

How Reliable is Galectin-3 Immunohistochemical Expression for Differentiation Between Metastatic and Benign Thyroid Neoplasms

Umm-e-Farwa *

Department of Histopathology, Foundation University Medical College, Islamabad, Pakistan

Corresponding author: Farwa U, Department of Histopathology, Foundation University Medical College, Islamabad, Pakistan, E-mail: farwam@gmail.com

Abstract

Galectin-3 has been reported to have substantial accuracy in detection or excluding malignant nodules with prior indeterminate FNAC and per operative findings. Keeping this fact in mind, Thus, Galectin-3 can have a pivotal role in separating benign from the malignant thyroid neoplasms. We aim to determine the frequency and intensity of Galectin-3 immunohistochemical expression studied in the benign and malignant thyroid neoplasms confirmed on histopathology. This descriptive, observational, cross-sectional study was conducted from 5th November 2017 to 4th May 2018 in the Department of Histopathology, Foundation University Medical College, Islamabad Campus and Department of Surgery, Fauji Foundation Hospital, Rawalpindi. We studied 78 thyroid specimens diagnosed with thyroid neoplasms on histopathology. Out of these 39 were benign cases (follicular adenoma and hurthle cell adenoma) and 39 were malignant cases (papillary thyroid carcinomas, follicular carcinoma, medullary carcinoma and poorly differentiated carcinoma). Each specimen was examined grossly and microscopically and checked for immunohistochemical staining pattern of Galectin-3 under the microscope. Age range in this study was from 15 to 65 years with mean age of 44.97 ± 10.78 years. Out of these 78 patients, 17 (21.79%) were male and 61 (78.21%) were female with male to female ratio of 1:3.6. Frequency of positive Galectin-3 immuno histochemical expression among thyroid neoplasms was found in 32 (41.03%) cases with Galectin-3 showing positive staining in 21 (53.85%) of all malignant and 11 (28.21%) of all benign cases. Among the malignant neoplasms, positivity was seen most frequently in papillary thyroid carcinomas as compared to the other malignancies.

This study concluded that positive Galectin-3 immunohistochemical expression is seen both in benign and malignant thyroid neoplasm, but its expression is more in malignant thyroid neoplasms (53.85%) as compare to the benign lesions (28.21%). Therefore, we recommend that Galectin-3 immunohistochemical marker cannot be used alone for the routine diagnosis of malignant thyroid lesions as it has shown less sensitivity and specificity. Moreover it also has shown no significant role in differentiating between the benign and the malignant thyroid neoplasms.

Keywords:

Neoplasm; Marker; Malignant; Benign

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Introduction

Thyroid gland is an important part of the endocrine system located at the base of the neck. It is chiefly composed of two types of cells, follicular and parafollicular cells. The follicular cells make thyroxine, which has important functional impacts on various systems and general metabolism. The parafollicular cells, also known as C cells arise from the neural crest and are involved in the calcitonin production, which has vital role in maintaining calcium homeostasis. Thyroid neoplasms including both benign and malignant lesions are common entities encountered in daily clinical practice. Most of the lesions (95%) arise from the follicular epithelial cells of the thyroid gland. Thyroid cancer is the most common among the endocrine tumors and its incidence has been increasing in the last three decades. An estimated mortality rate of thyroid cancer is 0.5 to 10 cases per 100,000. The annual male and female percentage is 6.3% and 7.1% for white population, 4.3% and 8.4% for blacks and for Asian population patients it is 3.4% and 6.4% respectively.³ These tumors can clinically present as a solitary nodule along with the normal thyroid gland or as a dominant nodule in the background of a multinodular goiter. 5% of the solitary thyroid nodules are found to be neoplastic.

