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The Part Immunohistochemical Markers (CK19 AND CD56) Play in Distinguishing Papillary Thyroid Carcinoma from Other Pathological Imitators

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ABSTRACT

Background: The most prevalent type of thyroid cancer is papillary thyroid carcinoma. PTC's unique nuclear properties are the primary diagnostic indicator. It is difficult to make a diagnosis since similar lesions are focally present in other thyroid lesions. **Aim:** The ability of the immunohistochemical markers CD56 and CK19 (cytokeratin 19) to identify papillary thyroid carcinoma from similar thyroid lesions.

Methods: We looked at 122 instances of various thyroid lesions in the population of Iraq where CK19 and CD56 immunoexpression was detected. The study was carried out on the clinical evaluation, responsiveness, precision, and predictive positive and negative values.

Results: CK19 was strongly expressed in 89.7% of the PTC group and strongly negative in 85.7% of the NPTC group, per the statistical findings (P<0.001). CD56 expression was significantly (P<0.001) reduced in more individuals of the PTC group by 97.2%). CK19 was the most sensitive marker and CD56 was the most specific marker when comparing papillary carcinoma with thyroid hyperplastic diseases, and diagnostic accuracy was improved when both markers were combined. In terms of papillary Micro carcinoma stain, CK 19 had 100% prevalence and 100% sensitivity, whereas CD56 had 100% specificity. **Conclusion:** In order to diagnose papillary thyroid carcinoma and distinguish it from its mimics, immunohistochemistry using the markers CK19 and CD56 is a recognized effective supplementary method in questionable cases.

Keywords: CK19, CD56 antigen, immunohistochemistry, Papillary thyroid carcinoma.

INTRODUCTION

Thyroid cancer is the most frequent malignant tumor of the endocrine system [(1)]. Hematoxylin and eosin staining is now employed in histopathologic evaluation to identify thyroid nodules and cancers[(2)]. Evidently, there is debate about whether or not particular situations fall under the purview of PTC or not. When a few of the PTC nuclear diagnostic criteria are met, diagnoses as follicular adenoma and the follicular variant of PTC might develop. Unfortunately, even among knowledgeable thyroid pathologists there is disagreement of this kind. Information abounds on the differences between pathologists[(3)].

We applied the major and minor aspects of Chan's [(4)] histologic criteria for PTC, the following notable traits:

1. The oval-shaped nucleus.
2. The nuclei are clustered.
3. Either transparent or light chromatin can be found in nuclei.
4. Detection of psammoma bodies. If one of the four qualities was absent, four or more of the following qualities may be present instead:

a) presence of papillae that have aborted.
b) Follicles with an abnormal shape.
c) Colloid that darkly stains.
d) Nuclear pseudoinclusions are present.
e) Multinucleated histiocytic in the lumen of the follicle.

When follicles bordered by cells with PTC nuclear characteristics made up all or almost all (99%) of a tumor, the tumor was categorized as FVPC [(5)]. When PTC-N was incomplete, Williams coined the phrase "well-differentiated tumour of undetermined malignant potential" (WDT-UMP) for tumours [(6)]. A subset of well-circumscribed/encapsulated FV-PTC without invasion indicators is NIFTP. [(7)].

The diagnostic issues with thyroid pathology are far more frequently associated with the follicular type of PTC than with typical PTC (FVPTC) [(8)], It has PTC-type nuclei and a follicular development pattern that is virtually entirely present. The encapsulation of these tumors might be partial or total, or they cannot be encapsulated. [(8)]

The term "papillary thyroid microcarcinomas" was used to describe accidentally discovered PTCs that were 1 cm or less. [(8)].

Given that various thyroid lesions may include papillary processes with nuclear characteristics, PTC diagnosis is difficult. [(5)] PTC may be mistaken with multinodular goitre (MNG), which has delicate papillary budding and localized nuclear clearance [(9)]. In rare circumstances, a morphologic examination-based objective diagnosis may not be attainable. [(10)]

Although none of these is currently convincing, CD56, Hector Battifora mesothelial (HBME-1), galectin-3 (Gal-3), and CK19 are a few interesting markers for the differential diagnosis of thyroid abnormalities. Research

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is being done on an increasing number of immunohistochemistry (IHC) markers. [11,12].

