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Diagnostic value of **galectin-3** and **hector battifora mesothelial epitope (hbme)-1** as a **marker** for **malignancy** in the **diagnosis** of **thyroid lesions**.

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Background: The differential diagnosis between malignant and benign lesions of the thyroid gland has always been in a problematic issue. Morphologic similarities of benign and malignant lesions, especially in follicular lesions, and subjectivity of morphologic criteria has led pathologists to search for more objective additional diagnostic markers.

Aim: To investigate the usefulness of immunohistochemical expression of galectin-3 and HBME-1 as markers in the differentiation between benign and malignant thyroid lesions.

Methods: We evaluated 64 thyroid lesions including 48 neoplastic lesions [16 papillary carcinomas (PC), 16 follicular carcinomas (FC), 16 follicular adenomas (FA)] and 16 non-neoplastic lesions/nodular goiter (NG) using immunohistochemical methods. The expression of Galectin-3 and HBME-1 were scored and analysed.

Results: The expression of galectin-3 was positive in 100% of PC, 62.5% of FC, 18.8% of FA and negative in NG. The expression of HBME-1 was positive in 93.8% of PC, 81.3 % of FC, 25% of FA and negative in NG. Significant differences were found between benign and malignant lesions, and also between the subgroups of benign and malignant lesions ($p<0.001$) for both galectin-3 and HBME-1. The sensitivity, specificity, positive predictive values and negative predictive values were 81.25%, 90.62%, 89.65% and 82.85%, respectively, for galectin-3 and 87.5% in each value for HBME-1; whereas the results for both markers combined were 93.75%, 81.25%, 83.33% and 92.85%. The diagnostic accuracy of the staining was 85.93% for galectin-3, 86.15% for HBME-1 and 87.50% for both markers.

Conclusion: Galectin-3 and HBME-1 have an excellent sensitivity and specificity for malignant thyroid lesions. Combining these markers could increase sensitivity and could be useful as an adjunct to distinguish benign from malignant thyroid lesion

Introduction

Thyroid gland is the biggest endocrine gland in our body with almost mainly nodules were found inside of it. Thyroid nodules were commonly occurs as benign tumors, some cases were found in carcinoma and rarely as sarcoma. *International Agency for Research on Cancer (IARC)* were estimated that 163.000 new cases of thyroid cancer worldwide in 2008 (1). Meanwhile, in *American Cancer Society* data, there will be 44.670 cases of thyroid carcinoma in 2010 (2). In Indonesia, there is not still yet new data with explain the exact number of insidens and mortalities of thyroid carcinoma. According to the data from Cancer registration council of Indonesia Cancer Foundation in 2005, it reveals that thyroid cancer took place in 9^{th} of 10-commonest of cancer in Indonesia after colon cancer. A study from Anatomical pathology Department of Hasanuddin university has showed that in 2010, there were 609 cases of thyroid cancer.

Galectin-3 is a 31 kDa molecular weight protein and belong to β-galactoside binding protein family which has coded in a gene that located in 14q21-22 chromosome.(3,4) This protein was involved in many biologic process such as cell to cell and cell to matrix interactions, *pre-mRNA splicing*, cell proliferation, cycle cell regulation, angiogenesis, tumorigenesis and metastasis. (3-5) There were so many previously studies which reported that galectin-3 has a main role in most of cancer types like breast cancer, malignant melanoma, colon cancer, pancreatic and gastric cancer, limfoma and glioblastoma. In this cases, overexpression of galectin-3 were correlated with the capability of tumor cells to invade and metastase. Model of breast cancer by in vitro assay has showed that galectin-3 overexpression give a protection to tumor cells from apoptotic that induced by *loss of anchorage*. It will support for availability of tumor cells when they move from their primary location. (6)

Many studies in recent years has reported that galectin-3 expression were increased in malignant thyroid lesions, meanwhile it were decreased or not expressed in benign thyroid lesions. (7-9). In cells, galectin-3 plays a role as *scaffold* protein of K-Ras, where K-Ras has a function as *binary switches alternating* between *Guanosine diphosphate (GDP)-binding* (inactive) and *Guanosine Triphosphate (GTP)-binding* (active) form. It will activate some effectors such as Raf, phosphatidylinositol-3-OH kinase (PI3-K) and Raf-guanine nucleotide exchange factors, which turn to collaborate and regulate cell proliferation and differentiation, survival and apoptosis. Overall, galectin-3 plays a role in thyroid carcinoma by K-Ras GTP and Ras signal pathway. (10)

Thyroid lesions were found by clinician in simple nodule or multionodular goiter form, which is defined as thyroid enlarged that caused by thyrocyte proferation. (11) Previously, diagnose and histologic classification of thyroid lesions were still depend on microscopically pattern which can be shown in specimen that stained with standard (haematoxylin-eosin/H.E.) method. In this way, the overlapping pattern between benign and malignant lesions is usually found and it makes a different interpretation in between pathologists. So it is important to find another marker to get a diagnose more accurately. In this study, we proposed galectin-3 as alternative protein that could be help to distinguish between benign or malignant thyroid lesions.

