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GALECTIN-3 THE STAND- ALONE MARKER IN DIFFERENTIATING BENIGN AND MALIGNANT THYROID NEOPLASM

Pathology

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ABSTRACT

INTRODUCTION: Thyroid carcinoma is the most common endocrine malignancy. FNACs are the initial diagnostic test for presurgical evaluation of all the thyroid lesions. Thyroid tumor with overlapping cytomorphology poses great diagnostic challenge even on histopathology. Galectin-3 represents the most accurate stand alone immuno-histochemical (IHC) marker to differentiate benign and malignant thyroid neoplasm.
AIM: The study aims to analyze the concordance between cytological and histological diagnosis of thyroid neoplasm. We will analyze Gal-3 expression in differentiating benign and malignant thyroid neoplasm and in predicting tumor metastatic potential.
MATERIALS AND METHODS: Concordance between cytological and histological diagnosis of thyroid neoplasm in collaboration with sonological features were assessed. Gal-3 scoring was done according to standard immunoperoxidase technique.
RESULTS: All the malignant cases had diffuse Gal-3 positivity. Benign lesions excepting two cases were negative for Gal-3. All the metastatic lesions had peripheral Gal-3 over expression compared to non metastatic lesions that did not have peripheral Gal-3 over expression.
CONCLUSION: Gal-3 is an useful marker to differentiate benign and malignant thyroid neoplasm. Peripheral Gal-3 over expression is associated with increased tumor metastatic potential. Targeted therapy against Gal-3 can positively impact the current management of thyroid neoplastic diseases.

KEYWORDS

Thyroid neoplasm, FNACs, Galectin-3.

INTRODUCTION

Thyroid carcinoma is the most frequently occurring endocrine malignancy. About 230,000 new cases of thyroid cancer were estimated in 2012 among women and 70,000 among men, with an age standardized (world-population) rate of 6.10/100,000 women and 1.90/100,000 men[1].

Fine needle aspiration cytology (FNAC) are the initial diagnostic test for pre-surgical evaluation of all thyroid neoplasm. In a review of more than 18,000 thyroid FNACs performed at the Mayo Clinic, FNACs had a reported sensitivity of 83%, specificity of 92%, and in about 15% of all the cases, the diagnosis remain unequivocal [2]. Diagnosing a thyroid neoplasm may not be straightforward even on histopathology even to the experienced pathologists. Equivocal nuclear clearing, equivocal capsular & vascular invasion remains a subject of debate and is subjected to inter observer variability. There arises the quest of a Immunohistochemical (IHC) marker that can confidently differentiate benign from malignant thyroid neoplasm. Galectin-3 (Gal-3) has emerged as the most accurate stand-alone marker for thyroid cancer diagnosis in the largest thyroid cancer study when compared with a panel of 56 other molecular markers including Gal-3, Cytokeratin 19, VEGF, Androgen receptor, p16, Aurora-A, and Hector Battifora Mesothelial Antigen-1 (HBME-1). The performance of Gal-3 alone (accuracy of 86.9%) was almost as good as the best multimarker panel (accuracy of 91.0%) determined by a Random Forests algorithm using marker combinations from the entire molecular marker panel[3,4,5].

LITERATURE SURVEY:

FNACs are initial diagnostic test for evaluation of thyroid neoplasm. For clarity of communications among pathologists and clinicians, the Bethesda system for reporting thyroid cytopathology (BSRTC), recommends that each thyroid FNAC report belong to a general diagnostic category with each group having its own intrinsic cancer risk, ranging from 0-3% in the benign category to virtually 100% for the malignant category[6,7,8]. Unfortunately, around 25% of FNACs render an indeterminate cytological diagnosis. Thyroid cytology in combination with USG thyroid gland will further help in reducing the number of diagnostic surgeries and will also help in selecting which patients should have a total thyroidectomy than a diagnostic lobectomy at the initial setting itself. Some sonographic features have been consistently associated with an increased risk of malignancy including (from least to most specific): increased internal vascularity (Odds Ratio * 3.5), solid composition (OR * 4.5), hypoechogenicity (OR*5), irregular margins (OR approximately *6), microcalcifications (OR * 7), and shape taller than wide in the transverse view (OR * 10)[9,10,11].

