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Original Research Article

Role of galectin 3 and thyroid peroxidase as diagnostic tool in thyroid carcinoma with clinicopathological correlation: a cohort study of 94 cases

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ABSTRACT

Background: Thyroid neoplasms constitute the most commonly occurring endocrine tumors worldwide. With ultrasonography, detection has been increased up to 30%. Thyroid neoplasms have a wide spectrum of clinical behaviour and varied therapeutic responsiveness. Thus, early diagnosis of thyroid tumors and appropriate management will prolong the survival rate of patients. So, this study focuses on thyroid tumors which have overlapping morphological features and exact diagnosis is essential for surgical and post- operative management of patients. The aim and objective of the study was to determine the role of Galectin 3 and Thyroid Peroxidase in malignant tumors of thyroid as diagnostic marker.
Methods: We evaluated the patients for detailed history, physical examination, fine needle aspiration cytology (FNAC) and biopsy or excision of thyroid gland and subsequently histological examination and Immunohistochemistry for galectin 3 and thyroid peroxidase antibody (TPO).
Results: A total of 94 cases were enrolled in the study. Out of these 94 cases, 70 cases (74%) were followed by histopathology and immunohistochemistry (IHC). Out of 70 cases, benign were 44 (63%) and malignant were 26 (37%). Among benign cases, most common were colloid goitre, 22 cases (31%). Among malignant cases, most common were papillary carcinoma, 15 cases (21%). For all thyroid carcinoma, both Galectin 3 and TPO showed sensitivity of 81% when used alone. However combination of galectin 3 and TPO increased the sensitivity up to 92%.
Conclusions: Galectin 3 and TPO alone has high sensitivity for all thyroid carcinoma but sensitivity was markedly increased when combination of the two markers were used.

Keywords: Galectin 3, Thyroid peroxidase antibodies, Thyroid neoplasms

INTRODUCTION

Thyroid neoplasms constitute the most commonly occurring endocrine tumors worldwide. Worldwide, thyroid cancer is the 18th most common cancer type among both the genders. The estimated new cases and deaths for thyroid cancer in 2020 are 586,202 (3.0%) and 43,646 (0.4%) respectively according to GLOBACON 2020.[1] Thyroid tumors are more common in developed countries. The incidence of thyroid tumors has increased in past two decades, predominantly papillary carcinoma of thyroid.[2] The deliberate criteria for diagnosis of papillary carcinoma of thyroid and detection of small tumors by imaging techniques and environmental factors

led to increase in the incidence of thyroid tumors.[3] Fine-needle aspiration cytology (FNAC) is the gold standard method for screening of thyroid nodules.[3,6]

As there is morphological overlap between many thyroid tumors with follicular pattern as seen in follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma and the nuclear features characteristic of papillary carcinoma like nuclear grooves and inclusions are also seen in multinodular goiter with papillary hyperplasia and hyalinising trabecular adenoma, immunohistochemistry is helpful in differentiating the tumors. Thyroid neoplasms with follicular architecture can have overlapping morphologic features and pose diagnostic confusion among pathologists. In this study, an attempt is made to evaluate the prospective value of combination of two immunohistochemical markers in thyroid neoplasms namely, galectin-3 and thyroid peroxidase (TPO) which may be helpful for the diagnosis and prognostication of these patients.

Galectin-3 (Gal-3), which has received significant recent attention for its utility as a diagnostic marker for thyroid cancer, represents the most well-studied molecular candidate for thyroid cancer diagnosis but its sensitivity and specificity in detecting malignant neoplasms should be accurately confirmed and analysed.

TPO is one of the main thyroid autoantigens. TPO, which is expressed on the thyroid cell surface as well as in the cytoplasm, is the cell surface antigen involved in complement-mediated cytotoxicity. TPO reflects normal thyroid function and, therefore, should not be expressed in malignant tissue. A number of studies have been published demonstrating the value of TPO in the diagnosis of thyroid lesions, and the sensitivities have ranged between 97% and 100%.[11]

Aim

The aim of the study was to evaluate the role of immunohistochemical expression of Gal-3 and TPO on the malignant lesions of thyroid with confidence and thereby, helping the clinicians to decide the optimal management approach for the patient.

