

Original Research Article

Diagnostic value of Galectin-3 expression in papillary thyroid carcinoma and its variants

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Abstract: Galectin-3 is a member of beta-galactoside binding protein family and function as modulator of cell growth through galectoside-protein correlated with the occurrence and diagnostic value of papillary thyroid carcinoma (PTC). The study performed in 67 known histopathological cases of institution including neoplastic and non-neoplastic cases of thyroid. The immunohistochemistry performed and result showed that, cases of papillary thyroid carcinoma in spite of any variant give 2 to 3+ positive for majority of case. The other neoplastic and non-neoplastic cases which give negative or lesser positive for galectin-3. The author conclude that galectin-3 could be a valuable antibody for diagnosis of papillary thyroid carcinoma and a potential immunomarker, in making a preoperative distinction between PTC and non-PTC patients. In addition galectin-3 may provide more significant contributions in distinguishing PTC with or without lymph node metastasis.

Keywords: Galectin-3, immunohistochemistry, papillary thyroid carcinoma

INTRODUCTION

Thyroid nodules are extremely common in the general population and usually discovered during routine medical check-up. However, the widely used of new techniques like ultrasound and fine needle aspiration cytology has led the discovery of an infra-clinical reservoir of thyroid nodules in 20 to 76 % of population [1]. In which most of the thyroid nodule are benign, while thyroid cancer represents only 2-4%. Out of which papillary thyroid carcinoma is by far the most common type of thyroid malignancy (85%) and it's characterized by distinctive nuclear features [2]. The diagnosis of benign lesion, such as hyperplastic nodules, colloid nodules and auto-immune disease like thyroiditis, can be cytologically established [3]. However, to distinguish between malignant and benign lesion in some instance like papillary and microfollicular pattern lesion is challenging in cytology and even in histopathology. Exclusive follicular pattern in adenomatoid nodule and disruption of capsule in adenomas create difficulties in diagnosis by histopathology [4]. However papillary carcinomas are prone to diagnostic discrepancies among pathologists

[5]. In this regard, the diagnostic approach to these tumours should include IHC (immunohistochemistry) markers that can aid the better assessment of morphologic details. There are several studies have shown that immunohistochemical may provide additional support in the evaluation of diagnosis of thyroid discrepancies lesions. Many IHC markers including Galectin-3 have been evaluated in this regard. Galectin-3 is a beta-galactosyl-binding lectin that is normally expressed in macrophages, mast cells, langerhans cells and various malignant cells, including thyroid cells [6]. It has been suggested that galectin-3 could also play a role in the malignant transformation of thyroid cells and many studies have shown that PTC (papillary thyroid carcinoma) cases are characterized by strong, intense Gal-3 expression [7-10]. In this study we aimed to determine the diagnostic value of galectin-3 in papillary thyroid carcinoma and its variants.

MATERIALS AND METHODS

Inclusion Criteria

Histopathologically diagnosed non-neoplastic and neoplastic (benign and malignant) cases of thyroid

- Substrate Chromogen Solution- Concentrated diaminobenzidine solution (DAB) was diluted with substrate buffer (500 µL of substrate buffer + 2 drops of DAB). Diluted DAB solution was added to smears for 5-10 minutes followed by Tris buffer washing.
- Counterstain and Mounting- Presence of brown coloured end product at the site of target antigen was indicative of positive reactivity. Positive tissue control (Carcinoma Prostate) and negative tissue control (Normal thyroid tissue) were taken. For negative reagent control, % non-immune serum was used in place of primary antibody; the rest of steps are the same as for the positive control.

Immunohistochemical scoring of Gal-3

Staining pattern of Gal -3 protein detected in many tissues and cell types, localized in the cytoplasm and / the nucleus, on the cell surface, or in the extracellular environment.

Interpretation of Gal-3 staining and corelation with histopathology features

Interpretation was done according to the criteria defined by Fabio Orlandi *et al.* in 1998 [13] and Katie B Weber *et al.* in 2004^[14] in a research paper titled (Gal-3 is a presurgical marker in Human Thyroid Carcinoma and The use of a combination of Gal-3 and Thyroid peroxidase for the diagnosis and prognosis of Thyroid Cancer respectively.