Prasad ML and Huang Y et al opined Gal-3 focal positivity in follicular adenoma/ FA may indicate the potential of incipient malignant transformation to either Follicular variant of papillary thyroid carcinoma/ FVPTC or represents an ongoing oncocytic metaplasia. In case of Hashimoto's thyroiditis, this may be an early indicator for the future malignant transformation to Papillary thyroid carcinoma/ PTCs[12].

Different journals available report variable Gal-3 expression in medullary thyroid carcinoma/MTC, Hurthle cell carcinoma, and poorly differentiated thyroid cancer/ PDTC that usually has 100% expression. Well differentiated thyroid carcinoma of uncertain malignant potential/ WDT-UMP expressed moderate Gal-3 positivity in up to 50% of tumor population cells, indicating their ambiguous morphologic position between follicular adenomas on one side and well-differentiated carcinomas on the other[13,14].

Ito et al correlated peripheral Gal-3 over expression with vascular or capsular invasion in cases of PTC, and Cvejic et al and Faggiano et al correlated peripheral Gal-3 over expression with lymph node metastasis in MTC. Similar findings are reported by Inohara et al in cases of Anaplastic thyroid carcinoma/ ATC[15,16,17,18].

METHODOLOGY:

This is an observational study, conducted in the Department of Pathology, Nil Ratan Sircar Medical College and Hospital/ NRS MCH, Kolkata, from a period of February 2017 to July 2018.

STUDY POPULATION: Persons presenting with thyroid nodules / anterior / midline neck swelling and underwent surgical interventions by removal of either part or entire gland either for diagnostic purpose or as a part of treatment or because of inconclusive cytology. The specimens include excision of thyroid nodules, lobectomy, isthmusectomy, hemi-thyroidectomy, sub-total thyroidectomy, near total and total thyroidectomy with or without neck dissection.

SAMPLE SIZE: The total number of cases in the present study is 57.

INCLUSION CRITERIA: All patients with thyroid nodules / swellings presented to the cytology OPD and later underwent surgical interventions by removal of either part or entire gland are included.

- Cases of thyroid carcinoma diagnosed by histopathological examinations were followed retrospectively for presurgical cytological and radiological evaluation.

EXCLUSION CRITERIA: Neck swelling other than thyroid swelling.

- Cases diagnosed on cytology as benign lesions that did not have any surgical interventions.
- Patients not giving consent.

GALECTIN-3 SCORING: The staining intensity was graded on a scale of 0 to 3 where 0, 1+, 2+, and 3+ denote no staining, weak/ slight staining, moderate staining and intense staining respectively, and the proportion of stained cells were interpreted as 1+ (< 5% of cells), 2+ (5% to 50% of cells) and 3+ (>50% of cells). Cases with specific cytoplasmic staining of more than 5% of the tumor cells, regardless of the intensity, were scored as positive for Gal-3.

RESULTS AND DISCUSSION:

Statistical Analysis: Statistical Analysis was performed with help of Epi Info (TM) 7.2.2.2 which is a trademark of the Centers for Disease Control and Prevention (CDC). Using this software, basic cross-tabulation and frequency distributions were prepared. 2χ test was used to test the association between different study variables under study. Corrected test was used in case of any one of cell frequency was found less than 5 in the bivariate frequency distribution. Test of proportion (Z-test) was used to test the significant difference between two proportions. t-test was used to compare two means. $p \leq 0.05$ was considered statistically significant.

A total of 57 cases are studied in the present study, of which 49 are female and 8 are male subjects.

Most of the malignant lesions are clustered in the elderly population whereas benign cases had a wide age spectrum from 20 to 60 years of age.

We analyzed the USG features of malignant thyroid cases and found 23 (54.76%) cases having thyroid nodule >2 cm in size, 27 (64.28%) cases having disorganized internal vasculature, 34 (80.9%) cases having internal solid composition, 36 cases (85.71%) with heterogeneous hypoechogenecity, 35 (83.33%) cases with irregular margins, 31 (73.80%) cases with microcalcifications, 36 (85.71%) cases having shape that is taller than width. (Table:1)

