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The role of CK19 and CD56 immunohistochemical staining in differentiating papillary thyroid carcinoma from other thyroid lesions

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Abstract
Background: The most prevalent thyroid cancer is papillary. PTC's nuclear features are the major diagnostic sign. Due to their localised presence in other thyroid lesions, diagnosis is difficult.
The idea is to use CD56 and CK19 (cytokeratin 19) immunohistochemical markers to identify thyroid cancer from similar thyroid lesions. The study aims to determine if CD56 and CK19 immunostains can distinguish papillary thyroid carcinoma from other thyroid lesions that look like it.
Methods: Cross-sectional examination of 60 thyroid lesions from April 2022 to November 2023 in AL-Hilla Teaching Hospital, AL-Sadiq Teaching Hospital, and private laboratories discovered immunoexpression of CK19 and CD56. Patients' charts and correlations were investigated.
Results: $p<0.001$ indicates that CK19 was highly expressed in 100% of the PTC group and considerably negative in the non-neoplastic thyroid nodule group. PTC cases showed significantly ($p<0.05$) increased CD56 expression levels. Comparing thyroid hyperplastic diseases to papillary cancer, CK19 was the most specific marker and CD56 the most sensitive. Combining markers improved diagnosis accuracy. In papillary thyroid carcinoma staining, CD56 exhibited 100% specificity and sensitivity, whereas CK 19 had 100%.
Conclusion: In dubious situations, immunohistochemistry using CK19 and CD56 markers can diagnose papillary thyroid carcinoma and other follicular lesions electively.

Keywords: The Role, CK19, CD56 immunohistochemical, differentiating, papillary thyroid carcinoma

Introduction
Thyroid carcinoma is recognized as the most prevalent malignant tumor of the endocrine system [1]. The identification of thyroid nodules and malignancies is now commonly performed histopathologically using hematoxylin and eosin staining [2]. Despite advancements in diagnostic techniques, disagreements persist among pathologists regarding specific diagnostic criteria for papillary thyroid carcinoma (PTC). Follicular adenoma and the follicular form of PTC may be diagnosed when certain nuclear criteria specific to PTC are met, but even among experienced thyroid pathologists, there is variability in diagnostic conclusions [3]. Chan has delineated the major and minor histopathological criteria for diagnosing PTC, which include: oval-shaped nuclei, nuclear clustering, nuclei with transparent or light chromatin, and the presence of psammoma bodies. The absence of any one of these four attributes may lead to a less definitive diagnosis [4]. Additionally, a combination of at least four of the following characteristics can support the diagnosis: the presence of abortive papillae, abnormally shaped follicles, deeply staining colloid, and nuclear pseudo inclusions [5, 6]. The combination of multiple signs is suggested to provide a more accurate diagnosis of PTC, as no single feature has demonstrated absolute sensitivity and specificity [7]. Neural cell adhesion protein CD56 is typically expressed in normal thyroid tissues but is notably absent in malignant thyroid tumors, particularly PTC [8]. A meta-regression analysis has shown a significant reduction in CD56 expression in malignant thyroid lesions compared to benign ones [1]. Consequently, measuring CD56 expression can be instrumental in differentiating between benign and malignant thyroid conditions, such as follicular adenoma, benign follicular nodules, and Hashimoto's thyroiditis [7]. Cytokeratin 19 (CK19), a type I intermediate filament protein, is commonly found in simple epithelial cells and has been observed to be strongly and widely positive in malignant thyroid tumors through various tests, including traditional PTC diagnostics [9].

The intensity and extent of CK19 staining can vary, reflecting different patterns of expression in both malignant and benign tumors [9]. Numerous studies have explored the utility of CK19 as a predictive marker for thyroid lesions, with varying levels of effectiveness [10]. CK19 shows a staining pattern that is broadly expressed in thyroid malignancies and weakly localized in benign nodules, making it a valuable tool in this diagnostic field. There is ongoing debate about whether the combination of multiple markers can enhance the accuracy of PTC diagnosis. The expression of CD56 and CK19 in both malignant and nonmalignant lesions necessitates further evaluation to determine their combined diagnostic value [10]. Aim of the study to assess the efficacy of (CD56 and CK19) immunostains as diagnostic indicators for differentiating papillary thyroid cancer from other thyroid lesions that mimic it.