In Pakistan, thyroid neoplasms are common especially in the northern areas, which are mainly attributable to the iodine deficiency or excess. Thyroid cancer accounts for 1.2% of all the malignancies diagnosed in our country with the papillary thyroid carcinoma being most common. The female to male ratio in Pakistan is reported as 2.2:1. Patients can present with both the features of hyper and hypothyroidism in both benign and malignant lesion. This makes it clinically difficult to diagnose the exact underlying cause. Here comes the role of histopathology, which can correctly diagnose the lesion, but there are some neoplasms that have very confusing morphological details and these cannot be exactly categorized into benign or malignant, only on the basis of histopathology. This scenario is mostly seen in the follicular and the Hurthle cell neoplasms. The gross appearance and the microscopic details are perplexing for a pathologist. Moreover, the cytological details are also much overlapping in various benign versus malignant lesions.

The final diagnosis of the lesion being benign and malignant has profound effects on the clinical outcome and prognosis of the patient. Several articles have reported the significance of immunohistochemical markers to solve this problem. Galectin-3, p63 and Ki67 have been reported quite accurate to detect or exclude malignancy in nodules with prior indeterminate FNAC and per operative findings. In this study, role of Galectin-3 will be quantified to differentiate and classify the thyroid lesions into benign and malignant categories. Galectin-3 belongs to the family of lectins. Galectin-3 is synthesized in both the nucleus and cytoplasm, and also expressed at the cell surface. It is also found extracellularly in the general circulation. Galectin-3 specifically binds to the beta galactoside containing intracellular, extracellular and cell surface associated glycol conjugates so it is over expressed in oncogenic pathology of

thyroid. In Pakistan, limited data is available regarding the role of galectin-3 as a diagnostic tool to differentiate malignant thyroid neoplasms from benign lesions. So, this study can have beneficial effects in the diagnostics and further treatment of such lesions.

Materials and Methods

There was a total of 78 thyroid specimens included in this study (39 benign and 39 malignant neoplasms). All of these patients were operated at the Department of Surgery, Fauji Foundation Hospital Rawalpindi during a period of six months from 5th November 2017 to 4th May 2018. The specimens were processed in the department of Histopathology, Foundation University Medical College Islamabad. The benign conditions included Follicular adenoma and Hurthle cell adenoma. The malignant conditions included Papillary thyroid carcinoma (both classic type and follicular variant), Follicular thyroid carcinoma, Medullary thyroid carcinoma and poorly differentiated carcinoma. The hospital ethical committee granted the approval for data collection. The data included patient's demographic details, clinical presentation, previous laboratory test record and clinical suspicion. The specimens were examined both grossly and microscopically in the laboratory. The thyroid specimens were fixed in 10% formalin and were sliced properly. The representative sections were processed in the tissue processor for the paraffin sectioning. After this step, 4-5 μm thick sections were cut using rotatory microtome (SAKURA ACCU-CUT MODEL SRM 200 CW). Hematoxylin and eosin stain (H and E) was used for staining the slides and get them ready to see under the microscope. For the immunohistochemistry, representative histological sections of the thyroid neoplasm were used. The sections were deparaffinised by xylene and then were rehydrated by ethanol. Tri-sodium citrate buffer (pH 6.0 to 6.2) was used for the antigen retrieval. When the slides came back to room temperature, endogenous peroxidase activity was blocked by 0.6% H₂O₂. After this step lyophilized mouse monoclonal Galectin-3 antibody in the dilution of 1:100 was applied for an hour. Washing was done with Tris- Buffered Saline (TBS). Then for 20 minutes super enhancer was added. Polymer Horseradish Peroxidase (HRP) was applied for 30 minutes as a secondary antibody and washing was done again with TBS. Subsequently Diamine Benzidine (DAB) chromogen was applied for 5 minutes. Mayer's Haematoxylin was used for counter staining followed by clearing and mounting. Positive and negative controls were also applied. Two consultant histopathologists examined the H and E stain and immunohistochemical marker (Galectin-3) under the Olympus light microscope. The sections with the best staining were selected for examination and reported likewise. Morphology and staining was noted and grading of Galectin-3 was done and Hermann ME et al guidelines. The intensity and distribution of Galectin-3 staining (cytoplasmic) on a scale of 0 to 3 was done as follows:

0 No staining

- 1+ Weak/slight staining
- 2+ Moderate staining
- 3+ Intense staining

The proportion of stained cells was interpreted as;

- 1+ < 5% of cells
- 2+ 5% to 50% of cells
- 3+ > 50% of cells

The lesions with the particular cytoplasmic staining of more than 5% of the tumor cells was taken as positive for Galectin-3 regardless of its intensity.

