Original Article

Immunohistochemical Expression of CK19 and CD56 in Differentiating Malignant Neoplasms of Thyroid from Its Benign Mimickers

Tanu Priya[1,*], Monika B Gathwal[1], Swaran Kaur[1], Vijyendra Puri Goswami[1], Chiranjeev Gathwal[2]

[1]Department of Pathology, Bhagat Phool Singh Government Medical College (W), Sonipat, Haryana, India.
[2]Department of Radiology, Bhagat Phool Singh Government Medical College (W), Sonipat, Haryana, India.

***Correspondence:** tanupriyasheoran@gmail.com

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Abstract

Background: Papillary formation is often observed in benign and malignant thyroid diseases, meaning that it is difficult to distinguish between benign and malignant lesions; hence, diagnosis based only on histopathology is often challenging, even in the hands of experienced pathologists. Thus, immunohistochemistry (IHC) is also essential in the diagnosis. This study aims to observe the utility of IHC markers (CK19 and CD56) in thyroid neoplasms.

Methods: This cross-sectional study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khnapur Kalan, Sonipat, Haryana. A total of 55 specimens, including 27 benign neoplasms and 28 malignant neoplasms, were subjected to IHC (CD56 and CK19).

Results: Out of 55 thyroid neoplasm cases, 27 were benign and 28 were malignant. Immunohistochemistry CK19 was positive in 78.5% (22/28) of malignant cases, while negative in 78% (21/27) of benign cases with a significant p-value of <0.001; while immunohistochemistry for CD56 was positive in 92.6% (25/27) of benign cases, and 67.9% (19/28) of malignant cases showed loss of CD56 expression with a highly significant p-value of <0.001. The expression of CK19 was found to have 75% sensitivity and 74.1% specificity, while loss of CD56 expression was found to have 90.4% sensitivity and 73.5% specificity in differentiating malignant from benign thyroid neoplasms.

Conclusion: CK19 is a specific marker for thyroid cancer, especially useful for identifying PTC and FVPTC. CD56 serves as a sensitive marker, with loss of expression indicating malignant potential, enhancing diagnostic accuracy in ambiguous cases.

Keywords: Immunohistochemistry; CK19; CD56; thyroid neoplasms

Introduction

Thyroid swellings are diagnosed in 4%–8% of adults by palpation and in 13%–67% of adults when ultrasound (USG) detection is used. The differential diagnosis of the thyroid nodule includes numerous entities, non-neoplastic and neoplastic, benign and malignant [1]. The benign neoplasms are mainly adenomas. Malignant thyroid neoplasms are mainly carcinomas, accounting for about 1.5% of all cancers. According to Global Cancer Observatory (GLOBOCAN 2022), there were 8,21,214 new cases of thyroid cancer worldwide, and it ranks seventh among the top ten malignancies in the world [2].

Preliminary investigations like USG and fine needle aspiration cytology are important to differentiate among benign and malignant thyroid lesions. But the gold standard diagnostic tool for determining the behaviour of thyroid nodules is histopathological examination [1]. Papillary formation is often observed in benign and malignant thyroid diseases, meaning that it is difficult to distinguish between benign and malignant lesions, and there always exists morphologic overlapping

between FVPTC and other follicular lesions of thyroid; hence, diagnosis based only on histopathology is often challenging, even in the hands of experienced pathologists. Thus, immunohistochemistry (IHC) is also essential in the diagnosis [3].

CK19 has been described with a useful role in the diagnosis of papillary thyroid carcinoma (PTC), as it is synthesized in simple and stratified epithelia with diffuse and strong cytoplasmic staining. It is hypothesized that CK19 expression results from dedifferentiation of tumor cells during malignant transformation, and it has no active role in the steps of carcinogenesis itself [4].

CD56 is present in follicular epithelial cells of normal thyroid tissue. This protein is thought to regulate cell motility and reduces tumor invasion. Loss of CD56 expression was associated with metastatic potential and poor prognostic clinical course [5].