Qualitative criteria

Score	Pattern
0	No staining
1	Light / weak staining
2	Moderate staining
3	Intense staining

Quantitative criteria

Score *	Pattern
+++	>60% of neoplastic cells in cytoplasm / nucleus/ both (cytoplasm and nucleus) /extracellular matrix
++	30 - 60% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix
+	<30% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix
-	No reactivity

*In our study, only those cases have been taken who had scoring grade ++ and +++

Statistical Tools Employed

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) statistical Analysis Software. The values were represented in Number (%) and Mean±SD. The Statistical formulas were used ie. Chi square test and Interobserver differences (Kappa) used. P <0.05 was considered statistically Significant And κ=0.20-0.40 is considered as Fair agreement.

University, Lucknow with an aim to evaluate Galectin-3 as ancillary test in diagnosis of thyroid Papillary carcinoma and correlation of Gal-3 with the histopathology of Thyroid neoplasia.

For this purpose a total of 67 histopathologically diagnosed neoplastic (benign and malignant) and non-neoplastic cases of thyroid lesions were evaluated.

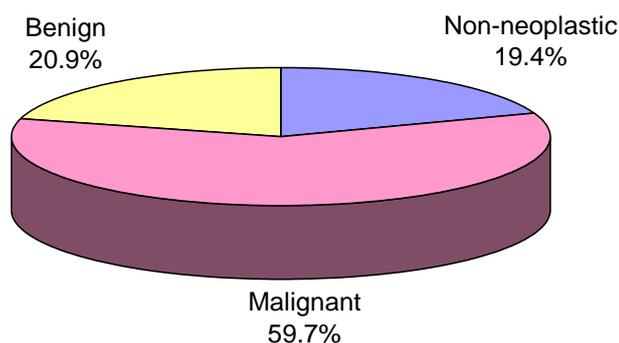
RESULTS

The present study was carried out at Department of Pathology, King George,s Medical

Distribution of specimen according to the final diagnosis has been shown in Table 4 below

Table-2: Distribution of Specimen according to the Final Diagnosis (n=67)

S.No.	Final Diagnosis	No. of cases	Percentage
1.	Neoplastic	54	80.6
	Malignant	40	59.7
	Benign	14	20.9
2.	Non-neoplastic	13	19.4



Majority of cases (n=54; 80.6%) were finally diagnosed as neoplastic. There were a total of 13 (19.4%) cases which were diagnosed as non-neoplastic. Among cases diagnosed as neoplastic – a total of 40 (74.1%) were malignant and 14 (25.9%) were benign whereas among those 13 cases diagnosed as non-neoplastic, a total of 8 (61.5%) were hyperplastic and remaining 5 (38.5%) were inflammatory lesions.

Maximum number of patients in non-neoplastic group was aged between 31-40 years whereas maximum numbers of malignant cases were aged between 41 to 50 years and maximum numbers of benign cases were aged between 21-30 years.

Intensity and Diagnosis

For scoring, it was observed that as compared to non-neoplastic, neoplastic lesions had higher scores (p=0.012). Similarly, more than three-fourth (77.5%) of malignant cases had scores ++ and +++ as compared to only 35.7% of benign and 23.1% of non-neoplastic cases, thus malignant cases showed a statistically significant difference from both benign as well as non-neoplastic lesions (p=0.042 and 0.002). With increasing intensity, a significant association with malignancy was evident. There were 31/40 (77.5%) of malignant cases, 5/14(35.7%) of benign cases and 3/13(23.1%) of non-neoplastic cases accorded with intensity 2 and 3.

