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Original Research Article

Can Immunohistochemical Exhibition of Galectin-3 help solve Conundrums of Thyroid?

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Abstract

Background: Belonging to a family of lectins, Galectin-3 has garnered a lot of attention for its active part in the cancerous metamorphosis of various organs including thyroid gland.

Aim: To use immunohistochemical expression of Galectin-3 to discern carcinomas from benign nodules of thyroid in histological samples.

Materials and methods: Immunohistochemical staining of galectin-3 was observed in a spectrum of benign as well as malignant neoplasms of thyroid. These included 20 specimens of follicular adenoma, 25 of papillary Carcinoma, 3 of follicular Carcinoma & 2 of medullary Carcinoma.

Results: Malignant thyroid carcinomas presented with significantly higher galectin-3 immunoreactivity as compared to benign neoplasms($p<0.05$). It presented with a sensitivity of 83.3%, specificity of 90%, positive predictive value of 92.6% and negative predictive value of 78.3% in distinguishing malignant from benign tumours.

Conclusion: Immunochemical staining for galectin-3 helps in discerning benign lesions from malignant carcinomas. It could help in recognition of tumours of thyroid with ambiguous morphologic attributes.

Keywords: Galectin-3, Immunohistochemical, Thyroid Gland

Introduction

Tumours of thyroid constitute 90% of the endocrine related tumours in human population [1]. Presence of a thyroid nodule is a common clinical presentation, usually identified by palpation or by ultrasonography. Fine needle aspiration cytology is the initial investigation done for diagnosis of nodules. However, this investigation has its own limitations. Follicular-patterned thyroid lesions remain a diagnostic dilemma, even with histological analysis. Capsular disruption in adenomas & presence of follicular pattern in adenomatoid nodule create a cause of concern in histopathological study [2]. To address these diagnostic challenges, several immunohistochemical markers are being evaluated. Galectins belong to a family of animal lectins that can exist intracellular or extracellular. Within a cell, they travel

between the nucleus and cytoplasm. They participate in important processes like pre-mRNA splicing, regulating cell growth, cell-cycle progression as well as apoptosis [3]. Galectin-3 could contribute significantly as a diagnostic tool, being significantly expressed in malignancies in contrast to benign tumours.

Aim

Use of immunohistochemical expression of galectin-3 to distinguish malignant from benign nodules of thyroid in histological samples

Materials & Methods

This study was accomplished in Dept of Pathology of MKCG medical college & hospital during the period of 2017 to 2019. Specimens were collected from fifty patients who underwent operation for both benign & malignant thyroid lesions in our institute. Detailed clinical history was taken regarding age, sex, size of tumour as well as local & distant metastasis. This study was conducted after obtaining consent from the Institutional Ethics Committee. All the gross specimens were fixed in 10% formalin, processed in automated tissue processor, embedded in paraffin wax & subjected to staining with haematoxylin & eosin stain. Histopathological evaluations were performed on the specimens received and the final diagnosis was made in accordance with World Health Organization classification. The histopathological report was regarded as gold standard for statistical purposes. The histopathological interpretation of the thyroid lesions was as follows: 20 cases of follicular adenoma, 25 of papillary carcinoma, 3 of follicular carcinoma & 2 of medullary carcinoma.

Inclusion criteria: Histopathologically diagnosed benign & malignant neoplastic cases of thyroid.

Exclusion criteria: Inadequate biopsies, tissue samples with inadequate material or with necrosis or haemorrhage & patient denying consent.

All 50 specimens were exposed to immunohistochemical assessment utilizing anti-human Galectin-3 mouse monoclonal antibody. Sections were deparaffinised & rehydrated. Antigen retrieval agent used was Tris buffered saline (adjusted to pH7.4) in a domestic pressure cooker. Slides were then allowed to come to room temperature. Peroxidase block was performed. Later primary antibody i.e anti-human monoclonal galectin-3 antibody (ready to use) was put in for 60mins & washed. This was followed by secondary antibody i.e. polymer horseradish peroxidase (HRP) for 30mins, followed by washing. In the end, Diaminobenzidine tetrahydrochloride (DAB) chromogen was applied for few minutes & counter staining was accomplished using Harry's Haematoxylin. Slides were mounted in DPX & observed under a microscope.

Positive control - papillary carcinoma of thyroid.

