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Evaluation of Diagnostic Utility of Immunohistochemical Marker CD56 in the Diagnosis of Thyroid Tumors

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KEYWORDS

Follicular adenoma, Papillary thyroid carcinoma, CD56, Follicular carcinoma.

ABSTRACT:

Introduction: Thyroid cancer is the most prevalent endocrine malignancy, with papillary carcinoma being the most common type and the follicular variant being the most common subtype. Hematoxylin and eosin staining is the standard method for diagnosing thyroid nodules, but the overlapping morphological features among follicular lesions can make diagnosis challenging. CD56, a neural cell adhesion molecule typically expressed in thyroid follicular cells, is lost or reduced in papillary, follicular, and anaplastic carcinomas. This study aims to assess the diagnostic value of CD56, an immunohistochemical marker, in differentiating benign and malignant thyroid tumors.

Materials & methods: This retrospective study was carried out in the Department of Pathology at a tertiary care hospital in Kancheepuram over a period of one month. The institutional ethical committee approved the study with reference number MMCH & RI IEC/PG/14/MAY/24. Thirty cases of benign and malignant thyroid tumors were analyzed. Following routine histopathological examination, the expression of CD56 was investigated and statistically evaluated.

Results: Statistical significant difference($P<0.05$) was found between the malignant thyroid tumors and benign lesions with respect to CD56 expression.

Conclusion: CD56 can be used as a valuable immunohistochemical marker in differentiating malignant thyroid tumours from the benign lesions.

INTRODUCTION:

The American Thyroid Association(ATA) defines thyroid nodules as a discrete lesion within the thyroid gland that affects 4-7% of the population. More than 90% of the nodules are benign lesions and 5 to 6.5% of the nodules are due to thyroid carcinoma[1]. There are two types of thyroid nodules: neoplastic and non-neoplastic. Benign neoplastic nodules include follicular adenoma and hurthle cell adenoma. Hyperplastic and inflammatory nodules are included in Non-neoplastic nodules[2]. Thyroid carcinomas develops from follicular cells and give to rise papillary, follicular and anaplastic carcinomas , whereas medullary carcinomas arise from Parafollicular C cells. Papillary carcinomas accounts for about 80% of thyroid carcinomas, follicular carcinomas accounts for 10%, medullary carcinoma constitutes 2-3% and anaplastic carcinoma constitutes 1-2% [1,2] . Papillary carcinoma of thyroid is characterized by the presence of distinct nuclear features such as nuclear enlargement/elongation, nuclear overlapping, nuclear grooving, nuclear membrane irregularity , powdery chromatin and intranuclear inclusions. Some of the variants include follicular(most common), tall cell, cribriform and solid etc., The standard diagnosis of thyroid lesions is based on histomorphological features on routine hematoxylin and eosin stained slides, however due to the presence of overlapping features , it is difficult to differentiate follicular variant of papillary carcinoma, follicular carcinoma and follicular adenoma from each other[3,4]. CD56 is a neural cell adhesion molecule which is normally expressed in thyroid follicular cells, while its expression is lost or reduced in papillary, follicular and anaplastic carcinomas[5]. The objective of this study is to assess the diagnostic value of CD56, an immunohistochemical marker in distinguishing between benign and malignant thyroid tumors and to correlate its findings with clinicopathological parameters.

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MATERIAL AND METHODS:

This study was conducted retrospectively in the Department of Pathology at a tertiary care hospital in Kancheepuram over a one-month period, with approval from the Institutional Ethical Committee (Reference number: MMCH & RI IEC/PG/14/MAY/24). A total of 30 hemithyroidectomy and total thyroidectomy specimens were examined. The study included cases with benign non-neoplastic lesions and benign tumors, such as nodular hyperplasia, autoimmune thyroiditis, follicular adenoma with hurthle cell adenoma. Additionally, malignant tumors, including papillary carcinoma of the thyroid, follicular carcinoma, and medullary thyroid carcinoma, were also evaluated. During our study period, no cases of anaplastic carcinoma were reported at our institute. The patient's clinicopathological information was gathered from medical records and entered into a Microsoft Excel sheet. To determine statistical significance, we used the Chi-square test with SPSS version 22 software, and a "P" value of less than 0.05 was considered statistically significant.

