Role of CD56 and Cytokeratin 19 in the diagnosis of Papillary and Follicular Neoplasms of Thyroid

Abilash Sasidharannair Chandrakumari[1], Pammy Sinha[2]

1.   Research Scholar
Bharath Institute of Higher Education and research
2.   Professor of Pathology
Bharath Institute of Higher Education and research

Abstract

Background: Neoplasms of thyroid are the commonest type of the endocrine neoplasms across the globe. Papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid neoplasms. In spite of extensive studies in the understanding of thyroid neoplasms, there are great concerns about the definite diagnosis of thyroid neoplasm. Morphological overlapping always exists between follicular patterned thyroid neoplasms. This study is carried out to evaluate the applicability of CD56 and CK19 expressions in discriminating the PTC including the follicular variant from other follicular thyroid neoplasms. **Materials & Methods:** This cross-sectional study was conducted in a tertiary hospital for a period of one year. A total of 55 surgically removed thyroidectomy cases including follicular adenoma (FA), follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC) were subjected to immunohistochemical staining. **Results:** PTC showed strong and diffuse staining of CK19 expression and loss of CD56 staining in in 87.5% and 81.25% of cases respectively. Follicular Carcinoma (FC) showed strong and diffuse staining of CD56 in 80% cases.

Keywords: CD56, CK19, Papillary Thyroid Carcinoma, Follicular Neoplasm, Immunohistochemistry.

1.   INTRODUCTION

Thyroid nodules are significantly common worldwide and are usually detected during routine clinical examination. Among adult populations the prevalence of palpable thyroid nodules constitute about 4–7%. [1, 2] Neoplasms of thyroid are the commonest type of the endocrine neoplasms and it constitute about 1% of overall malignancies. Among the malignant neoplasms of thyroid, PTC constitutes about 80%. [2] At present, in spite of extensive studies in the understanding of thyroid neoplasms, there are great concerns about the definite diagnosis of thyroid neoplasm. The gold standard tool for the diagnosis of thyroid neoplasm is the histopathological examination by using the hematoxylin and eosin (H&E) stain. The wide variety of the histological subtypes and aggressiveness of the neoplasm often complicate the diagnosis. There always exists morphological overlapping between follicular

variant papillary thyroid carcinoma (FVPTC) and other follicular lesions of thyroid; hence diagnosis based only on histopathology is often impractical and challenging even in the hands of experienced pathologists. [2, 3] Consequently, there are cases where the present histopathological criteria do not allow clear distinction between benign and malignant follicular-patterned thyroid lesions, thus making the diagnosis between these two groups are quite subtle and challenging. [4-6]

Several immunohistochemical markers viz Hector Battifora Mesothelial Cell-1 (HBME-1), Galectin-3 (Gal-3), Cytokeratin-19 (CK19) etc. have been examined along the time in differentiating follicular patterned thyroid neoplasms. Even though these markers have shown wide range of sensitivity and specificity values none of them either as single or in combination gave conclusive results. [4]

CD56 is a glycoprotein representing neural cell adhesion molecule which is expressed in neural tissue, natural killer (NK) cells, activated T-lymphocyte. Recently, studies have shown that CD56 expression is detected in the follicular cells of normal and benign thyroid lesions, its expression is lost in all thyroid carcinomas except PTC. [3, 7]

The aim of the current study is to evaluate the applicability of CD56 and CK19 expressions in discriminating the PTC including the follicular variant from other follicular thyroid neoplasms. Vast majority of the studies have examined the expression of these markers separately, only very few studies have mentioned about the combined expression of these two markers and they also failed to put forth conclusive and conforming results. Hence the current study has been taken up to back the literature with our results and attempt to make a significant contribution to the current practice.

II. MATERIALS & METHODS

The study was a cross-sectional study carried out in the pathology department of a tertiary care hospital over a period of one year from August 2016 to July 2017. After obtaining approval from Institutional ethical committee, a total of 55 surgically removed, formalin-fixed thyroid specimens were studied which included FA and its variants, FTC, PTC and its variants. Thyroidectomy specimens of patients with thyroiditis, colloid goitre, medullary thyroid carcinoma, anaplastic thyroid carcinomas and metastatic thyroid carcinomas were excluded from the study. After thorough gross examination, the specimens were processed by routine histopathological techniques stained by using hematoxylin and

eosin (H&E) stain, subjected to histopathological evaluation by the pathologists and final diagnosis were recorded separately for each case.