Results

Age range in this study was from 15 to 65 years with mean age of 44.97 ± 10.78 years as shown in (Table:1). Out of

these 78 patients, 61 (78.21%) were female and 17 (21.79%) were male with female to male ratio of 3.6:1 (Figure 1 and Table 1).

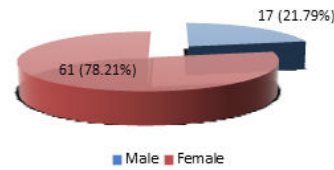


Figure 1: Distribution of patients according to gender (n=78).

Table 1: Age distribution of patients (n=78), having Mean \pm SD = 44.97 ± 10.78 years.

Age (in years)	No. of Patients	%age
15-40	27	34.62
41-65	51	65.38
Total	78	100

On the basis of histopathological diagnosis half (39) cases belonged to benign neoplasms and other half (39) were diagnosed as malignant neoplasms as shown in (Figure 2).

78 (41.03%) cases while 46 out of total 78 (58.97%) were showing negative galectin-3 staining (Figure 3).

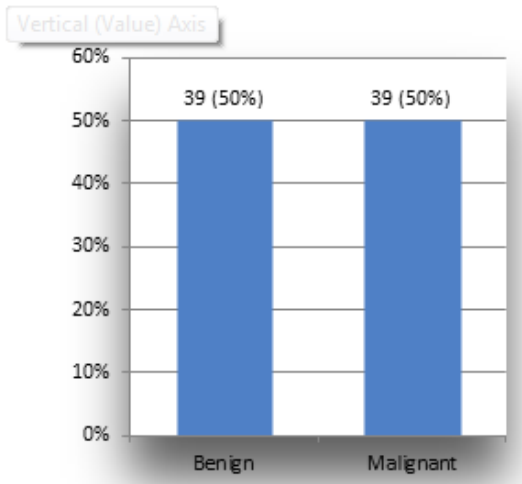


Figure 2: Distribution of patients according to histopathological features (n=78).

Frequency of positive Galectin-3 immunohistochemical expression among thyroid neoplasms was found in 32 out of

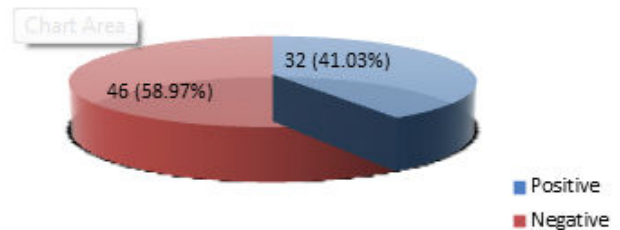


Figure 3: Frequency of Galectin-3 immunohistochemical expression among thyroid neoplasms confirmed on histopathology (n=78).

A detailed look at the further breakdown of galectin-3 staining among benign neoplasms reveal that 11(28.1%) among 39 benign cases were positive for the stain. For the malignant neoplasms, total 21(53.85%) among 39 cases were positive. On the other side 28(71.79%) benign cases and 18(46.15%) malignant cases showed negative galectin-3 staining. The p-value calculated was 0.021 which is not significant (Table: 2)

Table 2: Stratification of Galectin-3 immunohistochemical expression with respect to age groups.

	Galectin-3 immunohistochemical expression		p-value
	Positive	Negative	
Male	8	9	0.567
Female	24	37	

The Stratification of Galectin-3 immunohistochemical expression with respect to age groups showed total 27 cases within the age range of 15- 40 years out of which 10 cases were positive. Total 51 cases belonged to the age range of

41-65 years out of which 22 showed positive galectin-3 staining. The p-value calculated was 0.602 which is again insignificant (Table: 3)

Table 3: Stratification of Galectin-3 immunohistochemical expression with respect to gender.

