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RESEARCH

Strong Expression of HBME-1 Associates with High-Risk Clinicopathological Factors of Papillary Thyroid Carcinoma

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Abstract Thyroid cancer comprises a heterogeneous group of lesions with great diversity of biological behaviour. Markers which could help clinicians to identify high-risk patients for tailored optimization of clinical management are of crucial importance. HBME-1 protein level was analysed immunohistochemically using routinely prepared archival tissue sections of a broad range of papillary thyroid carcinoma (PTC) variants and in corresponding lymph node metastases (LNM). The results were evaluated in comparison with clinicopathological features of PTC. Positive immunoreaction was noticed in most classical (83/92; 90.2 %), follicular (60/71; 84.5 %) and trabecular (4/5; 80.0 %) variants of PTC. All cases of macrofollicular, Warthin-like and diffuse sclerosing PTC variants were HBME-1 positive (4/4, 3/3, 2/2; 100 % respectively). Tall cell and solid PTC variants showed diversity of staining (2/3; 66.67 % and 13/23; 56.52 % respectively), while PTCs with mixed histological pattern containing insular areas were mainly weakly positive (2/5; 40.0 %). A single case of clear cell PTC variant showed no reaction. Moreover, all matched metastatic PTC into lymph nodes (LNM) were HBME-1 positive (17/17; 100 %) and expressed HBME-1 in a similar pattern to the matched primary tumour. We also found a statistically significant association between high HBME-1 expression and the presence of lymph node metastasis, advanced pT status and pTNM stage ($P<0.05$), but only a tendency for association with extrathyroidal invasion of the tumour ($P=0.058$). Therefore, we recommend using immunoexpression of HBME-1 as useful mean to increase the likelihood of detecting most PTC variants and to predict some unfavourable clinical parameters in these patients.

Keywords Papillary thyroid carcinoma · HBME-1 · Diagnosis · Prognosis · Lymph node metastasis · pT · pTNM

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Introduction

Thyroid cancer is the most prevalent type of endocrine malignancy with increasing incidence in many countries in the last decade [1]. The most frequent type is papillary carcinoma of the thyroid gland (PTC), which accounts for 70–80 % of all thyroid cancers. Although papillary carcinomas are relatively indolent and generally curable with surgery (near total or total thyroidectomy), radioiodine ablation and hormone therapy, a small proportion of them show aggressive behaviour. These patients have a poor prognosis. There is recurrence in 10–15 % of cases, usually in regional lymph nodes or at the cervical level in the thyroid bed. In these patients, treatment based on thyroidectomy with neck dissection, followed by radioiodine ablation and total hormonal suppression of TSH, is highly recommended. This brings up a pressing need to identify high-risk patients for tailored optimization of clinical management.

Many studies have described numerous factors influencing prognosis and long term survival of thyroid cancer, but the

most relevant are age and sex of the patient, tumour size, histopathological variant, multifocality, the presence of lymph node metastasis (LNM) and invasiveness [2–5]. However, in the absence of these features, predicting outcomes in thyroid cancer is difficult.

Therefore, discovering molecular markers could be useful for the detection, diagnosis and monitoring of diverse types of thyroid carcinomas, as well as for identifying subsets of patients at high risk for a poor prognosis. This has currently made this area of research one of the busiest in the world of endocrine oncology [6–10]. On the basis of published findings, one promising marker is Hector Battifora mesothelial antigen-1 (HBME-1).

Monoclonal HBME-1 antibody was originally developed against the microvillus surface of mesothelial cells, but subsequently it has been applied for identification of malignant thyroid tumours (reviewed by Raphael et al. [9]). Nowadays, the unknown protein recognized by HBME-1 antibody has been indicated as a sensitive marker of thyroid malignancy [7–13]. There are many studies showing that high and uniform expression of HBME-1 strongly suggests a diagnosis of differentiated thyroid carcinoma (DTC), particularly PTC [14–18], while poor or negative immunostaining of HBME-1 does not rule out malignancy in undifferentiated PTC [19, 20]. On the other hand, only scanty data have been reported on the immunohistochemical evaluation of HBME-1 content in uncommon PTC variants. Also, there is poor information about HBME-1 immunostaining in PTC metastases in lymph nodes. According to the occasional studies, all LNM of papillary carcinomas show strong HBME-1 positivity [21, 22]. In addition, the few results concerning the association of HBME-1 expression with prognostic clinicopathological factors in thyroid carcinoma patients are controversial [9, 23, 24].

