Original Article

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Diagnostic Utility of Immunohistochemistry Markers Galectin-3, CK19 and CD56 in Thyroid Neoplasms: A Descriptive Study

Pathology Section

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ABSTRACT

Introduction: The spectrum of follicular patterned thyroid lesions vary from benign to malignant, their categorisation based solely on morphology can often be equivocal. Diagnostic value of immunohistochemistry as a useful ancillary technique is researched in detail, but there is no consensus for use of a marker of diagnostic utility in differentiated thyroid carcinoma.

Aim: To evaluate the differential immunohistochemical expression of galectin-3, CK19 and CD56 in benign and malignant thyroid neoplasms.

Materials and Methods: The present descriptive cross-sectional study was carried out in the Department of Pathology of a tertiary care centre, Government Medical College, Ernakulam, Kerala, India, from January 2018 to January 2019. Immunohistochemistry staining of galectin-3, Cytokeratin 19 (CK19) and Cluster Differentiation 56 (CD56) was done in 47 thyroid neoplasms. Cytoplasmic and nuclear staining of galectin-3, cytoplasmic or membranous staining of CK19 and loss of membranous expression of CD56 in more than 10% neoplastic cells were taken as positive expression. The data was analysed using IBM Statistical Package for the Social Sciences (SPSS) software version 22.0. Diagnostic test evaluation for markers done by calculating sensitivity and specificity.

Results: Out of total 47 neoplasms, 26 were malignant and 21 were benign neoplasms. Of these, galectin-3 positivity was seen in 22 (84.61%) malignant neoplasms and in 2 (9.52%) benign neoplasms. Cytokeratin 19 positivity was seen in 26 (100%) malignant neoplasms and in 7 (33.33%) benign neoplasms. Loss of CD56 expression was observed in 24 (92.3%) malignant neoplasms and in 4 (19.04%) benign neoplasms. Considering histopathology as the gold standard, the sensitivity for detecting malignancy for the three markers, galectin-3, CK19 and CD56 was 84.62%, 100%, 92.3% and specificity was 90.48%, 66.67%, 80.95%, respectively. The diagnostic accuracy of galectin-3, CK19 and CD56 was 87.23%, 85.10%, 87.23%, respectively. Diagnostic Odd's ratio for Galectin-3 was 2.3% in the present study.

Conclusion: Galectin-3 was found to be a reliable marker for thyroid papillary carcinoma and for differentiating malignancy. The panel consisting of galectin-3 and CD56, is valuable and complementary when used in two marker combination. CK19 was found to be the least specific diagnostic marker of thyroid malignancy.

Keywords: Cluster differentiation 56, Cytokeratin 19, Papillary carcinoma, Thyroid biomarker

INTRODUCTION

Diseases of the thyroid gland are common in clinical practice with thyroid neoplasms being the most common endocrine neoplasms. According to Global Cancer (GLOBOCAN) 2020 global cancer burden estimates of cancer incidence, thyroid cancer is responsible for 586,000 cases worldwide, ranking in ninth place for incidence in 2020. The global incidence rate of thyroid cancer in males and in females are 3.1 and 10.1, respectively per 100,000 [1]. Majority of the thyroid lesions are non neoplastic. The small subset of neoplastic lesions, need to be accurately diagnosed for timely surgical management. Ultrasonography and fine needle aspiration cytology diagnosis are valuable diagnostic modalities for preoperative assessment of thyroid cancer [2].

Histopathology is considered the gold standard in diagnosis and classification of thyroid lesions. The spectrum of lesions in thyroid surgical pathology varies from benign goitre to follicular patterned malignant lesions. Impairment of thyroid hormone synthesis leads to compensatory increase in the serum Thyroid Stimulating Hormone (TSH) level, which in turn causes hypertrophy and hyperplasia of thyroid follicular cells, resulting in diffuse goitre. Recurrent episodes of hyperplasia and involution produce irregular enlargement termed multinodular goitre. Genetic abnormalities can lead to a few cells becoming clones of proliferating cells, this results in autonomous growth and formation of a hyperplastic nodule [3]. These lesions were referred to as cellular colloid nodule, adenomatoid nodule or adenomatous hyperplasia until recently. Studies have shown that these nodules are frequently but not always clonal, some are adenomas, while others are hyperplastic [4-6]. A new terminology for these lesions thyroid Follicular Nodular Disease (FND) has achieved consensus support from the World Health Organisation (WHO) editorial board and is now included under benign neoplasms of thyroid [6].