All of the samples were fixed using 10% neutral buffered formalin for 24 hours, and then embedded in paraffin. Four-micron-thick sections were cut and stained with Hematoxylin and Eosin (H&E), and a histopathological diagnosis was given according to the WHO thyroid tumor classification by a pathologist.

Immunohistochemical analysis was conducted on all 30 samples. The 3-micron-thick sections on charged slides were incubated overnight at 37°C, deparaffinized with xylene, and hydrated through two exchanges of absolute alcohol and two exchanges of distilled water. Antigen retrieval was performed with the pressure cooker method using TRIS EDTA buffer for 15 minutes, and the slides were then washed with two changes of distilled water. Three percent hydrogen peroxide was added, followed by an immunewash buffer, and then the primary antibody (CD56 monoclonal antibody supplied by Dako) and polyexcel target binder reagent were added. A secondary antibody (HRP reagent supplied by Dako) was added, followed by an immunewash buffer, DAB chromogen, and a 30-second counterstain with Hematoxylin. Finally, the slides were mounted and underwent immunohistochemical analysis.

According to Park et al., Strong and complete membranous expression of CD56 with or without the cytoplasmic staining of the cells were considered positive[7].

With respect to percentage of tumor cells , the results were expressed in semiquantitative manner. Scoring from 0 to 3 was given based on the percentage of tumor staining[Table 1] . Score 0 was considered as negative and score of 1-3 were considered as positive for CD56. Normal thyroid tissue was taken as a control and stained[17].

TABLE 1: Percentage of tumor cells stained:

SCORE	PERCENTAGE OF TUMOR CELLS STAINED
0	<10%
1+	10-25 %
2+	26-50 %
3+	>50 %

INCLUSION CRITERIA:

All cases of benign and malignant thyroid tumors.

Availability of adequate tissue material in paraffin blocks.

EXCLUSION CRITERIA:

Tissue blocks of preoperative chemotherapy or radiotherapy patients.

Not enough tissue material in paraffin embedded block.

RESULTS:

The expression of CD56 was analyzed in all 30 histopathologically diagnosed cases, along with positive and negative controls. The study comprised 4 cases of hyperplastic nodules, 4 cases of autoimmune thyroiditis, 4 cases of follicular adenomas, 3 cases of Hürthle cell adenomas, 8 cases of papillary carcinomas, 5 cases of follicular carcinomas, and 2 cases of medullary

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carcinomas. Of the 30 cases, 22 were female and 8 were male, with an average age range of 20-65 years.

CD56 expression in benign thyroid cases: Out of 15 benign lesions, CD56 was expressed in 3/4(75%) cases of nodular hyperplasia(Figure 1), 4/4(100%) cases of follicular adenoma(Figure 2), 3/4(75%) , 3/3(100%)cases of hurthle cell adenoma(Figure 3) and 4/4(100%) cases of autoimmune thyroiditis with a overall expression of 14/15(93.3%)cases. Out of 15 cases, only one case had a score of 0, 1 case had a Score of 1+ , 5 cases had a score of 2+ and 8 cases had a score of 3+(Table 2).

TABLE 2: Expression of CD56 in benign thyroid lesions

Type of benign lesions of thyroid	CD56 negative	CD56 Positive			Total
		Score 1+	Score 2+	Score 3+	
Follicular adenoma	0/4 (0%)	1/4 (25%)	1/4 (25%)	2/4 (50%)	4/4 (100%)
Hurthle cell adenoma	0/3 (0%)	0/3 (0%)	1/3 (25%)	2/3 (75%)	3/3 (100%)
Nodular hyperplasia	1/4 (25%)	0/4 (0%)	1/4 (25%)	2/4 (50%)	3/4 (75%)
Autoimmune thyroiditis	0/4 (0%)	0/4 (0%)	2/4 (50%)	2/4 (50%)	4/4 100%
Total	1/15 (6.6%)	1/15 (6.6%)	5/15 (33.3%)	8/15 (53.3%)	14/15 (93.3%)

