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Research Article

DIAGNOSTIC ROLE OF GALECTIN-3 EXPRESSION IN BENIGN FOLLICULAR PATTERNED THYROID LESIONS, NON-INVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES (NIFTP), AND INFILTRATIVE FOLLICULAR VARIANT PAPILLARY THYROID CARCINOMA (IFVPTC)

TAMILSELVI V[1], LAKSHMY VENUGOPAL[1], KRISHNAN RAVIKUMAR[2], DIVYA RAJENDRAN[3]

[1]Department of Pathology, Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. [2]Department of Surgery, Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. [3]Department of Pathology, Government Karur Medical College, Karur, Tamil Nadu, India. Email: tamilselviaruna16@gmail.com

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ABSTRACT

Objectives: The objective of the study was to determine the role of Galectin-3 expression in distinguishing Benign follicular patterned thyroid lesions, non-Invasive follicular thyroid neoplasm with papillary-like nuclear Features (NIFTP), and invasive follicular variant of papillary thyroid carcinoma (IFVPTC).

Methods: The Institutional Human Ethics Committee reference number is 271/pathology/09/2022. A total of 85 cases were included in the study after the histopathological evaluations based on strictly defined inclusion and exclusion criteria. Study groups were created as nodular hyperplasia, follicular adenoma, follicular carcinoma, NIFTP, invasive EFVPTCs, and classical papillary thyroid carcinomas. Cytoplasmic Galectin-3 Immunohistochemistry (IHC) expression was evaluated in these cases. Galectin-3 IHC scores data were analyzed using IBM SPSS statistics. The Chi-square test was used to determine the association between the variables. $p<0.05$ was considered statistically significant.

Results: Cytoplasmic galectin-3 IHC expression was significantly increased in malignant follicular patterned thyroid lesions compared to benign lesions with $p<0.00001$. Similarly, cytoplasmic galectin-3 IHC expression was significantly increased in IFVPTC when compared to NIFTP with a p-value of 0.01358. The Odds Ratio showed the positive cytoplasmic Galectin-3 expression in IFVPTC with a 7.5 times higher risk of having adverse outcome when compared to NIFTP.

Conclusion: Cytoplasmic Galectin-3 IHC expression may serve as a useful biomarker in predicting the invasiveness of FVPTC and distinguishing NIFTP from infiltrative FVPTC.

Keywords: Galectin-3, Immunohistochemistry, Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, Follicular variant papillary thyroid carcinoma.

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INTRODUCTION

Thyroid cancers have the most rapidly increasing incidence rates among all major cancers, the increasing incidence being attributable to the enhanced vigilant screening and also partly due to the evolution of histologic criteria in the diagnosis as well as the increased recognition of Follicular variant Papillary Thyroid Carcinoma (FVPTC) [1,2]. The FVPTC is categorized into two main subtypes: encapsulated and infiltrative. Encapsulated FVPTC is a challenging diagnosis in the pathology of thyroid nodules [2]. The introduction of Non-Invasive follicular thyroid neoplasm with Papillary-like nuclear Features (NIFTP) to replace the encapsulated FV-PTC was proposed by a group of experts in endocrine pathology led by Nikiforov in 2016 [3-5]. The mean rate of incidence of NIFTP in Asian countries was found to be 6.3% which is much less when compared to the Western nations [3-5].

NIFTP was addressed to reduce the overtreatment of indolent thyroid tumors and to eliminate the word "carcinoma" [5,6].

NIFTP presents with the following characteristics: encapsulated tumor with nuclear characteristics of papillary carcinoma of thyroid; no papillae; no psammoma bodies; follicular growth pattern; lack of invasion; and no tumor necrosis. It is recently recommended that there should be no well-formed papillae within the NIFTP spectrum [7-9].

Several studies point toward the fact that NIFTP is a highly indolent tumor with <1% overall risk of recurrence [9,10].

