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ORIGINAL PAPER

The Significance Of Galectin-3 Expression in the Immunocytochemical Evaluation of Thyroid Fine Needle Aspiration Cytology

Safak Ersoz · Hikmet Sert · Mustafa Yandi · Cihangir Erem · Sevdegul Mungan · Halil Onder Ersoz · Umit Cobanoglu · Arif Hacihasanoglu

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Abstract The aim of this study is to evaluate the significance of immunohistochemical expression of Galectin-3 in the differential diagnosis of benign and malignant thyroid nodules. We studied the fine needle aspiration specimens of 38 patients who had evaluated for nodular goiter and undergone a thyroid surgery between 2004–2005. Slides had been stained immunocytochemically with Galectin-3. The cytoplasmic staining of Galectin-3 was analyzed. Three cases of five follicular carcinomas had positive staining for Galectin-3, while two had not. Two cases with follicular adenomas were negative for Galectin-3. Five cases of six papillary carcinomas had positive staining for Galectin-3, while one case (the case with a papillary microcarcinoma) had not. The single cases with medullary and anaplastic carcinomas were negative for Galectin-3. None of the cases with a benign thyroid pathology had positive staining for Galectin-3. Galectin-3 immunocytochemical staining, had a sensitivity of 61.5%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 83.3% for thyroid malignancies. For the evaluation of follicular neoplasm, Galectin-3 immunocytochemical staining had a sensitivity of 60%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 50%. Galectin-3 expression in thyrocytes is a strong indicator of a malignant proliferative lesion especially for papillary and to an extent in follicular thyroid neoplasms. Galectin-3 could be used as a supplementary marker for cytological diagnosis.

Keywords Thyroid neoplasms · Galectin-3

S. Ersoz (✉) · S. Mungan · U. Cobanoglu
Department of Pathology, Faculty of Medicine,
Karadeniz Technical University,
61080 Trabzon, Turkey
e-mail: ersozs@yahoo.com

H. Sert · M. Yandi
Department of General Surgery, Faculty of Medicine,
Karadeniz Technical University,
61080 Trabzon, Turkey

C. Erem · H. O. Ersoz · A. Hacihasanoglu
Department of Endocrinology, Faculty of Medicine,
Karadeniz Technical University,
61080 Trabzon, Turkey

Introduction

Thyroid carcinoma is the most common endocrine malignancy [1]. Fine needle aspiration cytology (FNAC) is a very informative procedure for the diagnosis of malignancy in thyroid nodules. Although having a 90% sensitivity and specificity for the evaluation of a thyroid nodule, FNAC has several limitations [2, 3]. Inadequate sampling and same morphologic changes shared by both benign and malignant nodules, cause difficulties in the diagnosis. False negative results are not uncommon in the diagnosis of papillary thyroid carcinoma [4, 5]. Galectin-3, a member of B-galactosidase binding lectin family, has important functions in physiological and pathological processes such as cellular adhesion, inflammation, apoptosis, and neoplastic transformation [6–8]. Some authors pointed the importance of immunohistochemical staining of Galectin-3 for the differential diagnosis of suspicious cytology of fine needle aspiration biopsy specimens [9, 10]. In contrast to the results demonstrating the specificity of Galectin-3 expres-

Table 1 Comparison of cytopathological and histopathological diagnosis of the study cases

FNAB/biopsy	Malign lesions	Benign lesions	Total
Malign cytology	6	0	6
Benign cytology	1	21	22
Suspicious cytology	6	4	10
Total	13	25	38

sion in thyroid carcinomas, Cvejic et al. have reported false positivity in follicular adenomas and false negativity in thyroid carcinomas [11]. The aim of this study is to evaluate the significance of immunohistochemical expression of Galectin-3 in the differential diagnosis of benign and malignant thyroid nodules.

Material and Method

We studied the fine needle aspiration specimens of 38 patients who had evaluated for nodular goiter and undergone a thyroid surgery between 2004–2005 at the departments of Endocrinology and General Surgery of Karadeniz Technical University Medical Faculty Hospital. The study was approved by local ethics committee and all patients gave written informed consent. Two specimens had been stained immunocytochemically with Galectin-3 before microscopic examination. Other specimens were fixed and stained with Papanicolaou and Giemsa.