Since no one marker has been demonstrated to have 100% sensitivity and 100% specificity, a combination of indicators may offer a more precise diagnosis of PTC than any one symptom. [8].

It was shown that CD56, a neural cell adhesion molecule [13], is frequently expressed in thyroid tissues that are normal but is absent from malignant thyroid tumours, especially in the PTC [10, 11]. Between benign and malignant thyroid lesions, there was a statistically significant difference in the reduction of CD56 expression, according to a meta-regression test. For separating benign from cancerous thyroid, such as follicular adenoma, benign follicular nodules, and Hashimoto's thyroiditis, a further test examining the lack of CD56 expression may be helpful. [1].

Simple epithelial cells frequently contain the type I intermediate filament protein cytokeratin 19. (CK19). Strong and diffuse positivity in malignant thyroid tumours was found in several tests, including the conventional PTC, FVPC, and FC. Although it is not unique to cancer, it is important to keep in mind that the amount and intensity of staining reveal various patterns of expression in connection to benign tumours and their cancerous counterparts. The utility of CK19 as a prognostic tool for thyroid lesions has been the subject of several research, with varying degrees of success [14]. Also helpful in this field is CK19, which exhibits a staining pattern that is weak localized in benign nodules and vast, broad expression in thyroid cancers [15].

AIM OF STUDY

To investigate the effectiveness of CD56 & CK19 immunohistochemical stains in distinguishing papillary thyroid carcinoma from similar thyroid lesions.

PATIENTS & METHODS

Tissue specimens

The Pathology departments provided the materials for this experiment, including Medical city Teaching Complex, Teaching laboratories, Medical City Health Directorate, AL-SHARIQA specialized lab and AL-SHARIQA Diagnostic Center, during a period from January 2019 to May 2022. In this retrospective analysis, 122 cases of thyroid lesions that had been surgically resected, formalin-fixed, and embedded in paraffin were included. The facilities where we obtained the samples processed the tissue according to a standard procedure, and the overall histological results were as previously described by **Ozolins *et al.* [19]**. According to the WHO Categorization of Tumors of Endocrine Organs, Fourth Edition, released in 2017, it was done to identify and categorize thyroid pathology [21]. Hematoxylin and eosin (H&E)-stained slides and pathology reports were analyzed to confirm the treatment and histological type. Pathological and Clinical information concerning age, gender, and type of operation was recorded by two researchers from hospital/lab files. This project was certified by the Ethical Committee, Arab Board of Health Specializations, and Department of Surgical Pathology.

Immunohistochemical staining

1. Preparation of 2–3 um thickness Paraffin sections.
2. The immunohistochemical staining method is carried out in a Dako Autostainer utilizing antibodies against CK19 and Cd56 and the designated streptavidin-biotin peroxidase complicated system (LSAB2) (Dako, Carpinteria, CA, USA)
3. At 56°C, deparaffinize tissue slices in the oven.
4. Add a few drops of xylene.
5. Decreasing levels of alcohol and rehydrate.
6. 15-minute microwave boil in sodium citrate buffer for antigen retrieval (0.001 M, pH 6).
7. Block endogenous peroxidase activity by allowing cells to sit in 3% hydrogen peroxide for 10 minutes.
8. Use distilled water to rinse.
9. Slides should be incubated with the primary antibodies CD56/FLEX, Anti-Human, Clone 123C3, fully prepared already, Dako autostainer or Autostainer plus, and CK19/FLEX, Monoclonal mouse, Anti Human, Clone RCK 108, over the course of an overnight period at 4°C.
10. Slides should spend 30 minutes at room temperature being treated with biotinylated secondary antibodies.
11. Place tissue samples in an incubation solution containing streptavidin-biotin-peroxidase.
12. After performing 15 minutes of diaminobenzidine incubation, rinsing with PBS were performed.
13. Hematoxylin counterstaining for 3 minutes followed by a rinse with distilled water. In ascending series of alcohol concentrations, the slides were dehydrated.
14. Use xylene for clearing.