Table-1: USG findings of the patients with benign and malignant neoplasm

Findings of USG	*Benign (n=15)*	*Malignant (n=42)*	*Test Statistic*	*p-value*
Nodule				
Multiple Nodularity	15(100.0%	0 (0.0%)	14.12	<0.0001*
Single Nodule	0(0.0%)	42(100.0%)	14.12	<0.0001*
	Size of individual nodule (in cm)			
≤2	7(46.7%)	2(4.8%)	x^2 =14.59	<0.0001*
>2	8(53.3%)	40(95.2%)		
Mean±s.d.	2.40±0.43.	2.82±0.63	t_{55}=2.38	<0.0001*
Median	2.0	2.5		
Range	2 – 3	0.5 – 4		
	Internal vascularity			
Disorganized internal vasculature Row % Col %	0 0.0 0.0	27 100.0 64.3	x^2=44.10	<0.0001*
Increased Row % Col %	3 16.7 20.0	15 83.3 35.7		
Normal Row % Col %	12 100.0 80.0	0 0.0 0.0		
Solid Composition (Present)	0(0.0%)	34 (81.0%)	Z=11.66	<0.0001*
	Echogenicity			
Internal Heterogenous Hypoechogenecity Row % Col %	0 0.0 0.0	36 100.0 85.7	x^2 =57.00	<0.0001*
Isoechogenicity Row % Col %	0 0.0 0.0	6 100.0 14.3		
Nil Row % Col %	15 100.0 100.0	0 0.0 0.0		
Irregular Margins	0(0.0%)	35(83.3%)	Z=11.94	<0.0001*
Microcalcifications	0(0.0%)	31(73.8%)	Z=10.73	<0.0001*
Shape Taller Than Width	0(0.0%)	35(83.3%)	Z=11.94	<0.0001*

All the BSRTC II (05 cases) lesions turned to be benign, they being nodular colloid goiter, hashimoto's thyroiditis. Cases of FA, hurthle cell adenoma, hyalinising trabecular adenoma & Non Invasive follicular thyroid neoplasm with papillary like nuclear features/ NIFTP are clustered in BSRTC-III (05 cases). Cases of adenomatoid goiter with papillary hyperplasia, FA, hurthle cell adenoma, follicular carcinoma are clustered in BSRTC-IV (09cases). The slightly elevated rate of malignancy in BSRTC-III, IV (20%, 33%) is due to limited study population that may get corrected on extrapolation of these datas on large population. Cases of well differentiated tumor of uncertain malignancy (WDT-UMP), FVPTC, hurthle cell carcinoma, MTC, PTC are clustered in BSRTC-V (09cases). Cases of PTC with its variants, MTC, PDTC, ATC are clustered in BSRTC-VI (29 cases). The rate of malignancy in BSRTC-V, VI in our study is as per the reported rate of original BSRTC. (Table:2) (Figure: 1)

Table-2: Comparison of present study with Original BSRTC

Bethesda Category	In this study			Original BSRTC
	Benign (n=15)	Malignant (n=42)	Total Cases / Rate Of Malignancy	Rate of malignancy
2 Row % Col %	5 100.0 33.3	0 0.0 0.0	5(0%) 100.0 8.8	0- 3%
3 Row % Col %	4 80.0 26.7	1 20.0 2.4	5(20%) 100.0 8.8	15%
4 Row % Col %	6 66.7 40.0	3 33.3 7.1	9(33.3%) 100.0 15.8	30%
5 Row % Col %	0 0.0 0.0	9 100.0 21.4	9(100%) 100.0 15.8	75%
6 Row % Col %	0 0.0 0.0	29 100.0 69.0	29(100%) 100.0 50.9	99%
TOTAL Row % Col %	15 26.3 100.0	42 73.7 100.0	57 100.0 100.0	

