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Original Article

Pathology Section

Immunohistochemical Expression of Galectin-3 and Cytokeratin 19 in the Spectrum of Thyroid Neoplasms

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ABSTRACT

Introduction: In systemic malignancies, thyroid carcinoma represents only 1%, but it is the most common endocrine malignancy with poor prognosis. Fine Needle Aspiration (FNA) is considered a requisite tool in providing a rational advent for the clinical management of these nodules. However, when solely based on cytopathological assessment, FNA leads to imprecise biopsy results in 10-20% of all cases. So, immunohistochemical markers, Galectin-3 (Gal-3) and Cytokeratin 19 (CK19) have received considerable attention as diagnostic marker for thyroid cancer.

Aim: To study the expression of Gal-3 and CK19 in different thyroid neoplasms.

Materials and Methods: This cross-sectional study was conducted at a tertiary care hospital from January 2019 to January 2021 on 120 cases. The thyroidectomy specimens received were fixed in 10% buffered formalin followed by gross examination. The paraffin embedded tissue blocks were subsequently stained for Haematoxylin and Eosin stain (H&E) followed by histopathological reporting. Qualitative immunohistochemical assessment of the marker Gal-3 and CK19 was performed on representative histologic sections of the thyroid neoplasms. Data were analysed using Graph Pad Instat 3.

Results: The positive expression of Gal-3 was significantly more in malignant tumours (87.5%) than in benign (36.0%) (p-value <0.001). In Papillary Thyroid Carcinomas (PTC) and in Follicular Variant of Papillary Thyroid Carcinomas (FVPTC) expression of Gal-3 was notably higher than in Follicular Adenoma (FA) (p-value=0.01 and p-value=0.0001, respectively). Follicular Thyroid Carcinomas (FTC) had higher expression FA (p-value=0.003). In malignant tumours (71.8%), positive expression of CK19 was significantly more than benign tumours (24.0%) (p-value <0.001). Significant difference in expression of CK19 was seen between PTC and FTC (p-value=0.019). Between PTC and FA, differences in expression were significant (p-value <0.001). Significant difference in expression was also seen between FVPTC and FTC (p-value=0.032) as well as with FA (p-value=0.028).

Conclusion: The most sensitive marker is Gal-3 for the diagnosis of thyroid malignancies. When combined with CK19, the specificity increases in identifying the thyroid cancers. More combination of markers together with Gal-3 and CK19 can be useful in the distinction between malignant and benign thyroid tumours because it is essential for further treatment and long-term management of the patient.

Keywords: Benign lesions, Fine needle aspiration, Malignant, Markers, Thyroidectomy

INTRODUCTION

In systemic malignancies, thyroid carcinoma represents only 1%, but it is the most common endocrine malignancy with poor prognosis [1]. To augment the survival rate and prolong the survival time of patients, prompt diagnosis and treatment of thyroid cancer is necessary. In the general population, thyroid nodules are very common, detected by palpation in 5% of patients and by Ultrasound (USG) examination in 50% of patients [2]. Fine Needle Aspiration (FNA) is considered a requisite tool in providing a rational advent for the clinical management of these nodules. Along with FNA, certain diagnostic tests like radionuclide scanning, high-resolution USG have been used, but FNA is most accurate and cost effective [3]. However, when solely based on cytopathological assessment, FNA leads to imprecise biopsy results in 10-20% of all cases [2].

So, immunohistochemical markers, Galectin-3 (Gal-3) and Cytokeratin 19 (CK19) have received considerable attention as diagnostic marker for thyroid cancer [4,5]. Gal-3 belongs to the family of lectins and is mostly confined within the cytoplasmic and nuclear compartment [6]. CK19 is a low molecular weight cytokeratin which presents widely in simple epithelia and basal cell layers of stratified epithelium [4].

The present study was conducted with the aim to study the expression of Gal-3 and CK19 in different thyroid neoplasms.

MATERIALS AND METHODS

This cross-sectional study was conducted at a tertiary care hospital of Institute of Postgraduate Medical Education and Research, Kolkata, West Bengal, India from January 2019 to January 2021 on 120 cases. The study was conducted after receiving approval from the Institutional Ethics Committee (Number: ECR/35/Inst/WB/2013).

Inclusion and Exclusion criteria: Patients presenting with neck swelling, symptoms of hypothyroidism like weight gain, menstrual abnormalities, symptoms of hyperthyroidism like exophthalmos, weight loss, tremor or palpitations were included in the study. Also patients with thyroid mass apparent on ultrasonography and those who gave consent were also included in the study. Patients who are uncooperative/not willing to do surgery, suffering from serious illness were excluded from the study.