The WHO classification of thyroid neoplasms has organised follicular cell-derived neoplasms into three categories: benign neoplasms, low-risk neoplasms, and malignant neoplasms [6]. Tumours exhibiting follicular cell differentiation comprise more than 90% of the thyroid cancers [7]. Though there are several potential markers, controversy exists regarding the correct combination of markers for diagnosing specific thyroid cancers. Immunomarkers most studied in thyroid pathology include organ specific immunomarkers such as Thyroid Transcription Factors (TTF) TTF1, TTF2, Paired box gene 8 (PAX) and Thyroglobulin (TGB). Immunomarkers for the differential diagnosis of thyroid neoplasms are Galectin-3, Cytokeratin 19, Hector Battifora mesothelial antigen-1 (HBME-1), Hepatocyte Growth Factor (HGF), CD57 and CD31. Loss of Thyroid Peroxidase (TPO) and CD56 expression are negative markers of diagnostic importance as they are lost in malignancy [8].

Galectin-3 is a member of the lectin family. Investigators have found Galectin-3 expression to be of value in discriminating between benign and malignant thyroid nodules [9]. *LGALS* 3 gene was found to be up

regulated in Papillary Thyroid Carcinoma (PTC) compared to normal thyroid. It is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. This protein has been shown to be involved in the following biological processes: cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle, and apoptosis [10].

Cytokeratin 19 (CK19) is a member of the keratin family. Keratin 19 (KRT19) gene is found to be up regulated in PTC compared to normal thyroid [11]. This cytoskeletal protein CK19 is significantly increased in PTC; but this finding remains controversial, since it is also positive in benign epithelium, chronic lymphocytic thyroiditis and at sites of reaction, usually in response to previous aspiration biopsy [12-14]. CD56 is a Neural Cell Adhesion Molecule (NCAM) present on follicular epithelial cells of the normal thyroid and it shows diffuse membranous staining [15]. Low or absent expression of CD56 was noted in PTC using immune histochemistry. Loss of CD56 expression has been noted in papillary carcinoma and such loss can serve as a specific and sensitive marker of PTC [15-17].

According to the American Thyroid Association (ATA) management guidelines, pathologic examination of thyroid samples establishes the diagnosis and provides important information for risk stratification of cancer and management [7]. Nodules with follicular pattern of growth can pose diagnostic difficulty. Immunohistochemistry (IHC) as an additional diagnostic tool to morphology can help making an unequivocal diagnosis. Individual studies of biomarkers show that some markers are more specific, some are more sensitive and all these markers may not be expressed in a single tumour. With combined use of markers, the diagnostic accuracy increases [17].

The present study aimed to describe the profile of surgically resected thyroid lesions and to find the differential immunohistochemical expression of galectin-3, CK19 and CD56 markers in thyroid neoplasms analysed during one year period.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted at a tertiary care centre in South India. Consecutive samples of thyroidectomy, from January 2018 till January 2019 were evaluated and neoplastic lesions selected for biomarker study. The study was approved by the Institutional Ethics Committee (IEC No. /04/17). Grossing and sampling of thyroidectomy specimens were done according to standard guidelines.

Sample size calculation: The sample size was calculated based on proportion (75.4%) of expression of CK19 in thyroid carcinoma in a study by Dunđerović D et al., [17]. Applying the formulae $4pq/d^2$, where prevalence (p)=75.4%, (q)=24.6%, precision (d)=16. The sample size calculated was 4×75.4×24.6/16×16=28.

Inclusion criteria: Cases that were histologically diagnosed as benign or malignant neoplastic lesions of thyroid follicular epithelial origin were included in the study.

Exclusion criteria: Benign cases diagnosed as lymphocytic thyroiditis, nodular goitre and neoplasms other than follicular derived origin were excluded from the study.

Study Procedure

Tissue sections were processed in automated tissue processing unit and Haematoxylin and Eosin (H&E) stained sections were prepared for microscopy. A total of 47 specimens, including 21 benign neoplasms (group1) and 26 malignant epithelial neoplasms (group 2) were subjected to immunohistochemistry staining for comparison of the three biomarker expression. The diagnosis and typing of thyroid pathology was done as per the World Health Organisation (WHO) classification guidelines of thyroid neoplasms [6].