As neoplasm develops more specific alterations at the genomic level, the molecular marker-based ancillary test may be a great asset in improving the diagnostic accuracy of the NIFTP diagnosis [10]. Galectin-3, a member of the beta-galactoside binding protein family is involved in cell proliferation, progression of the tumor, and cancer metastasis. The aberrant expression of galectin-3 blocks the apoptotic proteins, thereby promoting the development of cancer. Galectin-3 is over expressed in a high proportion of carcinomas, especially of the papillary type but nearly absent in benign thyroid lesions suggesting its major role in the malignant transformation of thyroid cells [11,12].

Our study was undertaken to determine the diagnostic value of galectin-3 expression in histological tissues as a useful biomarker to distinguish the invasive follicular variant of papillary thyroid carcinoma (IFVPTC) from the indolent NIFTP.

METHODS

This observational study was performed at the Department of Pathology, KFMSR, Coimbatore, from June 2019 to June 2022. The Institutional Human Ethics Committee reference number is 271/pathology/09/2022. A total of 85 cases were included in the study after the microscopic evaluations based on defined inclusion and exclusion criteria. Study groups were created as nodular hyperplasia (20), follicular adenoma (4), follicular carcinoma (5), NIFTP (21), invasive FVPTCs (10), and classical papillary thyroid carcinomas (PTC) (25).

Histological inclusion and exclusion criteria

For the NIFTP group, the inclusion criteria included encapsulation, follicular growth pattern, nuclear features of PTC (2–3 points of the 3 point nuclear scoring) – enlargement, crowding, grooves, pseudo-inclusions, chromatin clearing, and minor features: irregularly shaped follicles, dark colloid, sprinkling sign, follicle cleft from the stroma, and multinucleated giant cells within the follicles [11,12]. The exclusion criteria used for NIFTP were the presence of true papillae, psammoma bodies, infiltrative border, tumor necrosis, high mitotic activity, and morphologic characteristics of other histological variants of PTC.

Immunohistochemistry (IHC) staining

For IHC studies, 5-micron thickness representative sections were taken from each tissue block. The sections were stained with an avidin-biotin complex method using galectin-3 antibody. Sections of the classical variant of PTC with known galectin-3 positivity were included as a positive control and an irrelevant isotype-specific IgG in place of the primary antibody was used as a negative control.

Evaluation of IHC

IHC sections were scored as positive if epithelial cells showed immunoreactivity in the cytoplasm using a bright field microscope. Sections were scanned at low and high power for Galectin-3 scoring. Percentage positive scores were assigned according to the scale as follows: 0 (≤10%), 1 (11–30%), 2 (31–50%), 3 (51–70%), and 4 (>70%). Staining intensity was scored as: 0 (none), 1 (mild), 2 (moderate), and 3 (intense). The total score was obtained (0–7) by adding the percentage positivity and intensity scores [13,14].

Galectin-3 IHC scores data were analyzed using IBM SPSS statistics. The Chi-square test is used to determine association between the variables. $p<0.05$ is considered statistically significant.

RESULTS

Galectin-3 IHC analysis was carried out to determine its expression at the cellular level in thyroid benign nodules (Fig. 1), Follicular adenomas (Fig. 2), NIFTPs (Fig. 3), invasive FVPTCs (Fig. 4), and classical PTCs (Fig. 5).

There was no expression in cases of nodular hyperplasia, whereas 1 out of 4 cases in follicular adenoma-stained positive among the benign group. Out of the 40 cases in the malignant group, 29 cases stained positive with galectin-3. Among 21 cases of NIFTP, 5 cases expressed positive galectin-3 expression (Table 1).

There was a significant increase in the expression of galectin-3 IHC among the malignant group of thyroid nodules when compared with the benign nodules, $p<0.00001$ and a Chi-square value is 28.13 (Table 2).

The galectin-3 expression was increased in IFVPTC when compared to NIFTP with the Chi-square value being 6.09 and was found to be statistically significant ($p=0.01358$) (Table 3).

