The Utility Of Galectin-3 And Hector Battifora Mesothelial Epitope (HBME)-1 In Differentiating Thyroid Lesions

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Abstract. The aim of this study is to determine the utility of galectin-3 and HBME-1 in the differentiation between benign and malignant thyroid lesions. We evaluated the immunohistochemical expression of 64 thyroid lesions including non-neoplastic lesions and neoplastic lesions. Galectin-3 immunostaining was positive in 100% of papillary carcinomas, 62.5% of follicular carcinomas, 18.8% of follicular adenomas and negative in all nodular goiter as non -neoplastic lesions. HBME-1 immunostaining was positive in 93.8% of papillary carcinomas, 81.3% of follicular carcinomas, 25% of follicular adenomas and negative in all nodular goiter. There was a significant difference between benign and malignant thyroid lesion for galectin-3 and HBME-1 (p<0.001). The sensitivity, specificity, positive predictive values and negative predictive values for HBME-1 and galectin-3 is over 80%. When both markers are combined, the values of each predictive parameter were 93.75%, 81.25%, 83.33% and 92.85%. The diagnostic accuracy for both markers was 87.50%. Combining both markers will be more helpful to differentiate malignant and benign thyroid lesions.

1. INTRODUCTION

Thyroid lesions can be found as benign and malignant. Definitive diagnosis is not always easy to define because morphologic similarities between them are often frequent. Sometimes the same patterns could be found in both malignant and benign lesion. By using routine hematoxylin and eosin (HE), in some tumors, subjective interpretation and interobserver variation among pathologist are well documented. This condition led the pathologist to search for additional diagnostic markers to define more accurate diagnosis because the treatment of thyroid lesions depends on the diagnosis [1,2].

Galectin-3 translated by gene located on chromosome 14q21-22 categorized as a beta galactosidase binding protein [3,4]. This protein has a function in cycle cell, angiogenesis, carcinogenesis and metastasis. Galectin-3 involved in many tumors like carcinoma thyroid, glioblastoma, breast cancer, limfoma, malignant melanoma, pancreatic cancer, colorectal cancer and gastric cancer [3-6]. In thyroid carcinoma, galectin-3 was reported work through K-Ras GTP and Ras signal pathway [7]. Some studies showed that galectin-3 staining was detected more frequent in malignant thyroid lesions, meanwhile it was decreased or not detected in benign thyroid lesions but another studies reported that Galectin-3 alone did not enough as a single marker in distinguishing thyroid tumors [8-12].

Hector Battifora Mesothelial Epitop (HBME)-1 was also reported increase reactivity with mostly papillary and follicular thyroid carcinoma, while adenomas are mainly negative. It is expressed in cytoplasm and cytoplasmic membrane like galectin 3 [13,14].
Thyroid neoplasms usually found by clinician as a simple nodule or multinodular goiter form, which is defined as thyroid nodules [15]. As mention above that by microscopic examination using HE only, it sometimes makes a different interpretation between pathologists. So, in this research, we studied the immunoexpression of galectin-3 and HBME-1 as a single and combine additional markers to define the thyroid lesions.

2. MATERIAL AND METHODS

2.1. Samples

Sample collection of paraffin block and all research activities were done in Hasanuddin University Hospital, Makassar, Indonesia. Sixty four samples from surgical resection of thyroid lesion were used in this study.

2.2. Immunohistochemistry

Two consecutive 4 µm sections were cut for HE staining and immunohistochemical study. Immunohistochemical staining was performed using Envision peroxidase detection system (Dako, Denmark). Immunohistochemical staining was perform using the primary anti human galectin-3 antibody (Novocastra) and anti human HBME-1 antibody (Dako) with the same dilution (1:100) and then incubated with labeled polymer Envision (Dako) for 60 min.
Slide were then evaluated by 3 independent observer (UM, MD, HC). Immunoreactivity of galectin-3 and HBME-1 were graded in 4 scoring level according to number of positive cells in % and the intensity of DAB color in all area of thyroid lesion each slide. Both proteins were positive when stained in the cytoplasm and score as follows: score 0, +1, +2 and +3. Score 0 and +1 were categorized negative, whereas score 2 and 3 were grouped as positive.

3. RESULTS

Sixty four samples of thyroid lesions were evaluated in this study. It was found that range of patient's age were between 22 to 81 years old. Most patients were female (87.5%), only 12.5% of patients were male. Characteristic samples were found in Table 1. All samples of thyroid lesion were stained with galectin-3 and HBME-1 antibody and associated with the features microscopic diagnosis as shown in Table 2. This table showed that there were a significant difference between galectin-3 expression, HBME-1 expression and group of histopathology ($p<0.001$).