Procedure

The thyroidectomy specimens received were operated either for benign or malignant diseases which included lobectomy, hemithyroidectomy, subtotal, near total and total thyroidectomy. The specimens were fixed in 10% buffered formalin. Gross examination was done for consistency, necrosis, haemorrhage, calcification or any other gross abnormalities. The paraffin embedded tissue blocks were subsequently stained for Haematoxylin and Eosin stain (H&E) followed by histopathological reporting which was done in accordance with World Health Organisation (WHO) classification (2017) [7]. Information including the gender, age was collected according to the proforma.

Immunohistochemistry (IHC) assessment of the marker Gal-3 (Monoclonal Mouse Antibody; clone: 9C4, positive control: normal prostate, nuclear and cytoplasmic staining) and CK19 (Monoclonal Mouse Antibody; clone: BA17, positive control: skin, cytoplasmic staining) was performed on representative histologic sections of the thyroid neoplasms. Appropriate negative controls were used by exclusion of the primary antibody. These markers were positive when immunoreactivity was observed in their cell membrane and/or cytoplasm. For each antibody, immunoreactivity (no staining or weak staining less than 10% of the cells) was scored as negative and the other immunoreactivity was scored as positive [5]. According to Beesley classify method, immunoreactivity no staining or buffy staining (1+), 25%-50% of the cells and buffy staining (2+), more than 50% of the cells and deep brown staining (3+) [7].

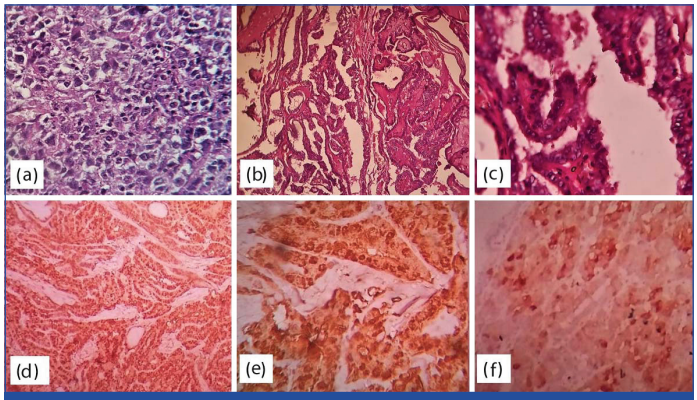
STATISTICAL ANALYSIS

At the end of the study, changes of all the parameters were presented in tabular form for each study group. All the data were recorded into Microsoft Excel 2016 and contingency tables were prepared. Data were analysed with the help of Graph Pad Instat 3. Chi-square test for independence was done. A p-value of <0.05 was taken as statistically significant.

RESULTS

This study comprised of 120 cases. The most common age group affected was 31-70 years with mean age of participants 55.9 years. Females (86.6%) were affected more than males (13.4%). The male: female ratio was 1:6.5.

Carcinomas: Malignant group involved 41 Papillary Thyroid Carcinomas (PTC), 20 Follicular Thyroid Carcinomas (FTC), one Medullary Thyroid Carcinoma (MTC) and two Anaplastic Thyroid Carcinomas (ATC) [Table/Fig-1a]. The PTCs [Table/Fig-1b,c] included 20 classic, 15 follicular, one oncocytic and five papillary microcarcinoma variants. Of 64 carcinomas, 56 cases (87.5%) expressed Gal-3 [Table/Fig-1d,e], 46 cases (71.8%) expressed CK19 [Table/Fig-1f] and 47 cases coexpressed Gal-3 and CK19. All MTC and ATC expressed Gal-3 (100%) [Table/Fig-2].



[Table/Fig-1]: Case of anaplastic thyroid carcinoma: a) Section shows highly pleomorphic cells with some tumour giant cells and increased mitotic figures (H&E,400X) Case of papillary thyroid carcinoma; b) Section shows complex branching papillae with fibrovascular cores (H&E,100X); c) Section shows cells with nuclear enlargement, chromatin clearing, ground glass nuclei (H&E,400X); d) IHC staining for Galectin-3 showing positivity (400X) Case of Follicular Variant of Papillary Thyroid Carcinomas (FVPTC); e) IHC staining for Galectin-3 showing positivity (400X) Case of papillary microcarcinoma of thyroid; f) IHC staining for CK19 showing positivity (400X).