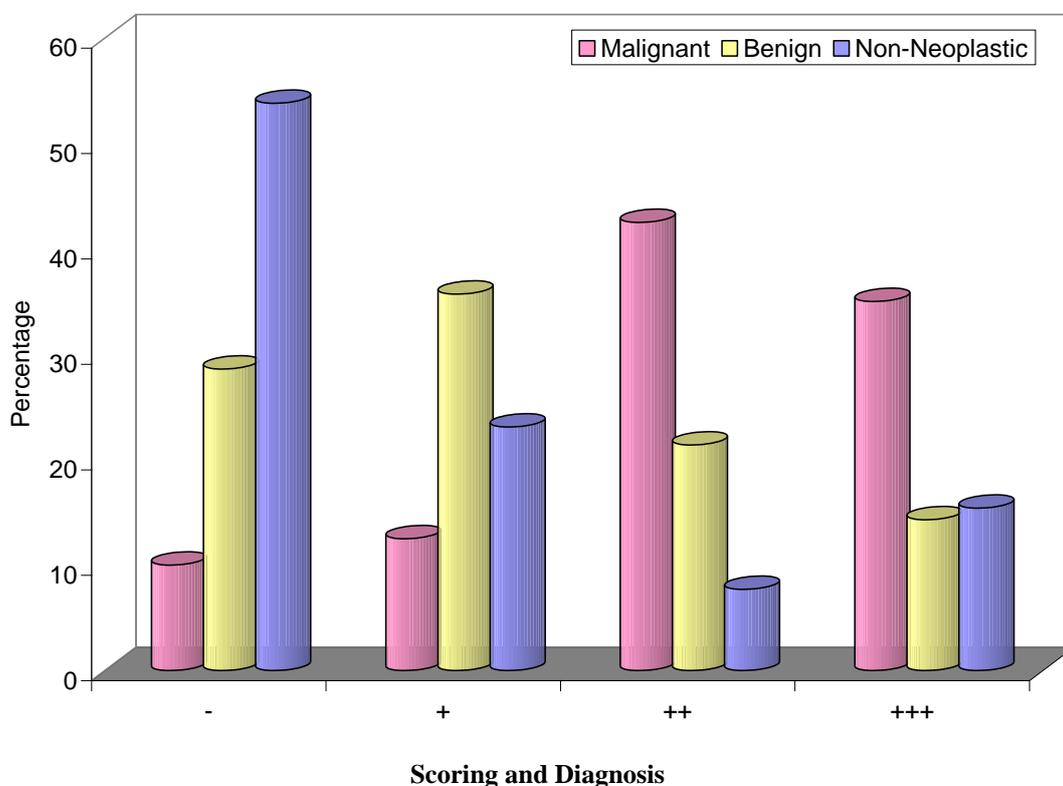


Table-3: Evaluation of IHC Gal-3 expression in thyroid neoplasm*

Diagnosis by Histopathology	Total No.	No. +ve of Gal-3 (%)	"p" value	Sens %	Spec %
A. Malignant Neoplasm	40	31 (77.5%)	<0.001	77.5	69.2
1. Papillary thyroid carcinoma					
Classical	09	07 (77.7%)	0.201	77.8	43.1
Follicular variant	18	13 (72.2%)	0.159	72.2	44.9
Solid variant	02	02 (100%)	0.224	100.0	39.7
Micropapillary	01	01 (100%)	0.393	100	40.9
2. Follicular thyroid carcinoma	10	08 (80%)	0.130	80	43.9
B. Benign Neoplasm (Follicular adenoma and Hurthle Cell adenoma)	14	05 (35.7%)	0.055	35.7	34.0
C. Non-neoplastic (Hyperplasia and inflammatory lesions)	13	03 (23.1%)	0.004	23.1	31.5

*Only those cases have been taken as positive who had scoring grade ++ or +++.

Gal-3 expression was identified in 76.7% (23/30) of PTC cases, and by histological subtypes of PTC Gal-3 expression was identified in classical, follicular variant, solid variant and micropapillary as 77.7% (7/9), 72.2% (13/18), 100% (2/2) and 100% (1/1) respectively. FTC 80% (8/10) cases showed positivity for Gal-3. Overall, Galectin-3 expression evaluated utilizing IHC techniques for thyroid malignancy shows sensitivity (77.5%). In benign and non-neoplastic lesion, Gal-3 expression was observed as 35.7% (5/14) and 23.1% (3/13) respectively.

Evaluation of IHC Gal-3 Expression in Staining Pattern of Thyroid lesions

Among malignancies, cytoplasmic Gal-3 expression was observed in 8/9 (88.8%) of classical

PTC, 14/18 (77.8%) of FVPTC, and 1/1(100%) of MPC. FTC showed 5/10(50%) Gal-3 expression, both (nuclear+cytoplasm) Gal-3 expression was observed in 2/11(11.1%) of FVPTC, 2/2 (100%) of solid variant PTC, and 3/10(30%) of FTC. Extracellular matrix Gal-3 expression was observed in 1/9(11.1%) of classical PTC and 1/18(9.09%) of FVPTC. No nuclear staining was observed. In Benign neoplasm and Non neoplastic lesions, cytoplasmic Gal-3 expression was observed in 7/14 (70%) and 6/13(46.2%) respectively. Both (nuclear+cytoplasm) Gal-3 expression was observed in 3/14 (30%) of benign neoplasm and extracellular matrix Gal-3 expression was observed in 2/13(15.4%) of non-neoplastic lesions. No nuclear staining was observed.