Our study showed no significant difference in the expression of Galectin-3 between NIFTP and the benign group of thyroid nodules. The Chi-square value was found to be 3.74 with the p-value being 0.053 (Table 4).

The cross-tabulation analysis of the odds ratio in our study showed the positive cytoplasmic Galectin-3 expression in IFVPTC with a 7.5 times higher risk of having adverse outcomes when compared to NIFTP (Table 5).

DISCUSSION

A subset of encapsulated follicular thyroid tumors, formerly considered to be Encapsulated Follicular Variant Papillary Thyroid Carcinoma (EFVPTC), has been reclassified under a new histological nomenclature, NIFTP (Non-invasive Follicular Thyroid neoplasm with Papillary-like nuclear features) based on the criteria proposed by Nikiforov *et al*. [15].

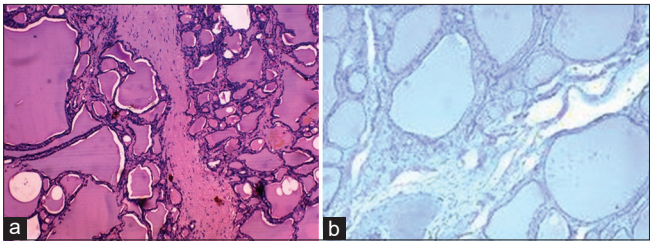

Fig. 1: (a) H&E ×400 colloid goitre, (b) ×400 shows negative galectin-3 expression

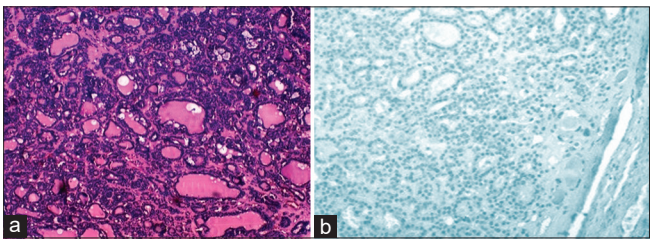

Fig. 2: (a) H&E ×400 follicular adenoma, (b) ×400 shows follicular adenoma with negative galectin-3 expression

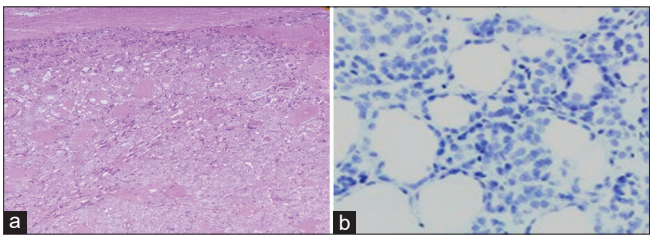

Fig. 3: (a) H&E ×100 shows NIFTP, (b) ×400 NIFTP exhibiting negative galectin-3 expression

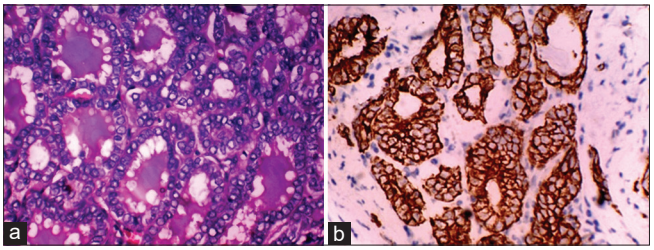

Fig. 4: (a) H&E ×400 infiltrative FVPTC, (b) ×400 IFVPTC showing strong cytoplasmic galectin-3 positivity