Scoring criteria

A light microscope with a 10x objective lens was used to evaluate the immunohistochemical staining, with a 20–40x objective lens being used only when necessary for confirmation. When CD56 and CK19 were expressed positively in the membrane of 10 percent more than of a neoplastic cells, with either cytoplasmic staining or not, a "positive (+)" classification was given to the case. Using a semi-quantitative scoring system, the membrane and cytoplasmic stains of CD56 and CK19, respectively, were used to evaluate whether the result was positive or negative. Both immunostains received scores of 0, 1, 2, or 3+ (zero, no staining, mild staining, strong staining, or 3+) for staining intensity. Normal thyroid tissue and colon mucosa served as the positive controls for CD56 and CK19 staining, respectively [18].

Ethical approval: It was attained by the Arab Board of Medical Specialization/Medical city/Baghdad/Iraq.

Analytical Statistics

For the study, SPSS version 26 was used (Statistical Package for Social Sciences). The data's mean, standard deviation, and ranges were presented. Expressed as frequencies and percentages for categorical data. To evaluate the statistical relationship between diagnostic indicators and diagnosis, the chi-square test by Pearson was performed. P values < 0.05 were used to designate significant levels.

RESULTS

Demographic and Clinical results

There were 122 total occurrences, ages 14 to 72, mean age 39.26 years, Standard Deviation (SD) 11.8 years. Most patients (38.5%) were between the ages of 30 and 39. There were 20 male cases (16.4%) compared to 102 female cases (83.6%), making a male to female ratio of 1:5.1. There were 106/122 (86.9%) specimens acquired by complete thyroidectomy, 12/122 (9.8%) through lobectomy, and 4/122 (3.3%) through partial thyroidectomy.

Histopathologic results

Among the 122 thyroid resection patients that were found, the PTC group comprised of 83 cases (68%), while the NPTC group number was 39 cases (32%).

The studied PTC group included 13 cases with classic papillary thyroid carcinoma (15.7%), 47 cases with papillary microcarcinoma (56.6%) (Fig. 1) ,1 case with hobnail variant of PTC (1.2%), 6 cases Follicular variant of PTC (7.2%), 11 cases encapsulated follicular variant and NIFTP(13.3%) and 3.6% of all cases were well-differentiated tumours with unknown malignant potential, which accounted for 3 instances. (Percentages are from the PTC group).

For the NPTC group we had 32 cases of thyroid hyperplastic disorders (82.1%) (Fig. 2), follicular carcinoma was found in 4 patients (10.3%). with one instance for each of the following, follicular adenoma of microfollicular type, Hurthle cell carcinoma, and poorly differentiated carcinoma (2.6% for each). (Percentages are from the NPTC group).

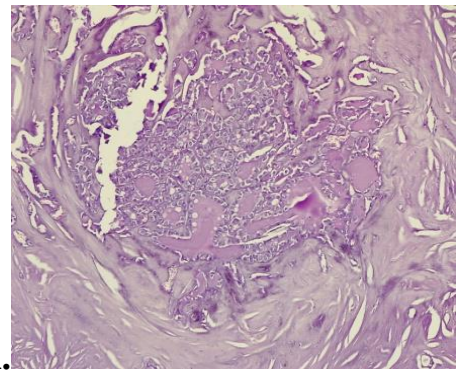
Total Immunohistochemical staining results

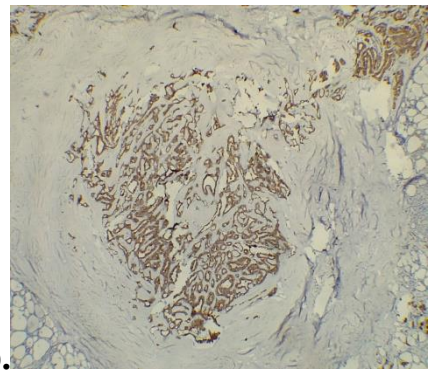
For total immunostain analysis CK19 was (71.3%) and (28.7%) positive and negative respectively of the total sample size while for CD 56 it was (41%) and (59%) positive and negative respectively of the total sample size. (Table 1).

Association between diagnostic markers and diagnosis

CK19 has been expressed in 89.7% of a PTC group but was only positive in 10.3 percent of the NPTC group, a statistically meaningful difference (P<0.001). (Table 2). In terms of CD56 expression, 97.2% of the PTC group had lost it, but just 2.8% of the (NPTC) group had negative CD56 expression, which was significantly different (P<0.001). (Table 2).

