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**RESEARCH ARTICLE**

# OVER EXPRESSION OF CYTOSOLIC GALECTIN-3 CORRELATES WITH ANTI-APOPTOTIC PROTEIN BCL-2: ITS SIGNIFICANCE IN THE DIFFERENTIAL DIAGNOSIS OF FOLLICULAR CELL DERIVED THYROID TUMORS IN FNAC

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**ABSTRACT**

Fine Needle Aspiration Cytology is considered as a reliable and cost effective method for the initial evaluation of thyroid nodules. But it poses a diagnostic challenge in differentiating benign follicular adenoma from follicular carcinoma and follicular variant of papillary carcinoma as they have similar cytological appearance. Present study focused on the identification of an immuno marker for the differential diagnosis of thyroid malignancy and to compare the expression of Galectins and Bcl-2 to explain its role in apoptosis. We performed immunohistochemistry of galectin-1, galectin-3 and bcl-2 on cell block prepared from FNA material and corresponding tissue sections on 289 samples. Galectin-3 mRNA expression was quantified using Real time PCR. Western blotting was carried out in surgically excised fresh tissue specimens. Pap stained slides were also subjected to immunocytochemistry without de-staining. Galectin-3 was found to be highly significant ($P<0.001$) in the differential diagnosis of malignancy from adenoma with high diagnostic accuracy of 97.8%. All the samples of papillary carcinoma with distant metastasis showed intense expression of galectin-3 and bcl-2. A negative correlation in the expression pattern of galectin-3 and bcl-2 was observed in the case of thyroiditis. QRT- PCR revealed a significant over expression of galectin-3 (2.4 fold) in papillary carcinoma. The present study observed a correlation for the over expression of Galectin-3 with its anti-apoptotic activity and suggests galectin-3 immunohistochemistry along with morphological evaluation and quantitative assessment to eliminate unwanted thyroidectomies for benign nodules.



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## INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is considered as a reliable, widely used and cost effective preoperative test for the evaluation of thyroid nodules. Though papillary thyroid carcinoma (PTC) is readily identified with FNAC, benign follicular adenoma (FTA), Follicular thyroid carcinoma (FTC) and follicular variant of papillary thyroid carcinoma (FVPC) cannot be distinguished, as they have similar cytological appearance (Freitas and Cerutti, 2010). Most experienced pathologist may pose a diagnostic challenge in differentiating these groups of follicular patterned thyroid lesions (De Matos *et al*., 2005) and cytologically interpreted as follicular neoplasm which leads to low diagnostic reproducibility. To address the problem of indiscriminate diagnosis of thyroid nodules several molecules involved in carcinogenic process such as oncogene product, altered enzymes, cadherins, lectins

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etc have been proposed as markers of thyroid malignancy (Serini *et al*., 1996; Ringel, 2000; Cohen *et al*., 2004). Among them galectins seems to be promising molecule in significantly increasing FNAC accuracy. Galectins are members of highly evolutionary conserved family of carbohydrate binding proteins found in multicellular organisms from fungi to mammals (Cooper and Barondes, 1999; Muller, 2001). To date 14 members have been identified, cloned and classified for their roles in various biological responses (Sacchettini *et al*., 2001). These are cytosolic proteins and may also be translocate into nucleus, into vesicle, or accumulate at sub-cytosolic sites (Hughes, 2001). Two members of this lectin family, galectin-1 and galectin-3 have received particular attention in the field of cancer biology for their tissue distribution and tissue specificity. Galectin-1 a monomeric or homodimeric protein composed by subunits of 14.5kDa associated with angiogenesis, tumor progression, immune escape and poor prognosis (Camby *et al*., 2006).

Up-regulation of galectin-1 is well documented in different types of tumors. Ras is an oncogene found to be mutated in many of the human tumors and promote malignant transformation of the cell. Increased expression of Galectin-1 results in the over expression of membrane associated Ras, Ras-GTP which in turns activates ERK results in the cell transformation (Paz *et al*., 2001). Galectin-3 is a β-galactosidase binding polypeptide having 31kDa molecular weight found to play a major role in biological processes such as cell growth, adhesion, differentiation and apoptosis (Mazurek *et al*., 2000). Although Galectin-3 is predominantly located in the cytoplasm, it has also been detected in the nucleus, on the cell surface or in the extra cellular environment, suggesting the multi-functionality of this molecule. Various studies of thyroid tumors have investigated the expression of Galectin-3, which has been shown to act as cell death suppressor interfering with an apoptotic pathway involving bcl-2 (Akahani *et al*., 1997). Bcl-2 family of proteins is a major intracellular regulator of apoptotic signalling; promote carcinogenesis not by accelerating proliferation but by protecting cells from a wide range of apoptotic stimuli (Mitsiades *et al.*, 2007).

