# RESEARCH ARTICLE

## GALECTIN-3 IMMUNOHISTOCHEMICAL EXPRESSION IN NEOPLASTIC AND NON-NEOPLASTIC LESIONS OF THYROID

**[1]Ajay Kr Singh, *,[2]Chumila Thinley Bhutia, [1]Raj Mehrotra, [1]Madhu Mati Goel, [1]Malti Kumari, [1]Mala Sagar and [1]Madhu Kumar**

[1]Department of Pathology, KGMU, Lucknow, UP, India
[2]Department of Pathology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India

**ABSTRACT**

**Background:** Thyroid cancer is the most common endocrine malignancy. The galectin-3 is a member of the beta-galectoside family that play an important role in cellular growth, differentiation and malignant transformation in various organs including thyroid.
**Aim:** To evaluate immunohistochemical expression of galectin-3 in neoplastic and non-neoplastic thyroid lesions and correlated with histopathology.
**Materials and Methods:** In this observational study we evaluated gal-3 expression in spectrum of 67 histopathological diagnosed sample including as follows: 30 PTC (included 9 classical variant, 18 follicular, 02 solid variant, 01 micropapillary), 10 FTC, 14 benign neoplasm (including follicular adenoma and hurthle cell adenoma) and 13 non neoplastic (includes hyperplasia and inflammatory lesions) cases. To rule out subjective differences during IHC, a 3- observe evaluation method was used.
**Statistical analysis:** The statistical analysis was done using SPSS (statistical package for social sciences) version 15.0 statistical analysis software.
**Results:** Galectin-3 expression was significantly higher in malignant thyroid neoplasm as compared to benign neoplasms (p<0.0001). Maximum number of malignant cases were aged between 41 to 50 years and majority of subjects were females. For scoring, it was observed that as compared to non-neoplastic, neoplastic lesions had higher scores (p=0.012).
**Conclusion:** The Gal-3 to be used as adjunct for aiding thyroid cancer diagnosis.

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## INTRODUCTION

The differential diagnosis of the thyroid nodule includes non-neoplastic and neoplastic (benign and malignant) (Fischer and Asa, 2008; Aiad *et al.*, 2008; Baloch and Livolsi, 2002; Weber *et al.*, 2004). Thyroid cancer is the most common endocrine malignancy and more then 95% of thyroid carcinoma originates from follicular epithelial cells. (Hundahi *et al.*, 1998) Worldwide, incidence rates of thyroid cancer may vary, possibly due to relation with inherent racial, ethnic differences, geographical, environmental differences (including iodine excess or deficiency), radiation exposure (Leonard Wartofsky, 2010) and specific mutations. various subtypes is providing novel diagnostic markers as well as targets for individualized therapies. (Kondo *et al.*, 2006) In India, the top five common sites of cancer in females are mouth, cervix, uterus, breast, thyroid and stomach. (Kalyani and Das, 2010) Survey also

detected a belt of thyroid cancer in women in coastal districts of Kerela, Karnataka and Goa. (Ganpati Mudur, 2005) The pathologists have challenging role in preoperative assessment of thyroid lesions through FNAC/ Core biopsy / lobectomy. (De Lellis and Williams, 2004) In many cases specially in follicular-patterned thyroid lesion even with histological analysis the distinction between benign and malignant neoplasm can be difficult. (Fischer and Asa, 2008; Hundahi *et al.*, 1998) For this reason, the approach to these challenging tumors should include ancillary techniques, immunohistochemistry and molecular profiling. The most useful markers for DTC (differentiated thyroid cancer) were Galectin-3(Gal-3), cytokeratin 19 (CK-19), Vascular endothelial growth factor, androgen receptor, p16, aurora-A, and HBME-1. (Connie *et al.*, 2010) In the largest thyroid cancer diagnostic marker panel study reported to date, Gal -3 was found to be the most accurate standard alone marker for DTC diagnosis when compared with a panel of 56 other molecular markers. (Wiseman *et al.*, 2008) Furthermore, the classification performance of Gal-3 alone (accuracy of 86.9%)

*Corresponding author: **Chumila Thinley Bhutia,***
Department of Pathology, Sikkim Manipal Institute of Medical sciences, Gangtok, Sikkim, India.

was almost as good as the best multimarker panel (accuracy of 91.0%). (Connie *et al.*, 2010)

## Aims and Objectives

To evaluate Galectin-3 expression in Thyroid Neoplasia in terms of intensity and proportion of tumour cell stained and correlated with histopathology.