Therefore, we aimed to evaluate the immunoexpression pattern of HBME-1 in various variants of PTC and their respective lymph node metastases. We also analyzed whether high HBME-1 expression in PTC associates with known unfavourable clinicopathological factors.

Material and Methods

Tissue Samples

The samples were selected from the database of the Centre for Endocrine Surgery, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Belgrade.

Histological diagnosis of hematoxylin and eosin stained sections of archival thyroid tissue blocks was made using the consensus opinion of two independent pathologists, according to widely accepted cytohistopathological criteria [25]. A total of 216 formalin-fixed, paraffin-embedded thyroid tissue samples comprised 209 PTC cases of diverse variants (92 classical PTC, 71 of follicular PTC variant, 5 cases of trabecular variant of PTC, 4 cases of macrofollicular variant of PTC, 3 Warthin-like variant of PTC, 3 tall cell variant of PTC, 2 diffuse sclerosing variant of PTC, 23 cases of solid PTC variant, 1 clear cell variant of PTC, 5 PTC with mixed histological pattern - comprising different histological variants (assorted follicular, solid and insular areas) and 17 randomly selected lymph node metastases of PTC (LNM)). All LNMs were matched with their primary tumour. Additionally, 126 cases among the 209 analyzed tumours included adjacent morphologically normal or hyperplastic thyroid tissue. Establishing the diagnosis of an aggressive variant of PTC or a poorly differentiated thyroid cancer was made according to Turin criteria [26].

Patients

All clinical specimens used in this study were approved by the Ethics Committee at the Centre for Endocrine Surgery, Clinical Centre of Serbia, Belgrade, and informed consent to use excess biological material for investigative purposes from all patients participating in the study was obtained.

Information concerning gender and age of the subjects, size, multifocality, lymph node metastases and extrathyroid invasion of tumours was retrieved by reviewing the clinical and pathology reports. LNMs were confirmed at the time of diagnosis. Carcinomas were staged according to pathological tumour-node-metastasis stage (pTNM) [27].

The group of PTC cases included 172 females (82.3 %) and 37 males (17.7 %) aged 10–76 years at diagnosis (46.7± 14.0 years, mean ± SD). Tumour size ranged from 2 to 150 mm (29.5±21.3 mm, mean ± SD), while 37 of them were over 40 mm (17.7 %). Multifocality was observed in 77 (36.8 %), lymph node metastasis in 49 (23.4 %) and extrathyroid invasion in 51 cases of primary PTC (24.4 %).

Follow-up data were mainly gathered from one institution and included findings for 23 PTC patients collected and updated by one researcher at the end of a follow-up period of 13 years.

Immunohistochemistry

Immunohistochemistry was performed as described previously [10]. The primary mouse monoclonal antibody against HBME-1 (Dako Cytomation, Carpinteria, CA, USA) was diluted 1:50. The signal was enhanced by the avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA), followed by visualization of the reaction with 3,3′- diaminobenzidine tetrahydrochloride (DAB) solution (Peroxidase Substrate Kit, Vector Laboratories, Burlingame, CA, USA). After counterstaining with hematoxylin, slides were examined using an Axio

Imager 1.0 microscope (Carl Zeiss, Jena, Germany) with a Canon A640 Digital Camera System.

Controls were incubated with PBS instead of the primary antibody and no positive staining was observed.