Galectin-3 is not a member of bcl-2 gene family, but it shares several significant structural properties with bcl-2. The lectin contains four amino acid motifs, Asp-Trp-Gly-Arg (NWGR), which is highly conserved sequence within the BH1 domain of bcl-2 family of proteins and is crucial for bcl-2 protein function in the inhibition of programmed cell death. Two sites in the N-terminal tail of Galectin-3 were shown to be relevant for its anti-apoptotic activity: the bcl-2 homology domain 1 with an anti-death motif, which is responsible for the inhibition of cytochrome *c* release from mitochondria and the second one, is the phosphorylation of the casein kinase 1 target Ser6 promotes Galectin-3 nuclear export and anti-apoptotic function (Cecchinelli *et al*., 2006. Due to the functional similarity of galectin-3 and bcl-2, it is possible that this lectin can replace or mimic bcl-2 protein. Since apoptosis can be regulated by bcl-2 protein, it is reasonable to assume that the expression of Galectins should exhibit a correlation with bcl-2 expression on thyroid malignancies. In the present study we analyzed galectin-1, galectin-3 and bcl-2 protein expression by immunohistochemistry in cell block preparation from FNA and in corresponding tissues to establish the correlation of Galectins with bcl-2 and to see whether bcl-2 expression can have any role in improving the diagnostic potential of Galectins. We have studied the possibility of Galectin immunostaining directly on pap stained slides to see whether the cytologist can study the protein expression on the same slide itself which was found suspicious on morphological evaluation. In order to confirm the immuno expressions we performed western blot analysis of Galectin-3 and bcl-2 in a spectrum of thyroid lesions. Relative expression levels of Galectin-3 mRNA in different thyroid lesions were assessed by Quantitative real time PCR.

## MATERIALS AND METHODS

### Tissue specimens and Cytology smears:

289 samples of cell block from FNA materials and corresponding tissue block from patients who had undergone surgery during the period 2008-2013 were selected for the study. The study protocol was approved by the Institutional Review Board as well as Ethical Committee (HEC No. 6/2009 dtd Ist May 2009) of 'Regional Cancer Centre', Thiruvananthapuram, Kerala . Samples selected for the study included 62 papillary thyroid carcinoma, 68 follicular variant of papillary thyroid carcinoma, 2 follicular carcinoma, 68 Follicular adenoma, 66 multi-nodular goitre and 23 thyroiditis. Fine needle aspiration was performed using 24 gauge needle and 5ml syringe in Division of Pathology, RCC after obtaining suitable informed consent from the patients. Each aspirate was smeared for conventional pap staining and remaining material was immediately fixed in acetic acid alcohol formalin (AAF).

An equal volume of AAF was added to the sample and were centrifuged twice at 10,000 rpm for 10 minutes and kept at room temperature overnight in slanting position. The cell button were wrapped in lens paper and processed for histopathology. Cell blocks with minimum five groups of cells were selected for immunohistochemistry. The hematoxylin and eosin (H&E) stained sections of cell block as well as tissues were evaluated for the presence of tumor cells and the information concerning patients were collected from medical records. For western blot analysis, surgically excised fresh tissues were obtained from Division of Surgical Oncology, RCC and snap frozen, kept at $-80^0$C for protein extraction.

### Immunohisto chemistry

Paraffin embedded sections were serially cut at 5µm thickness and transferred on to APES (3-aminopropyl triethoxy silane) coated slides. Following deparaffinization in xylene and rehydration in descending grades of alcohol, the slides were rinsed in deionised water. The Pap stained slides were incubated with 4mM sodium deoxycholate after removing the cover slip. For both tissue specimen and cell blocks, heat induced antigen retrieval was performed using 10mM citrate buffer (pH 6.0) for two consecutive cycles of 10 and 5 minutes each at high temperature. After blocking endogenous peroxidise activity the slides were rinsed in TBS buffer and incubated with protein block. Mouse monoclonal antibody

against human galectin-1, galectin-3 and bcl-2 were obtained from Novocastra Laboratories Ltd, UK. The sections were incubated with primary antibody against galectins and bcl-2 at $4^0$C overnight at a dilution of 1:150 for galectins and 1:80 for Bcl-2. This was followed by incubation with biotinylated secondary antibody for 30 minutes and thereafter Avidin biotin enzyme complex for 30 min. The reaction was visualized using DAB (3, 3'-Diaminobenzidine) as chromogen. After counter staining with Hematoxylin, slides were dehydrated, cleared and mounted with DPX. Corresponding positive controls were included in every batch. Negative controls were incubated with TBS in the place of Primary antibody.

**Scoring and statistical analysis**

Immunostainings were blindly scored by two independent investigators, without any prior knowledge of the histological and cytological diagnosis. The intensity of the expression patterns were evaluated on 0- 3+ scale (0-none, 1+-light, 2+-moderate and 3+ intense). Also the percentage of cells with each grade of staining was recorded. H-scores were then calculated for each of the antibodies as the product of intensity (0-3+) and distribution (0-100%) with H-score ranging from 0-300. By using histopathology as the gold standard the sensitivity and specificity of each of these markers were assessed. Statistical analyses were performed by using SPSS software. Chi-squared test or Fisher exact test were adopted to examine the relationship between the variables. The result was considered significant at $p<0.05$. Correlation between each marker and their corresponding cytology specimens were performed using Pearson's correlation.