Sections of three microns thickness prepared from selected tissue blocks in each case. One section stained by H&E, and other three sections mounted on poly-L-lysine-coated slides for IHC staining. Three markers for immunohistochemistry, galectin-3 (Gal 9C4, mouse monoclonal pathinsitu), Cytokeratin 19 (CK19-A53-B/A2.26+BA17 rabbit monoclonal, pathinsitu) and CD56 (NCAM1/795 mouse monoclonal Biomarq) antibodies were used. IHC procedure was manual, antigen retrieval done on Heat-induced Epitope Retrieval (HIER) with tris Ethylenediamine Tetraacetic Acid (EDTA) buffer at pH of 9. Positive and negative controls were used for each marker. The stained slides were examined by two pathologists, using dual head microscope and consensus on reading was reached in each case.

Immunohistochemistry (IHC) staining scores were given based on the proportion of cells staining positive and on the intensity of staining. Proportion score-0, for staining in <10% of the cells; score 1, staining in 11-25% of the cells; score 2, staining in 26-50% of the cells; score 3, staining in >51% of cells [14]. Immunoreactivity was considered positive when cytoplasmic and nuclear staining for galectin-3 was seen in more than 10% of neoplastic cells, cytoplasmic or membranous staining in CK19 more than 10% of was positive, and membranous CD56 expression was lost in tumour cells or was observed in <1% of tumour cells. The staining intensity for each antibody were scored as 0 for negative; Positive immunostaining 1+, 2+, 3+ for weak; moderate; and strong [17,18].

STATISTICAL ANALYSIS

Data was entered into Microsoft Excel data sheet and analysed using Statistical Package for the Social Sciences (SPSS) software version 22.0. Comparison of qualitative variables between groups were done using the Chi-square test. Probability values, p-value <0.05 was considered significant. The sensitivity, specificity, positive and negative predictive values for each marker were calculated for diagnostic test evaluation.

RESULTS

The present study on thyroid neoplasms included 47 cases from eight males and 39 female patients with a mean age of 41.57±15.24 years. Maximum number of malignant neoplasms were in the age group of 20-30 years. Gender wise distribution of cases in the study showed an overall predominance of thyroid neoplasms by 4.9 times in females. Male to female ratio was 1:4.2 for malignant neoplasms and 1:6 for benign neoplasms [Table/Fig-1].

Age group (years)	Benign (n=21)			Malignant (n=26)		
	Male (n)	Female (n)	Total (n)	Male (n)	Female (n)	Total (n)
20-30	-	2	2	2	8	10
31-40	1	5	6	-	6	6
41-50	-	5	5	1	2	3
51-60	1	3	4	1	2	3
61-70	-	2	2	-	3	3
71-80	1	1	2	1	-	1
Total	3	18	21	5	21	26
	Mean age: 41.57±15.24 years					

[Table/Fig-1]: Age group and prevalence of thyroid neoplasms (N=47).

Group 1, included 21 benign neoplasms and group 2 included 26 malignant neoplasms. Among the benign neoplasms there were 12 cases of thyroid FND and nine cases of follicular adenomas. The spectrum of malignant tumours was comprised of 23 PTC, two Follicular Thyroid Carcinoma (FTC), and one Poorly Differentiated/ Insular Thyroid Carcinoma (PDTC). PTC was the most common subtype (88.46%). The mean tumour size was 2.01 cm. Tumour size was between 1 cm-2 m in 38.46% cases and size more than 4 cm seen in 3 (11.53%) cases. Applying the American Joint Committee on Cancer- Tumour, Nodes and Metastases (AJCC-TNM) staging system, 21 (77.77%) carcinomas were stage 1 diseases [19]. Pathological characteristics of 26 malignant neoplasms are depicted in [Table/Fig-2].