Materials and methods

This study were performed in the laboratory of Anatomical pathology department, Hasanuddin university, Makassar. Eighty samples of thyroid tissues were used from patients with got surgery

for thyroid lesions. Tissue samples were then fixated in buffered-formalin and keep in paraffin block form. A serial cutting of paraffin block with 5-μm thickness were performed in order to make a specimen slide, which then stained with H.E methods to confirmed the previous diagnose.

Other specimen slides were stained immunohistochemically according to standard protocol by using NovoLink Polymer Detection System (Novocastra, New Castle, UK) and anti human galectin-3 monoclonal antibody (Novocastra, New Castle, UK) with 1 : 100 dilution.

Slide were then evaluate by two pathologist in blind methods. Expression of galectin-3 were positive when cytoplasm of cell give brown colour. Score of galectin-3 expression were defined according to percentage of positive cells in 10-randomized area (400X), as follows: 0, staining of less than 10% of tumor cells; 1, staining of 10 – 25% of cells; 2, staining of 25 – 50%; 3, staining of 50 – 75%; and 4, staining of more than 75%. Score 0 were then determined as negative, and score 1 to 4 were positive.

Statistical analysis was done with SPSS version 16 software. Kruskal-Wallis and Mann-Whitney-U test were used to analyze differences of histopathologic diagnosis in galectin-3 score. X^2 test was done to analyze differences of histopathologic diagnosis in galectin-3 expression. A p value ≤ 0.05 was considered statistically significant.

Results

In eighty patients with thyroid lesions, it was found that range of patient's age were between 6 to 81 years old (y.o.), with most frequent in age group between 31 to 55 y.o. (71.3%) (Table 1. Age). In sex, most of patients were female (88.8%); meanwhile, only 11.3% of patients with thyroid lesions were male (Table 1. Sex). In related with types of tumor, it was found that benign thyroid lesions were consist of nodular goiter and follicular adenoma; and malignant thyroid lesions were follicular and papillary carcinoma, with each of types has the same number and percentage (n = 20; 25%) (Table 1. Types of tumor).

Galectin-3 was mainly expressed in cytoplasm of cells (Figure 1). Positive control specimens showed galectin-3 score from 1 to 4, meanwhile in negative control specimens, galectin-3 was unstained (score 0). In related with galectin-3 expression, there were 36 (45%) unstained samples (score 0); 7 (8.8%) samples with score 1; 8 (10.8%) samples with score 2; 15 (18.8%) samples with score 3; and 14 (17.5%) samples with score 4. No significant differences were noted in each groups of sample characterstics.

Table 1.

Characteristic of samples

Characteristics		Number (%)
Age	6 – 30 years old (y.o.)	15 (18.8)
	31 – 55 y.o.	57 (71.3)
	56 – 81 y.o.	8 (10)
Sex	Male	9 (11.2%)
	Female	71 (88.8%)
Types of tumor	Jinak	20 (25)
	- Nodular goiter	20 (25)
	- Follicular adenoma	
	Ganas	20 (25)
	- Follicular carcinoma	
	- Papillar carcinoma	20 (25)
Galectin-3 expression	Positive	44 (55)
	Negative	36 (45)

X^2 test $p > 0.05$

Table 2.