**Protein Isolation and Western blotting**

Protein was extracted from surgically excised tissue specimens of representative lesion kept at $^{-}80^0$C. Adjacent normal epithelium was taken as negative control. Sample lysate was prepared with lysis buffer containing 1M Tris (pH7.4), 0.5M EDTA, triton X-100, 5M NaCl, glycerol and PMSF. The lysate was incubated in ice for 1hr and centrifuged at 12,000 rpm for 20 min at $4^0$C. Protein concentration was determined using Bradford assay kit (Sigma, USA). Equal amount of protein were diluted by adding 5X gel loading buffer. The mixture was denatured by placing the tissue extract in boiling water bath for 10 min at $95^0$C. The extracted proteins were resolved on 12% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) with 5% stacking gel. The separated proteins were then electroblotted to nitrocellulose (NC) membrane and quenched in 5% non fat dried milk in TBST for 30min. The membrane was incubated with primary antibody (Galectin-3, bcl-2 and β-actin) overnight at $4^0$C. After washing with TBST several times, the membrane were subjected to react with biotinylated anti-mouse immunoglobulin for 30min. Immunoreactivity was detected by using enhanced chemiluminescence (ECL) kit (Proteo Qwest, Sigma, USA) and analyzed using FluorChem M multiplex fluorescent imaging system.

**RNA isolation and Quantitative Real-time PCR**

Total RNA was extracted from aspirates collected during FNAC and from tumor tissue as well as adjacent normal epithelium of all samples as per manufactures instruction (Roche applied science, US). Eluted RNA was quantified spectrophotometrically and integrity was verified in agarose gel electrophoresis. cDNA was synthesized using random hexamer primer. SYBR Green-based quantitative real-time PCR assay for evaluating change in expression of Gal-3 gene was performed by taking normal adjacent epithelium as reference calibrator and GAPDH as endogenous control gene. Primers were designed using Primer 3 software and the nucleotide sequence were synthesised from Sigma Aldrich, Bangalore, India. Primer sequence were as follows: Galectin-3: sense 5' ATGCAAACAGAATTGC TTTAGATT3', antisense 5' AGTTTGCTGATTTCATTGAGTTTT3'(280bp); GAPDH: sense: 5'AGAAGGCTGGGGCTCATTTG-3' Antisense: 5' AGGGCCATCCACAGTCTTC-3' (258bp). Relative quantification of Gal-3 was performed according to the standard protocol using annealing temperature of $56^0$ C for 45 cycles in Light cycler 480 SW1.5.0 SP3 version 1.5.0.39. Melting curve analysis was performed at $95^0$ C for 5 seconds and final cooling at $40^0$ C. Relative gene expression was interpreted in ratios. PCR products were verified in 2% agarose gel stained with ethidium bromide.

## RESULTS

Majority of the participant in our study were women, with a male to female ratio of 1:3 and was most prevalent between 35 to 45 years of age in both sexes. A strong positive staining of Galectin-3 was noted in the cells of papillary thyroid carcinomas in both cell block as well as tissue sections (Fig 1. A). Many of the tumor cells showed strong expression of Galectin-3, mainly in the cytoplasm. Nuclear staining was rarely observed and only in pale intensity (Fig. 5). 12 cases of papillary carcinomas with distant metastasis showed intense expression. The mean H-score of Galectin-3 staining in papillary carcinoma was 240. The staining pattern was similar in follicular variant of papillary carcinoma also with a mean H-score of 220 (Fig 1: B). In the case of follicular carcinoma one of two cases was completely negative, while the other one was focal positive for Galectin-3 (Fig. 1. C).

Immunostaining of follicular adenoma showed mild staining in focal areas (Fig. 1. D). 34% of multinodular goitre showed mild positivity for Galectin-3 in focal areas. The mean H-score for follicular adenoma and MNG was 30. Twenty samples of thyroiditis showed very weak positivity and the remaining cases were completely negative. Galectin-3 positive staining was observed in the cytoplasm of Hurthle cells and macrophages of both thyroiditis and MNG (Fig. 1. E & F). Staining was uniform in all samples. Galectin-3 was found to be highly significant ($P<0.001$) in the differentiation of carcinoma from adenoma. The immuno - expression of galectin-3 in histology and cytology samples showed a very strong positive correlation ($r = .915, P<0.001$) in all the lesion of thyroid when compared with other proteins. The sensitivity and specificity of Galectin-3 for the differential diagnosis of follicular variant of papillary carcinoma were 99.7%, 100% and 92%, 100% for histology and cytology specimens respectively. The diagnostic accuracy of Galectin-3 was 97.8%. Intense expression of Galectin-3 was observed in the papillary carcinoma cells of Pap stained samples (Fig. 1. G).