Scoring of Staining and the Cut-Off Value

HBME-1 staining was cytoplasmic with membranous accentuation. Immunoreactivity, assessed by the consensus opinion of two independent observers, was graded using a semi-quantitative scoring method. By evaluating both the heterogeneous positive distribution and the differing intensity of staining simultaneously, we classified all cases into 5 categories: (0) less than 5 % positive epithelial cells (negative staining), (1) from 6 to 20 % positive (weak staining), (2) from 21 to 50 % positive (mild staining), (3) from 51 to 80 % positive (strong staining) and (4) more than 80 % positive cancer cells (diffusely strong staining). The cut-off value for considering a case as truly positive was set at 20 % of stained cancer cells as established previously [10] and based on analyzing the HBME-1 staining in differentiated thyroid carcinomas of follicular cell origin against benign thyroid tumours. For testing the association of HBME-1 immunostaining with known clinicopathological factors, the threshold value was set at 50 % positive malignant cells. Thus, the patients were divided into two groups: low HBME-1 expressing (if≤50 % of all cancer cells were HBME-1 immunopositive, the cases scored as (0), (1) and (2)), and high HBME-1 expressing (when>50 % of malignant cells were immunopositive, the cases scored as (3) and (4)).

Statistical Data

The diversity of HBME-1 immunostaining between PTC subgroups was tested by Pearsons' $\chi 2$-test. Receiver operating characteristic (ROC) curve analysis was applied for assessing the most optimal cut off value in testing the association of HBME-1 immunostaining with known clinicopathological factors (data not shown). The ability of HBME-1 to predict some clinicopathological characteristics of PTC was initially evaluated by univariate analysis (Fisher's Exact, $\chi 2$- or t- test). The $\chi 2$-test was applied to variables with expected counts equal to or greater than 5. If any expected count was less than 5, Fisher's Exact test was applied. The *t*-test was used for quantitative variables. The results were considered significant at $P<0.05$. Findings of statistical importance were further examined by a multivariate set of tests. Thus, logistic regression analysis was employed for variables with two possible outcomes, while discriminate analysis was applied for those with three or more ranking levels.

Statistical analysis was performed using SPSS software (SPSS 16.0, Chicago, Il, USA).

Results

Paraffin-embedded tissue samples from PTC patients were analyzed immunohistochemically, using a monoclonal antibody against HBME-1. Figure 1 shows some representative photomicrographs of HBME-1 immunostaining of thyroid tissue. The staining scores for the individual samples are presented in Table 1.

Statistical analysis of the immunostaining data showed that HBME-1 immunoreactivity was significantly more predominant in malignant than in nonmalignant thyroid tissue ($P<0.001$). In general, HBME-1 stained the majority of PTC cases diffusely, while there was absence or focal expression in nonmalignant thyroid tissue.

When present, normal follicular cells surrounding tumour nodules were HBME-1 immunonegative, but some thyrocytes located at the rims of sections occasionally showed weak focal reactivity. Expression of HBME-1 was sporadically noticed in less than 5 % of thyrocytes in hyperplastic areas of the peritumoral tissue. In addition, weak expression of HBME-1 was also occasionally found in follicle colloid and the connective tissue of tumours, with no differences regarding tumour type.

Although some diversity in staining patterns was noticed between the subgroups, most tested PTC cases showed intense and diffuse HBME-1 immunoreactions. The staining of malignant cells was cytoplasmic with membrane accentuation. When immunoreactivity in at least 20 % of epithelial follicular cells was taken as a positive result [10], a positive HBME-1 reaction was detected in 172 of the 209 tested PTC cases, giving an overall sensitivity of 82.3 %. A positive HBME-1 imunoreaction was observed in most cases of classical PTC (83/92; 90.2 %), the follicular (60/71; 84.5 %), trabecular (4/5; 80.0 %) and tall cell variant of PTC (2/3; 66.7 %). The majority of these cases had more than 75 % of the tumour area positive. There was no statistically significant difference in HBME-1 staining between the classical and follicular variants of PTC ($P=0.477$). Moreover, all tested cases of macrofollicular (4/4; 100 %) and Warthin-like PTC variants (3/3; 100 %), as well as both cases of diffuse sclerosing PTC variant were HBME-1 positive (2/2; 100 %).

