DOI: 10.7860/NJLM/2019/40370:2349

Original Article

Pathology Section

Intensity and Grading of Galectin-3 Expression as a Sole Marker to Differentiate Benign and Malignant Thyroid Neoplasms

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ABSTRACT

Introduction: Errors in categorization of thyroid neoplasms as benign or malignant has management implications. In several studies Galectin-3 was one of the most reliable markers for malignancy in thyroid.

Aim: To evaluate utility of Galectin-3 as a sole marker in differentiating benign and malignant thyroid neoplasm.

Materials and Methods: Forty one thyroidectomy cases were studied, which included 12 benign and 29 malignant thyroid neoplasms. Galectin-3 (CELL MARQUE Galectin-3 mouse monoclonal antibody, CMC25521020) expression was assessed. Grading based on intensity and extent (diffuse/focal) of Galectin-3 expression was also noted.

Results: The expression of Galectin-3 was significantly higher ($p<0.001$) in malignant thyroid neoplasms. Difference in Galectin-3 expression in benign thyroid neoplasms and PTC was significant ($p<0.001$). Galectin-3 expression within various follicular neoplasms was not useful when only positivity (>10% of cells positive) ($p>0.08$) and diffuse positivity (>50% of cells positive) ($p>0.\ 0.167$) were taken into consideration. However, with grade 3 intensity of Galectin- 3 staining in various follicular neoplasms there was a significant difference in Galectin-3 expression ($p<0.002$)

Conclusion: Galectin-3 is useful in differentiating benign and malignant thyroid neoplasm ($p<0.05$). It can be used as sole marker for differentiating Papillary thyroid carcinoma and benign thyroid neoplasms. Grade 3 intensity of Galectin-3 positivity could prove to be a useful marker in differentiating follicular patterned lesions.

Keywords: Follicular patterned lesions, Malignancy, Papillary thyroid carcinoma

INTRODUCTION

Of all endocrine neoplasms thyroid is most common site. Incidence of thyroid cancer is India is around 3 per 100,000 [1]. This incidence it likely to rise with routine thyroid screening by ultrasound implemented in practice and resultant increase in thyroidectomy [2,3]. Diagnosis of papillary thyroid carcinoma which accounts to 85% of thyroid carcinomas usually do not pose a challenge. However, in cases with papillary hyperplasia, suboptimal fixation and tissue processing may lead to altered nuclear staining, it can then be tough to rule out papillary thyroid carcinoma [4-6]. Follicular patterned lesions of thyroid are difficult to categorize. Common issue being focal minimal capsular invasion needing extensive blocks and examining of several deeper sections. Another recurrent issue with thyroid is fixation issues because thyroid is highly vascular tissue. This can lead to focal clearing of nuclei adding to confusion in diagnosis [7,8].

With increase in incidence of thyroidectomy specimens, number of lesions with diagnostic difficulties are likely to rise further. Hence there is a need to identify markers which will help in differentiating benign and malignant thyroid neoplasms to avoid erroneous diagnosis. In recent past several studies are done with Galectin-3, HBME-1, CK19, HMWCK, Cyclin D1, $p27^{kip1}$ loss, TG, Ki-67, Ret oncoprotein, CD56, p63 and others [9-13]. In most of the studies Galectin-3 was the most reliable marker for malignancy in thyroid. However, in several of these studies Galectin- 3 was used as a part of panel immunohistochemical markers. There is a need for more cost effective application of immunohistochemistry as sole marker or in algorithmic approach rather than panel of markers, particularly in our country with majority of hospitals having limited resources and catering to people with financial constraints. Hence, in present study we had evaluated utility of Galectin-3 as a sole marker to differentiate benign and malignant thyroid neoplasm.