Thyroid neoplasms are a heterogeneous group of tumors that pose significant diagnostic and therapeutic challenges. The accurate classification and diagnosis of thyroid neoplasms are crucial for determining prognosis and guiding treatment decisions. This study aims to observe the utility of IHC markers (CK19 and CD56) in thyroid neoplasms.

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khnapur Kalan, Sonipat, after obtaining approval from the Institutional Ethical Committee (refrence: BPSGMCW/RC891/IEC/23 dated 26/05/2023). It was a retrospective study from January 2022 to October 2024 that included 55 cases of thyroid neoplasms. All proven cases of thyroid neoplasms were included. Cases with history of chemotherapy and radiotherapy and whose blocks could not be retrieved were excluded.

Thyroidectomy specimens were received and then paraffin embedded blocks were made. Blocks of known cases of thyroid neoplasms, along with requisition forms, were retrieved. Patient confidentiality was ensured by deidentifying the data, and patients were assigned a unique numerical code. Additional clinical information and relevant investigations were collected from the case sheets and requisition forms.

Tissue sections were processed in an automated tissue processing unit, and Hematoxylin and Eosin (H&E) sections were prepared for microscopy. A total of 55 specimens, including 27 benign neoplasms and 28 malignant neoplasms, were subjected to IHC for comparison of two biomarker expression. The diagnosis and typing of thyroid pathology was done as per the World Health Organisation (WHO) classification, 2022 guidelines of thyroid neoplasms.

IHC staining scores were given based on the proportion of cells staining positive and on the intensity of staining. CK19 expression was considered positive when cells demonstrate brown cytoplasmic staining; the proportion of positivity in terms of percentage of cell showing staining was expressed as scores from 0 ($<$5% of the cells), +1 (5–30% of the cells), +2 (31–69% of the cells), +3 ($>$70% of the cells). The scoring of positively stained CD56 expressions were done as follows: 0 ($<$10% of the cells), +1 (11–25% of the cells), +2 (26–50% of the cells), +3 ($>$50% of the cells). The staining intensity for both the immunostains was graded as follows: 0 (negative staining), +1 (slight staining), +2 (diffuse staining), +3 (strong staining) [6].

Two independent pathologists performed the scoring. Blinding was done, and the final score was the mean aggregate of both the scores done by pathologists.

Statistical Methodology: Mean and standard deviation were calculated for a quantitative data. Percentage and proportion were calculated for qualitative data. Sensitivity and specificity were calculated for CK19 and CD56. Chi square test and Fischer's exact test were used for association between CK19, CD56, and thyroid neoplastic lesions using Stastical Package for the Social Sciences (SPSS) software version 26. A p value <0.05 was considered as statistically significant.

Results

This study was conducted on 55 cases of thyroid neoplasms. Gender-wise distribution of cases in this study showed an overall predominance of females (48, 87.4%) in thyroid neoplasms, with a male to female ratio of 6.8 times. Herein, in the present study, out of 55 cases, the mean age of the study subjects is 36.42$\pm$13.56 years, with a minimum and maximum age of 9 years and 68 years, respectively. Remarkably, most of the cases (n=21; 38.2%) involved young people, with the majority being in the 21–30 years of age group. The number of cases in benign (27 cases) and malignant neoplasms (28 cases) were almost same, with a ratio of 1:1.04.

Out of 27 cases of benign neoplasms, 23 cases were of follicular thyroid adenoma and 4 cases belonged to oncocytic adenoma. The malignant type neoplasm included 28 cases, which was further divided into 15 cases of PTC, 6 cases of follicular variant of papillary thyroid carcinoma (FVPTC), 4 cases of follicular thyroid carcinoma, 1 case of oncocytic carcinoma, and 2 cases of medullary carcinoma of thyroid. Out of 15 PTC cases, 13 were classical PTC, one was columnar

cell variant of PTC, and one was warthin tumor like variant of PTC.