In order to distinguish papillary thyroid carcinoma from its related lesions with greater diagnostic accuracy, we used both immunohistochemical markers.

a. 

b. 

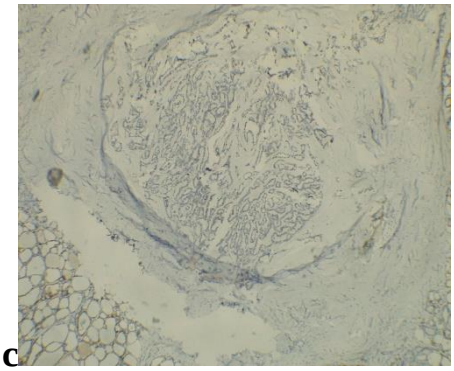
c 

Fig. 1. Papillary microcarcinoma A. H&E staining; B: Positive immunostain for the CK19 marker; C: Absence of CD56 expression, note that the remaining non-neoplastic thyrocytes, on the other hand, have widespread membranous CD56 staining. (All figures have been magnified by x40).

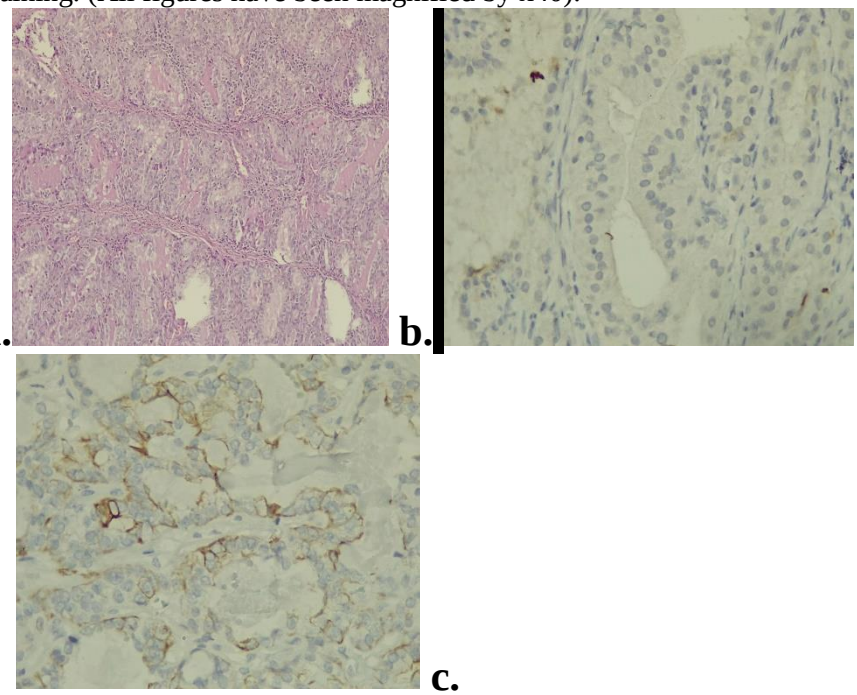


Fig. 2. Thyroid hyperplastic disorder. A: H&E stains (magnification x40); B: CK19 marker was absent in thyroid hyperplastic nodule; C: CD 56 shows positive membrane expression (c and d magnification is x :400).

Table 1: CK19 and CD56 markers total immunostain analysis

Variable		
CK19 %	**n=122**	
Positive	87	71.3
Negative	35	28.7
CD56		
Positive	50	41.0
Negative	72	59.0

Diagnostic indicators, Sensitivity, Specificity, and Accuracy

CK19 .3.5.1

The CK19 marker's precision, sensitivity, and specificity for PTC diagnosis are displayed in Table 3. The sensitivity of CK19 was = 94%, specificity = 76.9% and accuracy was 88.5%.
+ve predictive value was 89.7% while –ve predictive value was 85.7%

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CD56 .3.5.2

The CD56 marker's precision, sensitivity, and specificity for PTC diagnosis are displayed in Table 4. The sensitivity of CD56 was = 84.3%, specificity = 94.9% and accuracy was 87.7%.
+ve predictive value was 97.2% while –ve predictive value was 74%

No.(n=122)	Percentage (%)