Immunohistochemistry

All the 55 samples were subjected to immunohistochemical (IHC) staining with CK19 (Clone RCK108; Dako), and CD56 (Clone 1B6; Novocastra) antibodies. The sections were deparaffinised by treating with xylene and were rehydrated through graded alcohol. Antigen retrieval was done by using trisodium citrate, peroxidase block was done. Sections were washed in distilled water for 5minutes and washed in three changes of tris-buffered saline (TBS) for two minutes. Primary antibodies for CK19 and CD56 were applied to sections and incubated for one hour at 37°c. Secondary enhancers (diluted biotinylated secondary antibody) for 20 min, followed by super enhancers (tertiary) was applied for another 30 min. Finally one drop of DAB chromogen solution was applied and kept for 10 min then counterstained with Harris hematoxylin followed by dehydration, clearing, and mounting

Scoring Criteria for CD56 and cytokeratin 19

The results of immunohistochemical staining were evaluated and semi quantitative scoring was done as positive and negative based on the membranous and cytoplasmic staining property of CK19 and CD56. CK19 expression was considered as positive when cells demonstrate brown cytoplasmic staining, the proportion of positivity in terms of percentage of cell showing cytoplasmic 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). CD56 expression was considered as positive when cells showed membranous immunoreactivity. 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 grades as follows 0(negative staining), +1(slight staining), +2(diffuse staining), +3 (Strong staining).

Colonic mucosal and normal thyroid tissue were used as positive control for staining CK19 & CD56 respectively.

Statistical analysis

Data analysis was carried out by using IBM SPSS program version 21. Descriptive statistics have been shown in the form of arithmetic mean and the nominal variables have been shown as the number of cases and percentage (%). Pearson Chi-square test was applied to compare whether a significant difference exists between different variables. P value less than 0.05 was considered as statistically significant

III. RESULTS

The study consisted of 32 cases (58.18%) of PTC, which included 22 cases (68.75%) of Classical Papillary Thyroid Carcinoma (CPTC) and 10 cases (31.25%) of Follicular Variant of Papillary Thyroid Carcinoma (FV PTC), 18 cases (30%) of follicular adenomas which included two cases of Hurthle cell Adenoma (HC FA) and five cases of follicular thyroid carcinomas ([Table 1]. The ages of the patients were between 24 and 76 years, and the arithmetic mean of age for neoplastic lesions 43.38 (SD 12.97), arithmetic mean of age for benign and malignant cases were found be 38.8 (SD±7.57) and 45.34 (SD±14.34) respectively.

In the study, 80% ($n = 44$) were found to be female patients and 20 % of patients ($n = 11$) were male. Among both benign and malignant groups, female gender showed higher proportion, but there was no significant difference between the two groups in terms of gender.

About the 32 cases of PTC, 25% (n=8) and 9.37% (n=3) showed multifocality and extrathyroidal extension respectively, the tumour was encapsulated in 65.62% (n=21) and capsular invasion was noted in 34.38%. [fig 1]
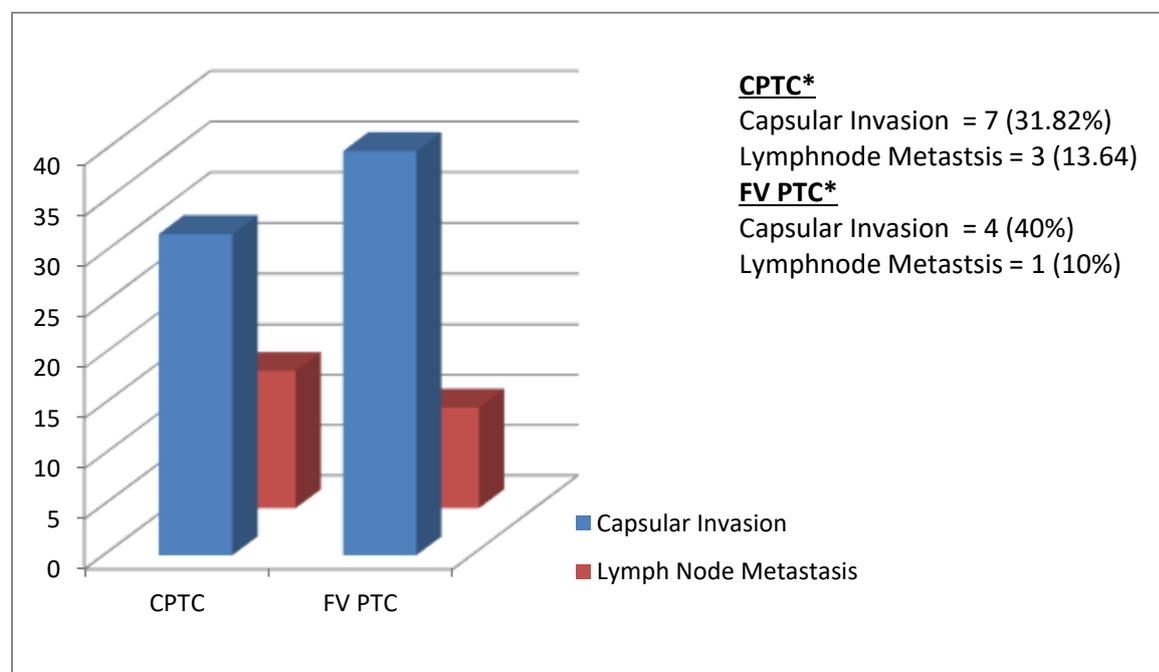
CPTC*
Capsular Invasion = 7 (31.82%)
Lymphnode Metastsis = 3 (13.64)
FV PTC*
Capsular Invasion = 4 (40%)
Lymphnode Metastsis = 1 (10%)