Table-4: Evaluation of IHC Gal-3 Expression in Staining Pattern of Thyroid lesions

Diagnosis by Histopathology	Total (n)	Cyto-plasm	Nucleus	Both (N+C)	ECM
A. Malignant Neoplasm	40				
1. Papillary thyroid carcinoma					
Classical	09	08 (88.8%)	-	-	01 (11.1%)
Follicular variant	18	14 (77.8%)	-	02 (11.1%)	1 (9.09%)
Solid variant	02	-	-	02 (100%)	-
Micropapillary	01	01 (100%)	-	-	-
2. Follicular thyroid carcinoma	10	05 (50%)	-	03 (30%)	-
B. Benign Neoplasm (Follicular adenoma, Hurthle cell adenoma)	14	07 (50%)	-	03 (21.4%)	-
C. Non-neoplastic (Hyperplasia and inflammatory lesions)	13	06 (46.2%)	-	-	02 (15.4%)

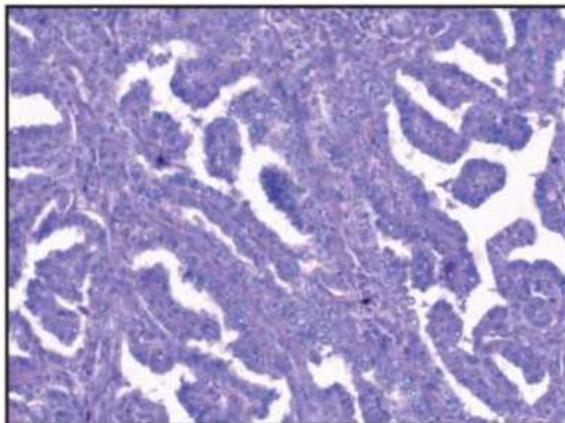


Fig-1: Microphotograph showing classical papillary thyroid carcinoma (H&E, 20X)

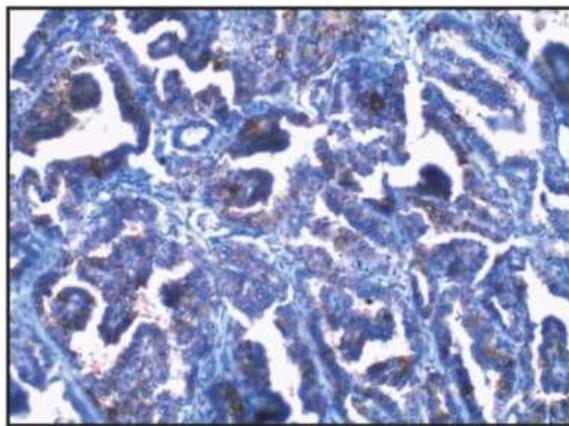


Fig-2: Microphotograph showing gal-3 expression in classical papillary thyroid carcinoma (IHC, 20X). Score - +++ , Intensity - 2, Pattern - Cytoplasm

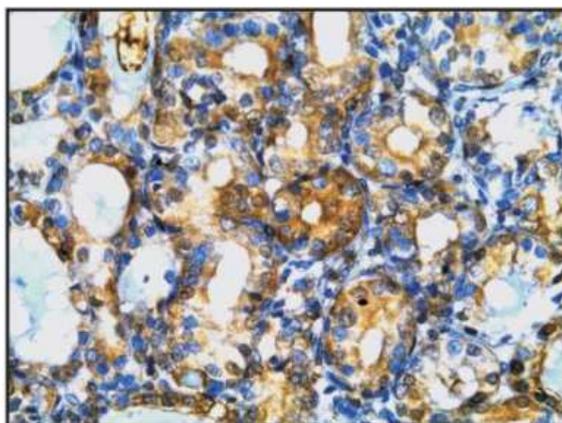


Fig-3: Microphotograph showing gal-3 expression in follicular variant of papillary thyroid carcinoma (FVPTC) (IHC 20x)

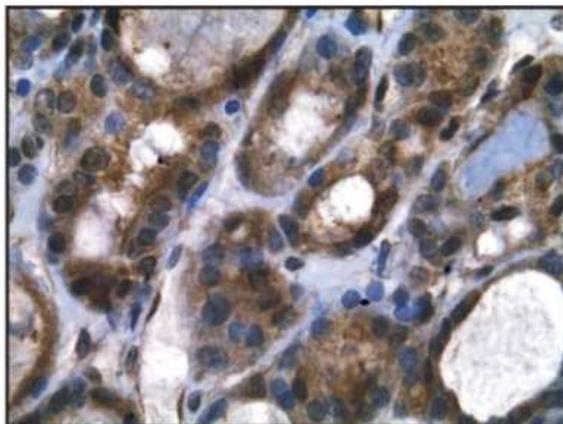


Fig-4: Microphotograph showing gal-3 expression in follicular variant papillary thyroid carcinoma (FVPTC) (IHC 40X), Score - +++, Intensity - 2, Pattern - Cytoplasm