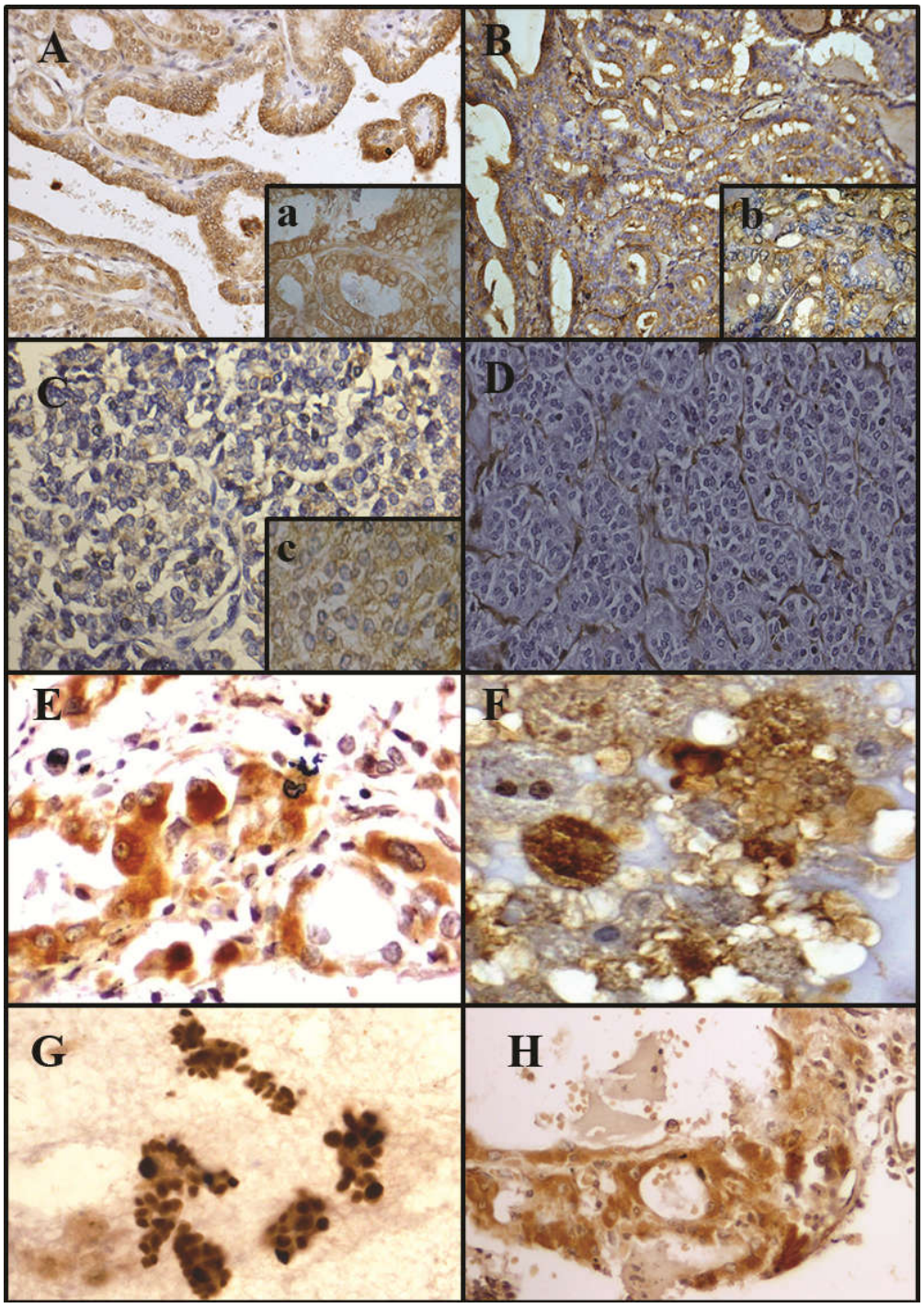


**Fig. 1(A-H): Immunostaining of Galectin-3 in different lesions of thyroid**

A: Intense expression of cytoplasmic Galectin-3 in papillary thyroid carcinoma (40X),
a: higher magnification (100X)
B: Galectin-3 expression in follicular variant of papillary carcinoma (40X)
(b): Cytoplasmic expression of Galectin-3 higher magnification (100X)
C: Focal staining of Galectin-3 in follicular thyroid carcinoma (40X) (c): 100X
D: Mild immunoexpression of Galectin-3 protein in follicular adenoma (40X)
E: Intense expression of Galectin-3 in hurthle cells of thyroiditis (40X)
F: Cystic macrophages of multi nodular goiter shows intense staining of Galectin-3 protein (40X)
G: Intense immunoexpression of Galectin-3 in PAP stained slides (40X)
H: Hurthle cells stained positive for Galectin-1 in thyroiditis (40X)

The staining pattern of Galectin-1 was predominantly cytoplasmic. Most of the PTC cases were strongly immunopositive (Fig. 2. A), with a mean H-score of 110.

In the case of FVPC, 54 of 68 cases showed moderate staining for Galectin-1 (Fig. 2. B). Follicular carcinoma was positive for one out of two cases and the mean H-score was 100 (Fig 2: C).