Although a positive reaction was detected in 13/23 (56.5 %) cases of solid PTC variant, no solid PTC variant had more than 80 % of malignant cells stained. Overall, malignant cells of the solid PTC variant had significantly weaker HBME-1 immunoreactivity in comparison with classical PTC ($P<0.001$) or its follicular variant ($P=0.0005$). While PTCs with a mixed histological pattern were rarely positive (2/5; 40.0 %), the insular foci of these cases were completely negative. The only case of clear cell PTC showed no reaction (0/1; 0 %).

The association between clinicopathological findings and HBME-1 expression is shown in Table 2. The specimens,

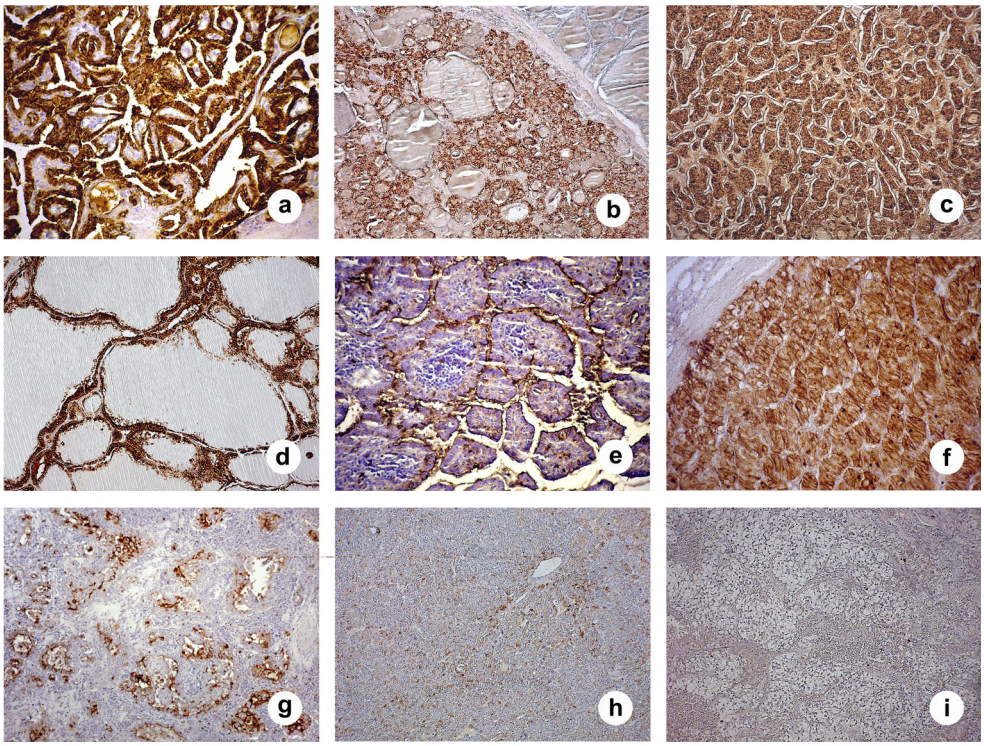

Fig. 1 Representative micrographs of HBME-1 immunohistochemical staining in diverse variants of papillary thyroid carcinoma. HBME-1 exhibited intense cytoplasmic and plasma membrane staining in classical papillary thyroid carcinoma (**a**), as well as in follicular (**b**), trabecular (**c**), macrofollicular (**d**), Warthin-like (**e**) and tall cell variants of papillary thyroid carcinoma (**f**). Immunopositive reaction in diffuse sclerosing (**g**) and solid variants of papillary thyroid carcinoma (**h**) with score 2. Negative immunoreaction in clear cell variant of PTC (**i**) and normal thyroid tissue (**b**). Indirect immunoperoxidase technique; hematoxylin diaminobenzidine counterstaining. Original magnifications: ×10 in all but×20 in **a**, **b**, **e** and **f**

which included 209 cases of PTCs, were divided into two categories: low HBME-1 expressing (68 cases) and high HBME-1 expressing (141 cases). The cut off score for low HBME-1 expression was set to≤50 % and the counterpart as high HBME-1 expression (>50 %).