	Galectin-3 immunohistochemical expression		p-value
	Positive	Negative	
15-40 years	10	17	
41-65 years	22	29	0.602

Similarly Table IV shows the breakdown of the cases according to gender. Total 8 out of 17 cases among male patients were positive for Galectin-3 and 24 out of 61 cases

of female patients were showing the positive staining. The p-value calculated was 0.567 which is again insignificant.

Table 4: Stratification of Galectin-3 immunohistochemical expression among benign and malignant thyroid neoplasms.

	Galectin-3 immunohistochemical expression		p-value
	Positive	Negative	
Benign	11 (28.21%)	28 (71.79%)	
Malignant	21 (53.85%)	18 (46.15%)	0.021

The breakdown of Galectin-3 positivity in the various histological types of malignant and benign thyroid neoplasms is also shown in (figure 4 and 5) respectively.

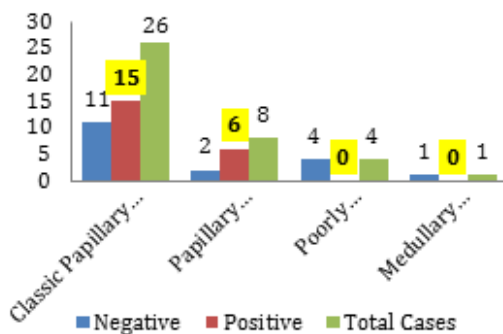


Figure 4: Galectin-3 staining in various histological types of Thyroid carcinomas.

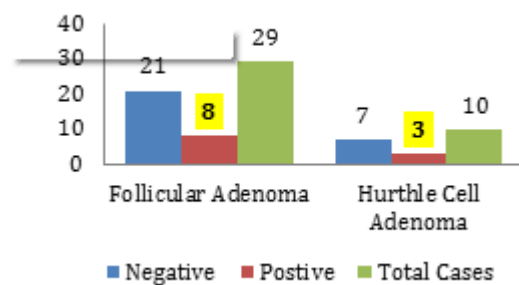


Figure 5: Galectin-3 staining in various histological types of Thyroid adenomas.

Discussion

Immunohistochemical markers have been extensively investigated for their potential diagnostic and prognostic utility in different thyroid tumors. Among these, they have deduced Galectin-3 to be a promising marker. Galectin-3 belongs to lectin family . And a constellation of other normal tissues and tumors masses express Galectin-3 . An intense nuclear localization of galectin-3 in tumors is seen in

malignant transformation of thyroid tissue . Research studies suggest Galectin-3 expression may serve as a potential diagnostic and prognostic marker of some cancers . However, galectin-3 has not been established as a universal and specific marker of thyroid neoplasia. Yet it can serve as a parameter in diagnostic approach for these tumors and for possible potential therapeutic target .

In 2015 a study was conducted in India regarding the staining pattern of Galectin-3 in thyroid neoplasms. The results showed 86% sensitivity and 85% specificity, with Galectin-3 showing positive staining in 87% of all malignant and 15% of all benign cases. In 2016, another study was conducted in Italy to check the diagnostic accuracy of the various immunohistochemical stains and they found galectin-3 to be 84.2% sensitive and 94.5% specific in detecting the thyroid neoplasms . In contrast, our study shows less frequency of positive Galectin-3 immunohistochemical expression among thyroid neoplasms i.e. total 32 (41.03%) cases with Galectin-3 showing positive staining in 53.85% of all malignant and 28.21% of all benign cases. In 2002 a study was conducted regarding the staining pattern of Galectin-3 in thyroid neoplasms. The results showed high frequency of staining in papillary thyroid carcinomas only and no significant staining in the other type of carcinomas. Moreover, it also showed positive results in follicular adenomas which made them conclude that galectin-3 is not a sensitive marker if used alone Our study also shows the same results.