CPTC: Classical Papillary Thyroid Carcinoma
FVPTC: Follicular Variant of Papillary Thyroid Carcinoma
Figure 1: Proportion of Capsular invasion and Lymphnode metastasis in CPTC & FVPTC

Immunohistochemical results

CK19. Among the PTC cases, strong and diffuse staining of CK19 was observed in 87.5% (n=28) and loss/slight staining of CK19 expression was noticed in 94.44% (n=17) cases of FA. All cases of FC showed negative expression with CK19 [Table 1&2]

Assessment of CD56 staining in the 32 PTC cases showed negative CD56 expression in 28 cases (87.5%). Among the 18 cases of FA except one case all 17 cases (94.44%) showed strong positive staining with CD56. Between benign and malignant groups, there found a significant difference for percentages of CK19, and CD56 staining with Chi-square test ($P <$ 0.001). [Table 1&2]

Lesions	No. of cases	CK19 +ve		CD56 +ve	
		n	%	n	%
Malignant	37				
CPTC	22	21	95.45%	04	18.18%
FVPTC	10	09	90.00%	02	20.00%
FC	05	00	00.00%	4	80.00%
Benign					
FA, HC FA	18	5	27.78%	17	94.44%

Table 1: Expression of CK 19 and CD56 in papillary thyroid carcinoma and benign lesion

Marker	Negative Response n (%)	+1 (%)	+2 (%)	+3 (%)	Total (%)	
CK19						
CPTC (n=22)	1 (4.55%)	1 (4.55%)	5 (22.72%)	15 (68.18%)	22(100%)	<0.001
FVPTC	1 (10%)	1 (10%)	4 (40%)	4 (40%)	10 (100%)	
(n=10)	5 (100%)	0	0	0	5 (100%)	
FC (n=05)	13 (72.22%)	4 (22.22%)	1 (5.56%)	0	18 (100%	
FA (n=18)						
CD56						
CPTC (n=22)	18 (81.82%)	3 (13.64%)	1 (4.54%)	0	22 (100%)	<0.001
FVPTC	8 ((80%)	1 (20%)	1 (20%)	0	10 (100%)	
(n=10)	1 (20%)	0	1 (20%)	3 (60%)	5 (100%)	
FC (n=05)	1 (5.56%)	0	4 (22.22%)	13 (72.22%)	18 (100%)	
FA (n=18)						

CK19 = Cytokeratin 19, CPTC= Classical Papillary Thyroid Carcinoma, FVPTC= Follicular Variant of Papillary Thyroid Carcinoma

Table 2: Scores of different thyroid lesions in terms of intensity for CK 19 and CD56 staining

IV. DISCUSSION

The gold standard diagnostic tool for determining the behaviour of thyroid nodules is histopathological examination. Since decades, the histopathological diagnosis of PTC is made based on nuclear features and papillary architecture. At times identification with these features alone will not be sufficient to confirm the diagnosis, because few benign lesions often exhibit nuclear features similar to PTC. Furthermore follicular Patterned thyroid tumours often create diagnostic dilemma, this is mainly because of challenges the pathologist come across while examining the follicular patterned tumours one of the main challenges involved is in determining the presence or absence of capsular invasion and another one is when such lesions displaying nuclear features of PTC without papillary architecture making it extremely challenging to discriminate FVPTC from adenomatous goitre. As a result application of immunohistochemical markers in the diagnosis of PTC and its mimics is a great yield in the field of diagnostic pathology. [8-10]