Negative control - elimination of the primary Galectin-3 antibody Interpretation of immunostaining [4,5]

Grade-1 was given when < 5% of cells stained positive for galectin-3, Grade-2 when <50% of cells stained positive & Grade-3 when proportion of stained cells were > 50%. The staining intensity was graded on a scale of 0 to 3 where 0 refers to no staining, 1+ weak staining, 2+ moderate staining & 3+ strong staining. It expressed itself in cytoplasm & nucleus as well. Cases that showed cytoplasmic and/or nuclear staining (>5% of tumour cells), irrespective of the intensity, were considered positive for Galectin-3. Calculations regarding sensitivity, specificity, positive and negative predictive values were done. Data analysis was done. Chi-square test was used to calculate P-value ($p<0.05$ was taken as statistically significant).

Results

In the current study, majority of the benign neoplasms were seen in 20-29 age group, while malignant neoplasms were seen in 30-39 age group. (Fig-1)

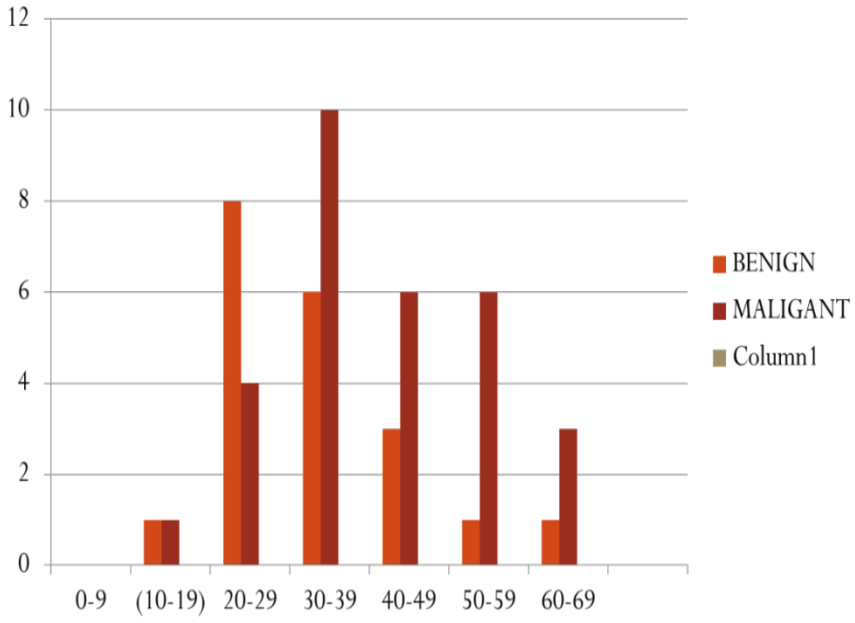


Figure 1: Graph Representative of Distribution of Age

Females outnumbered males in both benign (85%) & malignant cases (80%) (Fig-2)

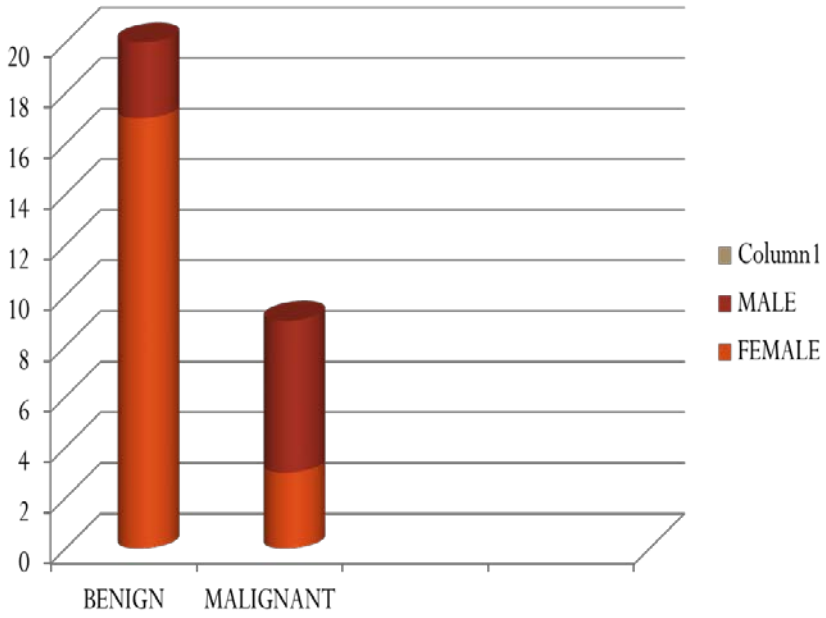


Figure 2: Sex distribution graph

In the present study, malignant neoplasms (30) were seen more commonly than benign neoplasms (20) (Fig-3). The predominant neoplasm was papillary carcinoma (25), followed by follicular adenoma (20), follicular carcinoma (3) & lastly medullary carcinoma (2).