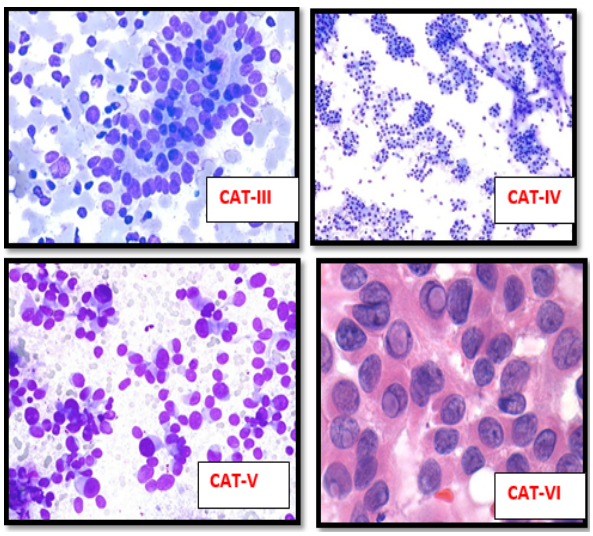


Figure: 1. Fnac Pictures Of Different Bsrtc Categories

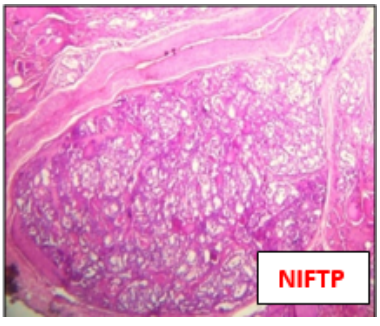
In our present study, we have 57 cases, out of which 12 are benign, rest 42 are malignant cases. (Table:3,4)

Table-3: Benign entities in present study

Histopathological Diagnosis of benign cases	Number of cases	Percentage %
Follicular Adenoma	6	40.0%
Hashimoto's Thyroiditis	3	20.0%
Hurthle Cell Adenoma	2	13.3%
Nodular Colloid Goitre	2	13.3%
Adenomatoid Goitre With Papillary Hyperplasia	1	6.7%
Hyalinizing Trabecular Tumor	1	6.7%
Total	15	100.0%

Table-4: Malignant entities in the present study

Histopathological Diagnosis	*Number of cases*	*Percentage%*
Papillary Thyroid Carcinoma	24	57.10%
Papillary Thyroid Microcarcinoma	1	2.40%
Papillary Thyroid Carcinoma, with focal squamous metaplasia	1	2.40%
Papillary Thyroid Carcinoma, tall cell variant	1	2.40%
Follicular Carcinoma, Minimally Invasive	1	2.40%
Follicular Carcinoma, Widely Invasive	2	4.80%
Follicular Variant Of Papillary Thyroid Carcinoma	2	4.80%
Non-Invasive Follicular Thyroid Neoplasm With Papillary Like Nuclear Features/ NIFTP	1	2.40%
Well differentiated tumor of uncertain malignant potential	2	4.80%
Medullary Thyroid Carcinoma	4	9.50%
Hurthle Cell Carcinoma	1	2.400%
Poorly Differentiated Carcinoma	1	2.40%
Anaplastic Thyroid Carcinoma	1	2.40%
Total	42	100.0%



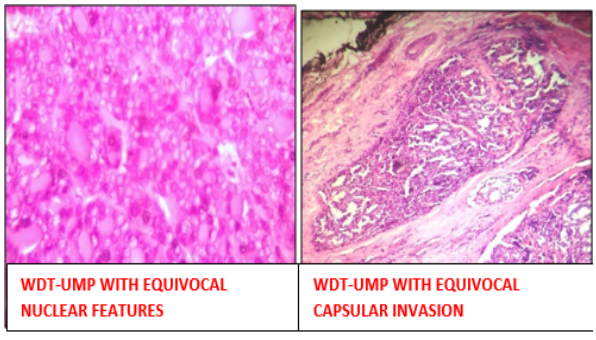
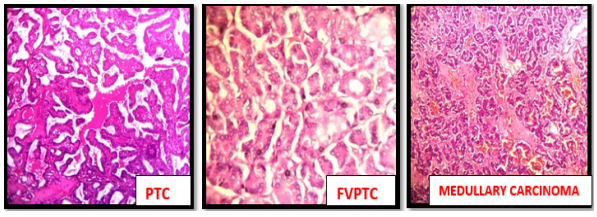
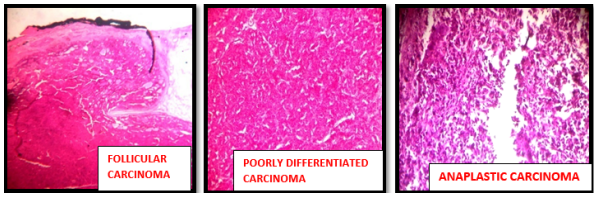


Figure:2 Histological pictures of various thyroid neoplasm





In our study, all the benign lesions are negative for Gal-3, excepting 1 follicular adenoma and 1 case of Hashimoto's thyroiditis gave score 1+ in <5 % tumor population cells. Among the malignant lesions, PTC, follicular carcinomas, hurthle cell carcinoma, FVPTC, poorly differentiated and anaplastic carcinoma show intense 3+ positivity in cytoplasm as well as on nucleus on > 50% of tumor population. All the medullary thyroid carcinomas, borderline lesions (WDT-UMP, NIFTP) gave 2+ cytoplasmic stain on nearly 25-50% of tumor population cells.