CK19: In the study, out of 37 malignant cases 30 cases (80.1%) showed positivity this finding matches with the study result by *Sanuvada R et al*. [11] 87.5% (n=28) of PTC and its variants showed diffuse and strong positivity for CK19 staining, these findings equate to the results demonstrated by *Abouhashem NS* [12] and *Calangiu et al* [13]. It was also proved in the study that 80% of FVPTC showed strong & diffuse CK19 positivity (>2+), the results were close to the findings reported by *Cheung CC et al* [14] and *Sahoo et al* [15]. None of the FC showed positivity for CK19, this finding is in concordance with other studies in the literature [11, 16], while the study done by Matos *et al* [17]. demonstrated 21% positivity among FC cases. Among the benign lesions the study showed positivity of 27.78% among FA the results are close to the study findings of Matos *et al* [17]

CD56: In the study loss of CD56 expression was observed in 72.97% (n=27) of malignant lesions. Absent or decreased CD56 expression was detected in 30 cases (93.75%) of PTC and diffuse expression was detected in 2 cases (6.25%) of PTC. The findings were in accord with the results demonstrated by other similar studies in the literature. [18, 19] Among the benign lesion 17 (94.44%) out of 18 cases of FA showed diffuse and strong positivity for CD56

expression. Similar results were demonstrated by studies conducted by *Erdogan-Durmus S et al* [16] and *Scarpino S et al.* [19] Among Follicular thyroid carcinoma 80% cases demonstrated diffuse and strong positivity for CD56, study done by *Mokhtari M* et al also showed similar findings. [20] In consonance with these findings CD56 is highly specific in determining malignant thyroid tumours.

In the study we found that CK19 and CD56 has same value of diagnostic accuracy in distinguishing follicular variant of PTC from follicular adenoma, but CK19 showed slightly higher sensitivity and NPV, simultaneously CD56 immunostaining showed higher values of specificity and PPV in distinguishing follicular variant of PTC from Follicular carcinoma. In distinguishing FVPTC from FA and FC CK19 expressed higher values of diagnostic accuracy and sensitivity, this finding is in consensus with the results of other similar studies in the literature. [21, 22] CD56 was more specific (94.4%) in distinguishing FVPTC from FA this value is higher than the results demonstrated by other similar studies. [12, 23]. [Table 3&4]

Markers	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
CK19	90% (55.5-99.8)	88.9% (65.3- 98.6)	81.8% (54.5-94.4)	94.1% (71.2- 99)	89.3% (71.8-97.7)
CD56	80% (44.4-97.5)	94.4% (72.7- 99.9)	88.9% (53.7-98.2)	89.5% (71- 96.7)	89.3% (71.8-97.7)

Table 3: Diagnostic performance of CD56 staining & CK19 staining in differentiating FVPTC from FA

Markers	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
CK19	90% (55.5-99.7)	80% (28.4- 99.5)	90% (60.6-98.1)	80% (37.2- 96.4)	86.7% (59.5-98.3)
CD56	80% (44.4-97.5)	80% (28.4- 99.5)	88.9% (57.4-97.9)	66.67% (34.9- 88.1)	80% (51.91-95.7)

Table 4: Diagnostic performance of CD56 staining & CK19 staining in differentiating FVPTC from FC

Limitations and Recommendations:

The study was conducted in a single hospital with a limited sample; so, the interpretations cannot be generalised. But taking this as reference, in future multicentric study with larger sample size can be planned

VI. CONCLUSION

In our study, we found that positive staining with CK19 and loss of expression of CD56 support malignancy. In case of in Follicular Adenoma it was observed that strong and diffuse immunoreactivity with CD56 staining. In the majority of PTC cases, CD56 was negative or there was a loss of expression in various degrees.

References

1.   Dobrinja C, Trevisan G, Liguori G, Romano A, Zanconati FJDc. Sensitivity evaluation of fine - needle aspiration cytology in thyroid lesions. 2009;37(3):230-5.

2.   DeLellis RA. Pathology and genetics of tumours of endocrine organs: IARC; 2004.

3.   El Atti RMA, Shash LSJJotENCI. Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma and solitary follicular thyroid nodules. 2012;24(4):175-84.

