Study of Galectin-3 Expression in Adenomatoid Nodule and Thyroid Neoplasms

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Abstract:

The thyroid is an essential endocrine organ that plays a pivotal role in the body's growth, development, and normal functioning.Most cases are diagnosed with histomorphology alone, a few lesions with overlapping histologic features require additional investigations like IHC to diagnose accurately. Aim of this study was to evaluate the expression of Galectin-3 in adenomatoid nodule and thyroid neoplasm and to correlate Galectin-3 immunoreactivity with the histopathological diagnosis. In this cross-sectional study, 44 histopathologically diagnosed neoplastic lesions of both benign and malignant and lesions with low-risk malignant potential were included. The degree of expression of Galectin-3 in the thyroid cells in the lesions was studied, and the expression percentage was determined based on cytoplasmic staining.

Results showed as the most common age group was 31-50 years with a female: male ratio of 6.3:1. 43.3% of thyroid carcinoma showed Galectin-3 expression and majority of them being Papillary carcinoma and was found to be progressively increasing from benign lesion to malignant lesion.The present study showed a correlation between Galectin-3 expression and lympho-vascular invasion. In conclusion Immunohistochemical Galectin-3 staining is diagnostically reliable in identifying tumour cells with high sensitivity and specificity. The decreased or absent staining of Galectin-3 indicates a benign lesion or intermediate lesion of the thyroid, and positive stain indicates malignancy, especially papillary carcinoma. It can be used with other IHC markers to confirm the diagnosis of other thyroid carcinomas. In the future, more studies have to be directed towards identifying specific markers of other carcinomas and lesions with low-risk malignant potential that have the potential to turn into a cancerous lesion or are lesions with a diagnostic dilemma, which will aid in predicting the early diagnosis and prognosis of the same.

Keywords: Galectin-3, Adenomatoid nodule, thyroid neoplasm, IHC expression.

Introduction:

Thyroid neoplasms are the most common endocrine malignancy in the world[1] . Prevalence of thyroid nodules in India is 12.2%[2] and the incidence of thyroid cancers has increased in recent years[3] . The challenges are differentiating and categorizing these nodules . The morphologic evaluation of thyroid nodule by fine needle aspiration cytology or surgical resection, followed by histopathological examination, provides the most definitive information of its nature. The pathological evaluation of thyroid nodules using hematoxylin and eosin staining remains the "gold standard" technique[4]. Histopathological diagnosis of thyroid lesions can be difficult at times due to overlapping morphologic features like microfollicular pattern, nuclear grooving and papillary architecture seen in benign and malignant thyroid nodules . Hence the role of a specific IHC marker that would help differentiate such lesions can be a boon to the pathologist.

Galectin-3, a member of the beta-galactoside-binding protein family, is a well-established histological marker of thyroid neoplasm are involved in various physiological and pathological processes. They are aberrantly expressed in Papillary carcinoma thyroid and are absent or least expressed by normal thyroid tissue[4] which can be assesed by western blotting, Real-time quantitative polymerase chain reaction and other

methods.

Thyroid lesions which show various morphological similarities that continue to complicate both diagnosis and management, the usage of Galectin-3 expression was progressively increased from benign neoplastic to malignant neoplasm , helping in categorization and early treatment.

Materials and Methods:

This study was designed to be a cross sectional study, carried out in the Department of Pathology at Sri Manakula Vinayagar Medical college and hospital, a tertiary care centre located at Kalitheerthalkuppam, Puducherry. Using open Epi software , considering 87% prevalence of galectin-3 expression in malignant neoplasms in a study done by Sumana BS et al [5] , the sample size was calculated to be 44, with 10% absolute precision and 95% confidence interval. The study was conducted for a period of 18 months which included both prospective and retrospective cases . The participants included the patients operated for various lesions of thyroid included Adenomatoid nodule , Follicular adenoma, thyroid malignancy and cases of colloid goitre , multi nodular goitre and Hashimoto's thyroiditis were excluded.

Study procedure:

The study was commenced after getting approval from the Institutional research committee and Institutional ethics committee (EC NO: EC/50/2022). After obtaining informed consent from the study participants, relevant patient information like hospital identification number, age, sex, relevant clinical details, investigation reports of thyroid profile were collected from the patient case records. Gross examination features like size, side and type of thyroid lesion were also included. The representative tissue specimen of corresponding cases were formalin fixed and paraffin embedded were cut into 2-4 mm thickness and IHC was performed.

Immunohistochemistry technique:

- Immunohistochemistry was done using polymer kit.

- The deparaffinised sections were heated in warmer for 15-20 minutes at 60 degree Celsius.

- Sections were rehydrated and kept in distilled water for 30 seconds.

- Slides were placed in a small box containing citrate buffer and heated in a pressure cooker for 8-10 minutes.

- Sections were covered with 3% Hydrogen peroxide for 15 minutes

- Washed in a buffer and kept for 5 minutes.

- Incubated in a block and covered with primary antibody

- Re-incubated with super enhancer for 20 minutes and washed with buffer.

