Expression of Galectin-3 in Thyroid Lesions; Immunohistochemical Analysis

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Abstract: Objectives: Evaluation of a single thyroid nodule is considered as one of the diagnostic challenges for pathologists. Recently Galectin-3 has been found to be a promising immunohistochemical marker for papillary carcinoma. The objective of this study is to test the value of Galectin-3 expression in differentiating PTC from other thyroid lesions.**Method:** In this study we evaluated a total of 74 cases of thyroid lesions which share a common clinical presentation as solitary thyroid nodule; 38 cases of papillary thyroid carcinoma, 5 cases of follicular thyroid carcinoma, 16 cases of thyroid adenoma, and 15 cases of hyperplasic thyroid nodules. The cases were immunohistochemically stained for Galectin-3 using the conventional biotin – avidin immunoenzymatic technique.
Results: Positive staining for Galectin-3 was detected in 35 cases out of thirty-eight (92%) of PTC (27 cases show moderate or strong diffuse staining). Of the three negative cases 2 were follicular variant of papillary carcinoma, and one case was conventional type. Weak or focal staining was detected in 2 out of 5 cases of FTC (40%), 5 out of 16 cases of follicular adenoma (31%), and 3 out of 15 of hyperplastic nodules (20%). None of the non papillary lesions show moderate or strong staining.**Conclusion**: Diffuse and strong immunohistochemical staining for Galectin-3 carries high significance in the diagnosis of PTC and differentiating benign from malignant tumors. However, we recommend its use with caution in diagnosing unconventional variants of PTC.
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Key words*:* Thyroid lesions, Galactin-3 expression, IHC, differential diagnosis.

1. Introduction

The burden of thyroid disease in the general population is enormous. As many as 50% of people in the community have microscopic nodules [1] whereas palpable nodules are encountered in 4% of the population of the United States between the ages of 30-60 years [2]. One of the challenging areas in surgical pathology is the differential diagnosis of encapsulated, follicular-patterned tumors with less-than-typical nuclei and equivocal signs of invasiveness. This necessitates the discrimination between; dominant nodule of nodular hyperplasia, follicular adenoma, minimally invasive follicular carcinoma, and the follicular variant of papillary carcinoma. Although the current diagnostic 'gold standard' for most thyroid lesions, is pathological evaluation using routine hematoxylin and eosin (H&E) stains by expert pathologists, yet, the diagnostic agreement among pathologists remains suboptimal. Malignancy of the thyroid gland is a common health problem worldwide [3-5]. In the Saudi Society, thyroid cancer is the second most common malignancy in females in all age groups (9.4%), being only preceded by breast cancer, as reported in Saudi Cancer Registry, 2004. PTC is the most common type of thyroid malignancy [6]. The histopathological diagnosis of PTC depends mainly on the appreciation of the characteristic nuclear features rather than the presence of true papillary fronds [7-9]. Recent studies pointed to some immunohistochemical markers questioning their diagnostic and prognostic utility in different thyroid tumors. Among these promising markers is Galectin-3. Galectin-3 is a unique member of an ancient lectin family [10]. Galectin-3 is also expressed in a variety of normal tissue and tumors [11] Malignant transformation of thyroid cells has been found to be accompanied with intense nuclear localization of galectin-3 [12]. Galectin-3 expression recently emerged as a potential diagnostic and/or prognostic marker of some cancers [13]. Although galectin-3 is not a universal and unambiguous marker of thyroid cancers, it could be a helpful parameter in diagnosis of these tumors as well as possible potential therapeutic target [14-17]. However the data is not very clear yet regarding the reliability of using Galectin-3 for confirmation of the diagnosis of PTC. In this study we test the value of Galectin-3 expression using immunohistochemistry in differentiating PTC from other thyroid lesions.

