



## Original Research Paper

# Evaluation of galectin-3 expression in non-neoplastic and neoplastic lesions of thyroid

Authors

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## Introduction

The burden of thyroid disease in general population is enormous. As many as 50% in the community have microscopic nodules<sup>1</sup> whereas palpable nodules are encountered in 4% of the population between the age of 30-60 years<sup>2</sup>. The vast majority of thyroid nodules are non-neoplastic or benign neoplasm. The distinction between these benign neoplasms cannot be based reliably on clinical presentation only. Several diagnostic tests; such as radionuclide scanning, high resolution USG, FNAC, biopsy & immunomarkers have been used for correct evaluation. Although current diagnostic "gold standard" for most thyroid lesions is histopathological evaluation using routine H&E stain, by expert pathologists, yet the diagnostic agreement remains controversial. One of the challenging area in surgical pathology is differential diagnosis of encapsulated follicular tumors with less than typical nuclei and equivocal signs of invasiveness<sup>3-4</sup>. This necessitates the discrimination between dominant nodule of nodular hyperplasia, follicular adenoma, minimally invasive follicular carcinoma and follicular variant of papillary carcinoma<sup>5-6</sup>. Recent

studies pointed to some IHC markers in different thyroid diseases in terms of diagnostic & prognostic utility where no vascular or capsular invasion can be observed but a transformation at molecular level can be considered. Galectin-3 represents the well-studied molecular candidate for this purpose<sup>7-9</sup>. This marker has been identified in both cytoplasmic and nuclear compartment & has been implicated in cellular proliferation, apoptosis as well as malignant transformation and metastasis of cancer cells<sup>10-14</sup>.

## Aims & Objectives

1. Histological assessment of different thyroid diseases & their categorization.
2. To study the expression of Galectin-3 in neoplastic & non-neoplastic thyroid lesions.

To provide clinician a platform for better diagnostic & targeted therapeutic management of patients

## Materials and Methods

- ✓ **Study Area:** The department of pathology, Hi-Tech medical college & Hospital, Bhubaneswar.

- ✓ **Study Design:** Cross sectional study of 2 years.
- ✓ **Study period:** 2 years from September 2018 to August 2020.
- ✓ **Selection of cases:** All patients undergoing thyroidectomy in the dept. of Surgery & dept. of ENT of Hitech-medical College & Hospital, Bhubaneswar, covering all race, age, sex & religion during study period.
- ✓ **Inclusion criteria-**patients with thyroid swelling, clinically diagnosed as thyroid disease. –undergone surgical resection for thyroid disease.
- ✓ **Exclusion criteria-** Unwilling patients for biopsy & surgery, patients who are previously diagnosed& undergoing therapy.
- ✓ **Statistical Analysis:** All the data will be analysed with special emphasis on Galectin-3 expression in patients with thyroid disease. At the end statistical analysis will be done to find significant results. Results will be compared with similar national and international works on galectin-3.

### Methodology

The detailed clinical history & result of relevant investigation done will be collected from the patient case file after obtaining informed consent from the patient. The thyroidectomy specimen will be fixed in 10% buffered formal saline & will be processed for histological examination according to standard protocol. Then slides will be stained by H & E staining for reporting & review. Immunohistological examination will be done on microsections mounted on 3-aminopropyltriethoxysilane coated slides, sections then will be boiled in Tris-buffered saline for antigen retrieval, at pH 6.0, blocking of endogenous peroxidase with aqueous 0.3% H<sub>2</sub>O<sub>2</sub> for 15 min. Sections will be then incubated with Galectin-3 for 1hr. The Ag-Ab immunoreactions will be carried out in room temperature.

### Observations and Results

The study was undertaken in the Department of Pathology, Hi-tech Medical College & Hospital, Bhubaneswar during a 2 year study from September 2018 to August 2020. A total of 70 thyroid biopsies are classified according to WHO. IHC for Galectin-3 is done for each category. The observations of the study are as follows

In the present study, most common non neoplastic lesion is Nodular/Multinodular Goitre (37.14%) and most common malignant neoplastic lesion is Papillary Thyroid Carcinoma (11.47%).

Most of the patients are females with 88.6% of our cases.