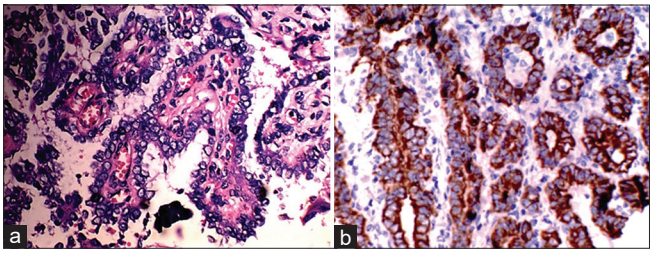

Fig. 5: (a) H&E ×400 classic papillary thyroid carcinoma, (b) ×400 classic PTC exhibiting strong cytoplasmic galectin-3 positive expression

Molecular marker testing has risen as an auxiliary tool to distinguish invasive EFVPTCs from the more indolent NIFTPs and benign nodules. Galectin-3 is recognized as a promising diagnostic marker due to its differential expression in thyroid malignancies and benign nodules [16].

Table 1: Positive IHC expression of Galectin-3 in our study

Thyroid lesions	Galectin-3 positivity
Benign (n=24)	1
Nodular Hyperplasia (n=20)	0
Follicular adenoma (n=4)	1
Malignant (n=40)	29
Follicular carcinoma (n=5)	0
Infiltrative FVPTC (n=10)	7
Classic PTC (n=25)	22
NIFTP (n=21)	5

FVPTC: Follicular variant papillary thyroid carcinoma, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features

Table 2: p-value and the distribution of the percentage of galectin-3 IHC expression in benign and malignant follicular patterned thyroid lesions

Galectin-3	Benign (n=24)	Malignant (n=40)	p-value
Negative	23	11	<0.00001
Positive	1	29	

Table 3: p-value and the distribution of the percentage of galectin-3 IHC expression with respect to NIFTP and infiltrative FVPTC (IFVPTC)

Galectin-3	NIFTP (n=21)	IFVPTC (n=10)	p-value
Negative	16	3	0.01358
Positive	5	7	

NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, IFVPTC: Infiltrative follicular variant papillary thyroid carcinoma

Table 4: p-value and the distribution of the percentage of galectin-3 IHC expression with respect to NIFTP and Benign thyroid nodules

Galectin-3	NIFTP (n=21)	Benign (n=24)	p-value
Negative	16	23	0.053
Positive	5	1	

NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features

Table 5: Cross tabulation analysis - Odds Ratio between NIFTP and IFVPTC

Galectin-3	NIFTP (n=21)	IFVPTC (n=10)	Odds Ratio
Negative	16	3	7.5
Positive	5	7	

NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, IFVPTC: Infiltrative follicular variant papillary thyroid carcinoma

In our study, the cytoplasmic galectin-3 expression was significantly high and increased in PTC and invasive FVPTCs when compared to benign thyroid nodules with a p-value being <0.00001 which is following the studies conducted by Huang *et al.* [17], Zhu *et al.* [18], and Chuang *et al.* [19].

Our study revealed no significant difference in cytoplasmic Galectin-3 expression between NIFTPs and benign nodules which is similar to the study done by Chuang *et al.* [19].

The cytoplasmic galectin-3 expression between NIFTP and infiltrative FVPTC was found to be statistically significant with p=0.01358 which is similar to the study done by Chuang *et al.* [19] but discordant with the study results of Cho *et al.* [20].

CONCLUSION

Our study demonstrates that increased cytoplasmic Galectin-3 expression can significantly distinguish infiltrative follicular variant papillary thyroid carcinomas (IFVPTCs) from the more indolent NIFTP, thereby playing as a supplementary tool and aiding clinicians to plan the treatment strategy. However, a larger patient cohort from various institutions with long-term follow-up is needed to further validate our observations.

AUTHOR'S CONTRIBUTION

Tamilselvi. V conceptualized the study, conceived, and designed the analysis along with manuscript writing. Data collection, data analysis, and statistical analysis done by Lakshmy Venugopal, Krishnan Ravikumar, and Divya Rajendran. Manuscript edited, finalized, and submitted for publication by Tamilselvi V.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

AUTHORS FUNDING

None.

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