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CD56 AS IMMUNOMARKERS IN THE DIAGNOSIS OF PAPILLARY THYROID CARCINOMA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Thyroid cancer is the most common malignant endocrine tumor. Nowadays tissue biopsy and pathological assessment are the best diagnostic modalities for thyroid lesions. Differential diagnosis between adenomas and follicular variant of papillary thyroid carcinoma (PTC) is an important issue in pathology. Papillary carcinoma typically stains for Thyroglobulin, TTF-1, Pan-Cytokeratin and PAX-8. An assortment of markers, such as Cytokeratin-19, HWCK, HBME-1, GAL-3, CD57, CITED-1, CD15, Fibronectin-1, CD44 and PDGF have been proposed to be of significance in the diagnosis of papillary carcinoma. Considering the above, the purpose of the study is to show CD56, a neural cell adhesion molecule can be used as immunomarkers in the diagnosis of PTC. Its expression may affect the migratory capability of tumor cells. Hence it is not surprising that loss of CD56 correlates with metastatic potentials and poor prognostic outcome in some malignancies. The thyroidectomy specimens of the 30 patients has been used and statistically analyzed in the present clinical case study. The results revealed the potential usage of CD56 expression in serving as immunomarkers in the diagnosis of papillary thyroid carcinoma.

Keywords: Thyroid cancer; malignant endocrine tumor; CD15; CD56.

1. INTRODUCTION

Thyroid neoplasms are the most frequently encountered endocrine neoplasms in clinical and surgical pathology practice [1]. Most of these neoplasms arise from follicular epithelial cells and they encompass a wide variety of benign & malignant neoplasms [2]. Papillary carcinoma typically stains for Thyroglobulin, TTF-1, Pan-Cytokeratin and PAX-8. An assortment of markers, such as Cytokeratin-19,

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HWCK, HBME-1, GAL-3, CD57, CITED-1, CD15, Fibronectin-1, CD44 and PDGF have been proposed to be of significance in the diagnosis of papillary carcinoma [3]. There has been a marked increase in the reported incidence of thyroid neoplasms globally [4], since the introduction of high-resolution imaging techniques (thyroid ultrasonography) [5]. The increased incidence may be the result of an actual increase in the incidence of PTC. However, a minor component has been attributed to over diagnosis of PTC [6,7]. The diagnostic criteria for PTC have been established for more than 50 years [8]. However, it appears that its application, especially with regard to quantization, is still not sufficiently established.

Inter-observer disagreements among pathologists have been documented quite frequently. Studies show that CD56 is expressed in normal thyroid follicular cells, as well as in benign and malignant follicular lesions, but not in PTC [9,10]. The loss of CD56 expression has displayed reasonably high sensitivity and specificity in differentiating PTC from other follicular neoplasms [11]. However, it is a negative marker for PTC. CK19, HBME-1 and P63, are positive markers, which have proven to be valuable in the distinction of PTC from other thyroid follicular lesions [12,13]. This study is aimed at evaluating the efficacy of CD56 as an immunomarker to aid in the diagnosis of papillary thyroid carcinoma. CD56 being a negative marker for PTC, CK19 has also been included in the study.

2. METHODS

Study Population: All the thyroidectomy specimens received for histopathological evaluation from the Department of General Surgery, Govt. Hospital, Chennai during the study period (2019-2021).

Sample size: A total of 30 cases of surgically resected follicular cell derived thyroid lesions that include both benign and malignant neoplasms were collected.

The following inclusion and exclusion criteria were adopted.

Inclusion Criteria:

The following follicular cell-derived lesions of the thyroid were included in the study:

- ✓ Classical Papillary Thyroid Carcinoma
- ✓ Papillary Thyroid Carcinoma – Follicular Variant
- ✓ Follicular Adenoma
- ✓ Follicular Carcinoma

Exclusion Criteria:

- ✓ Recently defined encapsulated follicular neoplasms with borderline behaviour
- ✓ (FTUMPs, WDTUMPs & NIFTP)
- ✓ Recurrent thyroid neoplasms
- ✓ Cases with inadequate material from the tumour
- ✓ Autolysed specimens
- ✓ Poorly processed material
- ✓ Cases with dense tissue necrosis

The list of materials used in the present study is presented in Table 1.

We attempt to reduce selection bias by conducting experimental research in which individuals are assigned to study or control groups at random (i.e. randomised controlled experiments).