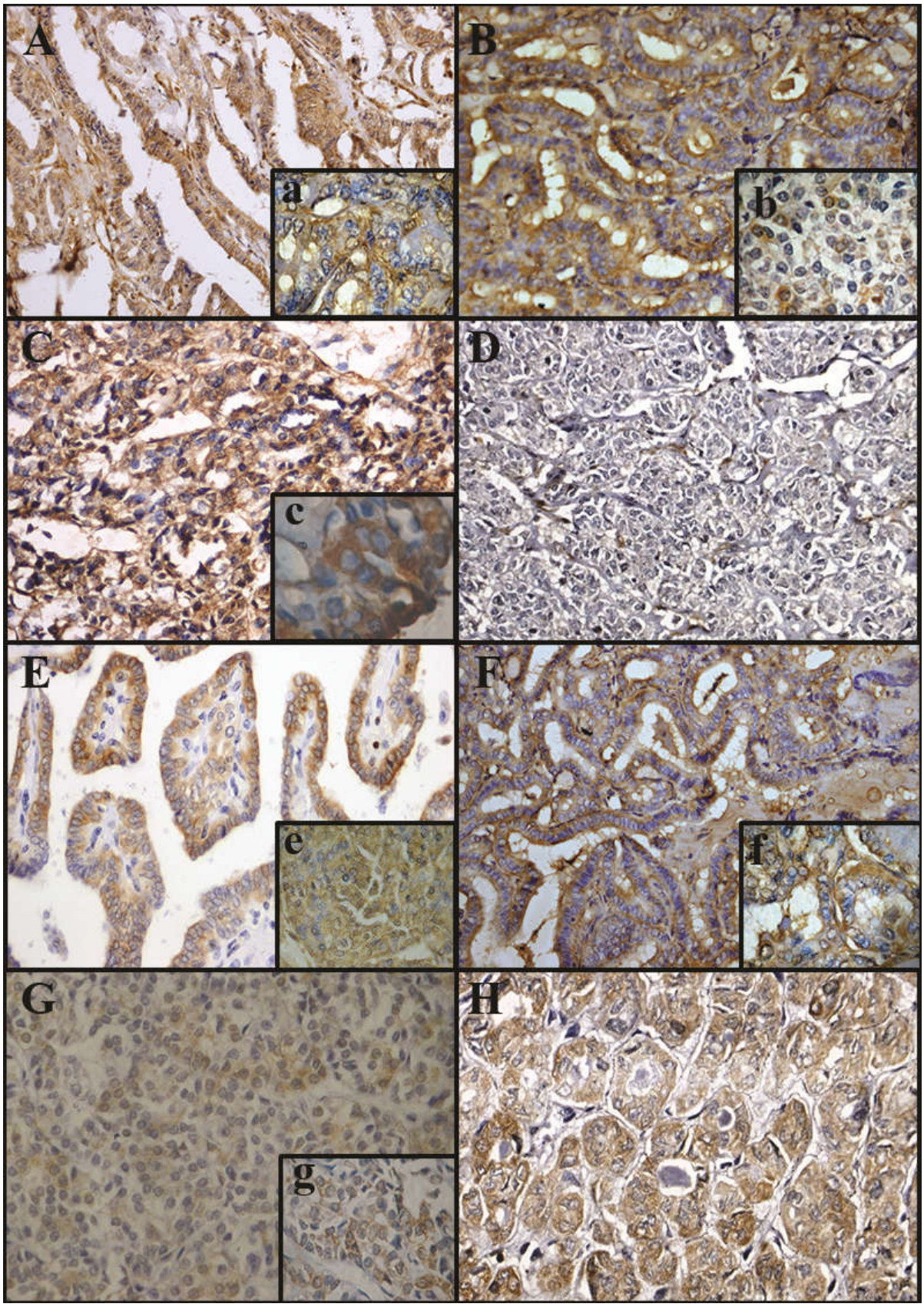


**Fig. 2(A-H). Varying levels of Immunostaining of Galectin-1 and bcl-2 proteins in different lesion of thyroid**

A: Papillary thyroid carcinoma shows intense expression of Galectin-1 (40X). (a): 100X.
B: Strong immuno expression of Galectin-1 protein was noted in follicular Variant of papillary carcinoma (40X).
b: corresponding higher magnification (100X)
C: Follicular thyroid carcinoma shows very weak immunostaining of Galectin1 protein (40X). c: corresponding 100X.
D: Moderate staining of Galectin-1 in follicular thyroid adenoma (40X). d: 100X
E: Immuno expression of bcl-2 protein in papillary thyroid carcinoma (40X).
F: Follicular variant of papillary carcinoma shows intense expression of bcl-2 (40X). f: 100X
G: Moderate expression of bcl-2 was observed in follicular thyroid carcinoma (40X). g: corresponding 100X.
H: Weak expression of bcl-2 in follicular adenoma (40X). h: and its corresponding higher magnification (100X).

Intense expression of Galectin-1 was observed in hurthle cells (Fig 1: H).

Only mild staining was observed in Follicular adenoma (Fig 2: D) and MNG with a mean H-score of 80 and 90 respectively.