CK19 was positive in 78.5% (22/28) of malignant cases, while 78% (21/27) of benign cases showed negative CK19 expression with a significant p-value of <0.001. CK19 showed +2 and +3 intensity in 19/28 (67.9%) cases of carcinoma. (Table 1)

CD56 was positive in 92.6% (25/27) of benign cases, while 67.9% (19/28) of malignant cases showed loss of CD56 expression with a highly significant p-value of <0.001. CD56, a negative marker of malignancy, was negative in 19/28 (67.9%) of carcinomas, while it showed +2 and +3 intensity in 22/27 (81.4%) cases of adenoma. (Table 1)

The expression of CK19 was found to have 75% sensitivity and 74.1% specificity in differentiating malignant from benign thyroid neoplasms, with a diagnostic accuracy of 74.5%. The loss of CD56 expression was found to have 90.4% sensitivity and 73.5% specificity in detecting malignant thyroid neoplasms, with a diagnostic accuracy of 80%.

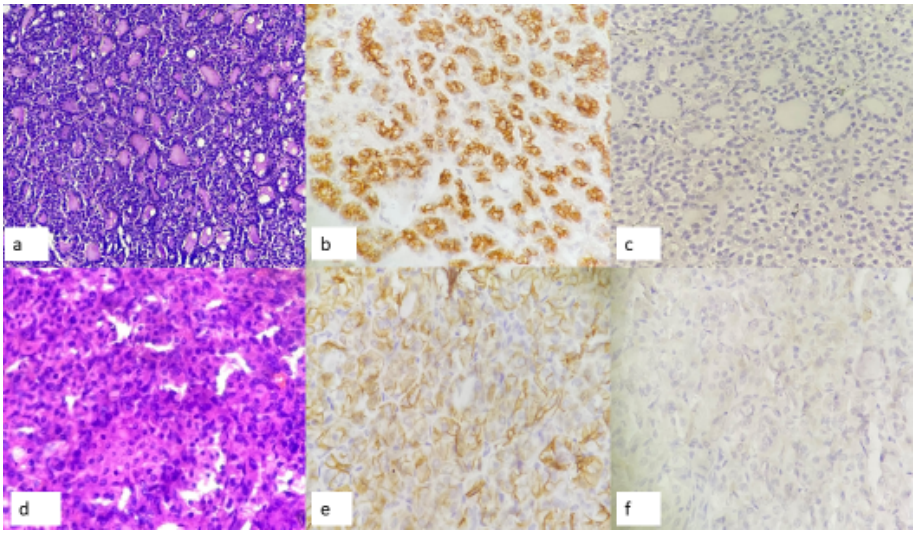

Figure 1: Benign thyroid neoplasms: a) Follicular adenoma showing microfollicular pattern [H&E, 400X], b) Positive CD56 (+3) expression in follicular adenoma [400X], c) Negative CK19 expression in follicular adenoma [400X] d) Oncocytic adenoma showing abundant eosinophilic cytoplasm [H&E, 400X], e) Positive CD56 immunostain in oncocytic adenoma [400X], f) Negative CK19 immunostain in oncocytic adenoma [400X].

Table 1: Association of CK19 and CD56 expression with thyroid neoplasms.

Histopathological diagnosis (n)	**CK19 expression**				**CD56 expression**			
	0	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**
Benign								
Follicular adenoma (23)	18	02	02	01	02	02	07	12
Oncocytic adenoma (04)	03	01	00	00	00	01	01	02
Total (27)	**21**	**–**	**–**	**06**	**02**	**–**	**–**	**25**
Malignant								
PTC (15)	02	00	04	09	15	00	00	00
FVPTC (06)	01	01	02	02	03	02	01	00
Follicular carcinoma (04)	01	01	01	01	00	01	01	02
Oncocytic carcinoma (01)	00	01	00	00	00	00	00	01
Medullary carcinoma (02)	02	00	00	00	01	00	00	01
Total (28)	**06**	**–**	**–**	**22**	**19**	**–**	**–**	**09**