Variables		n	%
Tumour type n=26	Classic Papillary Thyroid Carcinoma (CPTC)	10	88.46
	Papillary Thyroid Microcarcinoma (PTMC)	5	
	Follicular Variant PTC (FVPTC)	7	
	Diffuse sclerosing subtype	1	
	Follicular Carcinoma (FTC)	2	7.68
	Poorly differentiated (insular) carcinoma	1	3.84
Tumour size (Mean size: 2.01cm)	<1 cm (pT1a)	5	19.23
	1-2 cm (pT1b)	10	38.46
	2-4 cm (pT2)	8	30.76
	>4 cm (pT3)	3	11.52
Tumour location and extension	Unifocal	18	69.23
	Multifocal	8	30.76
	Capsular invasion	5	19.23
	Vascular invasion	1	3.84
	Extra thyroid extension	1	3.84
	Lymph nodes	2	7.68
Adjacent thyroid	Multinodular goiter	2	7.68
	Lymphocytic thyroiditis	3	11.52
AJCC stage groups [19]	Stage I (Age >55 years, any T, any N/and age >55 years, T1, T2, N0)	21	80.76
	Stage II (age >55 years, T1/T2, N1/T3, any N)	5	19.23
	Stage III (T4a, any N)	1	3.84

[Table/Fig-2]: Characteristics of malignant thyroid tumours in the study (n=26).
AJCC: American joint committee on cancer; T: Tumour; N: Node; M: Metastases

Immunohistochemistry staining scores 0, 1+, 2+, 3+ of each marker in neoplastic lesions are shown in separate tables [Table/Fig-3,4]. Benign neoplasms (n=21) in the present study showed negative expression to weak staining of score 1. Follicular adenomas showed positive staining with cytokeratin 19 in 6/9 cases, galectin-3 positivity and CD56 negative staining were seen in 2/9 cases and 3/9, respectively each with low intensity. All FND (n=12), were negative for galectin-3, whereas CK19 positivity and loss CD56 expression was seen in all 1/12 cases.

In thyroid carcinomas, biomarker expression was seen significantly high, with 2+ and 3+ staining scores. Positive staining of Galectin-3 was seen in 21 cases of classic PTC and subtypes. Two cases of encapsulated follicular variants of PTC were negative. Galectin expression in follicular carcinoma was seen as score 1 staining in one case, expression was negative in the other case. Poorly differentiated (insular) carcinoma (n=1) showed negative expression of Galectin.

Cytokeratin 19 showed positive staining with good intensity and proportion in all malignancy irrespective of the subtypes. CK19 showed 70% to 90% expression with 2 to 3 plus intensity noticed in all 26/26 cases of carcinoma.

CD56 expression, a negative marker of malignancy negative in 24/26 cases of PTC. One classic PTC and one follicular variant showed score 1 expression. Follicular carcinoma and poorly differentiated carcinoma showed negative expression.

Comparison of percentage immunostaining pattern of three antibodies in benign and malignant cases is shown in [Table/Fig-5]. On comparing the staining patterns in benign and malignant lesions, CK19 showed maximum true positives and maximum false positives. False positivity was lowest with galectin-3. Statistical values of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Diagnostic accuracy and diagnostic Odd's ratio were calculated for single marker and combinations are shown in [Table/Fig-6].

In general, the sensitivity for identifying malignancy for the three markers Galectin-3, CK19 and CD56 was 84.6%, 100%, and 92.3% and specificity were 90.48%, 66.67%, 80.95% respectively. Sensitivity for identifying PTC for the three markers were 91.3%, 100%, and 91.3% respectively and specificity 90.48%, 66.67%, 80.95%. Galectin-3 was found have the highest specificity of

	Galectin-3				CK19				CD56			
		Positive				Positive				Positive		
Benign thyroid neoplasms	Negative	+	2+	3+	Negative	+	2+	3+	Negative	+	2+	3+
Follicular nodular disease	12	-	-	-	11	1	-	-	1		2	9
Follicular adenoma	7	2	-	-	3	6	-	-	3	6	-	-
Total	19	2			14	7			4	17		

[Table/Fig-3]: Expression of Galectin-3, CK19, CD56 in benign thyroid neoplasms (n=21).

	Galectin-3				CK19				CD56			
		Positive				Positive				Positive		
Thyroid neoplasms	Negative	+	2+	3+	Negative	+	2+	3+	Negative	+	2+	3+
Follicular carcinoma	1	1		-	-	-	2	-	2	-	-	-
Classic papillary PTC	-	-	2	14	-	-	1	15	15	1	-	-
Follicular variant PTC	2	5	-	-	-	-	2	5	6	1	-	-
Insular carcinoma	1	-	-	-	-	1	-	-	1	-	-	-
Total	4	22			0	26			24	2		

[Table/Fig-4]: Expression of Galectin-3, CK19, CD56 in malignant thyroid neoplasms (n=26).