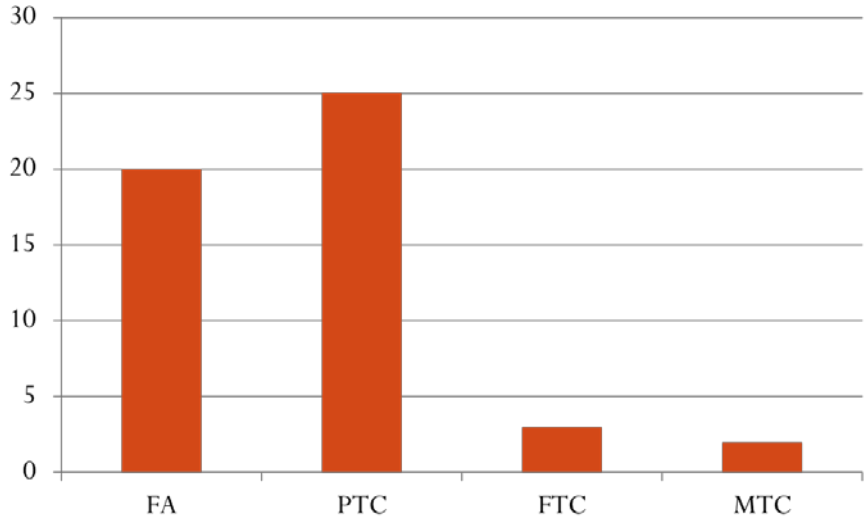
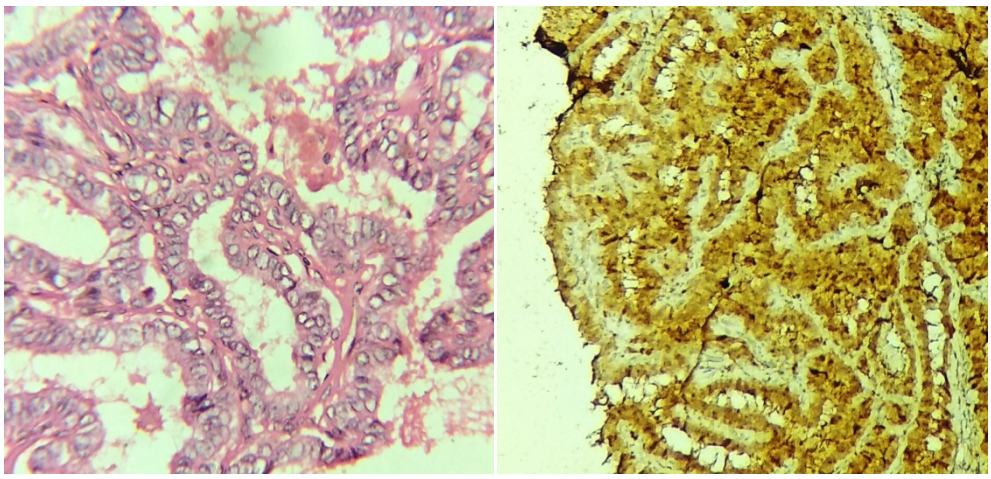
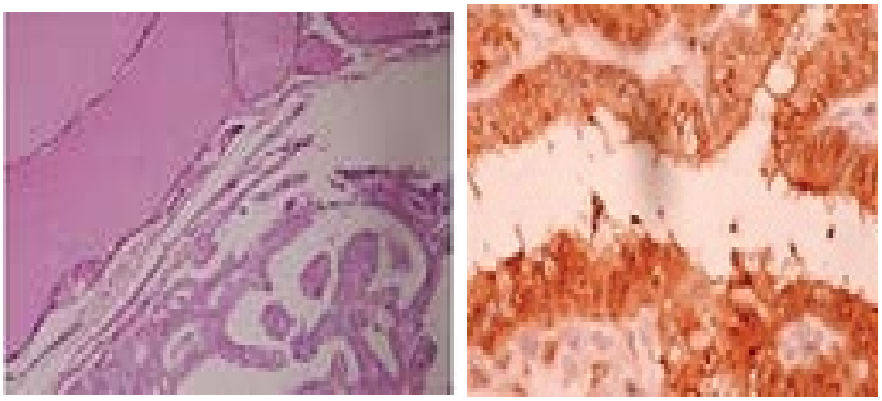