## MATERIALS AND METHODS

Histopathologically diagnosed non-neoplastic and neoplastic (benign and malignant) cases of thyroid lesions were taken from archives of department of pathology of King George,s Medical university, Lucknow. These surgical specimen were fixed in 10% formalin, routinely processed, paraffine-embedded and the sections were stained with hematoxylin and eosine stain. Study duration was two year ie one year retrospective and one year prospective. The histopathological evaluation were performed by three pathologists and final diagnosis of the tumour histotype and subtype was made in accordance with world health organization. (Paron *et al.*, 2003) For statistical purpose the histopathological diagnosis was considered as gold standard. The diagnosis of the neoplastic lesion were distributed as follows : 30 PTC (included 9 classical variant, 18 follicular, 02 solid variant, 01 micropapillary), 10 FTC, 14 benign neoplasm (including follicular adenoma and hurthle cell adenoma) and 13 non neoplastic (includes hyperplasia and inflammatory lesions) cases (Table-1).

## Study sample: (Table -1)

| Thyroid lesions | | No. of cases |
|---|---|---|
| Nonneoplastic | | |
| Inflammatory lesions : Hashimoto's Thyroiditis | | 2 |
| Lymphocytic thyroiditis | | 2 |
| Reidel's Thyroiditis | | 1 |
| Hyperplasia : Colloid Nodule /goiter | | 5 |
| Multinodular Goiter | | 2 |
| Adenomatous Colloid Goiter | | 1 |
| Neoplastic Benign : Follicular adenoma | | 12 |
| Hurthle cell adenoma | | 2 |
| Malignant : Papillary Thyroid Carcinoma | | |
| •Classical type | | |
| • Follicular variant | | 9 |
| • Solid variant | | 18 |
| • Micropapillary Carcinoma | | 2 |
| | | 1 |
| Follicular Thyroid Carcinoma | | 10 |
| Total | | 67 |

**Immunohistochemistry** (Dabbs J David, 2010) (Standard protocols were used) For immunohistochemical evaluation using Streptavidin Biotin immunoperoxidase method was done. Staining and evaluation using specific monoclonal antibody to Gal-3 was done as per following protocol: 1. Deparaffinization 2. Unmasking of antigenic sites by microwave antigen retrieval method 3. Peroxidase Treatment 4. Primary Antibody (Novocastra[TM] Lyophilized Mouse Monoclonal Antibody Galectin-3, product code : NCL-GAL-3 ; 1:100 dilution)- Slides were wiped off excess buffer and incubated overnight with primary antibody (monoclonal

antibody for Gal -3) 5. Biotinylated link antibody 6. Enzyme Conjugate- Enzyme conjugate (Streptavidin horse radish peroxidase) was applied for 30 minutes. 7. Substrate Chromogen Solution- Concentrated diaminobenzidine solution (DAB) used. 8. Counterstain and Mounting- Presence of brown coloured end product at the site of target antigen was indicative of positive reactivity. Positive tissue control (Carcinoma Prostate) and negative tissue control (Normal thyroid tissue) were taken. Immunohistochemical scoring of Gal-3 : Staining pattern of Gal -3 protein detected in many tissues and cell types, localized in the cytoplasm and / the nucleus, on the cell surface, or in the extracellular environment.