Table 1. Properties of the primary antibodies used

Primary Antibody	CD56	CK19
Source	Mouse Monoclonal	Rabbit Monoclonal
Clone	123C3	EP72
Class	IVD	IVD
Isotype	Mouse IgG1	Rabbit IgG
Tested Reactivity	Human FFPE	Human FFPE
Localization	Cytoplasmic Membranous	Cytoplasmic
Dilution	Prediluted Ready to use	Prediluted Ready to use
Manufacturer	PathnSitu	PathnSitu
Antigen Retrieval Buffer	Tris EDTA	Tris EDTA
TRIS Buffer *p*H	9	9
Visualization Kit Manufacturer	PathnSitu	PathnSitu
Positive Control	Pancreas	Colon

Processing of Specimens:

The specimens were received in 10% formalin. They were allowed to fix over a period of 24 hours. After formalin fixation, gross examination of the specimens was done. The role of immune-histochemical staining with CD56 and CK19 in differentiating papillary thyroid carcinoma and its variants from other follicular patterned thyroid lesions is evaluated and the results are compared with those reported in the literature.

3. RESULTS

This retrospective case-control study includes a total of 30 cases of follicular cell-derived thyroid neoplasms. We selected 15 cases of unequivocal PTCs as the study group. These included 9 cases of classical PTC and 6 cases of follicular variant of PTC. We selected 15 cases of follicular neoplasms (other than PTCs) as the control group. These included 11 cases of follicular adenoma and 4 cases of follicular carcinoma. For the purpose of this study, we have assigned scores 0 and 1 as lack of expression of the immunomarker. We have considered scores 2 and 3 as positive expression of the marker.

Distribution of age:

The mean age in our study was 43.53 years. The youngest patient in our study population was 23 years old and the oldest was 68 years old. The details were presented in Table 2.

Table 2. Distribution of age

Age	No. of Cases
< 30 Years	3
30 - 60 Years	23
> 60 Years	4
Total	30

Distribution of gender:

We studied the gender distribution in our study. The results are tabulated in Table 3.

Table 3. Distribution of Gender

Gender	No. of Cases
Female	23
Male	7
Total	30

Expression of CD56:

In the study group, CD56 was negatively expressed in 12 (80%) of the 15 cases of PTC. However, 3 (20%) of the 15 cases of PTC expressed CD56. The details of expression are shown in Table 4. The loss of expression of CD56 was found to be statistically significant, with a P-value of <0.001, in distinguishing PTCs from other follicular neoplasms.

Table 4. Expression of CD56

CD56	Study Group	Control Group	Total
Negative Expression	12	0	12
Positive Expression	3	15	18
Total	15	15	30

Expression of CK19:

In the study group, CK19 was positively expressed in 14 (93.3%) of the 15 cases of PTC and is presented in Table 5. The expression of CK19 was found to be statistically significant, with a P-value of <0.001, in distinguishing PTCs from other follicular neoplasms.

Table 5. Expression of CK19

CK19	Study Group	Control Group	Total
Positive Expression	14	4	18
Negative Expression	1	11	12
Total	15	15	30

Combined expression of CD56 & CK19

In the study group, 11 (73.33%) of the 15 cases of PTC showed CD56 (-) / CK19 (+) expression pattern; while 4 cases (26.67%) displayed other staining patterns. In contrast, all 15 cases (100%) in the control group showed other expression patterns (Table 6).

Table 6. Combined expression of CD56 & CK19

CD56 / CK19	Study Group	Control Group	Total
CD56 (-) / CK19 (+)	11	0	11
Other Expression Patterns	4	15	19
Total	15	15	30

Sensitivity:

CK19 was the most sensitive among the two markers studied, it demonstrated a sensitivity of 93.30% in diagnosing PTC (Table 7).

Table 7. Sensitivity of CD56, CK19 & CD56 / CK19 Panel

Immunomarker	CD56	CK19	CD56 / CK19
Sensitivity	80.00% (61.30% – 80.00%)	93.30% (73.20% – 99.60%)	73.30% (54.40% – 73.30%)

Specificity:

CD56 was highly specific for the diagnosis of PTC, with a specificity of 100% (Table 8).

Table 8. Specificity of CD56, CK19 & CD56 / CK19 Panel

Immunomarker	CD56	CK19	CD56 / CK19
Specificity	100.00% (81.30% – 100%)	73.30% (53.20% – 79.60%)	100.00% (81.10% – 100%)

Positive predictive value:

CD56 demonstrated an excellent positive predictive value (PPV) of 100% in diagnosing PTCs (Table 9).

Table 9. PPV of CD56, CK19 & CD56 / CK19 Panel

Immunomarker	CD56	CK19	CD56 / CK19
PPV	100.00% (76.60% – 100%)	77.80% (61.00% – 83.00%)	100.00% (74.20% – 100%)

Diagnostic accuracy:

CD56 recorded a very high diagnostic accuracy of 90.00%, while CK19 had a lower diagnostic accuracy of 83.30% (Table 10).

Table 10. Accuracy of CD56, CK19 & CD56 / CK19 Panel

Immunomarker	CD56	CK19	CD56 / CK19
Accuracy	90.00% (71.30% – 90.00%)	83.30% (63.20% – 89.60%)	86.70% (67.80% – 86.70%)

The staining patterns in the study group is shown in Fig. 1.