Other encapsulated follicular-patterned thyroid tumours: Some encapsulated follicular-patterned neoplasms of the thyroid causes diagnostic difficulties due to ambiguity in the PTC type of nuclear changes and about the presence of capsular or vascular invasion. This includes Follicular Tumour of Uncertain Malignant Potential (FT-UMP) (nuclear features of PTC and with questionable capsular or vascular invasion), Well-Differentiated Tumour of Uncertain Malignant Potential (WDT-UMP) (well-developed or partially developed PTC-type nuclear changes and with questionable capsular or vascular invasion), Non Invasive Follicular Thyroid Neoplasm with papillary-like nuclear features (NIFTP) (well-developed or partially developed PTC-type nuclear changes and absence of capsular or vascular invasion) [6]. Here, two FT-UMP, two WDT-UMP, two NIFTP were also included to see the Gal-3 and CK-19 expression. Of six cases, two FT-UMP one WDT-UMP and one NIFTP expressed Gal-3 (66.7%). One FT-UMP (16.7%) expressed CK19. Co-expression was seen in one FT-UMP and one WDT-UMP (33.3%) [Table/Fig-2].

Histologic diagnosis	Number of cases	Gal-3		CK19		Co-expression (Gal-3+CK19)	
		N	Positive rate (%)	N	Positive rate (%)	N	Positive rate (%)
Papillary thyroid carcinoma	41	38	92.6	34	82.9	39	95.1
Follicular thyroid carcinoma	20	15	75	11	55	8	40
Medullary thyroid carcinoma	1	1	100	0	0	0	0
Anaplastic thyroid carcinoma	2	2	100	1	50	0	0
Encapsulated follicular-patterned thyroid tumours (FT-UMP, WDT-UMP, NIFTP)	6	4	66.7	1	16.7	2	33.3
Follicular adenoma	47	17	36.2	11	23.4	6	12.7
Hurthle cell adenoma	3	1	33.3	1	33.3	0	0

[Table/Fig-2]: Positive Expression of Gal-3 and CK19 in different pathohistological entities (N=120).
FT-UMP: Follicular tumour of uncertain malignant potential; WDT-UMP: Well-differentiated tumour of uncertain malignant potential; NIFTP: Non invasive follicular thyroid neoplasm with papillary-like nuclear features

Adenomas: Benign lesions comprised 47 Follicular Adenoma (FA) and 3 Hurthle cell adenomas. Of 50 adenomas, 18 cases (36.0%) expressed Gal-3, 12 cases (24.0%) expressed CK19 and 6 cases (12.0%) coexpressed Gal-3 and CK19 [Table/Fig-2].

The positive expression of Gal-3 was significantly more in proportion in malignant tumours (87.5%) than in benign (36.0%) (p-value <0.001). In PTCs and in FVPTC expression of Gal-3 was notably higher than in FA (p-value=0.01, p-value=0.0001; respectively). No significant difference in expression was seen between PTC and FTC (p-value=0.054), neither between FVPTC and FTC (p-value=0.589). FTC had higher expression than FA (p-value=0.003).

In malignant tumours (71.8%), positive expression of CK19 was significantly more than benign tumours (24.0%) (p-value <0.001). Significant difference in expression of CK19 was seen between PTC and FTC (p-value=0.019). Between PTC and FA, differences in expression were significant (p-value <0.001). Significant difference in expression was also seen between FVPTC and FTC (p-value=0.032) as well as with FA (p-value=0.028). Between FA and FTC no significant difference was seen (p-value=0.542).

The coexpression of Gal-3 and CK19 was significantly more frequent in carcinomas than adenomas (p-value <0.001). Sensitivity, specificity, positive and negative likelihood ratios, disease prevalence, Positive Predictive Values (PPV) and Negative Predictive Values (NPV), accuracy and odds ratio for Gal-3 and CK19 and their combination in differentiating 64 malignant from 50 benign thyroid lesions was evaluated [Table/Fig-3].

Sensitivity and specificity of different combinations of histologic entities are shown in [Table/Fig-4].

