Diagnostic Value of TROP-2 and CK19 Expression in Papillary Thyroid Carcinoma in Both Surgical and Cytological Specimens

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ABSTRACT: Papillary thyroid carcinoma (PTC) represents the most common primary malignant thyroid tumor and its diagnosis is dependent on the presence of classic nuclear features that are sometimes seen in some non-neoplastic and benign lesions. Several immunohistochemical markers are used individually or in combination to help in differentiation of PTC from mimickers. The aim of the current study was to assess the diagnostic value of TROP-2 and cytokeratin 19 (CK19) expression in differentiating PTC from other mimickers both singly and in combination. The current study was carried out on 77 surgical specimens (56 PTC and 21 non-neoplastic cases) and 12 cytological specimens (4 THY2, 6 THY4, and 2 THY5). TROP-2 was negative in 81% of non-neoplastic surgical specimens and in 100% of THY2 cytological specimens while it was positive in 71.4% of PTC surgical specimens and 100% of THY4/THY5 cytological specimens. Sensitivity and specificity of TROP-2 positive expression for diagnosis of PTC in surgical specimens reached 71% and 81%, respectively, while it reached 100% for both in cytological specimens. Cytokeratin 19 showed positive expression in 85.7% of non-neoplastic surgical specimens and in 92.9% of PTC surgical specimens. Cytokeratin 19 showed negative expression in 75% of Thy2 cases while it was positive in all studied Thy4 and Thy5 cases. Sensitivity and specificity of CK19 total estimated score for diagnosis of PTC in surgical specimens were 78.6% and 66.7%, respectively, while it reached 100% and 75% in cytological specimens. Positive TROP-2 and CK19 expression in PTC were associated with lymph node metastasis. TROP-2 is a specific rather than sensitive marker while CK19 is a sensitive rather than specific marker in differentiating PTC from other mimickers in surgical specimens. The diagnostic validity of both markers was superior in diagnosis of classic PTC compared with follicular variant PTC. TROP-2 is superior to CK19 in diagnosis of PTC in cytological specimens. Both TROP-2 and CK19 could be used preoperatively in adjunct to hematoxylin and eosin for more confident diagnosis of thyroid cytology and along with radiology as predictors of lymph node metastasis.

KEYWORDS: PTC, diagnosis, CK19, TROP-2, cytology, immunohistochemistry

Introduction

Thyroid cancer is currently the third fastest rising cancer diagnosis in the United States representing 3.4% of all new cancer cases. About 56 870 new cases of thyroid cancer were reported in the United States in 2017.[1]

In Egypt, primary malignant thyroid neoplasms constituted 1.96% of malignant neoplasms at the Egyptian National Cancer Institute (NCI) and represented 74.7% of malignant endocrine tumors. Papillary thyroid carcinoma (PTC) represented the most common primary malignant thyroid tumors accounting for 70.94% of all thyroid malignancies.[2]

Papillary thyroid carcinoma diagnosis is based mainly on the presence of classic nuclear features, including elongated nuclei with inconspicuous eccentric nucleoli and irregular nuclear membranes, chromatin clearing, and intranuclear grooves. Although a majority of PTC can be diagnosed and classified on the basis of histopathologic criteria, there are benign thyroid lesions that show nuclear cytologic features or the architecture and growth pattern of PTC, posing diagnostic problems.[3] Also some variants of PTC do not show prominent nuclear features.[4]

Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is defined as a non-invasive neoplasm of thyroid follicular cells with a follicular pattern and nuclear features of PTC without evidence of capsular, lymphatic, or vascular invasion. This entity represents a diagnostic challenge, especially in cytological preparations.[5]

Several studies used immunohistochemical markers either individually or in combination to help in differentiation of PTC from mimickers, either neoplastic or non-neoplastic such as galectin-3, HBME-1, and cytokeratin 19 (CK19).[6] However, the use of these markers has several difficulties, such as low specificity due to staining of benign cases in addition to variability of sensitivity degree and the appearance of background staining.[7] Thus, we are in need of highly sensitive and specific markers to improve PTC diagnosis.

TROP-2 is a transmembrane glycoprotein encoded by the *Tacstd2* gene. It was originally identified in human trophoblast and choriocarcinoma cell lines.[8,9] TROP-2 was subsequently reported to be over-expressed in a variety of human carcinomas and only rarely in normal tissues.[9] TROP-2 over-expression in human carcinomas is associated with tumor aggressiveness and poor prognosis.[10,11] TROP-2 has been actively studied as a prognostic marker and an attractive immunotherapeutic target in human cancer treatment.[12]

Cytokeratin 19 is a low-molecular-weight cytokeratin found in a variety of simple or glandular epithelia, both normal

and their neoplastic counterparts. In the thyroid gland, normal follicular epithelium usually shows no detectable CK19 expression.[13,14] A few reports noted CK19 expression in normal thyroid tissue in a focal staining pattern, especially in inflamed tissue.[15] Using CK19 expression as a diagnostic marker for PTC gave controversial results in previous studies.[14,16,17]

The aim of the current study was to assess the diagnostic value of TROP-2 and CK19 expression in differentiating PTC from other mimickers both singly and in combination.