2. Material and Method:

Seventy four cases of different thyroid tumors were retrieved from the archival files of pathology department at king Abdulaziz University Hospital (KAUH). We selected 38 cases diagnosed as papillary thyroid carcinoma (PTC); 18 cases were

conventional type, 9 cases were follicular variants (FVPTC), 6 cases were encapsulated type, one case was tall cell variant, and 4 cases were microcarcinomas. Seven cases showed multifocal tumor. Four cases of PTC were associated with lymph node metastasis and one of the involved lymph nodes from each case was also stained with Galectin-3 to study the staining pattern in metastatic tumor as well. The study also included 5 cases diagnosed as minimally invasive follicular thyroid carcinoma (MIFTC), 16 cases of follicular adenoma, and 15 cases of hyperplastic nodules. The cases were reviewed by two pathologists, and stained with Galectin-3, using the conventional biotin – avidin immunoenzymatic technique as follows: Five-micrometer sections from selected tumor blocks are mounted on 3-aminopropyltriethoxysilane coated (Sigma, St. Louis, MO) slides, Sections are deparaffinized in xylene, rehydrated in graded alcohols, and rinsed in 0.05 M Tris-buffered saline (TBS), sections then boiled in 10 mM citrate buffer for antigen retrieval, at pH 6.0, blocking of endogenous peroxidase with aqueous 0.3% H_2O_2 for 15 min. Sections are then incubated for one hour with the monoclonal antibody; Galectin-3 (Novacastra, UK, clone 9C4). The dilution used was 1:100 as per the company instruction. The antigen-antibody immunoreactions are visualized using 3, 3'diaminobenzidine. All immunoreactions are carried out at room temperature. Negative control sections are made by exclusion of the primary antibody. Positive control sections are obtained from sections of normal prostatic tissue. Gal-3 gives nuclear and/or cytoplasmic staining. Tumor cells were considered positive only when the appropriate staining pattern was noted. Positive histological reaction for different antibodies used is visualized as follows; A semi quantitative scoring system is used to score immunohistochemical positivity; The extent of immunoreactivity was categorized as negative (0); less than 5% f the cells show positive staining; equivocal (+/-), focal or weak staining (1+); 5% to 10%, moderate (2+); 11% to 50%, strong and diffuse (3+); greater than 50% positivity of tumor cells.

3. Results:

The study investigated 74 cases of different thyroid lesions; 38 cases of papillary thyroid carcinoma (PTC), 5 cases of minimally invasive follicular thyroid carcinoma (FTC), 16 cases of thyroid adenoma, and 15 cases of hyperplasic nodule. All share a common clinical presentation as single thyroid nodule. The thirty-eight cases of PTC were excised from 33 females and 5 males (female to male ratio 6.6/1). The age ranged from 17 to 70 years with the mean age of 38 years. Only 3 cases out of 38 were negative for Galectin-3 immunostaining. The positive cases represented 92% of the total number of PTC (table 1 and figure 1). Of the three negative cases 2 were FVPTC, and one case was conventional type. As for the staining intensity; 13 cases stained strongly and diffuse (+3), 14 cases revealed moderate staining (+2), 7 cases stained weakly or focal (+1), while another one case revealed equivocal pattern of staining (+/-). The localization of staining was mainly cytoplasmic, both nuclear and cytoplasmic staining was encountered in (+3) cases. Neither The staining localization nor its intensity showed relation to specific type of the tumor. The study included 5 cases of minimally invasive FTC; three cases were females and 2 cases were males. The age ranged from 21 to 43 with mean age of 33 years. Three cases were negative for the Galectin-3 immunostaining, while two cases were positive (+1). The included 16 cases of adenoma were excised from 13 females and three male patients (female to male ratio was 4.3/1), the mean age was 34 years. Negativity was demonstrated in 11 cases (73 %). The 5 positive cases either weak (+) or equivocal (+/-), whereas in the hyperplasic nodules; 12 cases were negative (80%), three cases were weakly positive (+1). The female to male ratio in hyperplastic nodules' cases was 6.5/1, while the mean age was 33 years. The results showed significant Galactin-3 expression differences between the benign and malignant lesions in which the tendency of positive expression is mainly more towards the malignant cases as comparing to benign lesions (**$p<0.0001$**).