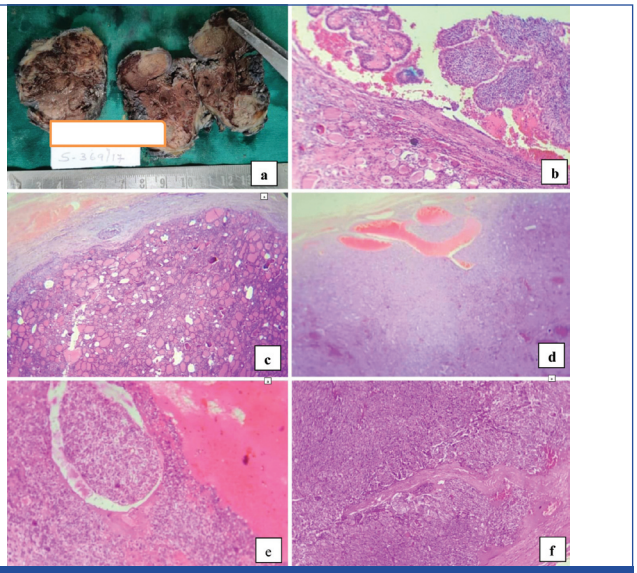
Biomarker	Expression	Benign neoplasms (n=21)	Malignant neoplasms (n=26)	True positive	False positive	False negative	True negative
Galectin-3	Positive	2 (9.52)	22 (84.61)	22	2	4	19
	Negative	19 (90.48)	4 (15.38)				
Ck-19	Positive	7 (33.33)	26 (100)	26	7	0	14
	Negative	14 (66.66)	0				
CD-56*	Positive	17 (80.95)	2 (7.69)	24	4	2	17
	Negative	4 (19.04)	24 (92.3)				

[Table/Fig-5]: Comparison of biomarker expression in benign and malignant of neoplasms (N=47).
*Loss of expression is considered positivity for the negative marker CD 56.

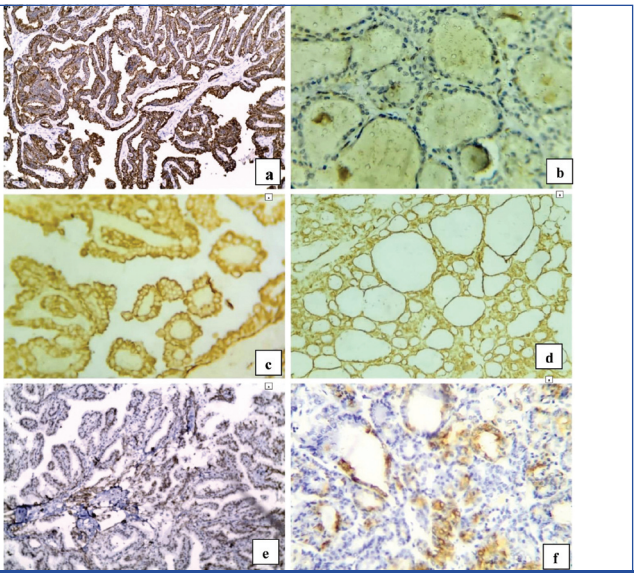
Diagnostic value in differentiating benign vs malignant	Single markers			Panel of immunohistochemistry markers			
	1. Galectin	2. CK19	3. CD56	1 and 2	1 and 3	2 and 3	1, 2 and 3
Sensitivity	84.62%	100%	92.30%	92.30%	88.46%	96.15%	92.3%
Specificity	90.48%	66.67%	80.95%	78.57%	85.71%	73.80%	79.36%
Diagnostic accuracy	87.23%	85.10%	87.23%	86.17%	87.23%	86.17%	86.52%
Positive predictive value	91.66%	78.78%	85.71%	84.21%	88.46%	81.96%	84.7%
Negative predictive value	82.6%	100%	89.47%	89.18%	85.71%	93.93%	89.28%
Positive likelihood ratio (LR+)	8.885	3	4.846	5.33	7.66	4.54	5.53
Negative likelihood ratio (LR-)	0.17	0.02	0.09	0.12	0.16	0.06	0.12
Diagnostic Odd's Ratio (DOR)	2.31	0.05	0.70	0.64	1.27	0.29	0.66

[Table/Fig-6]: Diagnostic values of Galectin-3, CK19, CD56, and panel of markers in differentiating benign from malignant neoplasms comparison to gold standard (N=47).