We analyzed the staining pattern of Gal-3. Borderline lesions (NIFTP, WDT-UMP) with equal intense stain in periphery and central portion of tumor do not have any involved neck nodes. Cases of PTCs (24 out of 29 PTC cases) with peripheral Gal-3 over expression had metastatic deposits in the neck nodes. Aggressive and undifferentiated (ATC, PDTC) tumors had the highest peripheral Gal-3 expression pattern and had bilateral involved neck nodes, indicating their highest metastatic potential. (Table:5)

Table 5: Galectin-3 distribution pattern and neck node status in malignant neoplasm

Preferrential Location Of Galectin-3 Stain	No Neck Node	Unilateral Neck Nodes	Bilateral Neck Nodes	Total
Equal Intensity Staining In Both Peripheral And Central Cells Row % Col %	6 75.0 50.0	2 25.0 14.3	0 0.0 0.0	8 100.0 19.0
Peripheral Tumor Cells Less Intense Stain Compared To Central Cells Row % Col %	6 100.0 50.0	0 0.0 0.0	0 0.0 0.0	6 100.0 14.3
Peripheral Tumor Cells More Intense Stain Compared To Central Cells Row % Col %	0 0.0 0.0	12 42.9 85.7	16 57.1 100.0	28 100.0 66.7
Total Row % Col %	16 38.1 100.0	12 28.6 100.0	14 33.3 100.0	42 100.0 100.0

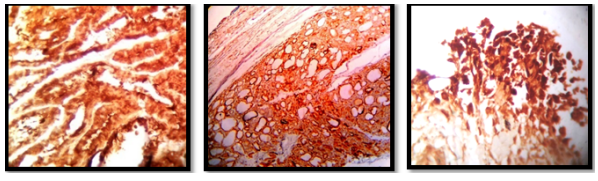
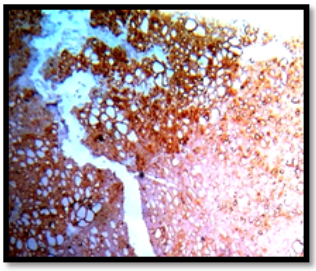


Figure 3: GALECTIN-3 IHC STAIN



PERIPHERAL MORE INTENSE STAIN IN AGGRESSIVE TUMOR

The specificity of Gal-3 in our study is 86.67%, sensitivity of 100%, positive predictive value of 95.45% and negative predictive value of 100%. So Gal-3 can be utilized to pick up all the malignant cases and benign cases with focal positivity should ideally be followed up for

their risk of incipient malignant transformation.

CONCLUSION:

- FNAC remains the most precise single test for the presurgical diagnosis of a thyroid neoplasm.
- Cytological indeterminate lesions when had suspicious USG features should undergo a total thyroidectomy at the initial setting itself rather than a diagnostic lobectomy and it does not represent a real overtreatment.
- Galectin-3 serve as the stand- alone marker to differentiate benign and malignant thyroid neoplasm.
- Focal positivity in subset of benign neoplasm may likely represent incipient risk for future malignant transformation. Surgical excision of these lesions advisable for Gal-3 expression drives the block of apoptosis, a biological feature that is not observed in normal cell.
- Peripheral Gal-3 overexpression is associated with a higher grade lesion with a greater metastatic potential.
-Histomorphology still remains the cornerstone for the diagnosis of thyroid lesions. Gal-3 can serve as an adjunct to solve dilemmas in cases with overlapping cytomorphology.

FUTURE DIRECTIONS:

-Studies have also focused on the use of Gal-3 expression as a tool for Immunocytochemistry (ICC) with reported a sensitivity of 78%, specificity of 93%[19].
- Estimation of serum levels of Gal-3 using ELISA as an adjunctive tool for thyroid carcinoma diagnosis has been recently reported[20].
- Gal-3 immuno-PET may potentially improve thyroid carcinoma diagnosis in the presence of multiple thyroid nodules, or the presence of suspicious subcentimetric lesions in deep mediastinal positions, or to distinguish thyroid cancer infiltration/ residual tumor nests and minimal normal thyroid tissue residues after thyroid surgery. This represents a common clinical problem during follow-up of patients after a thyroid cancer surgery[21].
- Gal-3 can potentially be exploited as a molecular target therapy for inhibition of apoptosis[22].

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