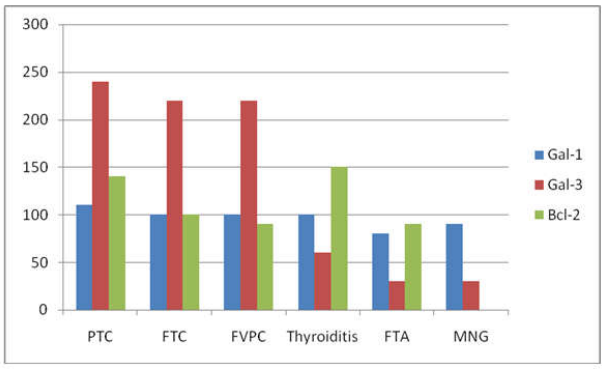


**Fig. 3. Mean H-score of immunostaining with gaectin-1, galectin-3 and bcl-2 in different thyroid lesions in cytology cell block**

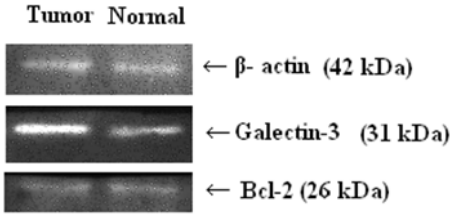


**Fig. 4. Western blot analysis of surgically excited thyroid tissue from papillary thyroid carcinoma shows higher expression of galectin-3 when compared with adjacent normal epithelium. Bcl-2 expression was similar in normal and tumor tissues. β-actin is used as positive control**

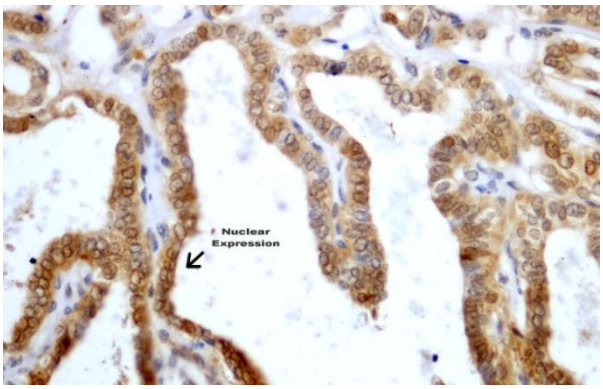


**Fig. 5. Nuclear staining of galectin-3 in papillary carcinoma thyroid with metastasis (100X)**

Galectin-1 was found to have a sensitivity of 70% and specificity of 74%. The expression pattern of Bcl-2 was membrane and cytoplasmic. The immunoreactivity was strong and uniformly positive in all the cases of PTC (Fig 2: E).

No nuclear staining was observed in any of these cases. All the cases of PTC with distant metastasis showed intense expression of bcl-2 compared to other cases. The mean H-score for PTC was 140. In the case of follicular carcinoma, only moderate staining (Fig 2: G) was observed in both the cases. In samples of FVPC, bcl-2 expression were positive in 51 cases and the others varied from mild to moderate (Fig 2: F) with a mean H-score of 90. Thyroiditis showed moderate expression in all the cases. The mean H-score of follicular adenoma was 90 with moderate expression in focal areas (Fig 2: H).