## Interpretation of Gal-3 staining and corelation with histopathology features

Interpretation was done according to the criteria defined by Fabio Orlandi *et al.* in 1998 (Orlandi *et al.*, 1998) and Katie B Weber *et al.* in 2004 (Weber *et al.*, 2004) in a research paper titled (Gal-3 is a presurgical marker in Human Thyroid Carcinoma and The use of a combination of Gal-3 and Thyroid peroxidase for the diagnosis and prognosis of Thyroid Cancer respectively.

### Qualitative criteria

| Score | Pattern |
|---|---|
| 0 | No staining |
| 1 | Light / weak staining |
| 2 | Moderate staining |
| 3 | Intense staining |

### Quantitative criteria

| Score * | Pattern |
|---|---|
| +++ | >60% of neoplastic cells in cytoplasm / nucleus/ both (cytoplasm and nucleus) /extracellular matrix |
| ++ | 30 - 60% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix |
| + | <30% of neoplastic cells in cytoplasm /nucleus/ both (cytoplasm and nucleus) /extracellular matrix |
| - | No reactivity |

*In our study, only those cases have been taken who had scoring grade ++ and +++

### Statistical tools employed

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) statistical Analysis Software. The values were represented in Number (%) and Mean±SD. The Statistical formulas were used ie. Chi square test and Interobserver differences (Kappa) used. P <0.05 was considered statistically Significant And κ=0.20-0.40 is considered as Fair agreement.

## RESULTS

Maximum number of patients in non-neoplastic group were aged between 31-40 years whereas maximum number of malignant cases were aged between 41 to 50 years and maximum no. of benign cases were aged between 21-30 years. Overall as well as for all the three groups individually, majority of subjects were females. Majority of cases (n=54; 80.6%) were finally diagnosed as neoplastic. There were a total of 13 (19.4%) cases which were diagnosed as non-

neoplastic. Among cases diagnosed as neoplastic – a total of 40 (74.1%) were malignant and 14 (25.9%) were benign whereas among those 13 cases diagnosed as non-neoplastic, a total of 8 (61.5%) were hyperplastic and remaining 5 (38.5%) were inflammatory lesions (Table-2).

**Table 2. Distribution of Specimen according to the Final Diagnosis (n=67)**

| S.No. | Final Diagnosis | No. of cases | Percentage |
|-------|-----------------|--------------|------------|
| 1. | Neoplastic | 54 | 80.6 |
| | Malignant | 40 | 59.7 |
| | Benign | 14 | 20.9 |
| 2. | Non-neoplastic | 13 | 19.4 |

**Multiple observer evaluation galectin-3 IHC**

To rule out subjective differences during IHC evaluation, 3-observer evaluation method was used - Assessment of Level of Agreement between Observer 1 and Observer 2 -The reliability of assessment was done using Kappa-statistic as follows: On scoring of staining the % of agreement (53.7%), order of agreement remained to be fair only (κ=0.369). On intensity of staining, total % agreement between two observers was observed to be 55.2% which was of fair order (κ=0.391). Assessment of Level of Agreement between Observer 1 and Observer 3- On scoring of staining the % of agreement (52.2%), order of agreement was found to be fair (κ=0.353). On intensity of staining, total % agreement between two observers was observed to be 47.8%, fair agreement (κ=0.288). Assessment of Level of Agreement between observer 2 and Observer 3- On scoring of staining the % of agreement (67.2%), category of agreement was moderate (κ=0.557). On intensity of staining, total % agreement between two observers was observed to be 53.7%, fair agreement (κ=0.372). As level of agreement between observers ranged from fair to moderate, the observations of single observer were not reliable. Hence, wherever grading was done either agreement of at least two observers and in case there was no agreement in all the three observers then the median grade of three observers was considered to be applicable for final assessment.