Table 1: Intensity of Galectin-3 positivity in different thyroid lesions:

	0	**+/-**	**+1**	**+2**	**+3**	**Total**
PTC	3	1	7	14	13	38
FTC	3	0	2	0	0	5
Adenoma	11	2	3	0	0	16
Hyperplastic	12	0	3	0	0	15
Total	29	3	15	14	13	**74**

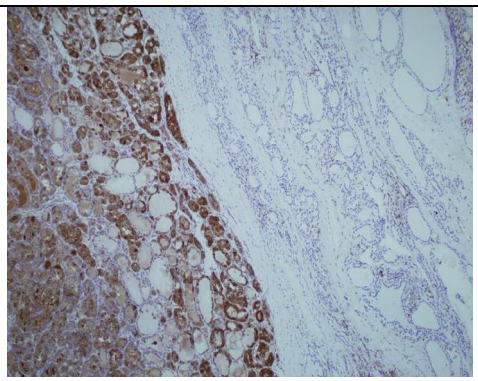

Fig. 1A) Gal-3 positivity in encapsulated FPTC against surrounding negatively stained normal thyroid tissue.

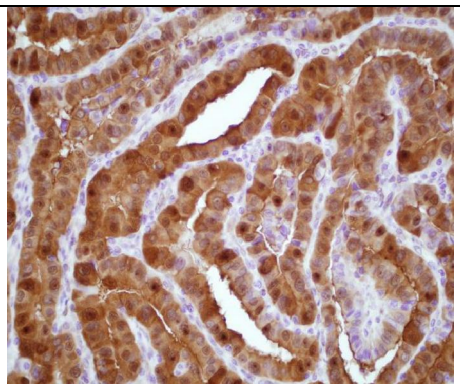

Fig. 1B) Pattern of Gal-3 staining (nuclear and cytoplasmic (+3) in PTC.

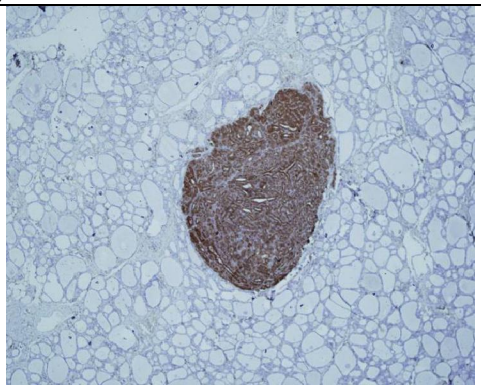

Fig. 1C) Gal-3 highlighting incidentally found papillay microcarcinoma background of normal thyroid with negative staining.

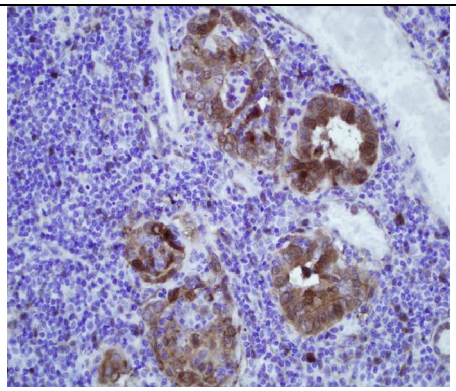

Fig. 1D) Gal-3 highlighting metastatic PTC in lymph node

4. Discussion:

Saxen et al [18] tested the reducibility of WHO classification of thyroid tumors on 696 cases and found only 58% agreement among five Nordic pathologists. A more recent study by Hirokawa et al. [19] who compared the diagnoses of 21 follicular nodules by four American and four Japanese pathologists; the agreement of benign versus malignant was encountered in only 62% of the nodules. Fassina et al.,[20], review of 200 thyroid tumors revealed good agreement for papillary and anaplastic thyroid carcinomas, moderate for medullary and poor for follicular thyroid carcinomas. In more recent review of 41 follicular carcinomas by five experienced French thyroid pathologists, the agreement for malignancy varied from 5% among all five pathologists to 56% between two pathologists. It is clear from these studies and others [21] that there is interobserver variation in the diagnosis of thyroid neoplasms.