Methods

This retrospective cross-sectional study was conducted at the Department of Pathology and Forensic Medicine, Faculty of Medicine, Babylon University. The study involved a sample of paraffin-embedded tissue blocks from thyroidectomy biopsies collected from archived material at Al-Hilla Teaching Hospital, Al-Sadiq Teaching Hospital, and private laboratories between April 2022 and November 2023. Sixty thyroid histopathology reports, slides, and tissue blocks were reviewed, with these cases diagnosed according to the WHO classification of thyroid tumors. The sample included 30 cases of papillary thyroid carcinoma (PTC) and 30 cases of other follicular lesions, all presenting with goiter as indicated by clinical data. Clinicopathological data retrieved included age, gender, residence, religion, smoking status, family history of thyroid diseases, postoperative histopathology report, and clinical history. Adults of both genders aged 18 and older with suspected thyroid conditions were included, specifically those with diffuse enlargement of the thyroid gland. Cases with malignancies of other organs, recurrent thyroid carcinoma, missing clinical data, or unfit paraffin blocks were excluded. Histopathological processing involved the use of a rotary microtome to cut representative sections from the paraffin-embedded blocks to a thickness of 5µm. Sections were then floated in a warm water bath, transferred to slides, and allowed to dry at room temperature. One section per case was stained with Hematoxylin and Eosin (H&E) to confirm the diagnosis, while another was used for immunohistochemical staining with CK19 and CD56 markers. The staining process began with deparaffinization in xylene and a series of alcohol rehydrations. Following this, heat-induced epitope retrieval was performed using an Immuno DNA Retriever with Citrate. The sections were then blocked for peroxidase activity, incubated with primary antibodies (CK19, CD56), and visualized using DAB chromogen and counterstaining with Mayer's hematoxylin. Slides were assessed for CK19 and CD56 expression using a semiquantitative H-score, which considered the intensity and proportion of stained cells. Statistical analyses were conducted using SPSS version 27. Variables were analyzed as frequencies, percentages, means±SD, and through Fisher's Exact Test and t-tests to examine associations and differences between groups. A p-value of ≤ 0.05 was set for statistical significance.

Results

Distribution of patients with thyroid mass according to age group. Patients with age (< 20 years) represent only 3 patients (5.0%), patients (20-30 years) represent 8 patients (13.3%), patients with age (30-40 years) represent 19 patients (31.7%), patients with age (40-50 years) represent 19 patients (31.7%), patients (50-60 years) represent 6 patients (10.0%) and patients with age (≥ 60 years) represent only 5 patients (8.3%). Mean age of patients was (39.63±12.38) years. Younger patient was 18 years and older patient was 74 years. As in Table 1.

Table 1: Distribution of patients with thyroid mass according to age (N=60)

Age (years)	Number	%
< 20 years	3	5.0%
20-30 years	8	13.3%
31-40 years	19	31.7%
41-50 years	19	31.7%
51-60 years	6	10.0%
≥ 60 years	5	8.3%
Total	60	100.0%

Illustrate the number of patients with thyroid mass according to diagnosis including (malignant and benign). Patients with malignant tumor (Papillary thyroid carcinoma) represent half of patients (N=30, 50.0%) and patients with benign lesion represent another half of cases (N=30, 50.0%). Majority of patients with benign lesion (N=11, 36.7%) presented with Follicular adenoma as in Table 2.

Table 2: Distribution of patients with thyroid mass according to diagnosis (N=60)

Study variables	Number	%
Diagnosis		
Malignant tumor (Papillary thyroid carcinoma)	30	50.0%
Benign lesion	30	50.0%
Total	60	100.0%
Type of benign lesion		
Follicular adenoma	11	36.7%
Graves' disease	8	26.7%
Lymphocytic thyroiditis	4	13.3%
Nodular colloid goiter	1	3.3%
Nodular hyperplasia	6	20.0%
Total	30	100.0%

Shows the pattern of CD56 immunostain expression in patient presented with thyroid mass. Patients with no staining (negative) represent (N=14, 23.3%, patient with weak (+) represent majority of cases (N=37, 61.7%), patient with moderate (++) represent (N=7, 11.7%) and patient with strong (+++) represent only two cases (3.3%). As in Table 3.

Table 3: Distribution of patients with thyroid mass according to CD 56 (N=60)

CD 56	Number	%
No staining (negative)	14	23.3%
Weak (+)	37	61.7%
Moderate (++)	7	11.7%
Strong (+++)	2	3.3%
Total	60	100.0%

Shows Distribution of patients with thyroid mass according to CK-19 including Patients with no staining (negative) represent (N=19, 31.7%), patient with weak (+) represent

(N=11, 18.3%), patient with moderate (++) represent (N=9, 15.0%) and patient with strong (+++) represent more than one third of patients (N=21, 35.0%). As in Table 4.