**Intensity and Diagnosis (Table-3)**

Similarly, more than three-fourth (77.5%) of malignant cases had scores ++ and +++ as compared to only 35.7% of benign and 23.1% of non-neoplastic cases, thus malignant cases showed a statistically significant difference from both benign as well as non-neoplastic lesions (p=0.042 and 0.002). With increasing intensity, a significant association with malignancy was evident. There were 31/40 (77.5%) of malignant cases, 5/14(35.7%) of benign cases and 3/13(23.1%) of non-neoplastic cases accorded with intensity 2 and 3. Gal-3 expression was identified in 76.7% (23/30) of PTC cases, and by histological subtypes of PTC Gal-3 expression was identified in classical, follicular variant, solid variant and micropapillary as 77.7%(7/9), 72.2% (13/18), 100%(2/2) and 100%(1/1) respectively (Figure-1,2) (Table-4). In FTC 80% (8/10) cases showed positivity for Gal-3 (Figure-3).

**Table 4. Evaluation of IHC Gal-3 expression in thyroid neoplasm∗**

| Diagnosis by Histopathology | Total No. | No. +ve of Gal-3 (%) | "p" value | Sens % | Spec % |
|-----------------------------|-----------|----------------------|-----------|--------|--------|
| A. Malignant Neoplasm | 40 | 31 (77.5%) | <0.001 | 77.5 | 69.2 |
| 1. Papillary thyroid carcinoma | | | | | |
| Classical | 09 | 07 (77.7%) | 0.201 | 77.8 | 43.1 |
| Follicular variant | 18 | 13 (72.2%) | 0.159 | 72.2 | 44.9 |
| Solid variant | 02 | 02 (100%) | 0.224 | 100.0 | 39.7 |
| Micropapillary | 01 | 01 (100%) | 0.393 | 100 | 40.9 |
| 2. Follicular thyroid carcinoma | 10 | 08 (80%) | 0.130 | 80 | 43.9 |
| B. Benign Neoplasm (Follicular adenoma and Hurthle Cell adenoma) | 14 | 05 (35.7%) | 0.055 | 35.7 | 34.0 |
| C. Non-neoplastic (Hyperplasia and inflammatory lesions) | 13 | 03 (23.1%) | 0.004 | 23.1 | 31.5 |

*Only those cases have been taken as positive who had scoring grade ++ or +++.

Overall, Galectin-3 expression evaluated utilizing IHC techniques for thyroid malignancy shows sensitivity (77.5%). In benign and non-neoplastic lesion , Gal-3 expression was observed as 35.7% (5/14) and 23.1% (3/13) respectively (Figure-4).

**Table 3. Association of IHC Findings with Final Histopathological Diagnosis**

| SN | Variable | Total Neoplastic (n=54) | | Malignant (n=40) | | Benign (n=14) | | Non-neoplastic (n=13) | |
|----|----------|------|------|------|------|------|------|------|------|
| | | No. | % | No. | % | No. | % | No. | % |
| **Intensity** | | | | | | | | | |
| 1. | 0 | 15 | 22.4 | 4 | 10.0 | 4 | 28.6 | 7 | 53.8 |
| 2. | 1 | 12 | 17.9 | 5 | 12.5 | 5 | 28.6 | 3 | 23.1 |
| 3. | 2 | 23 | 34.3 | 18 | 45.0 | 3 | 28.6 | 1 | 7.7 |
| 4. | 3 | 17 | 25.4 | 13 | 32.5 | 2 | 14.3 | 2 | 15.4 |
| **Scoring** | | | | | | | | | |
| 1. | - | 15 | 22.4 | 4 | 10.0 | 4 | 28.6 | 7 | 53.8 |
| 2. | + | 13 | 19.4 | 5 | 12.5 | 5 | 35.7 | 3 | 23.1 |
| 3. | ++ | 21 | 31.3 | 17 | 42.5 | 3 | 21.4 | 1 | 7.7 |
| 4. | +++ | 18 | 26.9 | 14 | 35.0 | 2 | 14.3 | 2 | 15.4 |
| Overall Scoring | | Neoplastic vs Non-neoplastic | | Malignant vs Benign | | Malignant vs Non-Neoplastic | | Benign vs Non-neoplastic | |
| $\chi^2$=18.515; p=0.005 | | $\chi^2$=10.912; p=0.012 | | $\chi^2$=8.177; p=0.042 | | $\chi^2$=14.566; p=0.002 | | $\chi^2$=2.284; p=0.516 | |

**Scoring and Diagnosis**

For scoring, it was observed that as compared to non-neoplastic, neoplastic lesions had higher scores (p=0.012).