Figure 3: Types of neoplasms

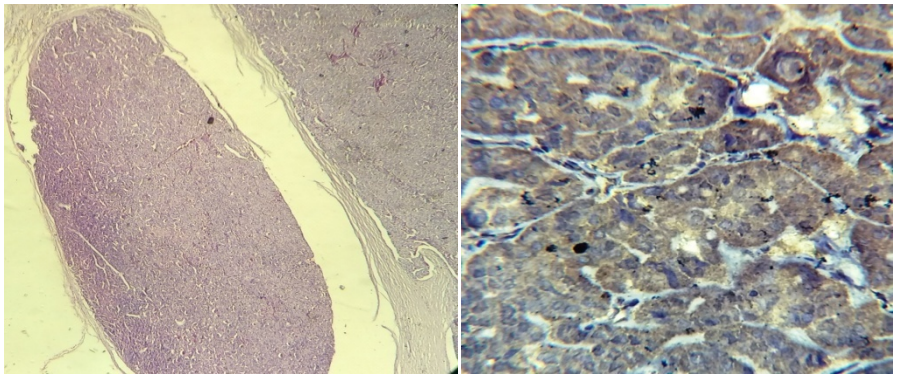
Papillary carcinoma of thyroid: H&E stain, 100X; Galectin-3 immunoexpression, 100X shows strong staining.



Papillary microcarcinoma: H&E,100X; Galectin-3 immunoexpression, 400X; highlights strong staining



Follicular carcinoma: H&E, 40X; capsular infiltration; Galectin-3 immunoexpression 400X; shows strong staining



Follicular adenoma: H&E, 400X; Galectin-3 immunoexpression ,400X; absent in tumour cells

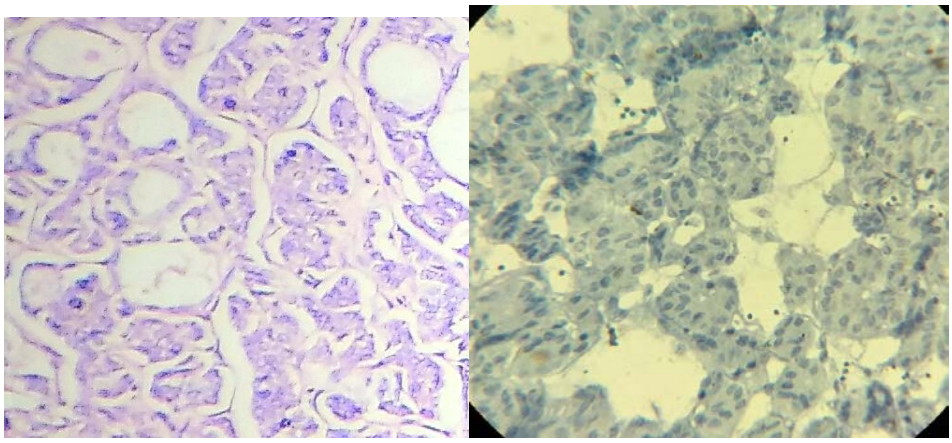


Table 1: Results of galectin-3 immunohistochemical staining

HISTOLOGIC DIAGNOSIS	NO OF CASES	I GRADE	P SCORE	INTERPRETATION
FOLLICULAR ADENOMA	18	0	0	NEGATIVE
(total-20)	1	3	2	POSITIVE
	1	2	2	POSITIVE
PAPILLARY CARCINOMA	18	3	3	POSITIVE
(total-25)	4	3	2	POSITIVE
	1	2	3	POSITIVE
	1	1	1	NEGATIVE
	1	0	0	NEGATIVE
FOLLICULAR CARCINOMA	1	1	1	NEGATIVE
	1	3	2	POSITIVE
	1	3	2	POSITIVE
MEDULLARY CARCINOMA	1	1	1	NEGATIVE
	1	0	0	NEGATIVE

Table 1 depicts all the neoplasms analysed for Galectin-3 immunostaining. Intensity grade & proportion score was also represented. Marked immunoexpression of Galectin-3 was seen in malignant lesions (25/30) in comparison to benign lesions (2/20). It was found to have a sensitivity of 83.3%, specificity of 90%, positive predictive value of 92.6% and negative predictive value of 78.3% in distinguishing malignant carcinomas from benign lesions.

Of the total no of papillary carcinomas (25), 23 cases (92%) presented with positive staining while 2 cases (8%) were negative.18 cases presented with strong staining (3+) of more than 50% of cells (grade 3), 4 cases with cells below 50% (grade-2) with intense staining (3+) & 1 case showed moderately intense staining (2+) of more than 50% of cells (grade-3). Of the total no of follicular carcinomas, 2 were positive for galectin-3 with cells below 50% (grade-2) showing strong staining (3+). 2 cases of Medullary carcinoma gave negative results. Immunoexpression for galectin-3 was absent in 18/20 cases (90%) of follicular adenoma, while positivity was observed in two cases (10%) which included one case with cells below 50% (grade-2) showing strong intensity (3+) & another case with cells below 50% (grade-2) showing moderate staining (2+).