Discussion

Histopathological examination is the gold standard diagnostic tool for determining the behaviour of thyroid nodules. For decades, the diagnosis of PTC has relied on identifying nuclear features and papillary architecture. However, these features alone are sometimes insufficient for a definitive diagnosis, as certain benign lesions can exhibit nuclear characteristics similar to PTC. Additionally, follicular-patterned thyroid tumors often pose diagnostic challenges, primarily due to the difficulties pathologists face in evaluating these structures. Key challenges include determining the presence or absence of capsular invasion and differentiating lesions with PTC-like nuclear features but without papillary architecture, which complicates

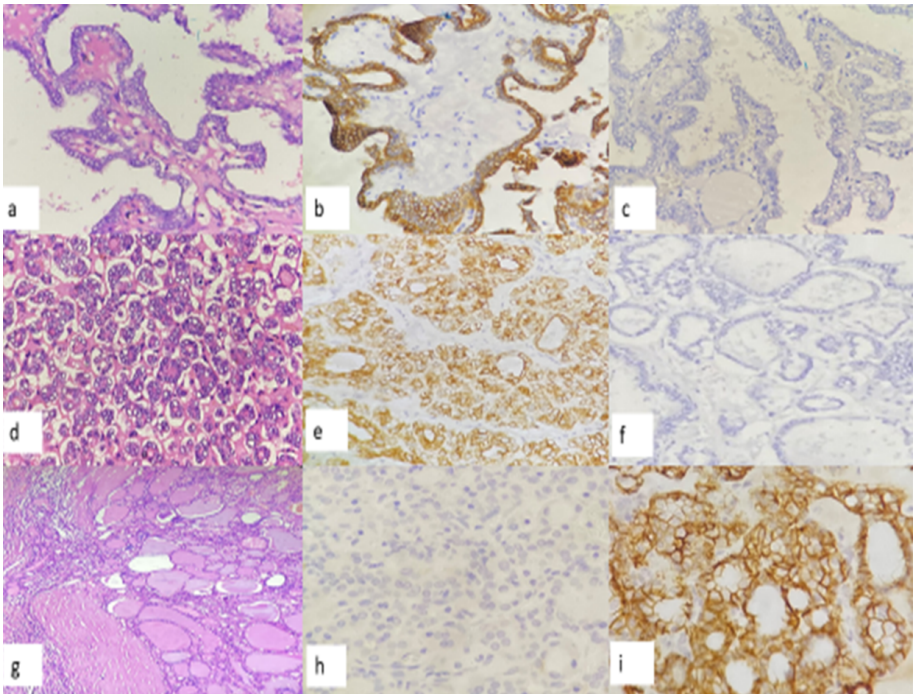

Figure 2: Malignant thyroid neoplasms: a) Papillary thyroid carcinoma with optically clear nuclei [H&E, 400X], b) Positive CK19 (+3) expression in PTC [400X], c) Negative CD56 expression in PTC [400X], d) Follicular variant of papillary thyroid carcinoma with papillary like nuclear features [H&E, 100X], e) Positive CK19 (+3) expression in FVPTC [400X], f) Negative CD56 expression in FVPTC [400X], g) Minimally invasive follicular carcinoma [H&E, 100X], h) Negative CK19 expression in follicular carcinoma [400X], f) Positive CD56 (+3) expression in follicular carcinoma [400X].

Table 2: Comparative analysis of CK19 and CD56 positivity in benign and malignant thyroid neoplasms with previous studies.