## DISCUSSION

Galectin-3 is a member of the beta-galactoside binding protein family which is normally expressed in macrophages, mast
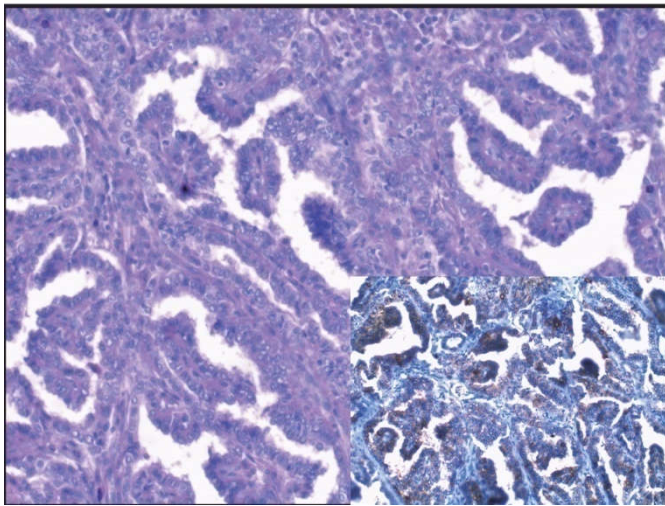
**Figure 1. (H&E 20X)- Photomicrographs showing classical papillary thyroid carcinoma, Inset- (IHC 20X)- showing gal-3 expression in classical papillary thyroid carcinoma. Score- ++, Intensity-2, Pattern- Cytoplasm**
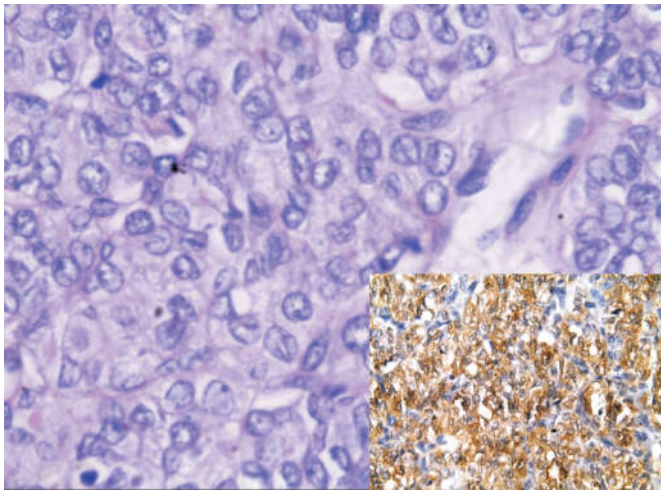


**Figure 2. (H&E 40X)- Photomicrograph showing papillary thyroid carcinoma (solid variant), Inset- (IHC 40X)- showing gal-3 expression in papillary thyroid carcinoma (solid variant). Score- +++, Intensity-3, Pattern- Cytoplasm**
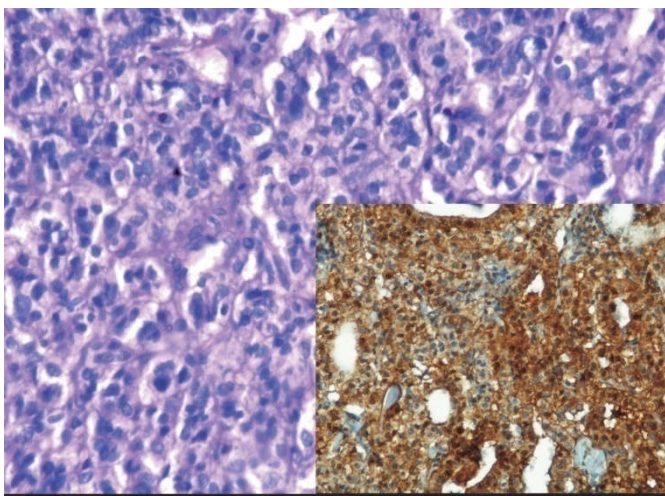