MATERIALS AND METHODS

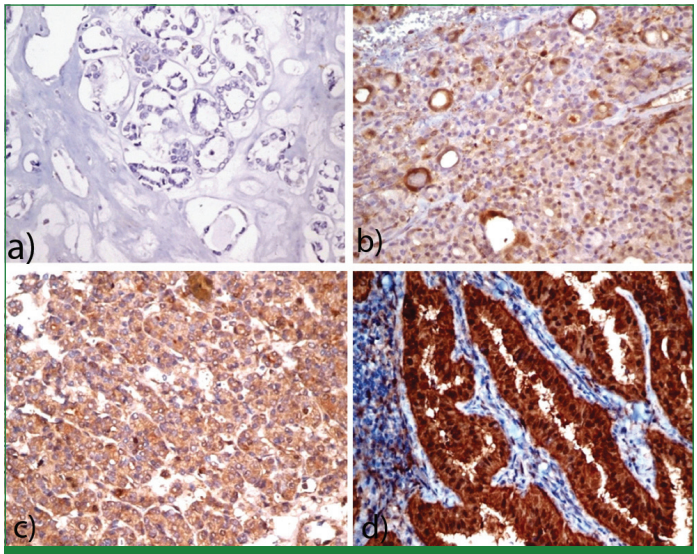
This was a prospective unbalanced case control study done from October 2010 to May 2012. Inclusion criteria for selection of cases included histopathological diagnosis of thyroid neoplasm and adequate tumour tissue available for further evaluation. A total of 124 thyroid specimens received in this duration, of these 46 were thyroid neoplasms. Histopathological diagnosis was confirmed after review of slides by two independent pathologists. In cases with interobserver variability slides were reviewed with additional senior pathologist. In five cases consensus in diagnosis was not reached even after review with three pathologists and hence excluded from the study. Finally, 41 cases were included, 12 benign and 29 malignant lesions. Detailed clinical findings were noted for all cases. The study was approved by the institutional ethics committee and scientific review committee. All patients gave a written informed consent.

Ten percent formalin-fixed, paraffin-embedded blocks routinely prepared from representative areas containing tumor and adjacent normal tissues were selected.

Two sections of 4 microns' thicknesses were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly-L-lysine coated slide for immunohistochemical staining. Standard procedure for H&E staining was employed using Harris Haematoxylin and aqueous Eosin.

The kits for GALECTIN-3 mouse monoclonal antibody immunohistochemical staining were obtained from CELL MARQUE (GALECTIN-3 mouse monoclonal antibody, CMC25521020). Staining was done according to the manufacturer's protocol. A positive control was included provided by the manufacturer. Cytoplasmic staining of Galectin-3 in cells was regarded as positive. Galectin- 3 staining was evaluated quantitatively as percentage of cells positive, qualitatively for intensity of staining. Also noted was

distribution of staining in tissue. No staining or weak staining in less than 10% of the cells, was scored as negative and any other cytoplasmic immunoreactivity was scored as positive [13]. Staining in 10%to 50% of the cells was defined as focal staining and staining more than 50% of the cells was defined as diffuse staining [9]. The staining intensity was graded as 0 (no staining), 1+ (slight staining), 2+ (moderate staining), or 3+ (intense staining) [14] [Table/Fig-1].



[Table/Fig-1]: Grading of intensity of Galectin-3 staining. (400X): (a) Grade 0; (b) Grade 1; (c) Grade 2; (d) Grade 3.

STATISTICAL ANALYSIS

Initial statistical feasibility of study was evaluated. Based on the positivity of marker observed in earlier publications and with 95% confidence and 80% power minimum sample size came to be 20 which would give 10 pairs of values. Statistical analysis was done by chi-square test and p-value calculated by SPSS.12 software. The p-value ≤0.05 was considered statistically significant.

RESULTS

Clinicopathological Characteristics

A total of 41 cases of thyroid neoplasms including 12 benign and 29 malignant lesions were studied. The benign cases were Follicular Adenomas (FA) (n=10) and hurtle cell adenomas (HA) (n=2). The malignant cases consisted of Papillary Thyroid Carcinomas (PTC) (n=19) of which 1 case was of distant metastases to bone, Follicular Variant of Papillary Thyroid Carcinomas (FVPTC) (n=6) of which 1 case was of distant metastases to brain, Follicular Thyroid Carcinoma (FTC) (n=3) and 1 Medullary Thyroid Carcinoma (MTC).

The highest incidence of thyroid neoplasms in present study was in 5th decade in males and in 4th and 5th decade in females. Incidence of thyroid neoplasms was higher in females (M: F; 1:13) up to 4th decade and from 5th decade onwards the incidence was observed increasing in males (M: F; 1:2.3). [Table/Fig-2].

Age group in years	Benign thyroid neoplasm		Malignant thyroid neoplasm		Total
	Male	Female	Male	Female	
20-29	0	1	0	4	5
30-39	1	5	0	3	9
40-49	0	2	5	6	13
50-59	1	1	1	3	6
60-70	0	1	1	5	7
70 and Above	0	0	0	1	1

[Table/Fig-2]: Age and sex distribution in the study population.

GALECTIN-3 Expression in Study Sample

In present study 12 benign neoplasms were included (10 FA and 2 HA). 3/10 (30%) cases of FA showed positivity for Galectin-3. One (10%) of these showed diffuse, grade three positivity and rest two (20%) showed focal, grade1/2 positivity. Seven out of ten (70%) of FA showed no Galectin-3 expression, grade 0. There were 2 cases of HA, 2/2 (100%) showed positive staining for Galectin-3. Of these one showed diffuse, grade 2 and other focal grade 2 positivity.