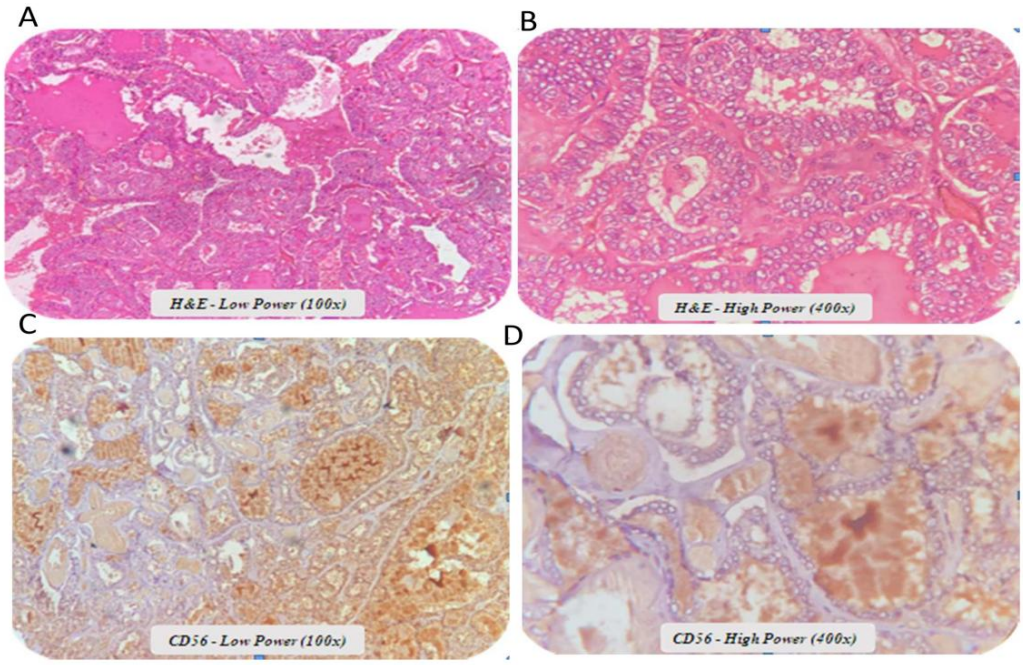

Fig. 1. A. Classical PTC with papillary architecture. B. Classical PTC with characteristic nuclear features. C. Negative expression of CD56 in classical PTC. D. Negative expression of cd56 in classical PTC

4. DISCUSSION

This study has suggested a possible explanation for the increased CD56 expression seen in some PTC instances. The extrapolation of this hypothesis points the probability of a more aggressive and metastatic phenotype being encountered in such cases [14]. A statistically significant difference between PTCs & other follicular neoplasms with regard to the CD56 expression ($p < 0.001$) is observed. Thus, the loss of CD56 expression in the FVPTCs can be utilized in distinguishing them from other follicular patterned lesions. However, Etem et al. found no statistically significant difference between his study group of FVPTCs and his control group of follicular tumours (Follicular adenomas, FTUMPs and follicular carcinomas) with regard to the expression of CD56 [15]. The validity of CD56 as a diagnostic immunomarker for PTCs is reported previously and shown in Table 11.

A fairly good sensitivity of 80% and negative predictive value of 83.30%is observed in our study. Ceyran et al. and Shin et al. reported similar values [16,17]. However, Ma et al. had reported a negative predictive value of 100% [18]. A better specificity (100%) and positive predictive value (100%) of CD56 in distinguishing PTCs from other follicular patterned lesions of the thyroid. While Ma et al. had reported a similar specificity in their study, and shown a much lower PPV of 64% [18]. In our study, CD56 is found to have a diagnostic accuracy of 90%, which was in close agreement with those reported [16,17].

CK19 has shown great promise as a diagnostic immunomarker for PTC, thus its expression in PTC has been studied extensively over the past two decades. While few studies have reported very good sensitivity as well as specificity with CK19, most studies have noted a poor specificity [19]. The diffuse positivity of CK19 in 14 (93.30%) of the 15 cases of PTC is observed in the present study. However, a positive expression of CK19 in 4 (26.67%) of the15 cases of other follicular neoplasms is studied. It has been suggested that though CK19 is also noted in follicular adenomas, the intensity and proportion of staining were different compared to PTCs. Cheung et al., Subramanian et al., and Noroozinia et al. reported the diffuse staining in PTCs and focal staining in Fas [20-24]. A similar trend is observed in our study, with 2+ staining noted in 4 (36.37%) out of the 11 cases of FA compared to the 3+ staining noted in most PTCs. Thus, if the study consider only diffuse staining pattern as positive expression, PTCs would be the one displaying CK19 positivity. So, a higher threshold for assigning positivity may improve the specificity of CK19.We compared the validity of CK19 as a diagnostic immunomarker for PTCs with results from similar studies done previously. The results from these studies, in comparison with our study, and have been tabulated (Table 12).