Comparison galectin-3 score in histopathologic types of thyroid lesions

Galectin-3 score	Histopathologic types			
	Nodular goiter	Follicular adenoma	Folliculary carcinoma	Papillary carcinoma
Mean	0.05	0.45	2.2	3.5
Median	0	0	2	4
Deviation standard	0.22	1.14	1	0.61
Minimun	0	0	1	2
Maximun	1	4	4	4

Kruskal-Wallis test df = 3 $p = 0.0001$ ($p < 0.05$)

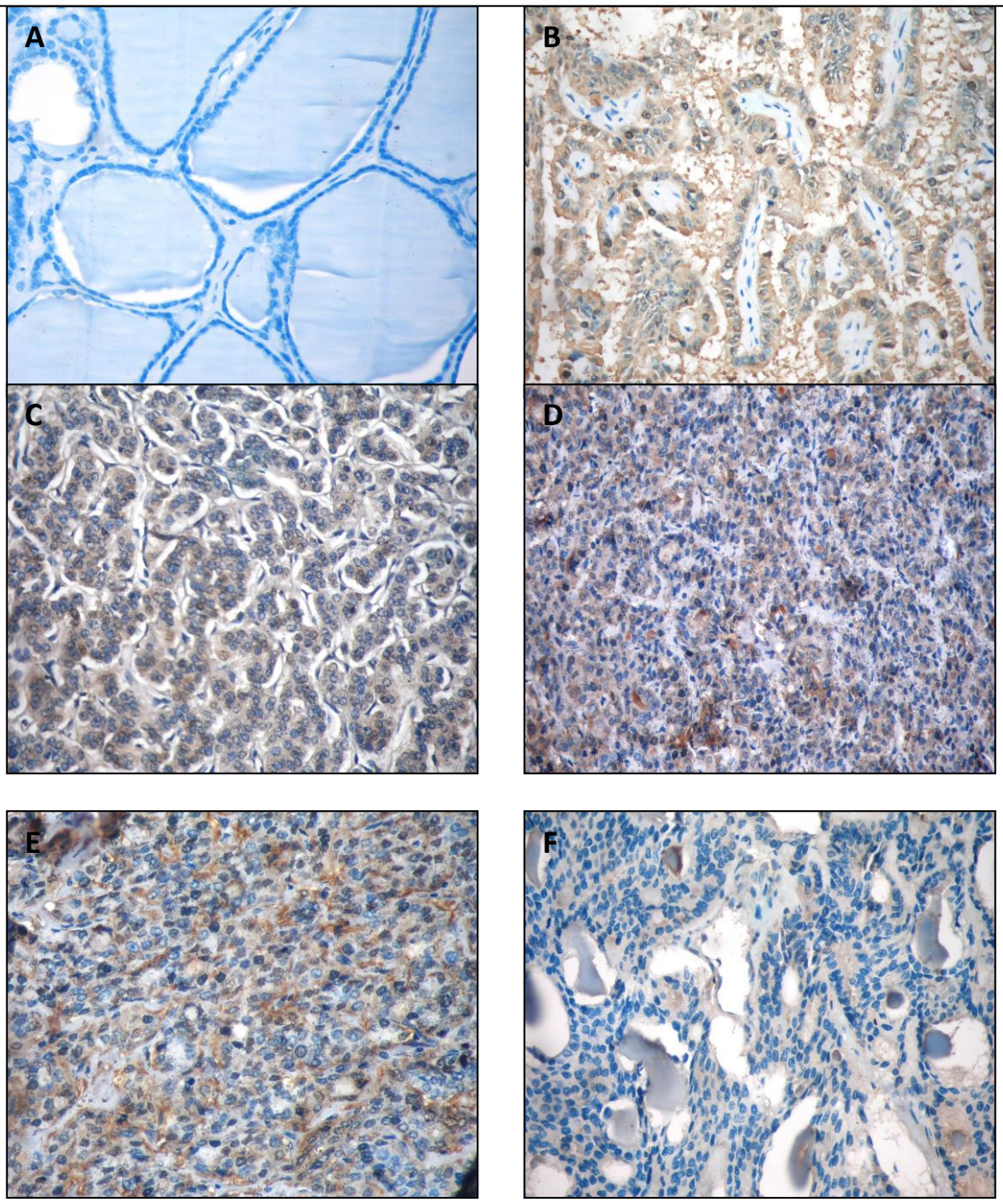

Figure 1. Galectin-3 expression in thyroid lesions (A) Nodular Goiter and (B) papillar carcinoma (score-4) (C) score-3 and (D) follicular carcinoma with score 1

Significant differences of histopathologic types of thyroid lesions in galectin-3 score

As noted in table 2, the mean of galectin-3 score was found highest in papillary carcinoma (3.5), with the lowest mean score was in goiter nodular (0.05). There is a significant difference of galectin-3 score between both types of carcinomas with two types of benign lesions. We also find

significant differences of galectin-3 score between papillary and follicular carcinomas, where papillary carcinoma has higher score than follicular carcinoma.