TABLE 3: Clinicopathological correlation of benign Non-neoplastic lesions/tumors with CD56 expression:

Clinico-pathological data	Parameters	Diagnosis				P Value of CD56
		Follicular adenoma	Hurthle cell adenoma	Autoimmune thyroiditis	Nodular hyperplasia	
Age	<50 years	2 (50%)	1 (25%)	3 (75%)	3 (75%)	0.61
	>50 years	2 (50%)	2 (75%)	1 (25%)	1 (25%)	
Gender	Female	3 (75%)	2 (75%)	4 (100%)	2 (50%)	0.45
	Male	1 (25%)	1 (25%)	0 (0%)	2 (50%)	
Total (15 cases)		4 (100%)	3 (100%)	4 (100%)	4 (100%)	

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CD56 expression in malignant thyroid cases: Out of 15 malignant lesions, CD56 was expressed in 1/8(12.5%) cases of papillary carcinoma of thyroid(Figure 4&5), (1/5(20%) cases of follicular carcinoma(Figure 6), 0/2(0%) cases of medullary carcinoma of thyroid with an overall expression of 2/15(13.3%). Out of 15 cases, 13 cases had a score of 0, 2 cases had a score of 1+, whereas score 2+ and score 3+ was not seen in any malignant lesions. CD56 expression was lost in 13/15(86.7%) cases in malignant thyroid lesions(Table 4).

TABLE 4: Expression of CD56 in Malignant thyroid lesions

Type of malignant lesions of thyroid	CD56 negative	CD56 Positive			Total
		Score 1+	Score 2+	Score 3+	
Papillary thyroid carcinoma	7/8 (87.5%)	1/8 (12.5%)	0/8 (0%)	0/8 (0%)	1/8 (12.5%)
Follicular carcinoma	4/5 (60%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	1/5 (20%)
Medullary thyroid carcinoma	2/2 (100%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
Total	13/15 (86.7%)	2/15 (13.3%)	0/15 (0%)	0/15 (0%)	2/15 (13.3%)

TABLE 5: Clinicopathological correlation of malignant thyroid lesions with CD56 expression:

Clinico-pathological data	Parameters	Diagnosis						P Value OF CD56
		Papillary thyroid carcinoma		Follicular carcinoma		Medullary thyroid carcinoma		
Age	<50 years	n	%	n	%	n	%	0.34
		4	50.0	2	40.0	2	100	
	>50 years	4	50.0	3	60.0	0	0.0	
Gender	Female	5	62.5	5	100	1	50.0	0.24
	Male	3	37.5	0	0.0	1	50.0	
Tumor stage	1	1	12.5	1	20.0	0	0.0	0.82
	2	3	37.5	3	60.0	1	50.0	
	3	4	50.0	1	20.0	1	50.0	
	4	0	0.0	0	0.0	0	0.0	
Lymph node status	Positive	6	75.0	0	0.0	2	100	0.15
	Negative	2	25.0	5	100	0	0.0	
Total (15 cases)		8	100	5	100	2	100	

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Both benign thyroid lesions and malignant thyroid tumors did not show any significant correlation of CD56 expression with respect to the age, gender, tumor staging and lymph node status(Table 3 &5).

The difference in expression of CD56 among benign and malignant thyroid lesions is statistically significant as the p Value is <0.05(Table 6).

TABLE 6: Correlation of cd56 expression of benign with malignant thyroid tumors:

TYPE OF CASES	POSITIVE CD56	NEGATIVE CD56	TOTAL CASES	P VALUE USING CHI-SQUARE TEST
Benign	14(93.3%)	1(6.7%)	15	<0.05
Malignant	2(13.3%)	13(86.7%)	15	
TOTAL	16	14	30	

DISCUSSION:

Follicular variant of papillary thyroid carcinoma is the most common subtype and it is characterized by follicular growth pattern combined with the usual nuclear features of papillary carcinoma of thyroid. Sometimes follicular adenoma which is an encapsulated nodule with a follicular growth pattern may exhibit clear nuclear feature and may cause difficulty in differentiating it from the follicular variant of papillary thyroid carcinoma.