Table 1. Characteristic of samples.

Characteristic of Samples		No. cases	%
Age	22-41 years old	40	62.5
	42-61 years old	18	28.1
	62-81 years old	6	9.4
Sex	Male	8	12.5
	Female	56	87.5
Diagnosis	Nodular Goiter	16	25
	Follicular Adenomas	16	25
	Follicular Carcinomas	16	25
	Papillary Carcinomas	16	25

Table 2. Galectin-3, HBME-1 and histopathology subgroup.												
	Histopathology									Total		*p*
		Nodular Goiter		Follicular Adenomas		Follicular Carcinomas		Papillary Carcinomas				
		n	%	n	%	n	%	n	%	n	%	
Galectin-3 Expression	Negative	16	100.0	13	81.3	6	37.5	0	0.0	35	54.7	<0.001
	Positive	0	0.0	3	18.8	10	62.5	16	100.0	29	45.3	
HBME-1 Expression	Negative	16	100.0	12	75.0	3	18.8	1	6.3	32	50.0	<0.001
	Positive	0	0.0	4	25.0	13	81.3	15	31.3	32	50.0	

In table 3, the histopathological diagnosis was categorized into 2 groups, benign and malignant lesion. Nodular goiter and follicular adenomas were categorized as benign thyroid lesion, follicular carcinomas and papillary carcinomas were labeled as malignant thyroid lesion. Galectin 3 was more frequent in carcinoma than nodular goiter and follicular adenomas. Both markers have the same results, $p<0.001$. Representative immunostaining for both markers were shown in Figure 1 and 2.

Table 3. Galectin-3, HBME-1 in thyroid lesions.

		Thyroid lesion						
		Malignant		Benign		Total		*p*
		n	%	n	%	n	%	
Galectin-3 Expression	Negative	6	18.8	29	90.6	35	54.7	<0.001
	Positive	26	81.3	3	9.4	29	45.3	
HBME-1 Expression	Negative	4	12.5	28	87.5	32	50.0	<0.001
	Positive	28	87.5	4	12.5	32	50.0	

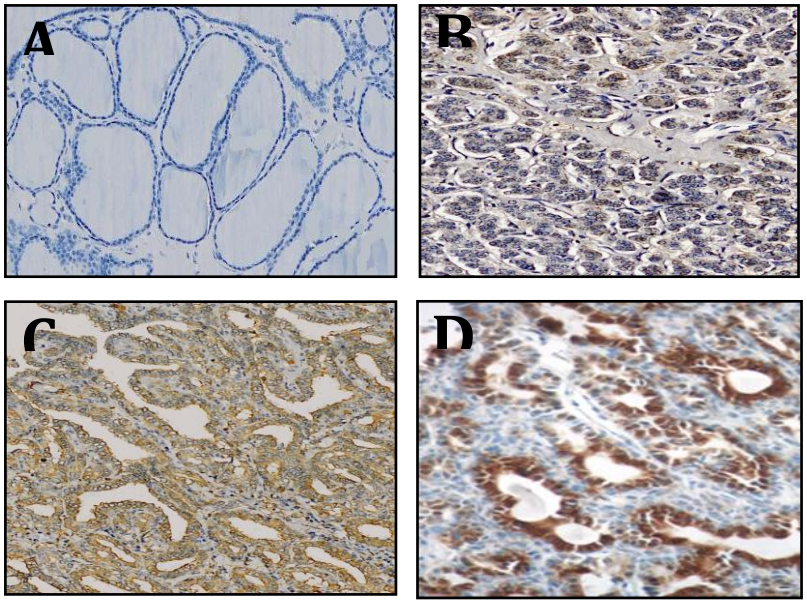

Figure 1 Immunostaining of Galectin-3 (A) Nodular goiter (-), (B) Follicular adenoma (+1), (C) Papillary carcinoma (+2), (D) Follicular carcinoma (+3)