**Figure 3. (H&E 20X)- Photomicrograph showing Follicular thyroid carcinoma, Inset- (IHC 20X)- showing gal-3 expression follicular thyroid carcinoma. Score-  +++, Intensity-3, Pattern- Cytoplasm**
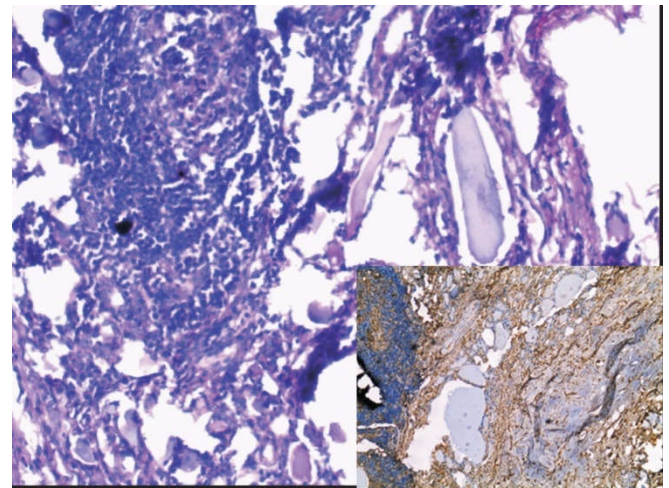


**Figure 4. (H&E 20X)- Photomicrograph showing lymphocytic thyroiditis, Inset - (IHC 20X)- showing  gal-3 expression in extracellular matrix in lymphocytic thyroiditis**

cells, langerhans cells and various malignant cells including thyroid gland cells (Fernandez *et al*., 1997). Galectin-3 possesses affinity for beta galactoside containing glycoconjugates which is found mainly in intracellular, extracellular and cell-surface and  they participates in cellular functions like cell proliferation, apoptosis, cellular transformation, tumour progression and metastasis of cancer cells (Cvejic *et al*., 2005; Nangia –Makkar *et al*., 2007).  In our study to analyze this protein by immunohistochemistry (IHC) in 67 histopathological samples, and for  IHC interpretation utilized a multi-pathologist consensus scoring strategy.   In which we have reported the 3 independent observer evalutaion of Gal-3 and which is more reliable determination and assess the interobserver variability**.**   As level of agreement between 3 observers ranged from slight to moderate.   Hence for each parameter (intensity and scoring), criteria of agreement of at least two observers was done. For scoring, it was observed that as compared to non-neoplastic, neoplastic lesions had higher scores (p=0.012). Similarly, more than three-fourth (77.5%) of malignant cases had scores ++ and +++ as compared to only 35.7% of benign and 23.1% of non-neoplastic cases.  Thus with increasing intensity, a  statistically significant association with malignancy was evident. The majority of studies reported Gal-3 positivity in 90% to 100% of PTC cases. [Connie *et al*., 2010] Very few studies have reported  Gal-3 expression in PTC by histological subtypes. Similarly, in our study, Gal-3 expression was  identified in 77% (23/30) of PTC cases , and by histological subtypes of PTC   Gal-3 expression was identified in classical, follicular variant, solid variant and micropapillary as 77.7%(7/9), 72.2% (13/18), 100%(2/2) and 100%(1/1) respectively. Expression of Gal-3 has also ranged from 20% to 100% in reported cases of FTC. The largest series, reported by Bartolazzi *et al*, identified Gal-3 expression in 95% (54/57) of FTC cases. (Bartolazzi *et al*., 2008) Similarly, in our study, 80% (8/10) cases of FTC showed positivity for Gal-3. Hence, Gal-3 is highly expressed in thyroid malignancies. There is no significant difference found in Gal-3 expression in PTC histological subtypes and FTC. Although it appears likely that a small proportion of follicular carcinomas are negative for Gal-3, likewise in our study 20% (2/10) of FTC cases were negative for Gal-3, it remains elusive whether the negativity is true or false. Orlandi *et al*. failed to detect Gal-3 positivity in some FTCs, where they succeeded in showing focal positivity in the same cases when analyzed with differently prepared sections. (Bartolazzi *et al*., 2008) Immunohistochemical