In some cases, hyperplastic nodule may also show papillary pattern making it difficult to distinguish from papillary carcinoma thyroid[6].

To help in the diagnosis of such problematic cases, many immunohistochemical markers were being studied. Some of the markers used in diagnosing and differentiating thyroid lesions are CD56, galectin-3, cytokeratin-19, HBME-1 and claudin etc[14].

CD56 is normally expressed in thyroid follicular cells, NK cells, lymphocytes, activated T cells, neurons, skeletal muscle and ovarian stromal cells[18].

CD56 helps in regulating cell motility and hemophilic binding among the neurons, so its expression will affect the migratory capacity of tumor cells. In some malignant lesions, loss of CD56 expression is correlated with metastatic potential and poor prognosis[12].

According to Demellawy [8], CD56 is a negative marker as its expression is lost in malignant thyroid lesions. Hence evaluating CD56 expression and its loss can be used as an ancillary test for differentiating benign and malignant thyroid lesions.

In our study, we observed that among the 15 benign thyroid lesions, the overall positive expression of CD56 is 93.3% . Shahebrahimi (2013) also noted a similar result of high CD56 positive expression of 92.3% in benign thyroid lesions[15]. Erdogen Durmus et all 2026 also noted a similar result.

Moderate to strong membranous expression with a score of 2+ and 3+ was observed in most of the benign positive cases. Atti(2012) and Alshenawy(2014) also showed a similar report of benign thyroid lesion showing strong and membranous CD56 expression[9,10] . There was no statistical significant difference observed among the benign thyroid lesions (P value = 0.72).

Among the 15 malignant lesions in our study , we found only 13.3% cases showing CD56 expression. Papillary carcinoma of thyroid showed CD56 expression of only 12.5%, followed by follicular carcinoma of 20% and medullary carcinoma of 0%.

Rasha M and Abd EL Atti(2012)., also showed a similar results of papillary carcinoma of thyroid showing low CD56 expression of 17.2%[12,16].

A statistically significant difference (P =<0.05)was also observed between the benign and malignant thyroid tumors . Park(2009) and Demellawy(2008) also showed a similar report of significant statistical

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difference between the benign and malignant tumors[7,8].

In our study, the results showed CD56 is either lost or less expressed in malignant tumors than the benign thyroid lesions , and found out to be the most sensitive negative marker which is in accordance with the previous studies by Nechifor-Boila(2014) and Dunderovic et al(2015)[5,14] .

CD56 was expressed less in papillary carcinoma than the follicular adenoma and follicular carcinoma. Hence CD56 can be used as a valuable marker to differentiate papillary carcinoma from other benign follicular lesions as well as differentiating follicular variant of papillary carcinoma from follicular adenoma in equivocal cases[17].

CONCLUSION:

The use of CD56 immunohistochemical marker in conjunction with histological and cytomorphological features can help overcome diagnostic challenges that arise when differentiating benign follicular lesions, such as follicular adenoma and hyperplastic nodules, from malignant thyroid lesions, particularly the follicular variant of papillary carcinoma and follicular carcinoma. This approach can provide valuable assistance in accurately identifying and distinguishing between these lesions.

LIMITATIONS:

The primary limitation of the study is its small sample size of 30 patients who underwent surgery at a tertiary hospital. Consequently, conducting a study with a larger population at a major center could provide additional insights and details.

CONFLICTS OF INTEREST:

The authors of the study documented that there are no conflicts of interest pertaining the publication of paper.