90.48% in diagnosing PTC and in differentiating malignancy. CK19 was found to be a marker with 100% sensitivity, but with lowest specificity of 66.67%. While using the panel of two markers in combination, galectin-3 and CD56 showed highest specificity and diagnostic accuracy. The panel of three markers in combination showed sensitivity of 92.3% and decreased specificity of 79.36%. Images of neoplastic thyroid lesions and images of biomarker staining in the study are shown in [Table/Fig-7(a-f)] and [Table/Fig-8 (a-f)] respectively.



[Table/Fig-7]: a) PTC with extra thyroid extension; b) PTC. H&E 100X; c) Follicular Adenoma. H&E 100X; d,e) FTC angioinvasion. H&E 100X, 400X; f) Insular carcinoma. H&E 100X.



[Table/Fig-8]: (a) PTC-IHC Gal-3,400X- score3 stain; (b) Follicular Adenoma IHC Gal-3,400X. -score0; (c) PTC. IHC CK19-400X. -score3 stain; (d) Follicular A IHC CK19, 200X. -score1; (e) IHC PTC-CD56,200X- score-0 stain; (f) IHC Follicular A. CD56,400X. score-1.

DISCUSSION

The present study is an evaluation of immunohistochemical profile of 47 neoplastic lesions. In the present study, malignant thyroid lesions were seen maximum in the younger age group of 20-30 years. Mean age of patients in the present study was 41.57 years, while mean age of cancer was 40.76 years. PTC in 23/26 (88.46%) patients was the commonest histologic subtype. Mean size of the tumour was 2.01 cm. Chirayath SR et al., in their study of 944 differentiated cancers of thyroid observed male to female ratio of 1: 2.6, mean age 43.8 years, tumour type was papillary carcinoma in CPTC in 48.3%, FVPTC 28.8%, PTMC 12%, follicular and Hurthle cell carcinoma in 10.1%. Mean size of the tumour was 2.7 cm in their study [20]. The data in present study was statistically significant (p-value <0.005).

Differential Expression Pattern of Three Biomarkers in Comparison to Other Studies

Galectin-3: In the present study, Galectin-3 positivity in malignant and benign neoplasms were 84.61% (23/26) and 9.52% (2/21) respectively (p-value <0.00001). Sensitivity and specificity of 84.62%, 90.48, respectively was observed for Galectin-3 in differentiating malignant from benign neoplastic lesions. Specificity was 91.3% in distinguishing PTC from benign. Galectin-3 was the only marker with diagnostic Odd's ratio >1 (2.31) in the present study indicating a good test performance level.

Huang L et al., reported galectin-3 positive rate of 95.8% in PTC, 93.8% in PTMC, 17.6% in nodular goitre, observing galectin-3 has the potential to be used as a diagnostic marker for thyroid cancer [21]. Saggiorato E et al., observed that galectin-3 had expression in 42/ 42 (100%) PTC, 29/33 FTC specimens [18]. Only 3/50 Follicular adenoma expressed immunopositivity. In discriminating benign and malignant lesions in their study, galectin-3 had sensitivity of 92%, specificity of 94%, diagnostic accuracy 92.8%. Most of the studies conducted on galectin-3 with higher sample size [12,13,16-18,21,22] show staining proportion in the range of 70%-99% in papillary carcinoma, 0-88% in FTC, 3%-40.7% in follicular adenoma and 0-17.6% in benign lesions, establishing the fact that Gal 3 is more useful for PTC. Comparison of Galectin-3 staining in other studies is shown in [Table/Fig-9].