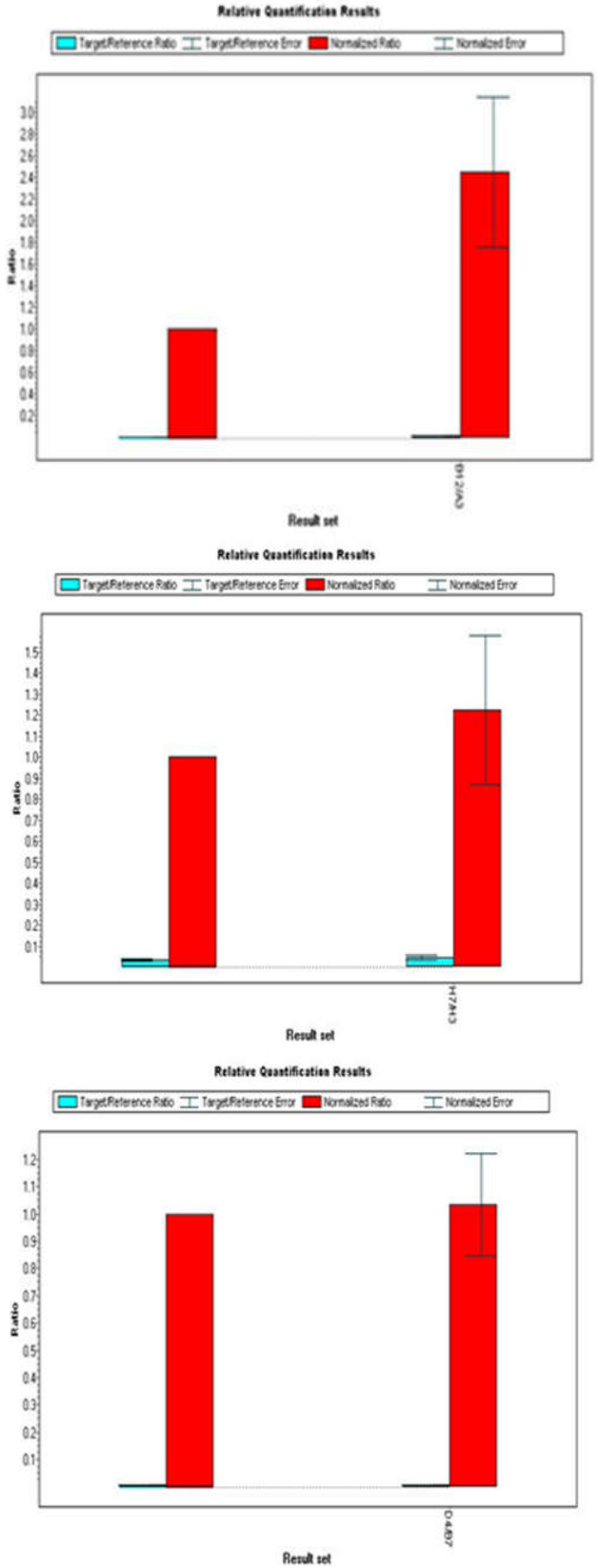


**Fig. 6. Quantitative Real time PCR results showing difference in the expression levels of Gal-3 in (A) Papillary thyroid carcinoma, (B) Follicular variant of papillary carcinoma and (C) Follicular thyroid adenoma**

In the case of MNG all the samples were negative for bcl-2.Bcl-2 was less sensitive when compared with Galectin-1 and Galectin-3. The mean H-score of different lesions of thyroid with Galectin-1, Galectin-3 and bcl-2 staining were shown in Fig 3. The expression pattern of galectin-3 showed a moderate positive correlation with the expression of galectin-1 ($r = .424$, $P<0.001$) and Bcl-2 ($r = .477$, $P<0.001$) in histology samples in the differential diagnosis of thyroid malignancy. The immunoblot analysis with protein extract of thyroid tumor tissues showed a higher expression of Galectin-3 and bcl-2(Fig: 4). Quantitative real time PCR product of 280bp indicated the expression of Galectin-3 transcripts in PTC, FVPC and FTC. An average of 2.4 fold and 1.4 fold increase in the expression of Gal-3 was found in PTC and FVPC respectively. In the case of thyroiditis it shows 1.2 fold increase in the expression level when compared with adjacent normal epithelium. In follicular adenoma it was only 1.035 fold increase. (Fig6). Galectin-3 was found highly significant ($P<0.001$) in the differential diagnosis of PTC from other thyroid lesions.

## DISCUSSION

Galectin-1 and galectin-3 are reported to express highly in thyroid carcinomas and are absent in normal and benign tumor tissues (Inohara *et al*., 1999). But so many contradictory reports are also available regarding these proteins. Mehrotra *et al*., 2004 reported that galectin-3 is not a reliable immunohistochemical marker to distinguish benign from malignant thyroid follicular lesions as moderate expression were seen in dominant nodule as well as adenomas. Desperate results in the literature prompted us to explore the molecular marker galectin-3 and galectin-1 in cytology samples and to compare the expression with a well established anti-apoptotic molecule bcl-2 to substantiate the correlation of the anti-apoptotic activity of galectins. The bcl-2 family of genes is well known regulators of apoptosis. There is evidence showing that Galectin-3 shares significant sequence similarity with bcl-2. The Galectin-3 functions by interacting with bcl-2 at mitochondria and is shown to bind bcl-2 *in vitro* (Hsu and Liu, 2004). Several studies reported that the expression of Galectin-3 by cancer cells consistently resulted in increased apoptosis resistance to a variety of apoptotic stimuli.

The presence of Galectin-3 in the extracellular location on the cell surface and in the extracellular milieu indicates its role in cell-cell and cell-matrix adhesion, whereas its intracellular localization is connected with its role in regulation of nuclear pre-mRNA splicing and protection against apoptosis (Krzeslak and Lipinska, 2004). Some studies demonstrate that Galectin-3 expressed on tumor cells may act as a modulator in different steps of the metastatic cascade and this molecule modulates attachment or detachment of cancer cell during tumor spreading (Rabinovich *et al*., 2002; Van *et al*., 1995). Moreover Galectin-3 is thought to be a cancer associated protein plays important role during carcinogenesis and/ or tumor progression (Nakahara *et al*., 2005) in relation to inhibition of certain apoptotic pathways (Liu and Rabinovich, 2005). The expression of endogenous Galectin-3 by a variety of primary cancers including thyroid, pituitary, lung and colon cancers has been shown to result in the apoptosis resistance in clinically more aggressive cancers.