Significant differences of galectin-3 score in between benign and malignant thyroid lesions

Table 3.

Differences of galectin-3 score in types of thyroid lesions

Galectin-3 score	Lesion types	
	Benign	Malignant
Mean	0.25	2.85
Median	0	3
Deviation standard	0.84	1.05
Minimun	0	1
Maximun	4	4

Mann-Whitney U test $z = 7.464$ $p = 0.0001$ ($p < 0.05$)

In table 3, it was noted that the mean of galectin-3 score was higher in malignant thyroid lesions (2.85) than in benign thyroid lesions (0.25). There were significant differences of galectin-3 score between benign and malignant thyroid lesions.

Significant differences of galectin-3 expression between benign and malignant thyroid lesions

Table 4.

Comparison of galectin-3 expression in tumor types

Galectin-3 expression	**Tumor types**		
	Benign	Malignant	Total
Positive	4 (10%)	40 (100%)	44
Negative	36 (90%)	0 (0%)	36
Total	40 (100%)	40 (100%)	80

X^2 test = 65.455, df = 1, $p = 0.0001$ ($p < 0.05$)

Table 4 showed that all of malignant thyroid lesions were positively stained for galectin-3 (100%), meanwhile mostly of benign thyroid lesions were unstained for galectin-3 (90%). Only four samples of benign lesions were positively stained (10%). The sensitivity, specificity, positive predictive value and negative predicitive value of the staining for galectin-3 were 100%, 90%, 90.9%, and 100%, respectively, on the basis of the percentage of positive-stained cells.

Discussion

In general, the most frequent insidens of thyroid nodules in our study were in between 31 to 50 years old, with the youngest cases was found in 6 years old and the oldest was in 81 years old. Both of them were diagnosed as folliculary carcinoma. The previous studies were reported that papillary thyroid carcinomas were commonly occur in patients between 20-50 years old, meanwhile folliculary carcinomas were mostly found in patients with 40 – 60 years old.(10)

Both types of thyroid lesions were largely found in female patients. In our study, all samples of goiter nodular and folliculary carcinomas were from female patients; meanwhile, only 18 (90%) of folliculary adenoma samples and 13 (65%) of papillary carcinoma samples were female. Other reports was noted that the number cases of thyroid carcinoma were 2 – 4 times higher in female. It was assumed that some genes which interact with androgen hormones receptors were involved in thyroid carcinogenesis.(10)

In our study, all of malignant lesions were expressed with galectin-3 and most of benign lesions were unstained. Other study were reported that galectin-3 was expressed in all malignant lesions and mostly of benign lesions did not express galectin-3; only a few (3 – 12%) samples were positive-stained (7, 13-15). However, another study has reported that galectin-3 was unexpressed in all benign thyroid lesions (16, 17). In our study, we found some benign lesion samples were positive-stained for galectin-3. We assume that these lesions were in malignant transformation which still occur in molecular level. Even these lesions give suspect malignancy histologic appearance such as hypercellularity, increase of nucleus and cytoplasm ratio, and mitosis, but there were no capsular and vascular invasion. Another explanation was some malignant focus were not found in these specimen. There might be some little focus of malignancy (occult cancer) that could be find in another area of tumor tissue but not in these evaluated-slides. In other words, galectin-3 expression could be helpful for pathologist as a guide to diagnose a folliculary adenoma which tend to a malignancy. This could be also an information for the clinician to consider a possibility of malignancy in suspected adenoma specimens.

In our study, we also find that galectin-3 score and expression were higher in papillary carcinoma than in folliculary carcinoma. Other study have reported that galectin-3 was involved in carcinogenesis process in many tissues. They recommended to galectin-3 as a sensitive and accurate marker for thyroid cancer, especially papillary carcinoma (12, 13, 18-20). However, there were no recent report that explain clearly for these differeances.

Since sensitivity, specificity, positive predictive value and negative predicitive value of the staining for galectin-3 in this study were almost 100%, it could be said that this method could become additional test beside H.E. staining as standard method in distinguishing between benign and malignant lesions, especially in folliculary or folliculary varian of papillary carcinoma with folliculary adenoma. Furthermore, if the morphological characteristic of carcinoma was so clear by H.E. method; immunostaining of galectin-3 was not required.

As conclusion in our study, galectin-3 expression has good sensitivity in malignant thyroid lesions. Galectin-3 expression could be used as additional marker to differentiate benign and malignant thyroid lesions, especially in some difficult cases with need special attention for pathologists.

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