Most of the patients are females with F:M ratio 7.7:1

Most common age group involved is 40-49 years, with 22 cases (31.42%)

In the present study the age of the patients ranged from 10-61years.

Most of the cases are non-neoplastic, seen in females in the age group of 20-29yrs

Maximum no. of malignant cases are seen in females in the age group of 40-49yrs.

### Interpretation of Galectin-3 Staining

**Table-1** Intensity Grade (Staining type)

SCORE	PATTERN
0	No staining
1+	Slight/Weak Staining
2+	Moderate Staining
3+	Intense Staining

**Table-2** Proportion Score (Proportion of stained cells)

SCORE	PATTERN
-	No Reactivity
1+	<5% of cells
2+	5% to 50% of cells
3+	>50% of cells

POSITIVE RESULT= Specific staining of more than 5% of cells (>1+) with slight, moderate or intense staining.

**Table-3** Results of cytoplasmic Galectin-3 IHC Staining

Histologic Diagnosis	No. of Cases	Intensity Grade	Proportion Score	Interpretation
NG/MNG(n=12)	12	0	0	Negative
NG with mfh (n=4)	4	2+	1+	Negative
NG with sc(n=2)	2	1+	1+	Negative
NG with ct (n=4)	3	3+	1+	Negative
	1	3+	2+	Positive
NG with ht(n=2)	2	2+	1+	Negative
MNG with cd(n=2)	2	0	0	Negative
CG (n=12)	12	0	0	Negative
HT/AT(n=4)	4	2+	1+	Negative
HA (n=2)	2	0	0	Negative
FA (n=4)	4	1+	1+	Negative
PTC (n=8)	7	3+	3+	Positive
	1	3+	2+	Positive
FVPTC (n=4)	4	3+	3+	Positive
FC (n=6)	6	3+	1+	Negative
MC (n=2)	2	1+	1+	Negative
AC (n=2)	2	3+	3+	Positive

The cases positive for cytoplasmic expression of Galectin-3 are Papillary thyroid carcinoma (100%), Follicular variant of PTC(100%) and Anaplastic carcinoma(100%) and Nodular goiter with chronic thyroiditis (2%).

**Table-4** Comparison of Gal-3 Expression in Malignant & Benign Thyroid Lesions

	Gal-3 Positive	Gal-3 Negative
Malignant(n=22)	14	01
Benign (n=48)	08	47

There is an association of expression of Galectin-3 towards malignant lesions of thyroid.

**Table-5** Calculation of Sensitivity, Specificity, PPV & NPV in Thyroid Lesions

SPSS 20.0	Malignant	Benign	Total
Gal-3 Positive	14	01	15
Gal-3 Negative	08	47	55
Total	22	48	70

Sensitivity= 63.63%

Specificity= 97.91%

PPV= 93.33%

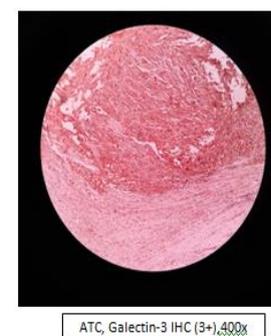
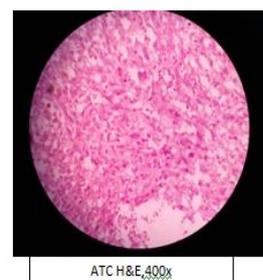
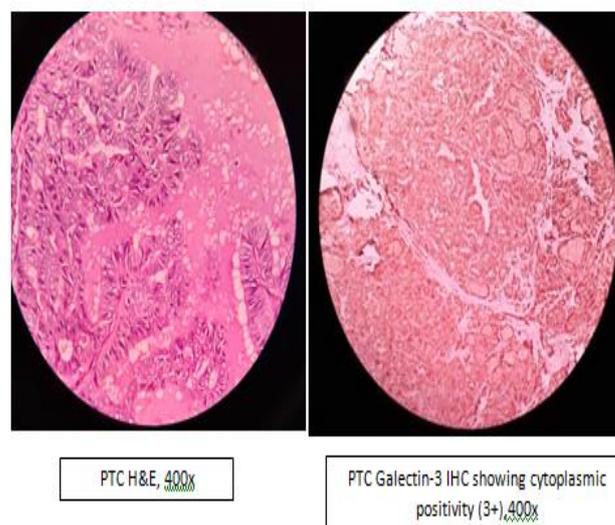
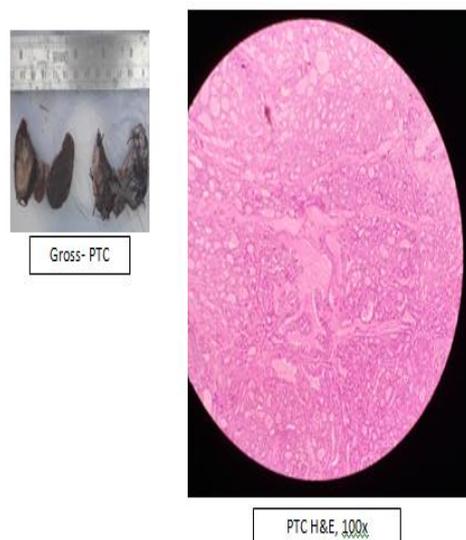
NPV= 81.81%