4.   Bose D, Das RN, Chatterjee U, Banerjee UJIjom, Medical poojoISo, Oncology P. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma. 2012;33(2):107.

5.   El Demellawy D, Nasr A, Alowami SJDp. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. 2008;3(1):5.

6.   Hirokawa M, Carney JA, Goellner JR, DeLellis RA, Heffess CS, Katoh R, et al. Observer variation of encapsulated follicular lesions of the thyroid gland. 2002;26(11):1508-14.

7.   Ceyran AB, Şenol S, Şimşek BÇ, Sağıroğlu J, Aydın AJIjoc, pathology e. Role of cd56 and e-cadherin expression in the differential diagnosis of papillary thyroid carcinoma and suspected follicular-patterned lesions of the thyroid: the prognostic importance of e-cadherin. 2015;8(4):3670.

8.   Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein A-LAJMP. Immunohistochemical markers in diagnosis of papillary thyroid carcinoma: utility of HBME1 combined with CK19 immunostaining. 2006;19(12):1631-7.

9.   Ozolins A, Narbuts Z, Strumfa I, Volanska G, Stepanovs K, Gardovskis JJM. Immunohistochemical expression of HBME-1, E-cadherin, and CD56 in the differential diagnosis of thyroid nodules. 2012;48(10):74.

10.   Song Q, Wang D, Lou Y, Li C, Fang C, He X, et al. Diagnostic significance of CK19, TG, Ki67 and galectin-3 expression for papillary thyroid carcinoma in the northeastern region of China. 2011;6(1):126.

11.   Sanuvada R, Nandyala R, Chowhan AK, Bobbidi P, Yootla M, Hulikal N, et al. Value of cytokeratin-19, Hector Battifora mesothelial-1 and galectin-3 immunostaining in the diagnosis of thyroid neoplasms. 2018;10(2):200.

12.   Abouhashem NS, Talaat SMJP-R, Practice. Diagnostic utility of CK19 and CD56 in the differentiation of thyroid papillary carcinoma from its mimics. 2017;213(5):509-17.

13.   Calangiu CM, Simionescu CE, Stepan AE, Cernea D, Zavoi RE, Margaritescu CJRJME. The expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas. 2014;55(3):919-25.

14.   Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SLJMp. Immunohistochemical diagnosis of papillary thyroid carcinoma. 2001;14(4):338-42.

15.   Sahoo S, Hoda SA, Rosai J, DeLellis RAJAjocp. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma: a note of caution. 2001;116(5):696-702.

16.   Erdogan-Durmus S, Ozcan D, Yarikkaya E, Kurt A, Arslan AJJorimstojoIUoMS. CD56, HBME-1 and cytokeratin 19 expressions in papillary thyroid carcinoma and nodular thyroid lesions. 2016;21.

17.   De Matos P, Ferreira A, de Oliveira Facuri F, Assumpcao L, Metze K, Ward LJH. Usefulness of HBME - 1, cytokeratin 19 and galectin - 3 immunostaining in the diagnosis of thyroid malignancy. 2005;47(4):391-401.

18.   Bizzarro T, Martini M, Marrocco C, D'Amato D, Traini E, Lombardi CP, et al. The role of CD56 in thyroid fine needle aspiration cytology: a pilot study performed on liquid based cytology. 2015;10(7).

19.　　Scarpino S, Di Napoli A, Melotti F, Talerico C, Cancrini A, Ruco LJTJoPAJotPSoGB, et al. Papillary carcinoma of the thyroid: low expression of NCAM (CD56) is associated with downregulation of VEGF - D production by tumour cells. 2007;212(4):411-9.

20.　　Mokhtari M, Eftekhari M, Tahririan RJJorimstojoIUoMS. Absent CD56 expression in papillary thyroid carcinoma: A finding of potential diagnostic value in problematic cases of thyroid pathology. 2013;18(12):1046.

21.　　Baloch ZW, Abraham S, Roberts S, LiVolsi VAJHp. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical study and its diagnostic utility. 1999;30(10):1166-71.

22.　　Beesley M, McLaren KJH. Cytokeratin 19 and galectin - 3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. 2002;41(3):236-43.

23.　　Nechifor-Boila A, Catana R, Loghin A, Radu TG, Borda AJRJME. Diagnostic value of HBME-1, CD56, Galectin-3 and Cytokeratin-19 in papillary thyroid carcinomas and thyroid tumors of uncertain malignant potential. 2014;55(1):49-56.