Studies	Sample size	CK19 expression		CD56 expression	
		Benign cases	Malignant cases	Benign cases	Malignant cases
Dundervoic D et al. [12]	201	29.1%	75.4%	92.4%	41.8%
Dalal N et al. [9]	24	41.17%	85.7%	58.8%	28.57%
Kammal WS et al. [10]	128	36.92%	81.48%	-	-
Tastekin E et al. [5]	120	30%	83.3%	92.5%	26.5%
Chandrakumari AS et al. [6]	44	27.28%	81%	94.44%	27.02%
Raman J et al. [8]	47	33.3%	100%	33.3%	7.6%
Priyadarshni P et al. [11]	36	11.76%	78.95%	82.35%	21%
Present study	**55**	**22%**	**78.5%**	**92.6%**	**32.1%**

Table 3: Comparative analysis of diagnostic value of CK19 in benign and malignant thyroid neoplasms with previous studies.

Studies	Positive CK19 expression				
	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
Dundervoic D et al. [12]	60.70%	70.89%	80%	65.12%	-
Dalal N et al. [9]	71.4%	100%	100%	94.70%	-
Kammal WS et al. [10]	81.48%	-	-	-	-
Chandrakumari AS et al. [6]	90%	88.9%	81.8%	94.8%	89.3%
Raman J et al. [8]	100%	66.67%	78.78%	85.17%	85.10%
Priyadarshni P et al. [11]	88%	79%	-	-	-
Present study	**75%**	**74.1%**	**75%**	**74.1%**	**74.5%**

distinguishing FVPTC from adenomatous goiter. As a result, application of IHC markers in the diagnosis of PTC and its mimics is a great yield in the field of diagnostic pathology.

Gender-wise distribution of cases in this study showed an overall predominance of females in thyroid neoplasms, with a male to female ratio of 6.8 times. Studies from various regions worldwide, including Zughaibi I et al. [7], Raman J et al. [8], Tastekin E et al. [5], Dalal N et al. [9], and Chandrakumari AS et al. [6] have consistently shown a higher prevalence of

Table 4: Comparative analysis of diagnostic value of CD56 in benign and malignant thyroid neoplasms with previous studies.

Studies	Loss of CD56 expression				
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Diagnostic accuracy**
Dundervoic D et al. [12]	58.2%	92.41%	92.21%	58.87%	-
Dalal N et al. [9]	71.4%	80.60%	41.70%	93.50%	-
Chandrakumari AS et al. [6]	80%	94.4%	88.9%	89.5%	89.3%
Raman J et al. [8]	92.3%	80.95%	100%	85.71%	87.23%
Priyadarshni P et al. [11]	82%	74%	-	-	-
Present study	**90.4%**	**73.5%**	**67.8%**	**92.6%**	**80%**

thyroid neoplasm in females compared to males.

CK19 was positive in 78.5% (22/28) of malignant cases and 22% (6/27) of benign cases. Raman J et al. [8] showed CK19 positive expression in all 26/26 (100%) cases of carcinoma. Chandrakumari AS et al. [6], Kammal WS et al. [10], Tastekin E et al. [5], and Dalal N et al. [9] showed CK19 expression in 81%, 81.48%, 83.3%, and 85.7% of malignant cases, respectively. (Table 2)

Priyadarshni P et al. [11], Dundervoic D et al. [12], Tastekin E et al. [5], and Chandrakumari AS et al. [6] described CD56 expression in 82.35%, 92.4%, 92.5%, and 94.4% of benign neoplasms, respectively. Our study also showed CD56 expression in benign cases (92.6%), which is in comparison with other studies. In terms of loss of CD56 expression in thyroid tumors, our study found that it is more common in malignant tumors than benign tumors, which is statistically significant. Inferences made in the studies by Dundervoic D et al. [12], Dalal N et al. [9], Tastekin E et al. [5], Chandrakumari AS et al. [6], Raman J et al. [8], and Priyadarshni P et al. [11] are similar. (Table 2)

In the present study, CK19 expression was seen in 13/15 (86.6%) of PTC, 5/6 (83.3%) of FVPTC, and 3/4 (75%) of FCa. In follicular adenoma and oncocytic adenoma, the expression of CK19 was weak, constituting 5/23 (21.7%) and 1/4 (25%), respectively. Priyadarshni P et al. [11] described CK19 expression in 92.3% of PTC, 66.6% of FCa, and only in 11.76% cases of follicular adenoma. Tastekin E et al. [5] described CK19 expression in 100% of PTC, 55% of FCa, and only in 20% cases of follicular adenoma.