Discussion

Galectin-3 seems like a novel molecular marker for evaluation of neoplasms of thyroid. Our study findings are similar to previous reports that have shown its overexpression in thyroid cancerous lesions [6-9]. As per studies done by Prasad et al [6] the sensitivity and specificity for discriminating between benign & cancerous lesions were 92% & 90% in comparison to 84% & 90% respectively in our study. Orlandi et al [7] examined its immunoexpression on 29 specimens of follicular adenomas, 17 of follicular carcinomas & 18 of papillary carcinomas. Malignant lesions presented with 100% positivity while only 9% of follicular adenomas showed positivity. Park et al [8] examined immunohistochemical appearance for 6 markers which included galectin-3 in 295 thyroid lesions and inferred that it was a useful marker to distinguish between cancerous & benign growth. Bartolazzi et al [9] deduced that its sensitivity and specificity in differentiating benign & cancerous lesions were more than 99% & 98% respectively after assessing 1009 lesions of thyroid. Previous studies show that it was overexpressed in 90-100% of cases of papillary carcinoma [6-10]. Our study also presented with significantly higher expression (92%) of galectin-3 in papillary carcinomas. Its positive expression was also spotted in a case of papillary micro carcinoma. Such findings were also detected by Cvejic et al [10] as well & it was assumed that it might be involved in malignant alteration. Comparative study of galectin-3 immuno expression in neoplasms of thyroid [4,6,7,8,9]

Table 2: Papillary, Follicular, Medullary carcinoma and Follicular adenoma

STUDY	Papillary carcinoma	Follicular carcinoma	Medullary carcinoma	Follicular adenoma
Weber KB et al	92%	44%		31%
Prasad et al	94%	66%		10%
Orlandi et al	100%	100%		10%
Park et al	99%	64%		2.9%
Bartolazzi et al	97%	96%	43%	3%
Present study	92%	67%	0%	10%

Bartolazzi et al & XU XC et el detected galectin-3 expression in 95% and 100% of follicular carcinoma cases respectively [9,11]. Its positive response was also reported in 64% of cases of follicular carcinoma as per Cvejic et al [10]. Our study shows 67% positivity. In the present study, there were 2 cases of medullary carcinomas without lymph node metastasis. Few studies have been done on galectin-3 immunoexpression on medullary carcinoma of thyroid [9,11]. Galectin-3 is not helpful for detecting medullary carcinoma, as they have a different line of origin(C-cell) [11]. Most studies identified galectin-3 expression to be positive in the range of 0-31% in reference to follicular adenomas [7,12,13,14,]. Our study shows galectin-3 positivity in 2 cases of follicular adenoma (10%). Aiad et al [15] presumed that its positive expression in adenomas may indicate an underlying cancerous transformation. Chiu et al [12] called for a standardized methodology for clinical application of Galectin-3 as an adjunct aid to histopathology for discriminating between cancerous and benign nodules. Since the thyroid follicular cells contain an increased value of endogenous biotin, studies suggest being cautious while employing avidin-based identification system in the absence of biotin blockade [5]. In the current study, polymeric HRP linker antibody conjugates have been employed. Galectin-3 immunoreactivity is quite high in malignant carcinomas in contrast to benign lesions ($p<0.05$). It expresses a sensitivity of 83.3%, specificity of 90%, positive predictive value of 92.6% and negative predictive value of 78.3%. Previous studies along with present study indicate that it is a sensitive immunohistochemical marker which can aid in discrimination between malignant & benign neoplasms. Even though present study showed decreased galectin-3 immunostaining in follicular adenoma in contrast to follicular carcinoma, the no of cases of follicular carcinomas are very few to arrive at a concrete agreement.[16]

Conclusion

Galectin-3 appears to be useful in discerning between benign lesions & malignant carcinomas. It is exceptionally useful in diagnosis of Papillary carcinoma & its variants. As per literature, it might help in preoperative diagnosis of neoplasms termed as "follicular tumour, suspected of neoplasm". Being highly expressed in thyroid cancer & uncommonly in non-cancerous lesions, it paves the path for future research regarding its role in therapy for thyroid cancer. Galectin-3 can't be used in isolation to detect thyroid malignancy. Standardized methodologies for immunohistochemical techniques & interpretation will surely affect its understanding in thyroid tumours.

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