Table 4: Immuno histochemically expression of CK19 in patient present with thyroid mass (N=60)

CK-19	Number	%
No staining (negative)	19	31.7%
Weak (+)	11	18.3%
Moderate (++)	9	15.0%
Strong (+++)	21	35.0%
Total	60	100.0%

Explanation relation between CD 56immunohistochemistry stain including diagnosis of thyroid mass including (malignant and benign). There was significant association between CD 56 results and diagnosis of thyroid mass. Majority of patients with positive CD56 (27) present in PTC. As in Table 5.

Table 5: The association between CD 56 and diagnosis of thyroid mass (N=60)

Diagnosis	CD 56				Total (N=60)	P-Value < 0.05
	No staining (N=14)	Weak (+), (N=37)	Moderate (++), (N=7)	Strong (+++), (N=2)		
Malignant Tumor (PTC)	3% (21.4)	21% (56.8)	4% (57.1)	2% (100.0)	30% (50.0)	0.045*
Benign lesion	11% (78.6)	16% (43.2)	3% (42.9)	0% (0.0)	30% (50.0)	
Total	14% (100.0)	37% (100.0)	7% (100.0)	2% (100.0)	60% (100.0)	

*p-value<0.05was significant

Relation between positive CD 56 including (weak (+), moderate (++) and strong (+++)) and diagnosis of thyroid mass including (malignant and benign lesion). There was no significant association between positive CD 56 and diagnosis of thyroid mass. As in Table 6.

Table 6: The association between positive CD 56 and diagnosis of thyroid mass (N=46)

Diagnosis	Positive CD 56			Total (N=60)	P-Value < 0.05
	Weak (+), (N=37)	Moderate (++), (N=7)	Strong (+++), (N=2)		
Malignant tumor (PTC)	21% (56.8)	4% (57.1)	2% (100.0)	27% (58.7)	0.712
Benign lesion	16% (43.2)	3% (42.9)	0% (0.0)	19% (41.3)	
Total	37% (100.0)	7% (100.0)	2% (100.0)	46% (100.0)	

The association between CK-19 immunohistochemistry stain including (no staining (negative), weak (+), moderate (++) and strong (+++)) and diagnosis of thyroid mass including (malignant and benign). There was significant association between CK-19 results and diagnosis of thyroid mass. All patients with strong (+++) (N=21, 100.0%) presented with malignant tumor (papillary thyroid carcinoma). As in Table 6.

Table 7: The association between CK-19 and diagnosis of thyroid mass (N=60)

Diagnosis	CK-19				Total (N=60)	P-Value < 0.05
	No staining (N=19)	Weak (+), (N=11)	Moderate (++), (N=9)	Strong (+++), (N=21)		
Malignant Tumor (PTC)	1% (5.3)	1% (9.1)	7% (77.8)	21% (100.0)	30% (50.0)	<0.001*
Benign lesion	18% (94.7)	10% (90.9)	2% (22.2)	0% (0.0)	30% (50.0)	
Total	19% (100.0)	11% (100.0)	9% (100.0)	21% (100.0)	60% (100.0)	

*p-value <0.05 was significant

Fig 1: Histopathology of papillary thyroid carcinoma, positive (diffuse strong) immunostaining for Ck19marker

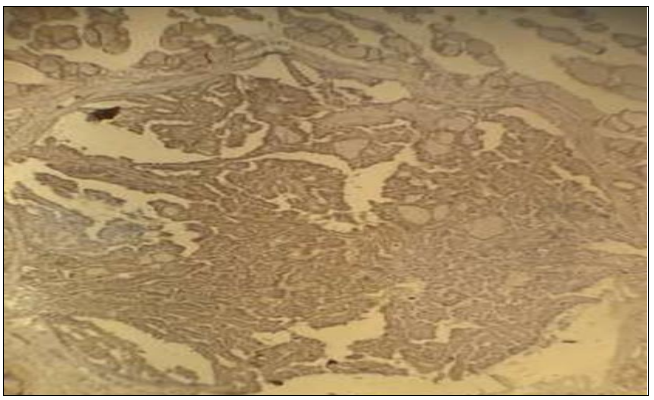

Fig 2: Papillary thyroid carcinoma, diffuse strong positive CD56 marker.