Both univariate and multivariate analyses showed close positive association of high HBME-1 expression and the presence of PTC metastasis in regional lymph nodes (P=0.012 by Fisher's Exact test, P=0.014 by Discriminate analysis), pT status (P=0.011 by χ2- test, P=0.010 by Discriminate analysis) and pTNM stage (P=0.024 by Fisher's Exact test, P=0.030 by Discriminate analysis). Thus, abundant HBME-1 expression was noticed in 83.7 % (41/49) of PTC carcinomas with LNM, 91.7 % (22/24) of PTC carcinomas of the most advanced pT status (pT4) and 92.6 % (25/27) of PTC carcinomas of the most advanced pTNM stage (IV). All statistical tests gave borderline significance for the association of HBME-1 expression and the presence of extrathyroid extension (P=0.055 by χ2- test, P=0.058 by Logistic regression). Finally, there were no statistically significant associations of high HBME-1 expression with patient gender and age, or with tumour diameter or multifocality.

Table 1 Levels of immunohistochemical expression of HBME-1 in thyroid tissue

Histotype	HBME-1 IHC staining (number of cases in staining category)					Total (n=335)
	0–5 % (score 0)	6–20 % (score 1)	21–50 % (score 2)	51–80 % (score 3)	81–100 % (score 4)	
NT	126	0	0	0	0	126
PTC cl	1	8	11	21	51	92
PTC fv	4	7	9	18	33	71
PTC trab	0	1	0	0	4	5
PTC macro	0	0	1	0	3	4
PTC w	0	0	1	2	0	3
PTC tc	0	1	1	0	1	3
PTC ds	0	0	2	0	0	2
PTC sol	6	4	5	8	0	23
PTC cc	1	0	0	0	0	1
PTC mix	1	2	2	0	0	5

NT normal thyroid tissue, *PTC* papillary thyroid carcinoma. *PTC cl* classical PTC. *PTC variants*: *fv* follicular, *trab* trabecular, *macro* macrofollicular, *w* Warthin-like, *tc* tall cell, *ds* diffuse sclerosing, *sol* solid and *cc* clear cell, *PTC mix* papillary thyroid carcinoma containing follicular, solid and insular areas

Table 2 Association between HBME-1 IHC expression and clinocopathological findings of PTC

Clinicopathological characteristics of thyroid carcinoma or staging system		IHC expression of HBME-1 (number of cases)		Statistical tests	
		Low (n=68)	High (n=141)	Univariate P value	Multivariate P value
Gender	M	12	25	0.988 $^{\chi 2}$	/
	F	56	116		
Age (years)	Range	10–72	11–76	0.534 ^{T}	/
	Mean	46	47		
	<45	32	60	0.539 $^{\chi 2}$	
	≥45	36	81		
Size (mm)	Range	2–60	3–150	0.389 ^{T}	/
	Mean	31.3	28.6		
	≤40	54	118	0.448 $^{\chi 2}$	
	>40	14	23		
pT status	T1a	8	31	**0.011* ^{F}**	**0.010* ^{D}**
	T1b	10	24		
	T2	27	34		
	T3	21	30		
	T4a	2	16		
	T4b	0	6		
Mf	No	45	87	0.530 $^{\chi 2}$	0.530 ^{L}
	Yes	23	54		
LNM	No	60	100	**0.012* ^{F}**	**0.014* ^{D}**
	Yes – central (a)	6	22		
	Yes – other (b)	2	19		
Ei	No	57	101	0.055 $^{\chi 2}$	0.058 ^{L}
	Yes	11	40		
pTNM stage	I	45	86	**0.024* ^{F}**	**0.030* ^{D}**
	II	8	15		
	III	13	15		
	IVa	2	20		
	IVb	0	5		