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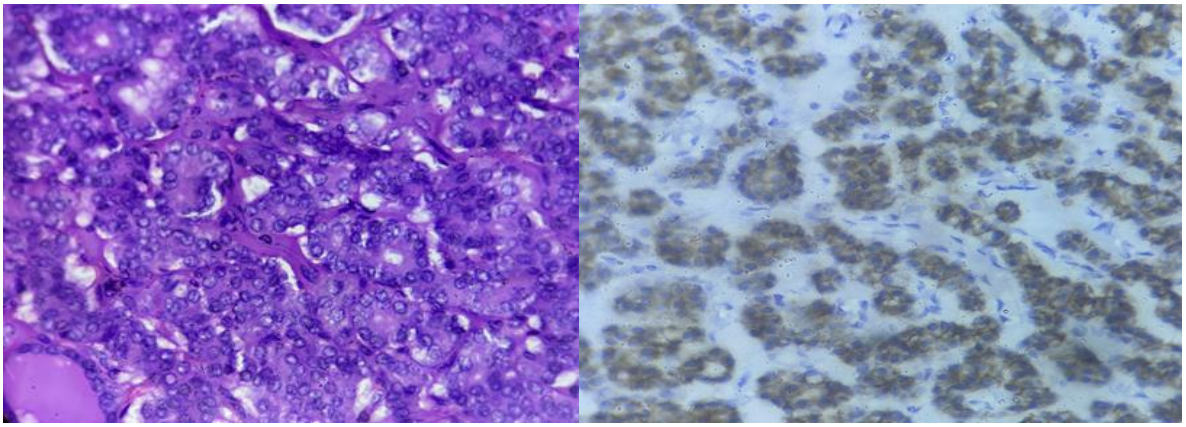

FIGURE 1: Nodular hyperplasia(H&E 40X) showing positive diffuse membranous CD56 expression(3+) (IHC, 40X)

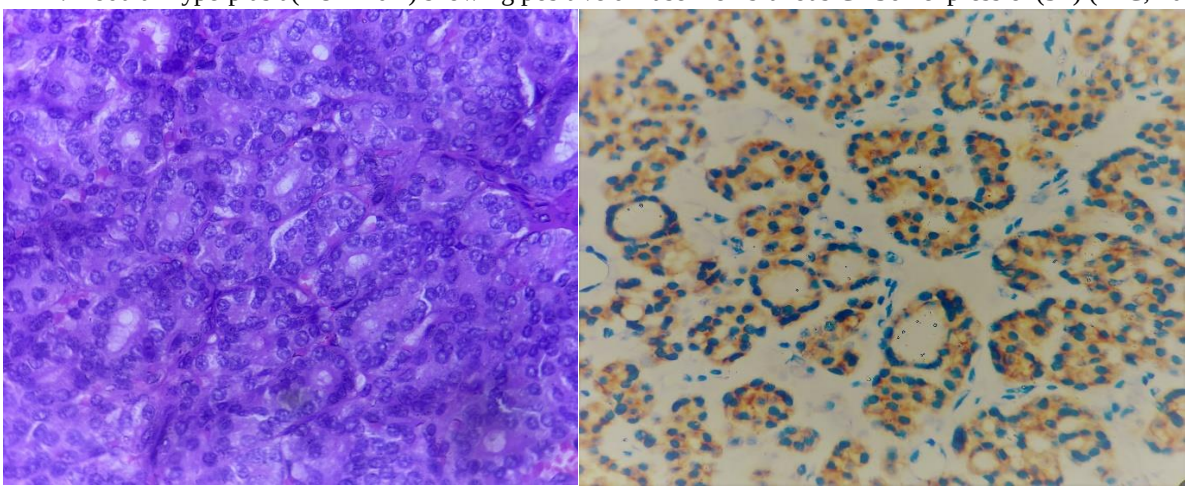

FIGURE 2: Follicular adenoma(H&E 40X) showing positive diffuse membranous CD56 expression(3+) in (IHC, 40X)

