat induced epitope retrieval using a microwave oven for pretreatment. The unstained slides were incubated in 0.1% trypsin in citrate buffer with a pH of 6 for 10 minutes at 37° C in microwave (600 w), followed by the standard ABC method. The HBME-1 antibody used in this study was obtained from Dako (code: M 3505). The immunohistochemical results were evaluated by two observers with light microscope. Localization and intensity and reactivity were evaluated, and only when reactivity was diffuse (both cytoplasmic or membranous) and strong it was classified as positive reaction.

Data were analyzed by Chi Square and Fisher Exact tests.

## RESULTS

Results are shown in tables 1 and 2. The majority of papillary carcinomas (28/31) including two of the follicular variants showed strong diffuse cytoplasmic and membrane staining. Three cases of papillary carcinoma failed to stain. The intense immunoreactivity in most of the carcinoma cases was useful to highlight small foci of the carcinoma within otherwise ‘benign’ thyroid tissue. Most follicular adenomas and nodular goiters were negative for HBME-1. However, three cases diagnosed as follicular adenomas showed focal membrane and cytoplasmic staining. Among the four examples of oxyphilic adenoma included in the study, none displayed membrane and cytoplasmic staining. Four cases of the follicular carcinoma (4/6) showed diffuse membrane and cytoplasmic staining. None of the 11 undifferentiated and poorly differentiated carcinomas showed staining (Table 2).

We evaluated a difference of staining between benign and malignant lesions of thyroid with HBME-1. We observed strong and diffuse HBME-1 staining in about 66.6% of the cases of follicular carcinomas (4/6). In the benign lesions, we observed that the majority of follicular adenomas were negative for HBME-1 (15/17, 88.3%); two cases showed weak focal staining.

We included in our study 13 cases of nodular goiter and 4 cases of Hashimoto’s thyroiditis and all of these cases were HBME-1 negative. We studied 4 cases of oxyphilic cell adenoma, and all of these were HBME-1 negative. Sensitivity and specificity of HBME-1 staining for diagnosis of malignant lesions were 71.1% and 95.5%, respectively. The positive and negative predictive values were 94% and 76.6%, respectively (Table 2).

**Table 1.** Staining results according to types of lesion

Staining	Lesion type		Total
	Benign	Malignant	
Positive	2	32	34
Negative	43	13	56
Total	45	45	90

**Table 2.** Sensitivity, specificity, and predictive values of HBME-1 staining

Lesion	Positivity	P value	Specificity	Sensitivity	NPV	PPV
Nodular goiter (n=13)	0	> 0.05	94.5	0	81.3	0
Follicular adenoma (n=17)	11.7	> 0.05	100	15.3	74.4	100
Oxyphilic adenoma (n=4)	0	> 0.05	95.1	0	90.6	0
Other benign lesions (n=11)	0	> 0.05	94.1	0	74.4	0
Papillary carcinoma (n=31)	90.4	< 0.001	71.4	90.3	76.9	87.5
Follicular carcinoma (n=6)	66.6	> 0.05	28.2	66.6	86.6	12.5
Other malignant lesions (n=8)	0	< 0.001	13.5	0	38.4	0

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

## DISCUSSION

Our results suggest that in follicular thyroid lesions, a strong and diffuse HBME-1 staining would support a diagnosis of follicular carcinoma or follicular variant of papillary carcinoma, but a negative result does not rule out a follicular carcinoma. These results indicate that a benign follicular thyroid lesion is usually HBME-1 negative and that immunoreactivity for HBME-1 in any such lesion should raise a suspicion of malignancy and the specimen should be thoroughly evaluated for capsular and/or vascular invasion. The results are similar to previous observations where most of the follicular adenomas were HBME-1 negative (18, 3).

HBME-1 monoclonal antibody was developed primarily to apply to the differential diagnosis of malignant mesothelioma and adenocarcinoma. Although somewhat useful for this purpose, HBME-1 is not entirely specific for mesothelial cells, and a number of adenocarcinomas of various sites may also show immunoreactivity (3, 17). Miettinen and Kovatich reported HBME-1 reactivity in all of the 25 cases of thyroid papillary carcinomas and in all 5 cases of follicular carcinoma (3). They found positive staining in 2 of the 17 follicular adenomas. In a later study, Miettinen and Karkkainen, tested a large number of cases and found all papillary (145/145) and all follicular carcinoma (27/27) to be HBME-1 positive whereas all anaplastic carcinoma (0/19) were negative, and most follicular adenomas were negative (53/74) (18, 2).

Our results confirm that the majority of papillary

carcinoma showed strong and diffuse HBME-1 reactivity. These results indicate that a strong and diffuse HBME-1 staining is useful in confirmation of the diagnosis of thyroid papillary carcinoma (2, 19-21).

In conclusion, HBME-1 monoclonal antibody is a useful adjunct immunostain to differentiate papillary and follicular carcinomas of thyroid from benign lesions. Benign lesions and follicular and oxyphilic cell adenomas are usually HBME-1 negative, and a follicular lesion with positive HBME-1 staining should raise a suspicion of malignancy and a careful evaluation of the specimens must be done to exclude invasion. It means that in a differentiated lesion in H&E staining, positive staining of the lesion is favorable of malignancy and though negative staining does not rule out malignancy, it is favorable of benignity. In a poorly differentiated lesion negative staining does not rule out malignancy.

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