Gender gender of patient (M-male, F-female), *Age* age of patient, *Size* size of tumour in millimeters, *pT* pT status of thyroid tumours (see Materials and Methods), *Mf* multifocality (no-tumours of one focus, *yes* there is more than one focus of tumour), *LNM* lymph node metastasis (no-no metastasis in regional lymph node, *yes* presence of metastasis of primary tumour in regional lymph nodes, central or other), *Ei* extrathyroid invasion (no-no extension, yes-presence of expansion), *pTNM stage* for detail see Materials and Methods

HBME 1expression Low if≤50 % of tumor cells are positive (cases scores as 0, 1 and 2), *High* if >50 % of tumor cells are positive (cases scored as 3 and 4)

*P value-statistical significance: *P< 0.05, **P< 0.01. Univariate analysis: χ^2-test (χ^2), t-test (T) and Fisher Exact test (F). Multivariate analysis: Logistic regression (L) and Discriminate analysis (D)*

Statistically significant associations are bolded

An association between level of HBME-1 expression and follow up data was sought in 23 cases (Table 3). As can be seen, 17 PTC patients were in remission, 5 had a recurrence, while there was one case of PTC-related death. High expression of HBME-1 was found in 12/17 PTC patients in remission (70.5 %), in all patients with recurrence (four of score 4 and one of 3) and in the single case of PTC-related death (score 4). However, the frequency of high HBME-1 expression did not differ between the primary PTC patient groups with recurrence and no recurrence (disease free) (P=0.324).

To evaluate HBME-1 as a marker of metastatic PTC in lymph nodes, 17 randomly selected cases of primary PTC together with their LNM were analysed. The results are shown in Fig. 2 and photomicrographs of primary tumour and matched LNM in Fig. 3.

All primary tumours were of the classical type (gray columns). Fourteen of them developed metastases that grew predominantly in the same pattern (cases 1–14, unspotted blue columns), while 3 developed metastases of follicular pattern (cases 15–17, spotted blue columns). All 17 lymph node metastases from 17 PTC patients showed positive HBME-1 immunoexpression, with concordant positive expression status in the primary tumour (17/17; 100 %). Additionally, strong staining was noticed in 14/17 (82.4 %) primary tumours and

Table 3 Association of follow up data of PTC patients and immunohistochemical expression of HBME-1 in primary tumour

PTC patients	IHC expression of HBME-1in primary tumour number of cases (%)		Total number of cases
	Low	High	
In remission	5 (29.5 %)	12 (70.5 %)	17
With recurrence	0	5 (100 %)	5
Death of disease	0	1 (100 %)	1

HBME-1 staining Low if≤50 % of tumor cells are positive (cases scored as 0, 1 and 2) High - if >50 % of tumor cells are positive (cases scored as 3 and 4)

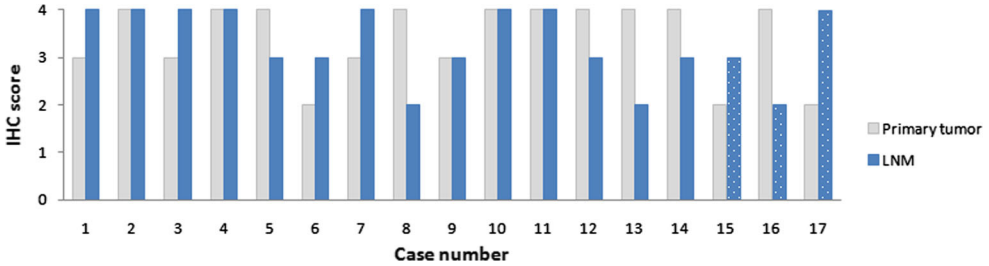

Fig. 2 Levels of immunohistochemical expression of HBME-1 in primary papillary thyroid carcinoma and matched lymph node metastasis (LNM). All primary tumours (grey columns) and 14 LNM were of classical type (cases 1–14, blue unspotted columns), while the remaining 3 LNM were of follicular type (cases 15–17, blue spotted columns)

14/17 (82.4 %) LNM, while the 3 remaining cases (17.6 %) showed positivity in 20–50 % of malignant thyrocytes.