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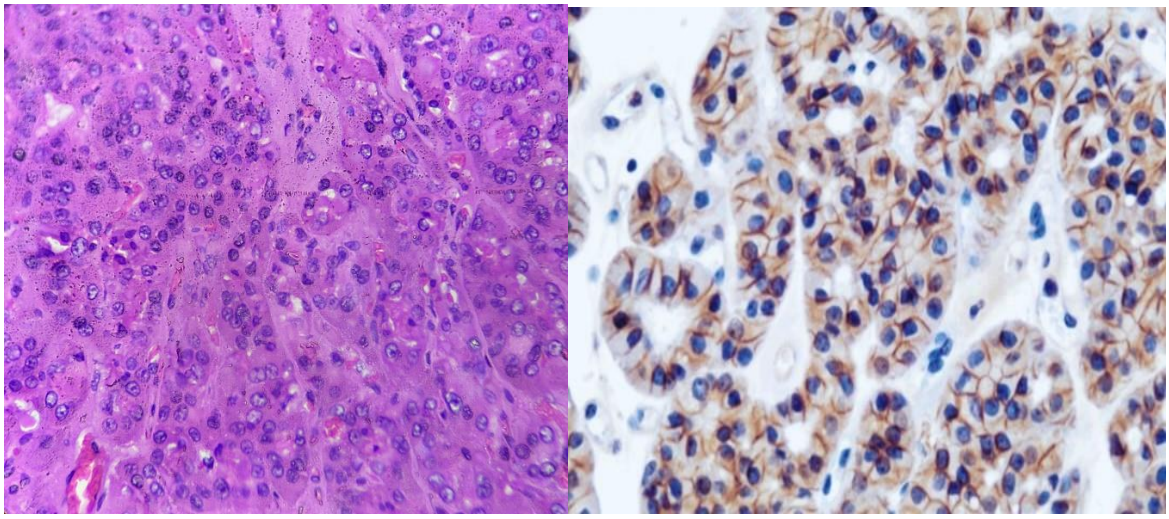

FIGURE 3: Hurthle cell adenoma(H&E 40X) showing positive membranous CD56 expression(2+) in Hurthle cell adenoma

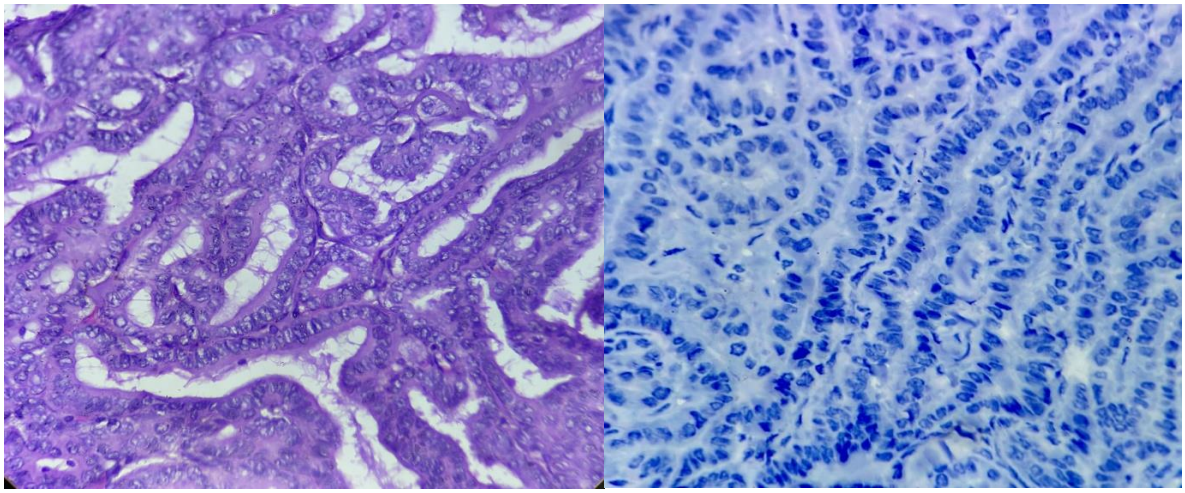

Figure 4: Papillary carcinoma of thyroid showing(H&E 40X) loss of CD56 expression(IHC, 40X)