For malignancy	Gal-3 (95% CI)	CK19 (95% CI)	Gal-3+CK19 (95% CI)
Sensitivity (%)	87.50 (76.85-94.45)	71.88 (59.24-82.40)	73.44 (60.91-83.70)
Specificity (%)	64.00 (49.19-77.08)	76.00 (61.83-86.94)	88.00 (75.69-95.47)
Positive likelihood ratio	2.43 (1.66-3.56)	2.99 (1.79-5.02)	6.12 (2.85-13.15)
Negative likelihood ratio	0.20 (0.10-0.39)	0.37 (0.24-0.56)	0.30 (0.20-0.46)
Disease prevalence (%)	56.14 (46.54-65.42)	56.14 (46.54-65.42)	56.14 (46.54-65.42)
Positive predictive value (%)	75.68 (68.00-81.99)	79.31 (69.58-86.53)	88.68 (78.47-94.39)
Negative predictive value (%)	80.00 (66.94-88.77)	67.86 (58.07-76.29)	72.13 (62.97-79.75)
Accuracy (%)	77.19 (68.40-84.53)	73.68 (64.61-81.49)	79.82 (71.28-86.76)
Odds ratio	12.44 (4.86-31.83)	8.09 (3.47-18.89)	20.27 (7.33-56.08)

[Table/Fig-3]: Diagnostic value of tests in discrimination of 64 malignant from 50 benign thyroid lesions.

Combination of histologic entities	Sensitivity (%)	Specificity (%)
PTC vs FA	95.12	87.23
FVPTC vs FA	60.00	87.23
FVPTC vs FTC	60.00	60.00
FTC vs FA	40.00	87.23

[Table/Fig-4]: Sensitivity and specificity of different combinations of histologic entities. PTC: Papillary thyroid carcinomas; FTC: Follicular thyroid carcinomas; FVPTC: Follicular variant of papillary thyroid carcinomas; FA: Follicular adenoma

DISCUSSION

The FNA and histopathology are the gold standards for a diagnosis of a thyroid nodule. But Pathologists still encounter difficulties to attain a precise differential diagnosis between benign and malignant thyroid nodules. To enhance disease identification, immunohistochemical markers, such as Gal-3, CK19 have been suggested and their efficacies for thyroid cancer diagnosis have been assessed.

Gal-3 is a structurally 31-kDa member of the galectin family and binds galactosides on cell glycoproteins and glycolipids [8]. In nucleus, Thyroid-specific transcription factor 1 transcriptional activity is upregulated by Gal-3 and these results in increased expression seen in highly proliferative state of these tumour cells [9]. Gal-3 regulates apoptosis as well as helps in cell motility and T-cell grozwth [8]. Gal-3 is associated with the pathogenesis of thyroid cancers [10-12].

CK-19 is a heterogeneous group of intermediate filaments which is normally expressed in ductal epithelium such as pancreas, bile ducts [13]. This has also been evaluated in cases of hepatocellular carcinomas, squamous carcinomas and colorectal adenocarcinoma and also an independent prognostic factor for pancreatic neuroendocrine tumours [13,14]. Strong and diffuse immunoreactivity of CK19 in PTCs and its weak or absent immunoreactivity in benign thyroid lesions has been described in several studies [5,11,15].

Majority of studies have reported Gal-3 positivity in 80% to 100% of PTCs [10,12,16-21].The present study showed significantly higher Gal-3 expression in PTC cases (92.6%). Bartolazzi A et al., and Xu XC et al., have found Gal-3 expression in 95% and 100% of FTC cases, respectively [12,22]. Cvejic D et al., and Prasad ML et al., have shown positivity in 64% and 66% cases, respectively whereas Fernandez PL et al., have reported positivity in 50% cases of FTC [16,18,23]. Dunđerović D et al., have found 73.3% positivity and this was comparable to this study (75%) [21].

In this study, only a single case of MTC was reported which showed positivity to Gal-3 expression (100%). MTC originates from a different cell line, as a result Gal-3 is not supposed to be useful [12,23]. Two cases of ATC reported here, strongly expressed Gal-3 (100%) which was similar to Prasad ML et al., where all the four cases stained positive [18]. In this study, due to small number of cases of MTC and ATC, there is varying expression of Gal-3. So diagnostic application in these rare entities require a large number of cases [12,23].

Xu XC et al., found Gal-3 expression in PTCs and FTCs but not in adenomas [22]. The Gal-3 positive expression in FA cases ranged from 0 to 30% in this study and was comparable to several studies [12,14,22-26,30]. Gal-3 positivity for FAs in the current study is 36.2%. Among the three cases of Hurthle cell adenoma, only a single case showed positive expression so as by Sumana BS et al., and none of the cases were positive in the study done by Prasad ML et al., but Dunđerović D et al., showed 50% positivity [10,18,21]. A significant difference in expression of Gal-3 between PTC (92.6%) with FTC (75%) was seen by some authors and so as in present study [19,21,24-26]. Some studies have not found these differences [10,18,23,26,27]. Immunoreactivity in PTC (92.6%) compared to FA (36.2%) was higher which is in concordant with this study [19,24-28]. Significant difference in expression of Gal-3 between FTC (75%) and FA (36.2%) was seen in this study as well as other studies [19,21,27].