Immunocytochemical staining The slides were fixed in methyl alcohol for 5 min. Some of the slides were restained with Galectin-3 after elimination of the routine stain. For antigen retrieval, the slides were heated in citrate buffer in microwave for 15 min. Indirect immunoperoxidase avidin–biotine complex technique was used for immunostaining. Slides were treated with Galectin-3 (Clone 9C4, Neomarkers) for 16 h. 3,3′-diaminobenzide tetrahydrochloride was used as chromogen. The slides were counterstained with Mayer Haematoxylin. Histiocytes were served as an internal control for Galectin-3 staining. Histopathological diagnosis was accepted as gold standard. Histopathological slides were also stained with Galectin-3 in order to compare cytopathological evaluation. The cytoplasmic staining of Galectin-3 was analyzed.

Statistical analysis As this is a methodological study, the specificity, sensitivity, negative and positive predictive value of Galectin-3 staining were evaluated.

Results

Classical evaluation of thyroid fine needle aspiration cytology of 38 cases revealed the following result: 22 (58%) benign cytology, six (16%) malign cytology, seven (18%) follicular neoplasm, and three (8%) suspicious for malignancy. These three suspicious cases were found to be one Riedel Thyroiditis, one nodular colloidal goiter, and one papillary thyroid carcinoma case after pathological evaluation of surgical material. Six cases with malignant cytological diagnosis were reported as papillary carcinoma in four, medullary carcinoma in one, and anaplastic carcinoma in one case after pathological evaluation. Five of seven cases with follicular neoplasm diagnosis had follicular carcinomas, while two had follicular adenomas. Twenty two patients had benign cytological diagnosis, 18 of those were found to be nodular colloidal goiter, while four had lymphocytic thyroiditis. Only in one patient with benign cytological diagnosis, pathological evaluation revealed a papillary microcarcinoma. A total number of 13 cases of 38 were found to be malignant, with five follicular carcinomas, six papillary carcinomas, one medullary carcinoma, and one anaplastic carcinoma (Table 1).

Galectin-3 staining of FNAC materials was as follows: three cases of five follicular carcinomas had positive

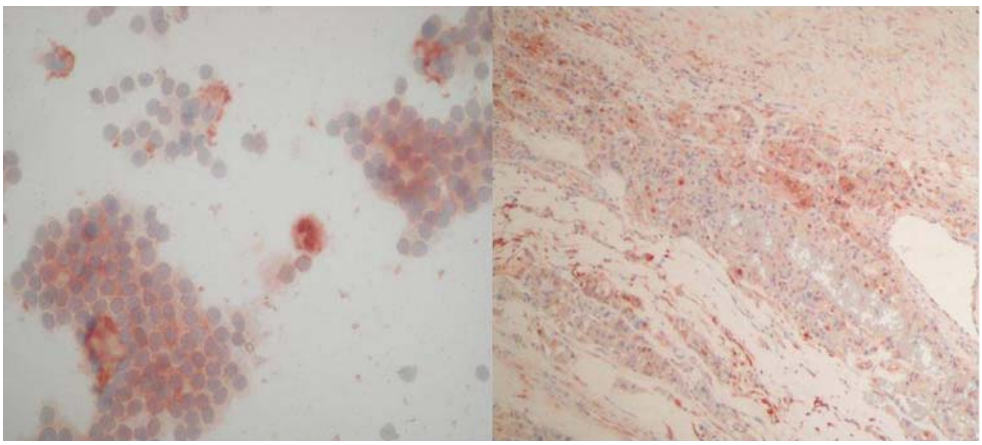

Fig. 1 Cytological (*left picture*, ×200) and histopathological (*right picture*, ×100) positivity of Galectin-3 in a case of follicular carcinoma

staining for Galectin-3 (Fig. 1), while two had not. Two cases with follicular adenomas were negative for Galectin-3. Five cases of six papillary carcinomas had positive staining for Galectin-3 (Fig. 2), while one case (the case with a papillary microcarcinoma) had not. However, in this case with papillary microcarcinoma, FNAC was performed from the dominant nodule, which did not show any malignant features. Moreover in the histopathological evaluation of thyroid surgical specimen of this case, papillary microcarcinoma area was significantly stained by Galectin-3. The single cases with medullary and anaplastic carcinomas were negative for Galectin-3. None of the cases with a benign thyroid pathology had positive staining for Galectin-3 (Table 2).

Galectin-3 immunocytochemical staining, when compared with histopathological evaluation as a gold standard, had a sensitivity of 61.5%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 83.3% for thyroid malignancies. For the evaluation of follicular neoplasm, Galectin-3 immunocytochemical staining had a sensitivity of 60%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 50%.