Objectives

The objective of the study was to determine the role of Galectin 3 and TPO in malignant tumors of thyroid as diagnostic markers

METHODS

This is a cohort study and was done in the Department of Pathology, LLRM Medical College, Meerut in collaboration with the department of ENT and Department of Surgery, SVBP Hospital, LLRM Medical College, Meerut. Purposive sampling was done in which hospital based convenient samples are taken from January 2020 to January 2022. All thyroid swelling specimens during period of January 2020 to January 2022 received in Department of Pathology, LLRM Medical College, Meerut were included. Patients with Diabetes mellitus, hypertension, tuberculosis, HIV, pregnant females, pediatric patients less than 10 years of age, patients whose consent was not obtained were excluded from study. All patients were subjected to standard diagnostic criteria, including detailed history, physical examination, fine needle aspiration cytology (FNAC) and biopsy or excision of thyroid gland and subsequently histological examination and immunohistochemistry.

Procedure for FNAC

Following the aspirate, three smears were prepared, of which two were stained with May Grunwald Giemsa and one with papanicolaou stain. These smears were then examined under the microscope. The specimens were fixed in 10% formalin for 24-48 hours and paraffin embedded as standard practice. For routine histopathological assessment, 4-6 µm sections were cut from appropriate block. These were deparaffinized in xylene and graded alcohol for rehydration. These were subsequently stained and mounted for microscopic examination. IHC was outsourced from oncquest labs, Gurgaon and Dr. Navani path labs, Mumbai. The results showing staining intensity and focality were analysed and then reported.

Grading of Gal-3 and TPO

The Grading of both the markers were done on the basis of degree of intensity which is achieved by the stain as well as area of staining achieved (Table 1). Cases that showed specific staining of more than 5% of the tumor cells, regardless of staining intensity, were scored as positive for TPO or Gal-3. The study was approved by Institutional ethical committee. Institutional review board approval was obtained for the staining and chart review portions of the study. Written informed consent was taken from all patients included in the study.

RESULTS

A total of 94 cases were enrolled during the study. Their demographic distribution were given in Table 2, 3, 4 respectively. All 94 cases underwent FNAC procedure for the diagnosis. Out of these, 74% (70) cases were followed for histopathology. 26% (24) cases were lost to follow up (Table 5). 93% (14/15) cases of papillary carcinoma thyroid showed positivity for Gal-3. 60% (3/5) cases of Follicular thyroid carcinoma showed positivity for Gal-3. While 67% (4/6) cases of Medullary thyroid carcinoma showed positivity for Galectin 3. Overall sensitivity Galectin 3 for malignant cases of thyroid was 81%. Only 16% (7/44) benign cases of thyroid showed positivity for Galectin 3 (Table 6). We applied Gal-3 in all the 70 cases of thyroid, while TPO in all the malignant cases of thyroid i.e.; 26 cases.

Table 1: Grading of Galectin and TPO.

Grade	Staining intensity	Focality
0	No staining	No cell involved
1+	Slight staining	<5% of cells
2+	Moderate staining	5%-50% cells
3+	Intense staining	>50% of cells

Table 2: Distribution of cases enrolled (n=94).

	N	%
Total cases enrolled	94	100
Cytology (FNAC)	94	100
Histopathology	70	74
Lost to follow up	24	26

Table 3: Age wise distribution of cases (n=94).

Age group (years)	N	%
11-20	09	10
20-29	18	19
30-39	29	31
40-49	21	22
50-59	10	11
>60	07	07
Total	n=94	100

Table 4: Distribution of cases according to gender (n=94).

Gender	No of cases	%
Male	11	12
Female	83	88
Total cases	94	100

Table 5: Distribution of total cases on histopathological diagnosis (n=70).