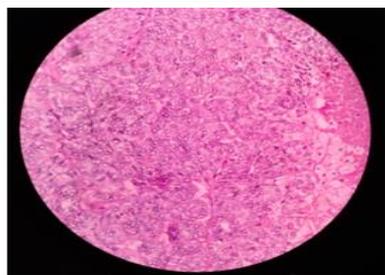
Chi square statistic for the above correlation =19.88 with 1degree of freedom.

Two tailed P value <0.01

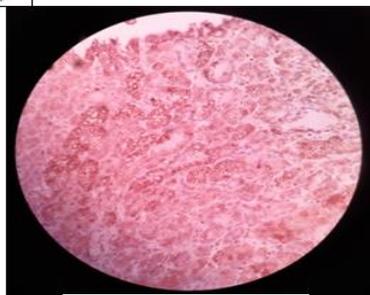
⇒ p<0.05

Hence, the relation between malignant and benign Thyroid Lesions is strongly statistically significant.

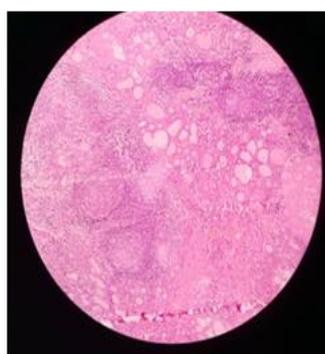




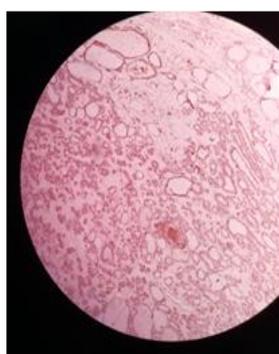
FVPTC, H&E, 400x



FVPTC, Galectin-3 IHC (3+), 400x



NG with CT (H&E, 100x)



NG with CT -Galectin-3 (+ve), 100x

**Discussion**

**Comparison of Female to Male ratio of the Present study with other studies**

Authors	Gupta et al	Basharat et al	Hussain Anwar	Bamanikar et al	Handa et al	Devechi et al	Present Study
F:M Ratio	11:1	5.2:1	6.9:1	8.6:1	7.3:1	4.3:1	7.7:1

Most of the studies show female predilection.

**Comparison of Sensitivity, Specificity, PPV, NPV of the Present study with other studies:**

Authors	Sensitivity	Specificity	PPV	NPV
Gharib& Pappini	83%	92%	75%	-
Gupta et al	80%	86.6%	80%	86%
Basharat et al	80%	97.7%	80%	97.7%
Bamanikar et al	50%	100%	-	-
Kessler et al	79%	98.5%	-	-
Dusko et al	88.52%	-	79.41 %	78.4%
Bolia et al	45.6%	100%	100	64.7
Gardiner et al	65%	91%	-	-
Altavilla et al	71%	100%	-	-
Burch et al	80%	73%	-	-
Smenov et al	100%	71%	-	-
<b>Present Study</b>	63.63%	97.91%	93.33 %	81.81%

Most of the reported sensitivity and specificity of thyroid FNA ranges between 45% and 100%. This large variation is attributable mainly to the manner in which data are analyzed.