- Again re-incubated with polymer linked secondary antibody for 30 minutes and washed with buffer.

- Covered with chromogen solution for 5 minutes

- Counter stained with Harris hematoxylin for 5 minutes and washed

- Cleared with xylene and mounted with DPX .

- After immune-histochemical staining of galectin-3, the slides were visualized and studied for the expression of galectin-3 and was correlated with histopathological diagnosis.

The degree of expression of Galectin-3 in the specimens was studied based on the extent of involvement as follows.

Table 1: The scoring was based on percentage of cells stained and intensity of staining.

Percentage of cells stained	Reaction
0	No visible reaction
01-May	1+
>5-25	2+
>25-75	3+
>75	4+

Table 2 : The scores were evaluated based on intensity of staining as follows.

Intensity of staining	Intensity scores
No staining	0
Weak	1
Moderate	2
Strong	3

RESULTS:

In the present study thyroid lesions were more common in fourth and fifth decades, with Male: female ratio of 1: 6.3. Lesion was more commonly found in right lobe of thyroid and majority of them presented in an euthyroid state followed by hypothyroidism. The proportion of papillary carcinoma among the thyroid carcinomas was higher in the current study. Galectin-3 was expressed predominantly in carcinoma and, more commonly, in papillary carcinoma when compared to benign thyroid lesions and lesions with low-risk malignant potential, similarly intensity of Galectin-3 was higher in papillary carcinoma of thyroid when compared to other lesions.

Table 3: The percentage of cells expressing Galectin-3 are tabulated:

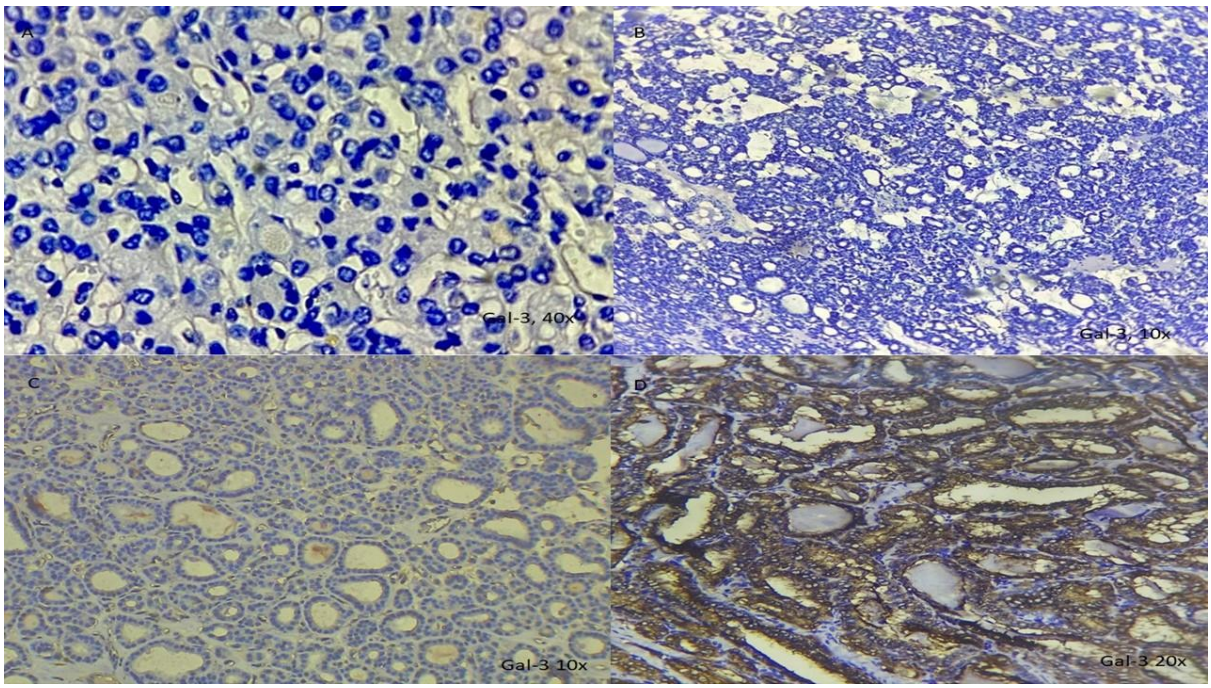
Galectin 3	**HISTOPATHDIAGNOSIS**						**Chi-square test**
	ADENOMATOID NODULE	FLUMP	NIFTP	PAPILLARY CARCINOMA	FOLLICULAR CARCINOMA	MEDULLARY CARCINOMA	
0	13	0	5	1	2	1	0.001
	59.1%	0.0%	22.7%	4.5%	9.1%	4.5%	
1+	0	1	5	0	0	0	
	0.0%	16.7%	83.3%	0.0%	0.0%	0.0%	
2+	0	0	1	2	0	0	
	0.0%	0.0%	33.3%	66.7%	0.0%	0.0%	
3+	0	0	0	8	0	0	
	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	
4+	0	0	0	5	0	0	
	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	
Total	13	1	11	16	2	1	