Histological diagnosis	N	%
Benign	44	63
Colloid goitre	22	31
Hashimoto's thyroiditis	19	27
Follicular adenoma	02	3
Hyperplastic nodule	01	2
Malignant	26	37
Papillary carcinoma	15	21
Medullary carcinoma	06	9
Follicular carcinoma	05	7
Total cases	70	100

Table 6: Immuno-expression of Galectin 3 (N=70).

Histological diagnosis	No of cases	%	Galectin 3 positivity (grade)	Interpretation (+/-)
Malignant (n=26)				
Papillary carcinoma of thyroid (n=15)	9	60	3+	+
	3	20	2+	+
	2	13	1+	+
	1	7	0	-
Follicular thyroid carcinoma (n=5)	1	20	3+	+
	2	40	2+	+
	1	20	1+	+
	20	20	0	-
Medullary thyroid carcinoma (n=6)	2	33	3+	+
	2	33	2+	+
	2	33	0	-
Benign cases (n=44)				
Colloid goiter (n=22)	2	09	2+	+
	20	91	0	-
Follicular adenoma (n=02)	1	50	1+	+
	1	50	0	-
Hashimoto's thyroiditis (n=19)	1	5	3+	+
	3	16	2+	+
	15	79	0	-
Hyperplastic nodule (n=1)	1	100	0	-
Total	70			

Table 7: Combined evaluation of Galectin 3 and TPO immunoexpression (n=26).

Malignant cases of thyroid	S. No of cases	Galectin 3	Thyroid peroxidase	Interpretation (+/-)
Papillary carcinoma thyroid (n=15)	1	+	-	+
	2	+	-	+
	3	+	-	+
	4	+	-	+
	5	+	-	+
	6	+	+	+
	7	+	+	+
	8	+	-	+
	9	+	-	+
	10	+	-	+
	11	+	-	+
	12	+	-	+
	13	-	-	+
	14	+	-	+
	15	+	-	+
Follicular thyroid carcinoma (n=5)	16	+	-	+
	17	+	-	+
	18	+	-	+
	19	-	+	-
	20	-	-	+
Medullary thyroid carcinoma (n=6)	21	+	-	+
	22	+	-	+
	23	-	+	-
	24	-	+	+
	25	+	-	+
	26	+	-	+
Total	26		-	

Immunohistochemistry was interpreted as a positive result for Galectin 3 and a negative result for Thyroid peroxidase was consistent with carcinoma. Therefore 92% (24/26) cases were interpreted as carcinoma (Table 7).

Table 8: Comparative evaluation of sensitivity of Galectin 3 and TPO alone and in combination (n=26).

Variables	Galectin 3	TPO	Galectin 3 and TPO as combination
All carcinoma (n=26)	81% (21)	81% (21)	92% (24)
Papillary (n=15)	93% (14)	87% (13)	100% (15)
Follicular (n=5)	60% (3)	80% (4)	80% (4)
Medullary (n=6)	67% (4)	67% (4)	83% (5)

Sensitivity was calculated for Galectin 3and Thyroid peroxidase individually and when used as combination.