In the present study immunohistochemical analysis of 70 thyroid specimens with anti-galectin-3 antibodies revealed that the epithelial cells in benign thyroid lesions did not express galectin-3 except one. However, most of the malignant cases show strong cytoplasmic positivity. Papillary and follicular variant of papillary and anaplastic carcinomas expressed high levels of galectin-3 which is similar to a study conducted by Chun Xu et al.

Connie G. Chiu et al mentions in his study that expression of Gal-3 ranges from 20% to 100% in reported cases of follicular thyroid carcinoma (FTC). The largest series, reported by Bartolazzi et al, identified Gal-3 expression in 95% (54/57) of FTC cases. Similarly, for the follicular variant of PTC, Gal-3 positivity ranged from 33% to 100% of cases. Jaudah Al-Maghrabi et al conclude that the role of Gal-3 in FTC is controversial. The present study also shows galectin-3 expression in 0% of follicular thyroid carcinoma cases and 100% of follicular variant of PTC.

The same above mentioned study shows in PTC, Gal-3 expression in n 58% to 100% of cases though according to most of other literatures it is 80% to 100%. The present study has galectin-3 expression in 100% PTC cases.

The present study shows Galectin-3 expression in 100% of anaplastic thyroid carcinoma cases which is similar to the expression of Gal-3 (75% to 100% of reported cases) of anaplastic thyroid cancer (ATC), Connie G. Chiu et al.

In the present study 04 cases of follicular adenomas were subjected to Gal-3 but neither of them showed positive expression. In a study conducted by Magdalena Jakubiak-Wielganowicz *et al* galectin-3 immunopositivity was noticed in the majority of follicular carcinomas and in a part of follicular adenomas. Thus, intense galectin-3 expression strongly suggests malignancy, while absence of this marker cannot exclude carcinoma. These results are concordant with a majority of previous reports.

Galectin-3-positive adenomas were also reported by others: Gasbarri et al. (1/37 cases), Aratake et al. (2/14) and Orlandi et al. (3/29). Again a few authors did not find galectin-3 expression in any adenoma. In the present study 2 cases of Hurthle cell adenoma didn't show Galectin-3 positivity.

Rita Beata Kovacs *et al.* (2003) reported all nodular goiter and normal thyroid tissue were negative for Galectin-3 IHC. Focal positivity was found in follicular adenoma. Also in all inflammatory cases a focal positivity was observed. In the present study 1/42 non-neoplastic case was found focally positive.

### Summary and Conclusion

Thyroid gland enlargement is a common clinical finding. In the present study 70 patients with enlarged thyroid referred from department of Surgery & ENT to our department over a period of two years. The cases were followed for histopathological findings and immunohistochemistry wherever possible.

HP study was done in all ages ranging from 10-61 yrs. with mean age being 35 yrs. Out of 70 cases 62 were females and 08 were males, with female to male ratio being 7.7:1.

In the present study 70 cases of thyroid swelling showed histological diagnosis of 68% benign and

32% malignant cases. Ratio of benign to malignant cases is 2.1:1.

Galectin-3 is expressed in 100% of Papillary carcinoma, Follicular variant of Papillary Carcinoma & Anaplastic Carcinoma cases & 2% case of Nodular goiter with chronic thyroiditis. The marker was negative for all other non-neoplastic & benign neoplasm.

Statistical analysis of benign lesions vs malignant lesions showed sensitivity, specificity, positive predictive value, and negative predictive value of IHC to be 63.63%, 97.91%, 93.33% and 81.81%, respectively

The differential expression of Galectin-3 in thyroid carcinoma compared with benign neoplasm represents a promising target for therapy of thyroid cancers. Gal-3 is a good marker of malignancy especially in Papillary carcinoma & its variants. A reliable and reproducible Gal-3 testing methodology & positive immunoeexpression criteria guidelines are necessary as it bears impact on the accurate and consistent reporting of Gal-3 expression in both non-neoplastic & neoplastic lesions of Thyroid.

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