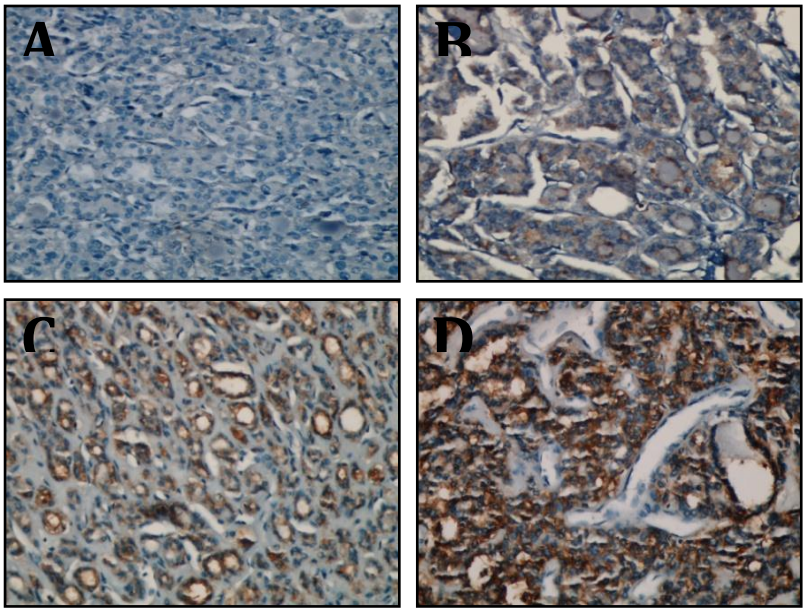

Figure 2 Immunostaining of HBME-1 (A) Nodular goiter (-), (B) Follicular adenoma (+1), (C) Follicular carcinoma (+2), (D) Papillary carcinoma (+3)

Diagnostic value for both markers was shown in Table 4. When combining both markers, the value of each parameter become 93.75% for sensitivity, 81.25% for specificity, 83.33% for positive predictive value (PPV), 92.85% for negative predictive value (NPV) and 87.50% for diagnostic accuracy. All value above 80% and some of them above 90%.

Table 4. Diagnostic value.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic Accuracy (%)
Galectin-3	81.25	90.62	89.65	82.85	85.93
HBME-1	87.50	87.50	87.50	87.50	86.15
Galectin-3 & HBME-1	93.75	81.25	83.33	92.85	87.50

4. DISCUSSION

In this study, we analysed the diagnostic accuracy of galectin-3 and HBME-1 in thyroid lesion. Both marker has been studied in previous study by using immunohistochemistry but the result is quite varied. They recommended Galectin-3 as an accurate marker for thyroid carcinoma, especially papillary carcinoma [14,16-20]. However, this marker still need to be elucidated.

Our study showed that Galectin-3 immunostaining predominantly positive in carcinoma, a few positive in adenomas and totally negative in nodular goiter. Our results consistent with some studies which reported that Galectin-3 immunoexpression was increased in all malignant neoplasms and only few samples (3 – 12%) of benign lesions were stained positively [2,7,14,18]. However, another study reported that Galectin-3 was totally undetected in all non-malignant lesions [21-22]. When we found that some samples of follicular adenomas were positive-stained for Galectin-3, we assume that these lesions might be in the level of in malignant transformation which still occur in molecular level not in phenotype level yet. Actually for diagnosing the follicular thyroid carcinoma, the histopathologic appearance not just enough with classical atypical performance such as hypercellularity, increase of nucleus and cytoplasm ratio, and mitosis, but also required a distinct vascular and capsular invasion. Another explanation may easy to understand that in those cutting specimens there was no malignant areas. There might be some little focus of malignancy that could be detected in another area of tumor tissue but not in these evaluated-slides. This condition sometimes found in papillary thyroid carcinoma as an occult cancer. In other words, Galectin-3 expression still could be helpful to define the diagnosis of follicular adenoma which tend to develop become follicular thyroid carcinoma or malignancy. This information from pathologist will help the clinician to consider a possibility of malignancy in suspected adenoma specimens and prepare more comprehensive management for the patients.
Compared with benign neoplasms, HBME-1 was highly detected in both carcinomas than follicular adenomas, but there was no significant difference between both malignant neoplasms. It means HBME-1 can be applicable as a marker for carcinomas, but not reliable to distinguish follicular carcinomas and papillary carcinomas.
We found that both markers were have excellent diagnostic value for thyroid malignancy. The first marker as a beta galactosidase binding protein was the most specific, especially for papillary thyroid carcinoma. The second marker seems to be a sensitive marker for both papillary carcinoma and follicular carcinoma. Combination of these markers increase sensitivity about 10% and become more applicable.
Since specificity and sensitivity of both markers in this study were almost 100%, it could be said that detection by immunohistochemical method may useful as an additional test beside H.E. staining as a standard method. Furthermore, if the morphological characteristic of carcinoma was so clear by Hematoxylin eosin staining, of course immunostaining of both markers, single or combine was not required.
In conclusion, Galectin-3 and HBME-1 could be applicable as additional staining in differentiating diagnosis of thyroid lesions.

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