Table 4: The intensity of cells expressing Galectin-3 are tabulated:

Intensity of galectin3	**HISTOPATHDIAGNOSIS**					
	ADENOMATOID NODULE	FLUMP	NIFTP	PAPILLARY CARCINOMA	FOLLICULAR CARCINOMA	MEDULLARY CARCINOMA
Grade 0	13	0	5	1	2	1
	59.1%	0.0%	22.7%	4.5%	9.1%	4.5%

Grade1	0	1	5	0	0	0
(Mild)	0.0%	16.7%	83.3%	0.0%	0.0%	0.0%
Grade2	0	0	1	5	0	0
(Moderate)	0.0%	0.0%	16.7%	83.3%	0.0%	0.0%
Grade3	0	0	0	10	0	0
(Severe)	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
Total	13	1	11	16	2	1

Thyroid malignancy with extrathyroidal extension and lymphovascular involvement also showed an increase in percentage and intensity of the cells stained. Though the lesions without extrathyroidal extension showed the intensity of all the grades, the lesions with extrathyroidal extension showed only maximum intensity, indicating it could be a sensitive marker for lesions with extrathyroidal extension. Lesions with margin involvement had no correlation with the Galectin-3 expression.



Galectin-3 expression (A) Negative expression in NIFTP (B) Negative expression in FLUMP (C) Negative expression in Adenomatoid nodule (D) Positive expression in Papillary carcinoma thyroid.

Discussion:

Galectin-3, a potential cytoplasmic marker, which binds lectin to its specific carbohydrate , is a regulator of normal cell proliferation and the overexpression of Gal-3 is seen in malignant transformation and metastasis. Out of the 44 benign neoplastic and malignant cases studied , Galectin-3 expression was found more in malignant lesion and more commonly in papillary thyroid carcinoma. Papillary carcinomas though a clear cut diagnosis , lesions such as NIFTP and Follicular variant of papillary carcinoma has a greater chances of misinterpretation in FNAC and biopsies. Galectin-3 aids in earlier diagnosis , categorization of such lesions which gives an approach of lesions to the clinicians for its treatment modality that results in a good prognosis of the patient.

In comparison to our study , a study done in Turkey by Ozcinar et al[6]. the present study had a preponderance of unifocal lesions and lesions with intact capsules but had a decreased number of cases with margin involvement. The present study was interpreted based on cytoplasmic staining; hence, nuclear with a background staining was considered negative as a study by Marco et al.[7] suggested that adenomas expressed erratic staining and have been categorised as carcinoma without any capsular/ vascular invasion priorly and indicating that Galectin-3 may be expressed in benign lesions which may progress to carcinoma and hence can be used as an aid in early detection of such lesions . The number of benign neoplasm staining for Galectin-3 was lower and in concordance with other studies indicating its specificity. Studies for Galectin-3 expression in correlation to lesions with low-risk malignant potential such as NIFTP or FLUMP are limited and was done in our study and compared to a study done by Tamilselvi et al[8] .In comparison the percentage of cells expressing Galectin-3 was lower in contrast to their study, and it mandates more studies have to be done in the future, as the treatment protocol, and the prognosis varies for the lesions of low risk malignant potential. Of all benign neoplastic and malignant lesions of thyroid , malignant lesions showed higher percentage and intensity of positivity in Galectin-3 with maximum percentage in a study by et al[7] with 88.8% of expression, followed by the present study (84.2%) in concordance with the study by Rameshbabu et al[9] that carried a significant p value. Studies done by Marco et al[7], Tamilselvi et at[8], Rameshbabu et al[9] and Chumila et al[10] · including the present study showed an increased percentage and intensity of staining in papillary thyroid carcinoma compared to other thyroid carcinomas, of which the classical variant contributed the majority with only Marco et al[7]. having an increased expression of Galectin-3 in follicular variant of Papillary thyroid carcinoma in his study.

However there are a few limitations in the present study as this was a cross-sectional study and was done in a single tertiary care centre, with unequal number of benign neoplastic and malignant cases and may not represent the prevalence or prpotion of cases in whole population . Galectin-3 expression was done using a visual analogue scale, which has the disadvantage of inter-observer bias, also the prognosis of papillary carcinoma could not be determined as there was no follow-up of the cases.

Conclusion:

Immunohistochemical Galectin-3 staining is diagnostically reliable in identifying tumour cells with high sensitivity and specificity. The decreased or absent staining of Galectin-3 indicates a benign lesion or intermediate lesion of the thyroid, and positive stain indicates malignancy, particularly papillary carcinoma. It can be used with other IHC markers to confirm the diagnosis of other thyroid carcinomas. In the future, more studies have to be directed towards identifying specific markers of other carcinomas , lesions with low-risk malignant potential and lesions with a diagnostic dilemma, which will aid in predicting the early diagnosis and prognosis of the same. We recommend a large-scale cohort study to analyse the increased expression of Galectin-3 in different thyroid lesions and the prognosis of thyroid malignancies which could help postulate better management protocols.

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