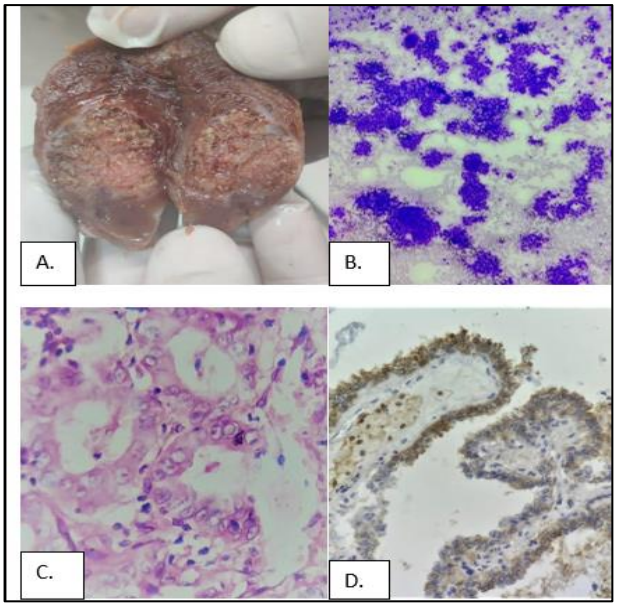

Figure 1: (A) Gross image of PTC. (B) 10X cytological image showing papillary arrangement of PTC. (C) 40X HPE image of PTC showing inclusion bodies. (D) 40X IHC image showing Galectin 3 positivity.

Sensitivity is defined as number of carcinoma with positive results (either Galectin 3 positive or TPO negative) to total number of carcinoma expressed in percentage (Table 8).

DISCUSSION

The present study enrolled a total of 94 cases with an aim to justify the role of Galectin 3 as a marker of malignancy in thyroid swellings and TPO antibodies as a marker of benign swellings, however when these two antibodies are used in combination, there is a significant increase in the sensitivity of detecting the positivity of thyroid malignancies.

Difficulties in the diagnosis of thyroid lesions, even with histologic analysis, are well known.[6,7] Follicular carcinoma presents a particular challenge, with one study showing diagnostic discrepancies in 57% of histologic cases of minimally invasive follicular carcinoma reviewed by 5 pathologists.[8] However, papillary carcinoma also can be a diagnostic dilemma. This was demonstrated by a study in which one group of pathologists diagnosed papillary carcinoma in 25% of a set of histologic thyroid samples, while a second group of pathologists diagnosed it in only 4% of the same specimens.[9]

Till now, swellings of thyroid have a challenging diagnostic dilemma due to its varying presentation to clinicians as well as pathologists. More classically, studies were conducted on various markers to improve and enhance the diagnostic ability of thyroid swellings; still, diagnostic tests of thyroid need robust studies and need immunohistological marker to enhance the diagnostic ability of thyroid swellings. In the present study, attempts have been made to enhance the diagnostic accuracy of various thyroid swellings pertaining to their benign and malignant nature using Gal-3 and TPO antibodies. Our results were in concordance with the previous studies of Weber et al and Savin et al which are showing sensitivity of 92% and 85.7% respectively for Galectin 3 and 100% for TPO.[10,11]

Recent studies of D. Kalfert et al also showed concordant results indicating lower sensitivity and specificity of both markers in single use for discrimination between benign and malignant thyroid lesions as well as distinct types of carcinomas.[12] However, the combined use of them has an excellent specificity (94.1%), relatively good sensitivity (81%) and good likelihood ratios as test parameters for diagnosis of papillary carcinoma of oncocytic and non-oncocytic variants.

In our opinion, epidemiology of thyroid lesions may be responsible for the difference in the proportion of the cases in the categories. Iodine deficiency disorders and colloid goiter are known to be endemic in India with a greater prevalence as compared to the western countries.[13] This may have lead to more proportion of cases being diagnosed as benign with consequent decrease in the other categories.

Advantages of study

Specific diagnosis like NIFTP and other variants resembling papillary carcinoma are difficult to diagnose on histopathology and need expertise opinion. IHC could be a tool where experienced pathologists and divergent views are warranted.

Limitations of the study

Feasibility of IHC is major limitation with widely equipped setups. However, histopathology could be a potent and feasible tool for diagnosis in most of cases with peculiar diagnosis.

CONCLUSION

Galectin 3 is highly sensitive for Papillary carcinoma of thyroid, but indeed could be sensitive in follicular and medullary carcinoma. Thyroid peroxidase antibodies, on the other hand, is a benign marker for thyroid neoplasms. So, TPO could be used for differentiating thyroid carcinoma as sensitivity is markedly increased and combination of these two markers could be used for differentiating malignancies in thyroid nodules due to their high sensitivity.

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