Table 2: Association between diagnostic markers and diagnosis

Diagnostic markers	Diagnosis		Total (%) n= 122	P – Value
	PTC (%) n= 83	NPTC (%) n= 39		
CK19				
Positive	78 (89.7)	9 (10.3)	87 (71.3)	**0.001**
Negative	5 (14.3)	30 (85.7)	35 (28.7)	
CD56				
Positive	13 (26.0)	37 (74.0)	50 (41.0)	**0.001**
Negative	70 (97.2)	2 (2.8)	72 (59.0)	

Table 3: Contingency table of Ck19 marker in diagnosing PTC

CK19 result	Diagnosis		
	PTC	NPTC	Total
Positive	78	9	87
Negative	5	30	35
Total	83	39	122

Table 4: Contingency table of CD56 marker in diagnosing PTC

CD56 result	Diagnosis		
	PTC	**NPTC**	**Total**
Negative	70	2	72
Positive	13	37	50
Total	83	39	122

DISCUSSION

Routine pathology examination is the main method for figuring out how thyroid nodules behave biologically. However, follicular and papillary features can be found in both benign and malignant tumours [(2)]. The examination of thyroid nodules has involved the investigation of several immunohistochemistry markers. A small number of markers have also shown promise in distinguishing between benign thyroid nodules [(19)]. Our decision to assess (CK19, CD56) indicators on histological specimens of benign and cancerous lesions was made in light of these information. Except for PTC, neoplastic follicular epithelium from **El Demellawy et al.** [(11)] large-scale CD56 expression (including follicular variants of PTC). In other words, it is very beneficial to distinguish PTC from other neoplastic lesions of follicular cell origin, when CD56 is not expressed in PTC. They concluded that that CD56 had 100% sensitivity and specificity for separating PTC from benign lesions. [(11,20)] A lower percentage was noticed in our study in that we found the sensitivity of CD56 was 84.3% and specificity was 94.9% in diagnosing papillary thyroid carcinoma a finding that is near to **Nechifor–Boila *et al.*** [(18)] finding of reduced CD 56 expression or absent in thyroid carcinomas with 81.8% sensitivity.

Pyo *et al.* [(1)] found that CD56 specificity is 95% and that is consistent with the results of the current study. In order to identify PTC and its follicular variants from the other thyroid neoplasms arising from follicular cells, CD56 demonstrated good specificity and sensitivity.

Only 14.2 percent of the total of the analyzed papillary carcinoma showed perceived positive CD56 expression, according to **Abouhashem *et al.*** [(16)], who compared papillary carcinoma with papillary hyperplasia, but 91.6 percent of total of papillary hyperplasia was reported to be have diffuse positivity. In our cases, sampling CD56 was positive in 26 percent of PTC and74 percent NPTC. However, it was negative in 67.2% of the PTC group.

In the same research [(16)], only 16.6 percent of all individuals with papillary hyperplasia exhibited positive CK19 expression, compared to 90.4 percent of papillary carcinoma cases who had positive CK19 staining. In our investigation, CK19 was found to be positive in 89% of instances of papillary carcinoma and in only 10.3% of cases of papillary hyperplastic tissue. This result is consistent with that of **El Demellawy *et al.*** [(11)] who found that CK 19 expression was present in 26% of NPTC lesions/tumors and 85% of PTC tumors.

The findings of this study showed that 89.7% of the patients as in PTC category stained positive for CK19 and 97.2 percent of them had lost CD56 expression.

In a study [(2)] there were 100% and 23.1%, respectively, of CD56 and CK19 staining cases. The most specific indicator for identifying benign and malignant tumours, according to these data, was CD56, whereas CK19 was the least precise marker. However, CK 19 showed 94% sensitivity and 76.9% specificity making CK 19 more sensitive compared to CD 56.

Ma *et al.* [(17)] Study showed similar results with CK19 being the most sensitive marker with 100% sensitivity for PTC group.

CONCLUSIONS

It is helpful to diagnose PTC using a plate of CD56 and Ck19, especially for the papillary microcarcinoma form. Negative expression of CD56 in PTC produced from follicular cells is specific and particular and positive expression of CK19 in PTC is more sensitive than CD56.

RECOMMENDATIONS

While H & E is the gold standard for identifying benign and malignant thyroid lesions, we also suggest utilizing immunohistochemistry with CK19 and CD56 markers to confirm papillary thyroid carcinoma in questionable cases.

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