DISCUSSION

Papillary thyroid carcinoma is the most frequent observed malignant tumour in the thyroid. In general the prognosis of PTC is favourable and ten-year survival rate for PTCs is greater than 90% [15]. However, about 20% of the differentiated thyroid cancer will present metastasis [16]. So accurate biomarker or immunohistochemical markers which can predict the aggressive behaviour of thyroid carcinoma is critical for clinical management [17]. Gal-3 could be an important tool for guiding therapeutic decision in patients with thyroid nodules [18]. On occasion cases of papillary thyroid hyperplasia can simulate papillary thyroid carcinoma and cause a diagnostic dilemma [19, 20]. In addition, PTC gives frequently rise to nodal metastasis via lymph vessels. Metastasis occurs in 20% of patients. In recent years, the development of PTC is influenced by many factors including genetic alterations, growth factors, and physical agents such as radiation. Galectin-3 a member of the B galactosyl binding lectin family, for which normal function include cell-cell regulation, growth and differentiation in some studies. Finding that the sensitivity and specificity of gal-3 were 93% and 100% respectively, for papillary thyroid carcinoma [21-23].

In studies of PTC, Gal-3 expression has been reported in 58% to 100%. However, the majority of studies reported Gal-3 positivity in 90% to 100% of PTC cases [24]. Very few studies have reported Gal-3 expression in PTC by histological subtypes. Similarly, in our study, Gal-3 expression was identified in 77% (23/30) of PTC cases, and by histological subtypes of PTC Gal-3 expression was identified in classical, follicular variant, solid variant and micropapillary as 77.7% (7/9), 72.2% (13/18), 100% (2/2) and 100% (1/1) respectively. Expression of Gal-3 has also ranged from 20% to 100% in reported cases of FTC. The largest series, reported by Bartolazzi *et al.*, identified Gal-3 expression in 95% (54/57) of FTC cases [25]. Similarly,

in our study, 80% (8/10) cases of FTC showed positivity for Gal-3.

Hence, Gal-3 is highly expressed in thyroid malignancies. There is no significant difference found in Gal-3 expression in PTC histological subtypes and FTC. Although it appears likely that a small proportion of follicular carcinomas are negative for Gal-3, likewise in our study 20% (2/10) of FTC cases were negative for Gal-3, it remains elusive whether the negativity is true or false. According to Kawachi *et al.* [26] primary lesions of papillary carcinomas with metastasis contained significantly higher concentrations of Gal-3 than tumours of this type without metastases. They explained further that Gal-3 down regulation in PTC may promote the release of some tumor cells from the primary tumor resulting in metastasis. In our single case of known metastatic PTC we found strong Gal-3 positivity. Follicular thyroid lesions have been rightly referred to as 'the bane of the pathologist' in a recent article by Baloch and LiVolsi [27]. The follicular variant of papillary thyroid carcinoma (PTCFV) often poses a diagnostic challenge in which the differential diagnosis includes other follicular patterned lesions such as follicular adenoma (FA) and follicular carcinoma. The distinction between these lesions is important because prognosis and management differ. Because FVPTC is diagnosed almost solely based on subjective cytologic criteria (nuclear grooves, nuclear clearing, nuclear overlap, and intranuclear pseudo inclusions) and no uniform minimal diagnostic criteria exist [28]. In our study, Gal-3 was expressed in 72.2% (13/18) of FVPTC, 80% (8/10) of FTC and 35.7% (5/14) of benign neoplasm (follicular and hurthle cell adenomas). It has been suggested that Galectin-3 could also play role in the malignant transformation of thyroid cells and many studies have shown that PTC cases are characterized by strong, intense Gal-3 expression. In few studies Gal-3 also differentiated from papillary thyroid carcinoma and papillary hyperplasia i.e. positive rates of gal-3 were

97.4% in PTC and 16.7% in papillary hyperplasia. Their study believed that galectin-3 could be a valuable antibody for differential diagnosis of papillary carcinoma and papillary hyperplasia [22, 23]. Finally conclude that galectin-3, a potential immunomarker, is accurate in making a preoperative distinction between PTC and non-PTC patients. In addition galectin-3 may provide more significant contributions in distinguishing PTC with or without lymph node metastasis. We suggest that a larger size and better studies should be conducted to confirm our results.

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