A systematic review and meta-analysis on galectin-3 as a biomarker found that it may be a potentially useful immunomarker to distinguish between patients with papillary thyroid carcinoma (PTC) and patients without PTC. In addition, lymph node metastasis is more frequently seen in PTC patients with positive expression of galectin-3 . Our study also gives us the result that although galectin-3 is sensitive for detecting papillary thyroid carcinomas but it is not much sensitive in detecting other carcinomas from benign lesions (adenomas) as shown in (figures 4 and 5). Galectin-3, HBME-1, and cytokeratin-19 may be helpful in diagnosis of malignant thyroid tumors as evidenced by a study, although the expression of these markers may be seen in benign lesions as well. However, cytokeratin-19 is investigated for its diagnostic prowess. It was found that the marker and its combinations with other markers have higher sensitivities in accurate diagnosis of papillary carcinoma than the other combinations. But these immunohistochemical markers have limited role in differentiation between benign and malignant lesions . Another important point which was noted in this study was that the carcinomas showing positive galectin-3 gave mostly focal positivity rather than the diffuse strong positivity in comparison to the results of the study done . That study, which was done in 2012, demonstrated that galectin-3 staining pattern is significant in differentiating benign from malignant follicular neoplasms as well as follicular variant of papillary thyroid carcinoma. Diffuse positivity for galectin-3 was associated with malignant thyroid follicular neoplasms while focal weak positivity

favours adenomas. On the other hand, a previous study have demonstrated that there was no marked difference in the staining intensity for intra cytoplasmic or intranuclear expression of galectin-3 in benign and malignant thyroid neoplasms .

Thin-Prep fine needle aspiration cytology showing increased expression levels of galectin-3 were seen with cellular hyperproliferation, hypertrophy, and pathophysiological situations associated with adenomas and thyroid carcinomas . A comparison of glypican-3 (a member of the glypican family of heparan-sulfate proteoglycans bound to the plasma membrane) with galectin-3 demonstrated that galectin-3 had higher sensitivity in diagnosing thyroid carcinoma; however, specificity is low for differentiating follicular-patterned neoplasm . These markers have also been investigated preoperatively and postoperatively, the preoperative serum galectin-3 level showed potential diagnostic value, as it was significantly higher in the cancer patients than in the control subjects ($p < 0.05$).

Galectin-3 is also used in combination with other biomarkers for a differential diagnosis of thyroid lesions. The most commonly combined biomarkers are Hector Battifora mesothelial epitope-1 (HBME-1) and cytokeratin-19 . However galectin-3 may not be used as single discriminators between follicular thyroid adenoma and carcinoma . Some studies show that galectin-3 and HBME-1 have an excellent sensitivity and specificity for malignant thyroid lesions (100 and 89.1%, respectively) . Despite core needle biopsies leading to the diagnosis of the majority of thyroid nodules, the accuracy is increased by also observing the galectin-3, cytokeratin-19 and HBME-1 panels, indicating their additional diagnostic value when combined with routine histology and not when used alone. It was also reported that galectin 3, Cluster Of Differentiation (CD) and, to an extent, HBME-1, are useful immunocytochemical parameters with the potential to support the fine needle aspiration cytology diagnosis of PTC, particularly in situations where the differential diagnoses is complicated .

Studies have noted variable Galectin-3 expression in poorly differentiated thyroid cancers also . However, in majority of cases (75% to 100% of reported cases) of anaplastic thyroid carcinoma, Galectin-3 positivity was identified suggesting that differentiated thyroid carcinoma can progress or undergo anaplastic transformation . In the present study and study by Herrmann et al., small number of cases of MTC and poorly differentiated carcinoma are reported with inconsistent Galectin-3 expression, making diagnostic application of Galectin-3 in these rare histological subgroups unlikely .

Conclusion

Galectin-3 immunohistochemical expression is found in the cases of both benign thyroid tumors and malignant neoplasms although more commonly seen in malignant ones. So, it cannot be used alone for the routine diagnosis of malignant thyroid lesions as it shows less sensitivity and

specificity. It also has expressed limited role in differentiating between the benign and the malignant thyroid neoplasms.

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