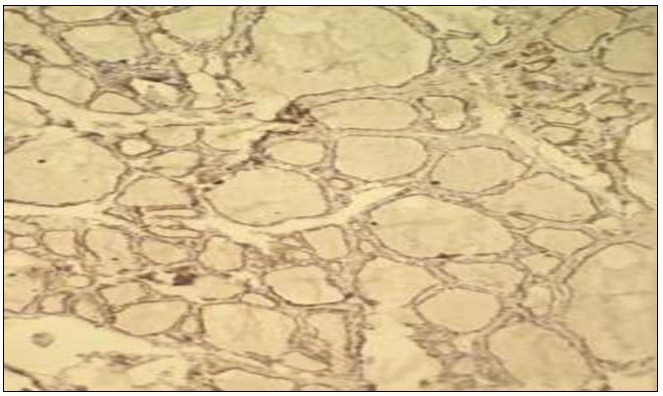

Fig 3: Adenomatous thyroiditis CD56 negative marker.

Discussion

The primary technique for determining the biological behavior of thyroid nodules remains routine pathological examination. Several immunohistochemical markers have been investigated to aid in the examination process, but differentiating between benign and malignant thyroid tumors, which can both exhibit follicular and papillary features, remains challenging [11]. The current study aimed to determine the efficacy of CD56 and CK19 immunostains in differentiating papillary thyroid carcinoma (PTC) from other thyroid lesions. The mean age of the cases in this study was 39.6±12.38 years, with the majority of patients (31.7%) falling within the 30 to 50-year age range, as shown in Table 3-1. This finding is consistent with previous studies, such as those by Ikram *et al*. (2022) in Iraq and Sumi *et al*. (2020) in India, which reported mean ages of 39.26±11.8 years and 45.71 years, respectively [11, 12]. These studies support the observation that the prevalence of thyroid nodules increases with age. Additionally, a prior study in Japan found that the prevalence of thyroid nodules was 35.3% among women over 40 years old, indicating an age-related increase in thyroid nodule prevalence [13]. In the current study, the mean age of patients with benign lesions was significantly lower than that of patients with malignant tumors (PTC), with a mean age of 43 years for PTC patients, as shown in Table 3-3. This finding aligns with the research by Ikram *et al*. and Sumi *et al*., reinforcing the trend that thyroid nodule prevalence rises with age [11]. The study included a total of 60 cases, with 20% being male and 80% female. This gender distribution was similar across both malignant and benign groups (p = 0.74), corroborating the findings of Dalia *et al*. (2020) in Iraq, where non-neoplastic lesions were more prevalent in females (94.7%) than males (5.3%) [14]. Other studies, such as those by Abdulla Al Mamun (2019) in Iraq and Ahmed Abdl El A Sultan *et al*. (2022) in Egypt, also reported higher incidences of thyroid disease in women [15, 16]. The results of the current study, displayed in Table 3-3, showed that a positive family history of thyroid lesions was statistically insignificant (p = 0.966). However, previous research, including studies by Ahmed Abd El Sultan *et al*. and Schneider *et al*., has indicated a familial component in certain PTC cases, even though most cases are sporadic [17, 18]. CD56 immunostaining revealed strong positive staining (100%) for PTC and negative staining (21.4%) for PTC, with a statistically significant association (p = 0.045) as shown in Table 3-8. This finding contrasts with the study by Sumi *et al*. (2020), which reported negative CD56 expression in all PTC patients [12]. Other studies, such as those by Demellawy *et al*. (2013) and Ozolins *et al*., have reported varying degrees of CD56 expression in PTC and non-neoplastic thyroid tissues [5, 19]. CK19, an intermediate filament protein in epithelial cells, showed high levels of expression in papillary thyroid carcinoma and was useful in diagnosing PTC, as reflected in Table 3-11. This finding is consistent with studies by Abouhashem *et al*. (2017) in Egypt, where 87.8% of the PTC group and 21.2% of the non-neoplastic thyroid carcinoma (NPTC) group expressed CK19 [20]. Similarly, Huang *et al*. (2018) in China reported that 116 out of 120 PTC patients had positive CK19 staining [21]. The current study demonstrated that CD56 and CK19 are valuable immunohistochemical markers for differentiating PTC from other thyroid lesions. The high specificity and sensitivity of these markers, as well as their combined use, can significantly improve the accuracy of PTC diagnosis in clinical settings.

Conclusion

In cases of PTC, all expressed CD56 stain intensely positive, whereas benign and malignant thyroid masses exhibit mild to moderate positive staining. Positive Ck-19 staining is indicative of benign disease, whereas significant diffuse positivity is indicative of PTC. This characteristic can be utilised to diagnose PTC in lesions that have an ambiguous morphological appearance. Strong positive CD56 expression is observed in all cases involving PTC, whereas benign and malignant thyroid masses exhibit mild to moderate positive immunostaining. Evaluate the ability of CD56 and CK19 to distinguish PTC from other thyroid lesions that resemble it.

Conflict of Interest

Not available

Financial Support

Not available

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