In this study, there were two cases of medullary carcinoma which were CK19-negative. Similarly, Priyadarshni P et al. [11] showed CK19 negativity in medullary carcinoma, but a study conducted by Kammal WS et al. [10] showed 83.3% of medullary thyroid carcinoma cases with positive CK19 expression.

In our study, CD56 expression was seen in 91.3% (21/23) cases of follicular adenoma and 100% (4/4) of oncocytic adenoma cases, while PTC showed loss of CD56 expression in all 15/15 (100%) cases. Three out of six cases (50%) of FVPTC showed positive CD56 immunostain. One out of two cases (50%) of medullary carcinoma and one case of oncocytic carcinoma included which showed +3 positive CD56 immunostaining.

Dundervoic D et al. [12], Tastekin E et al. [5], and Chandrakumari AS et al. [6] showed 96.30%, 90%, and 94.4% CD56 positivity in follicular adenoma, respectively. Dundervoic D et al. [12] showed CD56 expression in 90% of oncocytic adenoma cases, which is in concordance with the present study.

In the current study, CD56 was seen in 4/4 (100%) FCa cases, which is in concordance with Dalal N et al. [9], Tastekin E et al. [5], and Muthusamy et al. [13], while Raman J et al. [8] and Priyadarshni P et al. [11] showed CD56 positivity in 0% and 33.3% of FCa cases.

Sensitivity and specificity of CK19 in differentiating malignant from benign thyroid neoplasms was 75% and 74.1%, respectively. There was a wide range of sensitivity and specificity reported in various studies. It varies from 60.70% to 100% for sensitivity and 70.89% to 100% for specificity, which is in amalgamation with the present study. In our study, PPV was 75%, which showed similarity with Dundervoic D et al. [12], Priyadarshni P et al. [11], and Raman J et al. [8]. An NPV of 74.1% was obtained, which was in concordance with the other studies. The literature showed diagnostic accuracy of CK19 in differentiating malignant from benign thyroid neoplasm ranges from 85.10% to 89.3%. Our study also showed a diagnostic accuracy of 74.5%. (Table 3)

Sensitivity and specificity of loss of CD56 expression in differentiating malignant from benign thyroid neoplasms was 90.4% and 73.5%, respectively. Sensitivity and specificity of various studies ranges from 58.2% to 92.3% and 74% to 94.4%, respectively, which were similar with our study. In the present study, PPV was 67.8%, which showed similarity with Chandrakumari AS et al. [6], while Dalal N et al. [9] showed low PPV (41.70%). An NPV of 92.6% was obtained, which showed similarity with Dalal N et al. [9] (93.50%) and Chandrakumari AS et al. [6] (89.5%). The literature showed diagnostic accuracy of loss of CD56 expression in differentiating malignant from benign thyroid neoplasm ranges from

87.23% to 89.3%. Our study also showed a diagnostic accuracy of 80%. (Table 4)

Conclusion

Routine use of CK19 and CD56 IHC markers can aid in distinguishing malignant from benign thyroid neoplasms. CK19 is a specific marker for thyroid cancer, especially useful for identifying PTC and FVPTC. CD56 serves as a sensitive marker, with loss of expression indicating malignant potential, enhancing diagnostic accuracy in ambiguous cases. Overall, integrating CK19 and CD56 IHC used for diagnostic practice can improve the accuracy of thyroid cancer diagnoses, particularly in cases where histopathological features alone are inconclusive.

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