Discussion

There is broad variation in the frequency of HBME-1 expression in papillary carcinomas, ranging from 55 % [15] to 100 % [16]. In most reports, however, the frequency ranged from 85 to 95 % [12–14, 17–19, 28, 29]. Possible explanations for this variation are differences in sample characteristics, diverse methodological/technical procedures and applied cut-off values (range from 1 to 25 %). Therefore, with HBME-1 immunopositivity in 82.3 % of the primary papillary thyroid carcinomas and absence from normal thyroid tissue adjacent to tumour cells, our results are in line with previous findings (reviewed by de Matos [30]).

Nevertheless, while HBME-1 was often found in conventional PTC and the follicular variant of PTC, in this study, it was more or less frequent in some other PTC variants and was almost absent in areas of PTC with an insular pattern, which partly coincides with the observations of Choi et al. [19] and Rossi et al. [20]. Therefore, published results indicate that HBME-1 has a minor role in less differentiated thyroid cancer. Variants of PTC with a similar prognosis and level of differentiation to conventional PTC, like macrofollicular and Warthin-like type, had a similar staining pattern. To our knowledge, there is no study of HBME-1 staining in the Warthin-like variant of PTC, while there are only two reports concerning its expression in the macrofollicular variant of PTC with findings similar to ours [14, 31]. The macrofollicular variant of PTC is an uncommon tumour that can be confused with nodular goiter or follicular adenoma [32]. This variant, despite being described as having a good prognosis with a low incidence of metastases, can sometimes present as a highly aggressive tumour, making its detection and proper characterization of importance. Trabecular and solid variants of PTC, which exhibit a variable prognosis displayed diversity of staining, while the only clear cell case of PTC tested here was completely negative. Interestingly, we found no studies of HBME-1 immunoexpression in solid, trabecular and clear cell PTC variants. More aggressive variants of PTC recognized by WHO, such as tall cell and diffuse sclerosing, stained mainly with low intensity in our study. These cases had no unfavourable clinicopathological factors at presentation, such as extrathyroid invasion and higher pTNM grade. As documented previously for the tall cell variant confined to the thyroid [33], such cases have a slightly increased rate of disease recurrence when compared with classic PTC without extrathyroid extension. While there are no reports about HBME-1 immunoexpression in diffuse sclerosing PTC variant, the diversity of staining of tall cell cases in our series differs from the results of Rigau et al. [34], who found apical reactivity of HBME-1 in tall cell variant of PTC to be stronger than in classical PTC. Similarly, Rossi et al. [20] gave one case report about HBME-1 positive tall cell PTC variant. Anyway, whether HBME-1 is preserved or down-regulated in tall cell variant of PTC should be verified by

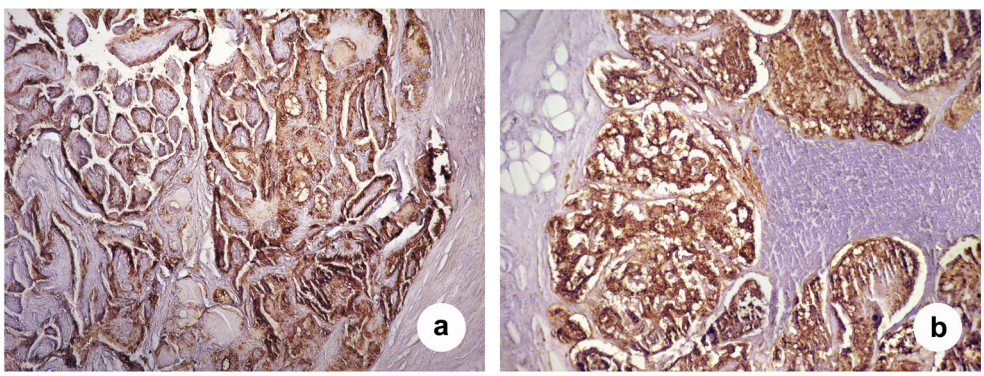

Fig. 3 Representative micrographs of high HBME-1 expression in primary papillary thyroid carcinoma (**a**) and matched lymph node metastasis (**b**). Indirect immunoperoxidase technique; hematoxylin diaminobenzidine counterstaining. Original magnifications×10