In breast cancer significant down regulation of Galectin-3 were found to be associated with axillary node metastasis and poor prognosis (Sujathan *et al*., 2011). Whereas Kawachi *et al*., 2000 reported that primary lesions of PTC with metastasis contained significantly higher concentration of galectins-3 than tumor of this type without metastases. The present study also observed a significant over expression of Galectin-3 in PTC with metastasis. A similar pattern of expression was noted in the case of Galectin-1 and bcl-2 also. Immunohistochemical analysis by Saggiorato *et al*., 2005 in FNA specimens as well as surgical samples reported higher expression of Galectin-3 in PTC and FTC, whereas only 3 out of 50 cases of follicular adenoma showed focal positivity, while remaining all were negative. Our study also documented similar observation. 59 out of 62 PTC showed strong positivity for Galectin-3 for both FNA specimens and their histological counter parts. Nuclear expression of Galectin-3 was also noted only in the case of PTC. This may denote that nuclear Galectin-3 is associated with specific pathway activation which is only present in PTC but not in other thyroid tumors (Weinberger *et al*., 2007).

The role of nuclear galectin in thyroid tumor progression is still unclear, whereas Gaudin *et al*., 2000 reported that cytoplasmic expression of Galectin-3 denotes the malignant transformation in various cell types and nuclear localisation is connected with cell proliferation. FTC and FVPC showed intense expression of Galectin-3 in 90% of FNAs as well as histological specimens whereas very low levels of Galectin-3 was expressed in normal follicular cells of benign nodules and thyroiditis. Western blot analysis also showed an increased expression of Galectin-3 in tumor tissues than adjacent normal epithelium. So we suggest that Galectin-3 to be reliable in the differential diagnosis of thyroid malignancies. Various authors also reported similar observations regarding galectin-3 in thyroid tumors, but in tissue samples (Orlandi *et al*., 1998; Saggiorato *et al*., 2001; Than *et al*., 2008). Saussez *et al*., 2008 suggested that relatively high level of serum Galectin-1 is found in patient with thyroid malignancy than normal. Immunohistochemistry and western blot analysis of FFPE tissue sections as well as human thyroid carcinoma cell lines by Chiariotti *et al*., 1995 reported that the neoplastic follicular cells contain higher concentration of galectin-1 than normal thyroid cells. Xu *et al*., 1995 reported that all thyroid malignancies of epithelial cell origin express high levels of both galectin-1 and galectin-3 but none of the benign thyroid adenoma expresses these proteins. Contradictory to this report we got a focal moderate expression of Galectin-1 in 12 cases out of 68 follicular adenomas.

Macrophages and hurthle cells were stained positive for all the cases of thyroiditis and multinodular goitre. We found Galectin-1 expression is not much significant in distinguishing benign from malignant thyroid lesions as moderate expression was seen in benign cells also. Unlike the previous reports we observed a similar pattern of expressions in cell blocks and the corresponding tissue samples. Matarrese *et al.,* 2000 reported that Galectin-3 is a mitochondrial associated apoptotic regulator in addition to anti-apoptotic molecule bcl-2. Yang R

*et al*., 2008 suggested that galectin-1 by binding with cell-surface glycoproteins induces apoptosis by triggering various apoptosis signalling pathways. So Galectin-1 is characterised as pro-apoptotic, whereas galectin-3 is well established as anti-apoptotic molecule. Immunohistochemical analysis by Mitselou *et al*., 2004 demonstrated prominent cytoplasmic bcl-2 expression in normal thyroid epithelium suggesting bcl-2 prevent apoptosis at normal levels of hormones. Aksoy M *et al*., 2005 reported that 96.7 of normal thyroid tissue express bcl-2 whereas PTC shows 86.6% immunostaining of bcl-2. So they suggests that apoptosis is prevalent in PTC and is infrequent when compared to normal thyroid tissue.

Our study also observed significant expression of bcl-2 in samples of PTC. However, its expression was not significant to differentiate the follicular variants of this tumor. 50% of thyroiditis samples showed strong immunoreactivity of bcl-2, possibly due to hurthle cell metaplasia. We have observed a negative correlation in the expression pattern of bcl-2 and Galectin-3 in the case of follicular adenoma and thyroiditis. In contrary to the previous reports pointing difficulties on demonstration of Galectin-3 in cytology samples (Martins *et al*., 2002; Niedziela *et al*., 2002), we have observed no such difficulty for preparing cell blocks from FNA material. Also we could immunostain the FNA smears stained with classical Pap stain, which will be very helpful for the routine cytopathology laboratories. Moreover immunohistochemical staining is observer dependent and leads to interpretation errors, quantitative measurement on mRNA level may serve as an objective way to distinguish benign from malignant thyroid nodules. Yatsuki *et al*., 2002 reported that quantitative evaluation of Gal-3 mRNA will differentiate thyroid carcinomas from adenomas and may helps in the pre-operative assessment of thyroid nodules. Our study also documented a significant over expression of Gal-3 in PTC and FVPC when compared with normal adjacent epithelium.

**Conclusion**

In conclusion our study has validated immunocytochemistry of galectin-3 along with morphological assessment and quantitative evaluation as an additional tool to help the cytopathologist for the differential diagnosis of thyroid malignancies in FNACs where as Galectin-1 and Bcl-2 has no significant diagnostic value in differentiating thyroid neoplasms, even though its expression correlates well in samples of papillary thyroid carcinoma. The up-regulation of Galectin-3 and bcl-2 in papillary carcinoma explains its anti-apoptotic activity.

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