Table 11. Diagnostic validity of CD56 in PTCs

CD56	Ma H et al.	Ceyran AB et al.	Mi Kyung Shin et al.	Our Study
Sensitivity	79.10%	91.10%	95%	80.00%
Specificity	100%	91.70%	72.73%	100.00%
PPV	64%	85.90%	92.68%	100.00%
NPV	100%	94.80%	80%	83.30%
Accuracy	84.70%	91.30%	90.20%	90.00%

Table 12. Diagnostic validity of CK19 in PTCs

CK19	Ma H et al.	Ceyran AB et al.	Mi Kyung Shin et al.	Our Study
Sensitivity	100%	84.20%	100%	93.30%
Specificity	56.25%	36.70%	36.36%	73.30%
PPV	86%	69.10%	85.11%	77.80%
NPV	100%	57.80%	100%	91.70%
Accuracy	88.10%	72.70%	86.27%	83.30%

The CK19 is found to be a highly sensitive marker for PTC, with a sensitivity of 93.30% and a high negative predictive value of 91.70%. Shin et al. & Ma et al. had both reported an excellent sensitivity, as well as an excellent negative predictive value, of 100% in their studies [17,18]. On the other hand, Ceyran et al. had reported a slightly lower sensitivity of 84.20% and a poor negative predictive value of 57.80% [16]. While CK19 was among the most sensitive markers for diagnosing PTC in most studies, the other diagnostic parameters were not as impressive. A poor specificity of 73.30% is recorded. In our study, CK19 had a positive predictive value of 71.10% and a diagnostic accuracy of 76.70%. The immunopanel of CD56 & CK19 demonstrated excellent specificity and positive predictive value of 100%. The panel had a sensitivity of 73.30%, negative predictive value of 78.90% and diagnostic accuracy of 86.70%. The efficacy of the panel may be attributed to the combination of two complementary markers. The high sensitivity of CK19 complements the highly specific CD56.

The negative expression of CD56 was found to be statistically significant ($p<0.001$) in distinguishing PTCs from follicular neoplasms. CD56 was negatively expressed in 12 (80%) of the 15 cases of PTC, while all 15 cases (100%) of follicular neoplasms diffusely expressed CD56. In our study, the loss of CD56 expression demonstrated a reasonably good sensitivity of 80% and negative predictive value of 83.30%.The negative expression of CD56 recorded excellent specificity (100%) and positive predictive value (100%) in distinguishing PTCs from follicular neoplasms. We calculated a diagnostic accuracy of 90% for CD56 in the diagnosis of PTC. The positive expression of CK19 was found to be statistically significant ($p<0.001$) in distinguishing PTCs from follicular neoplasms.

CK19 was diffusely positive in 14 (93.30%) of the 15 cases of PTC under study. CK19 also positively expressed in 4 (36.37%) of the 11 cases of follicular adenomas studied. However, the staining patterns were different. A lower proportion, as well as, intensity of staining, was noted. CK19 was a highly sensitive marker for PTC, with a sensitivity of 93.30% and a fairly good negative predictive value of 91.70%. CK19 demonstrated a poor specificity of 73. 30%, a satisfactory positive predictive value of 77.80% and a diagnostic accuracy of 83.30%.The immunopanel of CD56 & CK19 demonstrated excellent specificity and positive predictive value of 100%. The panel had a sensitivity of 73.30%, a negative predictive value of 78.90% and a diagnostic accuracy of 86.70% (Table 13).

Table 13. Summary of the diagnostic validity of the immunomarkers studied

Immunomarker	CD56	CK19	CD56 / CK19
Sensitivity	80.00%	93.30%	73.30%
Specificity	100.00%	73.30%	100.00%
PPV	100.00%	77.80%	100.00%
NPV	83.30%	91.70%	78.90%
Accuracy	90.00%	83.30%	86.70%

5. CONCLUSION

CK19 showed very good sensitivity; however, the other diagnostic parameters were quite poor. CK19 was expressed in follicular neoplasms but to a lesser degree. Setting the cut-off for positive expression of CK19 at 3+ would increase the specificity of the marker. We recorded excellent specificity & positive predictive values with CD56. The other diagnostic parameters were also quite impressive. Taking into account our relatively small sample size, we suggest evaluation of the immunohistochemical expression of CD56 and CK19 by a group of expert thyroid pathologists in a more extensive study, with focus on the recently introduced borderline categories of follicular neoplasms (FTUMPs, WDTUMPs & NIFTP).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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