further research, due to the small number of cases included here and in previous studies. The reported frequency of HBME-1 positivity in insular carcinomas varies widely among immunohistochemical investigations by different research groups [15, 20, 35]. In our cases of PTCs with a follicular/solid/insular histological pattern, the insular foci were completely immunonegative, although the HBME-1 result for whole sections could be positive. This result further illustrates the potential variability of HBME-1 expression in different histologic variants. Because HBME-1 is not expressed or only focally expressed at a low level in the normal thyroid, its aberrant expression in papillary cancer suggests a role in the development or progression of these tumours. Even more, our data demonstrated that the level of HBME-1 expression depends on PTC histotype and the extent of tumour differentiation. Similarly, other research groups found no or poor expression of HBME-1 in poorly and undifferentiated carcinomas of the thyroid gland [19].

Although we cannot say anything about its specific function in the process of carcinogenesis of thyroid tissue, HBME-1 protein expression (high) was associated with unfavourable clinical parameters of differentiated thyroid cancer and has potential as an indicator of prediction for PTC. In addition, recurrence and the PTC-related death in this study developed exclusively from PTC cases with a high HBME-1 IHC score. However, the frequency of high HBME-1 expression in PTCs that recurred (100 %) was not significantly different from the value for those who remained disease free (70 %) ($P>0.05$), which may partly be due to the small number of cases with follow-up data. Therefore, the results we report warrant further examination. This initial cohort will be followed and clinical outcomes, such as tumour recurrences, metastatic disease, or death from thyroid cancer, will be collected over the extensive period of time required for true outcome analysis in this disease. Several earlier studies documented a significant correlation between HBME-1 expression in primary tumour tissues and the presence of unfavourable clinicopathological factors in thyroid cancer [9] but such a finding was not invariably confirmed [23, 24]. According to our data, abundant expression of HBME-1 in the primary PTC might act as an independent predictive factor for the presence of lymph node metastases, advanced pT status and advanced pTNM stage of patients. Our results are in agreement with those of Cheng et al. [9] concerning an increased risk of lymph node metastasis in thyroid carcinoma patients with membranous staining of HBME-1, but on the other hand, the association with extrathyroid invasion in our work was of borderline significance. Liang et al. [23] documented elevated levels of HBME-1 expression in metastatic compared to non-metastatic thyroid carcinoma, but they were unable to assert HBME- 1 as an independent prognostic factor. Furthermore, Cui et al. [24] found no association between HBME-1 expression and thyroid carcinoma aggressive behaviour. The diversity of published results may be explained by the use of unequal sample sizes, different cut-off values for HBME- 1 or technical considerations in immunohistochemistry.

Furthermore, to identify expression changes of HBME-1 protein that occurs subsequent to thyroid LNM, we applied immunohistochemistry to primary PTC and matched LNM. All papillary carcinomas demonstrated positivity for HBME-1 in both the thyroid and lymph nodes, which corresponds to the observation of Torregossa et al. [21] and Hirokawa et al. [22]. Even more, in this study 82.4 % of primary tumours and their LNMs were highly HBME-1 positive. These findings emphasize the importance of HBME-1 in the prediction of metastatic papillary carcinoma and its possible use for the detection of LNM.

In summary, this study reveals HBME-1 as a sensitive marker of malignancy for most PTC variants, whether primary tumour or nodal metastases. In addition, an association of increased HBME-1 expression in primary PTC with the presence of LNM, advanced pT status and pTNM stage underlines the clinical relevance of its use as an independent predictive factor for a potentially poor prognosis.

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Conflict of Interest The authors declare that no conflict of interests exists.

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