In this study 19/19 (100%) cases of PTC showed positive expression with Galectin-3.18/19 (95%) of these showed diffuse, grade 3 positivity for Galectin-3. Only one (5%) case of PTC showed diffuse, but grade 2 positivity for Galectin-3.

All cases of FVPTC 6/6 (100%) showed positive staining, with Galectin-3, grade 3. 4/6 (67%) of these showed diffuse, grade 3 positivity. 2/6 (33%) cases showed focal, grade 3 positivity for Galectin-3.

Two third (67%) of FTC showed positive staining, with Galectin-3. One third (33%) showed diffuse, grade 3 positivity. 1/3 (33%) showed focal, grade 2 positivity, One third (33%) showed no Galectin-3 expression, grade 0.

There was only one case of MTC in this study and it showed no Galectin-3 expression, grade 0, 0/1 (0%).

Overall in this study, there were 29 malignant thyroid neoplasms. 27/29 (93%) showed positive staining for Galectin-3. A total of 24/29 (82%) showed diffuse positivity and 3/29 (10%) showed focal positivity. Grade 3 positivity was seen in 25/29 (86%) of cases and grade 2 positivity was seen in 2/29 (6.8%) of cases. Adjacent normal thyroid tissue was negative for Galectin-3 staining in all cases [Table/Fig-3,4].

HPE	Galectin-3		Galectin-3 Positivity				Total
	Negative	Positive	Diffuse	%	Focal	%	
FA	7	3	1	10%	2	20%	10
HA	0	2	1	50%	1	50%	2
PTC	0	19	19	100%	0	0%	19
FVPTC	0	6	4	67%	2	33%	6
FTC	1	2	1	33%	1	33%	3
MTC	1	0	0	0%	0	0%	1
NORMAL THYROID	41	0	0	0%	0	0%	41

[Table/Fig-3]: Galectin-3 expression in study sample.
FA-Follicular adenoma; HA- Hurthle cell Adenoma PTC- Papillary thyroid carcinoma; FVPTC-Follicular variant of Papillary thyroid carcinoma; FTC-Follicular thyroid carcinoma; MTC-Medullary thyroid carcinoma.

In present study there was significant difference ($p<0.001$) in expression of Galectin-3 in benign and malignant thyroid neoplasms. Similarly, differential Galectin-3 expression in benign thyroid neoplasms and PTC was significant ($p<0.001$).

In the present study there was no significant difference for Galectin-3 expression in follicular neoplasms when only positivity (>10% of cells positive) ($p>0.08$) and diffuse positivity (>50% of cells positive) ($p>0.$ 0.167) were taken into consideration. However, when positivity (>10% of cells positive) along with intensity of only grade 3 staining were considered as a criterion for differentiation of various follicular neoplasms there was a significant difference in Galectin-3 expression ($p<0.002$). [Table/Fig-5].

DISCUSSION

The current standard in the diagnosis of thyroid lesions is by histological examination of routine H&E stained sections. However, diagnostic dilemma in follicular patterned thyroid lesions is a pertinent issue. Over last decade several immunohistochemical markers and recently molecular markers are studied to identify ideal marker to aid in this situation. Galectin-3 is one of most sensitive marker of thyroid malignancy in studies evaluating panels of immunohistochemistry [9,11,12]. In present study we have evaluated Galectin-3 expression in thyroid neoplasms in order to evaluate its usefulness as a single marker

HPE	Galectin-3 Expression-Intensity				TOTAL
	0	1	2	3	
NORMAL THYROID	41	0	0	0	41
FA	7	1	2	0	10
HA	0	0	2	0	2
PTC	0	0	1	18	19
FVPTC	0	0	0	6	6
FTC	1	0	1	1	3
MTC	1	0	0	0	1

[Table/Fig-4]: Galectin-3 expression by intensity in study.
FA-Follicular adenoma; HA- Hurthle cell Adenoma; PTC- Papillary thyroid carcinoma; FVPTC-Follicular variant of Papillary thyroid carcinoma; FTC-Follicular thyroid carcinoma; MTC-Medullary thyroid carcinoma.

Histopathology	Galectin-3		X^2	p
	Positive	Negative		
BTN	5	7	10.277	<0.001
MTN	27	2		
NT	0	41	45.976	<0.001
BTN	5	7		
PTC	19	0		
FA	3	7	5.034	0.08
FVPTC	6	0		
FTC	2	1		
	Galectin-3			
	Diffuse	Focal/Negative		
FA	1	9	3.569	0.167
FVPTC	4	2		
FTC	1	2		
	GALECTIN-3 Grade 3 Intensity			
	3	<3		
FA	0	10	12.33	0.002
FVPTC	6	0		
FTC	1	2		

[Table/Fig-5]: Differential Galectin-3 expression and its significance.
BTN- Benign thyroid neoplasm; MTN-Malignant thyroid neoplasm; NT-Normal thyroid tissue; PTC- Papillary thyroid carcinoma; FA-Follicular adenoma; FVPTC-Follicular variant of Papillary thyroid carcinoma; FTC-Follicular thyroid carcinoma.

to distinguish benign and malignant thyroid neoplasms. Further detailed analysis of grading and intensity of expression was carried out to assess utility as a single marker particularly in follicular patterned lesions.