Among the recent ancillary techniques emerged, Galectin-3 has been found to be a promising marker with high specificity for papillary thyroid carcinoma (PTC). In the study of **Smenov, et al** who examined fourty cases of thyroid nodules, positive immunohistochemistry staining for Galectin-3 was found to be a highly sensitive oncomarker for PTC: sensitivity of 100%, specificity of 71%, diagnostic value of 85% [22]. This study was in agreement with ours which demonstrated positivity for galectin-3 in 90% cases of PTC including microcarcinoma and were positive in metastatic carcinoma in the lymph nodes in all the four cases with lymph nodes metastasis. This was also in concordance with the study of **Cvejic et al,** [23] who reported immunopositivity in 80.9% of papillary microcarcinomas.

Our study revealed different intensity of immunostaining among PTC cases ranging from (+1 to +3), and showed predominant cytoplasmic localization with or without nuclear staining. The strong diffuse (3+) positivity was seen only in papillary carcinoma and none of the other lesions show this strong intense staining pattern.

Our results were also in keeping with that of **Cvejic et al,** which revealed that localization of galectin-3 was mainly cytoplasmic, and the intensity of staining was variable, ranging from strong to weak. The

immunonegative cases in their study were of non-classical types lacking the papillary architecture. They didn't relate galectin-3 positivity to rate of growth or aggressiveness of the tumor, rather suggested its role in tumor biology [23]. Our study also demonstrated that two out of the three negative cases of PTC were of FVPTC, an observation that could be a feature of Galectin-3 specificity to tumors with papillary architecture.

In another study by **Cvejic et al** [24] conducted on wider scale of papillary thyroid cases (202 cases), they studied Galectin-3 expression in different histological patterns of PTC, cases with lymph node metastasis and cases with extrathyroid invasion. Their results revealed sensitivity of galectin-3 immunostaining for conventional histology in 98% (100 of 102) for classical PTC, 85.2% (46 of 54) for follicular variant, and 50% (23 of 46) for follicular/solid variant of PTC. They also demonstrated that Galectin-3 immunohistochemical expression itself is not an indicator of lymph node metastasis or extrathyroid invasion of PTC. They declared as well as other investigators [25-27] that Galectin-3 as an excellent marker for classical PTC yet recommended its use with caution in diagnosing unconventional variants of PTC because of the possibility of false-negative results. On the other hand, **Mehrotra et al** [28] found that galectin-3 immunopositivity is not only in PTC, but also in a large proportion of follicular adenomas (72%) and multinodular goitres (57%) which is in contrast to our study and other studies [29].

Few studies investigated Galectin-3 expression in follicular thyroid carcinoma (FTC), positivity was encountered in minimally invasive FTC [30], as well as in fully invasive FTC in a study of 260 cases [31] which also correlated the galectin-3 positivity to the degree of capsular or vascular invasion. They concluded that Galectin-3 expression level significantly increased with the degree of vascular or capsular invasion ($p<0.0001$). However, its diagnostic value for follicular carcinoma was not high because the sensitivity and specificity were 68.7% and 57.5%, respectively. In our study three cases out of five of FTC were negative for galectin-3 immunostaining and the two positive cases only show weak (+1) staining pattern.

In conclusion our results as well as those of the vast majority of researchers confirmed that galectin-3 is an excellent marker for supporting the diagnosis of papillary thyroid carcinoma and for distinguishing malignant from benign or hyperplasic lesions when the morphologic criteria are equivocal. Strong (3+) positivity for galectin-3 is almost characteristic for papillary carcinoma. Although the number of FVPTC is small, the data demonstrate that negative staining for galectin-3 does not exclude papillary carcinoma in those cases and the presence of strong diffuse staining (3+) is very suggestive of papillary carcinoma. Galectin-3 expression is also successful in highlighting metastasis in lymph nodes, yet its role in FTC is controversial. Further studies using combination of galectin-3 and other immunohistochemical markers such as HBME-1, CITED-1, CK-19 and fibronectin would be useful to establish a panel for the diagnosis thyroid neoplasms with higher sensitivity and specificity.

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