analysis of FTCs with focal Gal-3 expression in this kind of tumor, we could also possibly hypothesize that a false negative result yielded when the areas negative for Gal-3 are sampled. Also (a well-known fact in the literature is that poorly-differentiated FTC subtypes show a weaker Gal-3 expression or have absolutely no expression compared with the well differentiated carcinomas). (Hidenori *et al*., 1999) According to Kawachi *et al*. (2000) Primary lesions of papillary carcinomas with metastasis contained significantly higher concentrations of Gal-3 than tumours of this type without metastases. In our single case of known metastatic PTC we found strong Gal-3 positivity.

Follicular thyroid lesions have been rightly referred to as ' the bane of the pathologist' in a recent article by Baloch and LiVolsi (**2002**). The follicular variant of papillary thyroid carcinoma (PTCFV) often poses a diagnostic challenge in which the differential diagnosis includes other follicular patterned lesions such as follicular adenoma (FA) and follicular carcinoma. The distinction between these lesions is important because prognosis and management differ. Because FVPTC is diagnosed almost solely based on subjective cytologic criteria (nuclear grooves, nuclear clearing, nuclear overlap, and intranuclear pseudoinclusions) and no uniform minimal diagnostic criteria exist (Renshaw and Gould, 2002; Chan, 2002). It is not surprising that disagreements in the diagnosis of PTC have underscored the interobserver variability in the evaluation of these lesions. In our study, Gal-3 was expressed in 72.2%(13/18) of FVPTC, 80%(8/10) of FTC and 35.7%(5/14) of benign neoplasm (follicular and hurthle cell adenomas). In an attempt to resolve this common diagnostic difficulties, many immunohistochemical markers have been evaluated for their potential in distinguishing PTC from other follicular lesions, the main ones including Gal-3, cytokeratin (CK-19) and HBME-1. Gal-3 has recently generated much interest and has repeatedly been suggested as a suitable marker to fulfill this role (Boila *et al*., 2014; Cui *et al*., 2012). In several studies, Gal-3 expression was not identified in any thyroid goiter cases, with the exception of the reports from Beesely *et al*. (2002) and Prasad *et al*. (2005) found that Gal-3 positivity in 38% and 55% of goiter specimens respectively. Similarly in our study, Gal-3 was expressed in 23.1% (3/13) of non-neoplastic lesions. The most likely explanation for the Gal-3 expression of non-neoplastic follicular cells in an inflamed area are neosynthesis effected by cytokines secreted by inflammatory cells or the simple permeation of Gal-3 abundantly shed by lymphocytes into the neighboring follicular cells (LIoyd, 2001)

## Conclusion

Use of strict criteria and guidelines for histological grading can result in acceptable levels of interobserver agreement and also identify areas that might benefit from refinement. Gal-3 is highly expressed in thyroid malignancies (specially in supporting the diagnosis of PTC and its variants. Immunopanel evaluation with Gal-3, HBME-1 and CK-19 have the most commonly studied adjunctive markers and may further improve sensitivity for thyroid malignancies . No single marker by itself is 100% sensitive for malignancy. Gal-3 to be used as adjunct for aiding thyroid cancer diagnosis.

## Disclosure

The authors report no conflict of interest in this work.

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