Malignant thyroid neoplasms show higher Galectin-3 expression. Majority of the studies in literature since conceptualization of Galectin- 3 in thyroid have shown high rate of Galectin-3 expression in malignant thyroid neoplasms [10,11-14]. In our study too malignant thyroid neoplasms had significantly higher expression of Galectin- 3 in comparison to benign neoplasms. However, in studies by Barroeta JE et al., and Barut F et al., only 70% malignant lesions were positive for Galectin-3 [9,15].

Galectin-3 is expressed only in neoplastic thyroid tissue. Xu XC et al., analysed expression of Galectin-1 and Galectin-3 with immunohistochemical and immunoblotting techniques in thyroid neoplasms and normal thyroid tissue [16]. They found that Galectins are not expressed in normal thyroid tissue and benign adenomas. In our study too adjacent normal tissue in all cases was completely negative for Galectin-3. However, around a third of benign thyroid neoplasms in our study expressed in Galectin-3 which is consistent with current literature [12,13]. In light of these findings role of Galectin-3 in thyroid carcinogenesis appears to be at an early step.

HBME-1, CK 19, CD 56, and TPO are some of other reliable immunohistochemical markers for differentiating benign and malignant thyroid neoplasms [9-11,13-15,17].

Galectin-3 can be used as marker to distinguish benign and malignant thyroid neoplasm, particularly PTC. PTC show diffuse strong positive reaction with Galectin-3 in nearly 100% of cases. Currently PTC is recommended as positive control by some manufacturers for Galectin-3 antibody. In our study all PTC and FVPTC cases showed diffuse strong positivity for Galectin-3. We recommend it as sole marker to distinguish benign thyroid neoplasms from PTC based on positive staining alone.

Follicular patterned lesions of thyroid pose a diagnostic challenge particularly to exclude a minimally invasive follicular carcinoma in a follicular adenoma and also with newer entities like encapsulated variant of FVPTC and non-invasive follicular thyroid neoplasm with papillary like nuclei. Galectin-3 utility in this scenario is debated particularly in distinguishing follicular adenoma and follicular carcinoma. In study by Sahel HA et al., 19 out of 46 follicular adenomas and 18 out of 22 follicular carcinomas were reported to be positive for Galectin-3. Song Q et al., reported Galectin-3 positivity of 30% in follicular adenomas and 45% in follicular carcinoma [12,13]. We explored this issue further and found that mere positive Galectin-3 expression cannot reliably differentiate follicular patterned neoplasms. Interestingly, on contrary to past literature we found that along with positive reaction additional criteria of grade 3 intensity staining is more reliable marker to differentiate benign and malignant follicular patterned lesions of thyroid. This finding is encouraging and Galectin-3 grade 3 intensity positivity can be used to differentiate benign and malignant follicular patterned thyroid neoplasms. However, it cannot be used as a sole criterion.

LIMITATION

Our sample size was small and distribution of cases within sample were unequal. Thus would require a study on larger sample in regards to follicular patterned lesions of thyroid for the observations to be validated.

CONCLUSION

To conclude our study showed that Galectin-3 is useful as a sole marker in differentiating benign and malignant thyroid neoplasm ($p<0.05$). This is particularly true for differentiating PTC and benign thyroid neoplasms as sole marker.

In contrast Galectin-3 is not a useful marker for differentiation of follicular patterned lesions, when only positivity for Galectin-3 is taken as criteria. However, we found that if Galectin-3 positivity and along with grade 3 intensity was taken as criteria it could prove to be a useful marker along with panel immunohistochemistry in differentiating follicular patterned lesions.

Future recommendation: A study comparing Galectin-3 a sole marker to others biomarkers in future would be worthwhile as final diagnosis of malignant neoplasm of thyroid has management implications with further treatment and follow-up protocols.

Authors and Contributions: Conception, design: PVB, GK & SK, collection, analysis and interpretation of data: PVB. Planning, conduct and reporting: PVB. Drafting the article: PVB, GK & SK. Critical revision for important intellectual content: GK & SK. All authors read and approved the final manuscript.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 13, 2019**
Date of Peer Review: **Feb 12, 2019**
Date of Acceptance: **Apr 18, 2019**
Date of Publishing: **Jul 01, 2019**