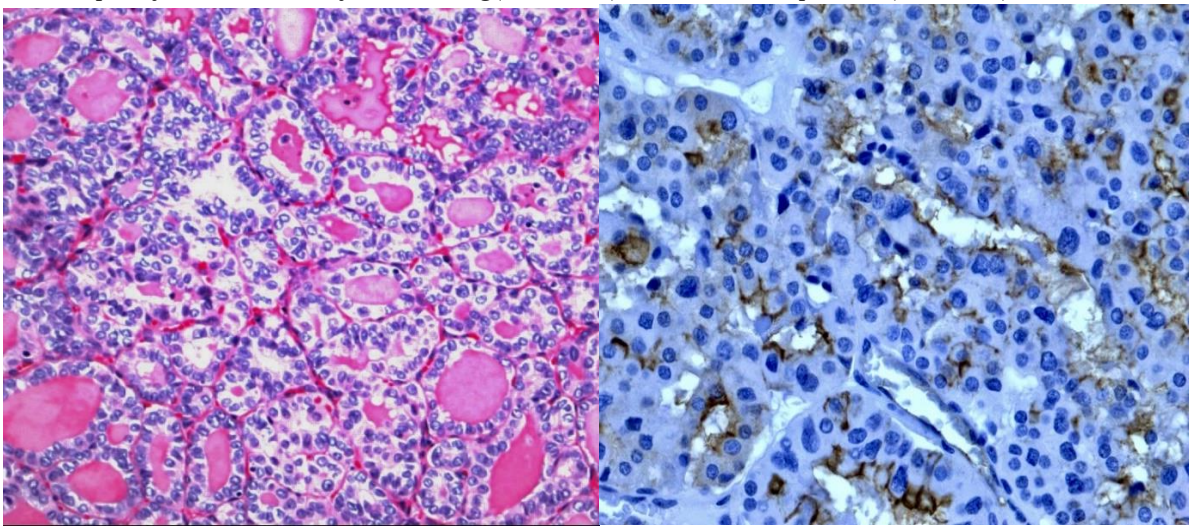

Figure 5: Follicular variant of papillary carcinoma(H&E 40X) showing focal(1+) CD56expression(IHC,40X)

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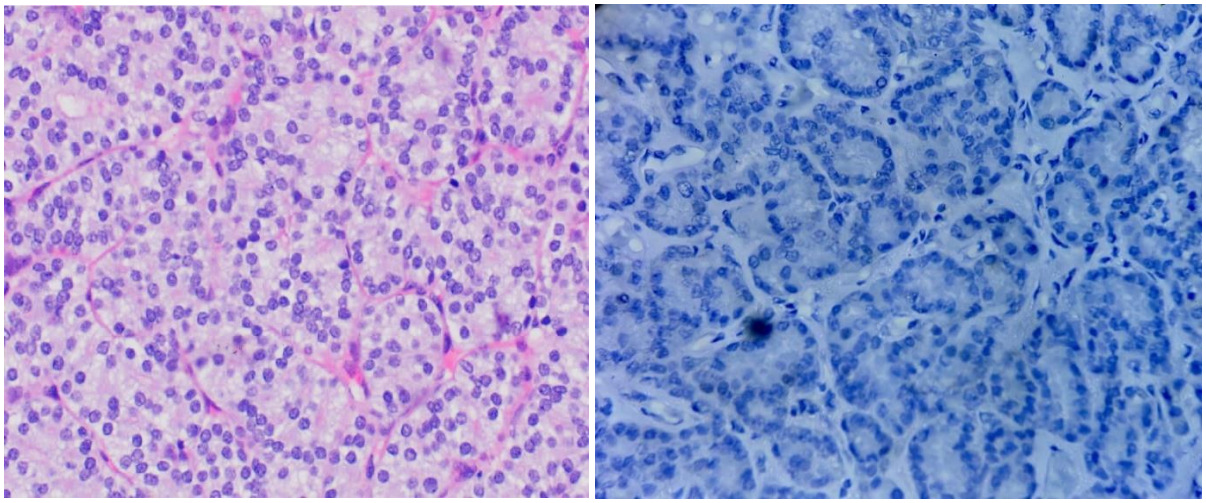

Figure 6: Follicular carcinoma(H&E 40X)showing loss of CD56 expression(IHC,40X)