Cytokeratin 19: CK19 reactivity in malignant neoplasms was 100% and 9.52% in benign lesions (p-value <0.00001). In follicular adenoma and FTC, the expression of cytokeratin was weak. FND (11/12) were negative for CK19 staining. We observed higher sensitivity 100% for CK19, and lowest specificity of 66.66% in differentiating malignant from benign neoplastic lesions. Huang L et al., described CK19 expression in 96.7% of PTC, 95.3% of PTMC, 5.9% of Nodular goitre [21]. CK19 expression is indicated to be valuable in the diagnosis of thyroid carcinoma. Saggiorato E et al., observed CK19 had expression in 37/42 cases of PTC, 23/33 FTC, 5/50 follicular adenomas in surgical specimens [18]. In discriminating benign and malignant lesions in their study, CK19 had a sensitivity of 76%, specificity of 90% and diagnostic accuracy was 81.6. Other studies with higher sample size showed CK19

Studies	Papillary thyroid carcinoma	Follicular thyroid carcinoma	Insular carcinoma	Follicular adenoma	*FND/NG/Hyperplastic nodule (benign)
Park YJ et al., (12)	179/181 (99%)	16/25 (64%)	-	1/35 (3%)	3/54 (5.6%NG)
Prasad ML et al., [13]	63/67 (94%)	4/6 (66%)	-	2/19 (10%)	16/88 (18%NG)
Tastekin E et al., [16]	28/40 (70%)	0/20 0	-	0/20 0	0/20 0
Dunđerović D et al., [17]	80/87 (92%)	11/15 (73.3%)	-	11/27 (40.7%)	4/30 (13.3%)
Saggiorato E et al., [18]	41/42 (97.6%)	27/33 (88%)	-	03/50 (6%)	-
Sumana BS et al., [22]	21/23 (91.3%)	½ (50%)	1/1 (100%)	1/17 (6%)	-
Present study (2022)	21/23 (91.3%)	01/2 (50%)	0/01 (0)	02/9 (22.2%)	0/12 0

[Table/Fig-9]: Comparative analysis of Galectin-3 positivity thyroid lesions in other studies.
FND: Follicular nodular disease; NG: Nodular goitre

expression with 72%-97.5% staining in papillary carcinoma, 33%-69.7% in FTC, 10%-28.6% in follicular adenoma and 9.3-40% in benign lesions [12,13,16-18, 21]. Comparison of CK19 staining in other studies is shown in [Table/Fig-10] [12,13,16-18, 21].

Studies	Papillary thyroid carcinoma	Follicular thyroid carcinoma	Insular carcinoma	Follicular adenoma	*FND/NG/Hyperplastic nodule (benign)
Park YJ et al., [12]	175/181 (96.7%)	11/25 (44.0%)	-	10//35 (28.6%)	5/54 (9.3%)
Prasad ML et al., [13]	48/67 (72%)	3/6 (50%)	-	2/19 (10%)	13/88 (15%)
Tastekin E et al., [16]	39/40 (97.5%)	11/20 55%	-	4/20 20%	8/20 40%
Dunđerović D et al., [17]	75/87 (86.2%)	5/15 33.3%	-	6/27 22.2%	11/30 (36.7%)
Saggiorato E et al., [18]	36/42 (85.7%)	23/33 (69.69%)	-	5/50 (10%)	
Huang L et al., [21]	116/120 (95.83%)	-	-	-	2/34 (17.6%)
Present study	23/23 (100%)	2/2 (100%)	1/1 (100%)	6/9 (66.66%)	1/11 (9.09%)

[Table/Fig-10]: Comparative analysis of CK19 positivity thyroid lesions in other studies [12,13,16-18, 21].
FND: Follicular nodular disease; NG: Nodular goitre

Cluster Differentiation 56: In the present study, CD56 expression was seen 80.95% in benign neoplasms, loss of CD56 expression was observed in 92.3%of the malignant group (p-value <0.00001). Previous studies reported high CD56 expression in normal thyroid tissue and benign thyroid follicular lesions [17, 23]. Huang L et al., described CD56 expression was detected in (12/120) of PTC, (3/64) of PTMC, and (33/34) of Nodular Goitre (NG) [21]. Pyo JS et al., in their meta-analysis described loss of CD56 immunohistochemistry expression of 87.8%, 79.1%, 11.9% and 25.5% in PTC, follicular carcinoma, follicular adenoma and in benign follicular nodule respectively [15]. Loss of CD56 expression in malignancy were observed in other studies as well [16, 17, 23]. The results of present study were comparable, CD56 is found to be both sensitive and specific for malignancy and PTC. CD56 staining in other studies are shown in [Table/Fig-11] [15-17, 21, 23].