Table 2 Galectin-3 expression in malign and benign lesions

Galectin-3/biopsy	Malign lesions	Benign lesions	Total
Galectin-3 (+)	7	0	7
Galectin-3 (−)	6	25	31
Total	13	25	38

Discussion

The differentiation of follicular adenoma or carcinoma could not be made certain without a thyroid surgery. Although thyroid fine needle aspiration cytology is still being the most important diagnostic tool for the evaluation of a thyroid nodule, inadequate sampling and same morphological features shared by follicular neoplasms (adenoma or carcinoma) limits the sensitivity and specificity of this procedure. Accurate evaluation of a thyroid nodule by FNAC could both decrease the rates of unnecessary operations that would lead to surgical risks and lifelong levothyroxine supplementation and also false negative results that would result in tumor progression and metastasis [4, 12, 13].

In this study, we evaluated the diagnostic value of Galectin-3 staining in addition to classical procedures, in FNAC of thyroid nodules. Galectin-3 seems to be a useful and promising marker for the diagnosis of papillary and follicular thyroid carcinomas. An exaggerated expression of Galectin-3 in follicular and papillary thyroid carcinomas had been reported previously by Xu et al., while benign nodules were not stained with this marker [14]. Bartolazzi et al. had also supported this result in their study [9]. However, following studies created a controversy in this issue causing a suspicion for the diagnostic role of Galectin-3 staining in the diagnosis of thyroid cancer. Positive staining of follicular adenomas had increased false positive rates and decreased the specificity of Galectin-3 as a marker for follicular carcinomas [15–17]. In our study, we did not observe any false positive staining in benign nodules, while all but one case of papillary carcinomas and more than half of follicular carcinomas were found to be positive for Galectin-3 staining. In a previous report by Kovacs et al., galectin-3 expression was shown strongly and diffusely in almost all papillary carcinomas [17]. We can conclude the same result for our study, because the only case with a Galectin-3 negative result for papillary carcinoma was the one with microcarcinoma whose FNAC was found to be negative for malignant cytopathology.

In this study, Galectin-3 staining was observed in more than half of follicular carcinoma patients while none of the patients with follicular adenomas were positive for this marker. Galectin-3 positivity could be considered as an indication for surgery. However as false negative rates are still high, Galectin-3 expression should not be accepted as a

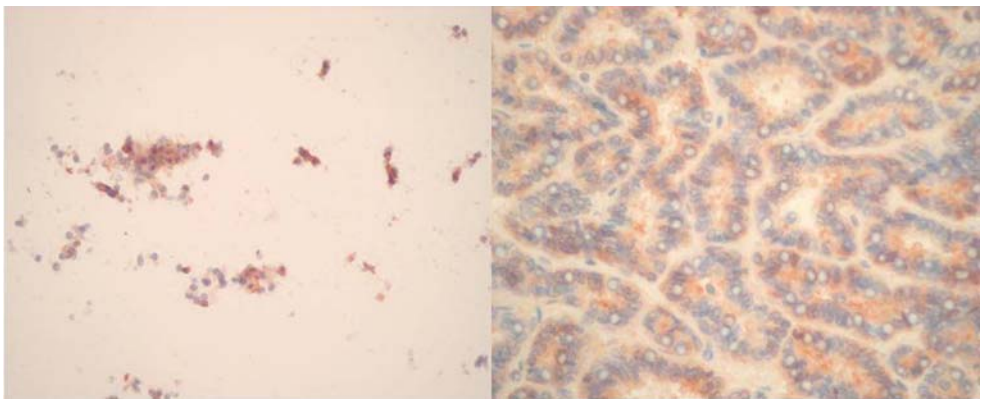

Fig. 2 This pictures show cytoplasmic expression of Galectin-3 in the thyroid papillary carcinoma in cytological (*left picture*, ×100) and histopathological (*right picture*, ×200) slides

sole indicator for the differentiation of follicular adenomas and carcinomas.

As medullary and anaplastic thyroid carcinomas were not stained with Galectin-3, this marker should be considered as a diagnostic tool for mostly papillary and follicular thyroid carcinomas. However in our study group, medullary and anaplastic carcinomas were represented as sole patients so this issue remains to be determined.

We did not observe any false positive cases, but that had been reported before by Kovacs et al., especially in follicular cells showing oncocytic changes in Hashimoto Thyroiditis [17].

As a result, Galectin-3 expression in thyrocytes is a strong indicator of a malignant proliferative lesion especially for papillary and to an extent in follicular thyroid neoplasms. Nevertheless a negative result should not exclude thyroid malignancy. Galectin-3 could be used as a supplementary marker for cytological diagnosis. However it should be kept in mind that it is not an absolute marker for the determination of a lesion as benign or malignant especially considering the follicular neoplasms.

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