Utility of CK19 in identifying PTCs has been researched in many studies [29-32]. The positive expression of CK19 for PTCs ranges from 70% to 100% and this was comparable to this study with positivity of 82.9% [5,15,18,23,33]. In FTCs, CK19 positivity was shown by Prasad ML et al., (50%) and Dunđerović D et al., (33.3%) and this study showed 55% positivity [18,21]. Here, among two cases of ATCs only one showed CK19 positivity which was comparable to Prasad ML et al., [18]. CK19 expression was also found in adenomatous nodular hyperplasia and FA [34,35]. In this study, FAs showed 23.4% CK19 positivity and this was comparable to other studies [18,21]. Among three cases of Hurthle cell adenomas, a single case strongly expressed CK19 as compared to Prasad ML et al., where none of the cases were positive and Dunđerović D et al., showed 60% positivity [18,21].

PTC and FVPTC showed significantly higher expression of CK19 than in FTC [26,27]. This study also showed the same findings. CK19 further helps in differentiating PTC from FA but not between FA and FTC which was similar to this study [8,19,25]. Other encapsulated follicular-patterned thyroid tumours showed intermediate protein expression pattern between adenomas and carcinomas. Amongst the six cases, Gal-3 was expressed in four and coexpression (Gal-3+CK19) was seen in two cases which defend that Gal-3 is a marker of early malignant transformation and minimally invasive carcinoma as proposed by some authors [36]. The results imply the 'indeterminate or borderline' nature of the tumours and this may be useful in selecting the tumours for further follow-up to evaluate their biologic nature [18,37].

The sensitivity, specificity, PPV and NPV of Gal-3, CK19 and coexpression of Gal-3 and CK19 are compared to other studies [Table/Fig-5,6]. The sensitivity and specificity of different combinations of histologic entities in this study was comparable to the study done by Dunđerović D et al., PTC vs FA (78.2, 88.9), FVPTC vs FA (70.0, 88.9), FVPTC vs FTC (60.0, 71.4), FTC vs FA (33.3, 88.9), respectively [21].

To summarise, still many laboratories face diagnostic difficulty to report thyroid pathology and so different immunohistochemical markers can be useful in the diagnosis. Gal-3 was the most specific marker for thyroid neoplasm and together with CK19, it gave more specific diagnosis. Also, the borderline tumours were identified, which is useful for follow-up.

Name of the study	Gal-3				CK19			
	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive values (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive values (%)
Dunđerović D et al., [21]	88.52	64.56	79.41	78.46	75.41	70.89	80.00	65.12
Zhu X et al., [26]	86	66	-	-	79	74	-	-
Prasad ML et al., [18]	92	85	-	-	66	89	-	-
Nechifor-Boila A et al., [36]	46	100	100	64.7	45	100	100	64.7
Arcolia V et al., [37]	97	83	85	96	98	76	80	98
Present study	87.5	64.0	75.68	80.00	71.88	76	79.31	67.86

[Table/Fig-5]: Sensitivity and specificity of Galectin 3 and CK19 for malignancy.

Name of the study	Gal-3+CK19			
	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive values (%)
Dunđerović D et al., [21]	68.03	88.61	90.22	64.22
Nechifor-Boila A et al., [36]	90.9	63.6	71.4	87.5
Arcolia V et al., [37]	92	99	98	93
Present study	73.44	88.00	88.68	72.13

[Table/Fig-6]: Sensitivity and specificity of Gal-3+CK19 for malignancy.

Limitation(s)

There are some limitations in this study. Firstly, small number of cases for a shorter time period. Secondly, combined histopathological and cytological evaluation would have provided further evidence to analyse the utility of Gal-3 and CK19 in thyroid cancers. Thirdly, only two immunohistochemical markers were studied.

CONCLUSION(S)

The most sensitive marker is Gal-3 for the diagnosis of thyroid malignancies. When combined with CK19, the specificity increases in identifying the thyroid cancers. More combination of markers together with Gal-3 and CK19 can be useful in the distinction between malignant and benign thyroid tumours because it is essential for further treatment and long-term management of the patient.

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