Studies	Papillary thyroid carcinoma	Follicular thyroid carcinoma	Insular cracinoma	Follicular adenoma	*FND/NG/Hyperplastic nodule (benign)
Pyo JS et al., (meta-analysis) [15]	(87.8% loss) 12.2%	(79.1% loss) 20.9%		(11.9% loss) 88.1%	(25.5% loss) 74.5%
Tastekin E et al., [16]	3/40 7.5%	18/20 90%		18/20 90%	19/20 95%
Dunđerović D et al., [17]	65/87 25.3%	4/15 73.3%		1/27 96.3%	4/30 86.7%
Huang L et al., [21]	12/120 10%	-	-	-	33/34 97.1%
Alshenawy et al., HA [23]	3/22 13.63%	2/15 13.33%		6/7 85.71%	4/5 80%
Present study	2/23 8.69%	0/2 0%	0/1 0%	6/9 66.66%	11/12 91.66%

[Table/Fig-11]: Comparative analysis of CD56 positivity thyroid lesions in other studies [15-17, 21, 23].
FND: Follicular nodular disease; NG: Nodular goitre

Previous studies show a heterogeneity in terms of sample size, comparison group and markers studied. A comparison of results of sensitivity and specificity of three markers in various studies shown in [Table/Fig-12] [12,14,17,23,24]. In differentiating malignancy from benign, Sensitivity of galectin-3 in studies shown varied from 85% to 94.7%, specificity was seen in the range 64.5% to 95.5%. Sensitivity of CK19 varied from 60.7% to 90.3%, specificity in the range of 71% to 83%. Sensitivity of CD56 varied from 58.2% to 81.8% and specificity was seen in the range 63.6% to 92.4%.

Comparative analysis of previous studies in general show that the three markers when used in single show sensitivity and specificity in the range of 80-95%. Results of expression of the marker Galectin-3 in the present study were similar to other studies, with a diagnostic Odd's ratio 2.31. Sensitivity of CK19 and CD56 were found to be high in the present study, with a low specificity for CK19 when compared to data in other studies. Addition of two or three markers in combination decreased specificity in diagnosis. When three markers were used in combination, specificity was found to

Statistical values benign vs malignant	Galectin-3				CK19				CD56			
	Park YJ et al., [12]	de Matos LL et al., [14]	Dunđerović D et al., [17]	Present study	Park YJ et al., [12]	de Matos LL et al., [14]	Dunđerović D et al., [17]	Present study	Dunđerović D et al., [17]	Alshenawy HA [23]	Nechifor-Boilă A et al., [24]	Present study
Sensitivity%	94.7	85	88.52	84.62	90.3	80	60.70	100	58.2	80	81.8	92.3
Specificity%	95.5	90	64.56	90.48	83.1	79	70.89	66.67	92.41	90	63.6	80.95
Diagnostic accuracy%	94.8	-	-	87.23	88.1		-	85.10	-	-	-	87.23
Diagnostic odd's ratio	-	64.18	14.05	2.31	-	30.31	7.46	0. 05	16.93	-	-	0.70

[Table/Fig-12]: Diagnostic values of GAL3, CK19, CD 56-comparison of results in other studies [12,14,17,23,24].

be decreased to 79.3%. Dunđerović D et al., in the meta-analysis concluded that more than one marker could be co-expressed even in benign lesions, which oblige us to continue the quest of searching for more diagnostic markers [17].

Limitation(s)

Major limitations are the small sample size with limited number of lesions in subsets of each category. No correlation with molecular testing was available.

CONCLUSION(S)

Galectin-3 was found to be a reliable marker for thyroid papillary carcinoma and for differentiating malignancy. The panel of Galectin-3 and/or CD56, is valuable and complementary when used in two marker combination with improved sensitivity and specificity in differentiating malignant from benign neoplasms. Immunoreactivity in most of the follicular lesions irrespective of the type makes CK19, equivocal as a diagnostic marker with low specificity in our experience. The usage of biomarkers, adopting standardised techniques and interpretation will help and can complement the morphology diagnosis in ambiguous cases.

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