Materials and Methods

This retrospective study was conducted on 77 thyroid gland surgical specimens (56 PTC and 21 non-malignant suspicious cases), collected from the archives of the Pathology Department, Faculty of Medicine, Menoufia University spanning the period between January 2014 and January 2018. They were selected according to the histopathological diagnosis. Twelve fine needle aspiration cytology (FNAC) specimens were also included, where cell blocks were prepared to obtain material suitable for immunostaining.

Evaluation of Non-Malignant (Suspicious Group)

Selection of the suspicious group was based on the presence of microscopic findings resembling or confusing with PTC, such as papillary architecture or presence of nuclear features like pale nuclei, nuclear grooves, and inclusions.

Evaluation of Malignant Group

Clinical data. Clinical data were collected from the patient's medical and pathology reports, including age, gender, type of operation (hemi-thyroidectomy, total thyroidectomy, and total thyroidectomy with neck dissection), size of the tumor (maximal diameter), T Stage (according to the TNM staging of thyroid cancer),[18] lymph node status, and focality.

Histopathological examination. Histopathological examination of hematoxylin and eosin (H&E) stained sections was performed to confirm the diagnosis and to determine histopathologic variants and background (multinodular goiter [MNG] or thyroiditis)

Cytological Specimens

Cytological specimens were assessed according to the Thy system of thyroid cytology specimens of the UK Royal College of Pathologists.[19] These cases were then followed for final histological diagnosis in excised surgical specimens.

Immunohistochemistry

Multiple paraffin sections, 4 μm in thickness from each case, were cut and stained by immunohistochemical method (streptavidin–biotin amplified system) using a DAKO automated immunostainer system (Autostainer Link 48).

Two Primary Antibodies Used

Trophoblastic cell surface antigen-2 (TROP-2)

Monoclonal antibody clone (F-5), mouse sc-376181 (Santa Cruz, Inc, California, USA), was received as a concentrated vial containing 200 μg IgG1 in 1.0 mL of phosphate buffered saline (PBS) with <0.1% sodium azide and 0.1% gelatin and diluted in PBS at a dilution of 1:50 according to instructions in the supplied pamphlet. The positive control was normal urothelium.

CK19

Mouse monoclonal antibody Keratin 19 Ab-1 (clone A53-B/A2.26) (LabVision, USA) was received as a ready to use 7 mL vial. The positive control was normal pancreatic tissue.

Immunostaining Interpretation

For both TROP-2 and CK19

Strong complete membranous staining in >5% of cells was considered positive. Nuclear or cytoplasmic staining was not taken into consideration for TROP-2.[20] For CK19, membranous +/− cytoplasmic staining was accepted.[21] Then for each marker, the following was performed:

1. Total estimated score (TES): The staining intensity was analyzed in 4 categories as negative (0), weak (1), moderate (2), and strong (3), and the staining percentage was analyzed in 5 categories, (0): <1%, (1): 1% to 25%, (2): 26% to 50%, (3): 51% to 75%, and (4): 76% to 100%.[22] Total estimated score was calculated by summing both the intensity and the percentage scores for each case. But due to marked heterogeneity of staining intensity within the same tumor; a modification has been made by calculating the percentage of different intensities (weak, moderate, and strong) and summing them together.
2. Percentage of expression expressed as mean, median, and range.
3. H score system: H score system was applied according to Bychkov et al,[23] where both the intensity and percentage of positivity were considered using the following formula: H score = (3 ×% of strong intensity) + (2 ×% of moderate intensity) + (1 ×% of mild intensity). The maximum score was 3 × 100 = 300.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with the "Statistical Package for the Social Sciences" (SPSS) version 22 program. Fisher's exact and chi-square tests were used for evaluation of qualitative data while Mann-Whitney test was used for evaluation of quantitative data. Accuracy, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were used to assess diagnostic values of tested markers. Receiver operating characteristic (ROC) curves were used to assess the diagnostic
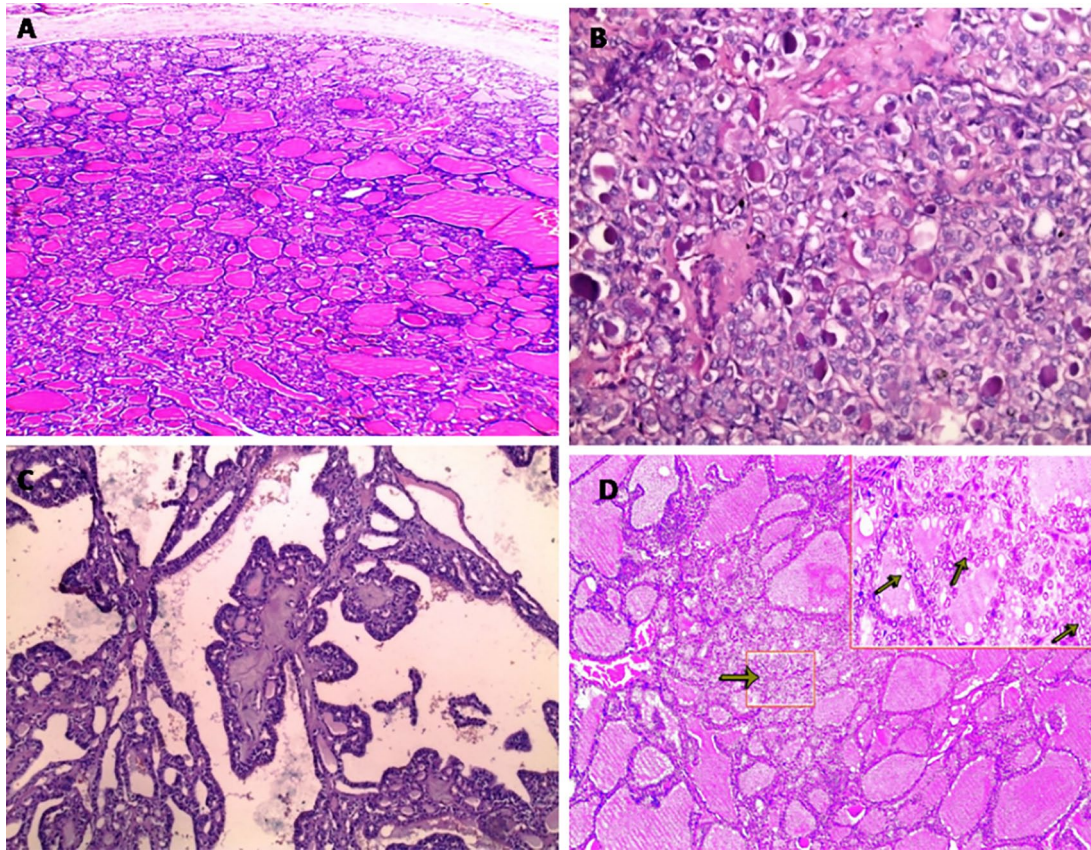
Figure 1. Capsulated follicular neoplasm (A) with areas of questionable nuclear changes of PTC (B), multinodular goiter with hyperplastic papillae (C) and focal nuclear changes of PTC (D) (hematoxylin and eosin staining ×40 for A, ×200 for B, and ×100 for C and D; arrows refer to pale nuclei, inset ×400). PTC indicates papillary thyroid carcinoma.

values of quantitative data and selection of the best cutoff point. $P < .05$ was considered significant.

The Clinicopathological Characteristics of Non-Malignant (Suspicious) and PTC Groups

The age of non-malignant cases ranged between 19 and 75 years with a median of 31 and a mean of 36.8 ± 17.45. They were 18 females and 3 males that included follicular adenoma (6 cases) (Figure 1A and B), MNG (8 cases) (Figure 1C and D), Hashimoto thyroiditis (5 cases) (Figure 2A), and Graves' disease (2 cases) (Figure 2B) (Table 1). The age of the PTC group ranged between 10 and 75 years with a median of 38 and a mean of 42.23 ± 16.82. They were 40 females and 16 males that included 35 classic PTC (Figure 2C) and 21 follicular variant PTC (Figure 2D). The background showed thyroiditis in 9 cases and MNG in 47 cases; 18 cases showed lymph node invasion and 18 cases showed multifocality. T1 stage was presented by 29 cases, T2 by 15 cases, and T3 by 12 cases (Table 2).

Results

Immunohistochemical expression of TROP-2 in the non-malignant (suspicious) group

TROP-2 was negative in 17 out of 21 non-malignant cases (81%) (Figure 3A and B) and only 4 cases (19%) were TROP-2 positive. One positive case was MNG with hyperplastic papillae (Figure 3C) and 3 out of these 4 positive cases were Hashimoto thyroiditis (Figure 3D). TROP-2 TES ranged from 0 to 7 with a mean $\pm$ SD of 0.81 ± 1.89 and a median of 0. The percentage of TROP-2 positivity ranged from 0% to 30% with a mean $\pm$ SD of 3.10 ± 7.66 and a median of 0. The calculated H score mean $\pm$ SD was 7.86 ± 19.59 and ranged from 0 to 70 with a median of 0.

Immunohistochemical expression of TROP-2 in total PTC cases

The positivity of TROP-2 was seen in 40 out of 56 malignant cases (71.4%) (35 classic) (Figure 4A) and 5 follicular variant papillary thyroid carcinoma (FVPTC) cases (Figure 4B and C) and was negative in 16 FVPTC cases (28.6%) (Figure 4D). The TES of TROP-2 ranged from 0 to 12 with a mean $\pm$ SD of 5.93 ± 4.56 and a median of 7. The percentage of TROP-2 expression ranged from 0% to 100% with a mean $\pm$ SD of 45.07 ± 41.64 and a median of 30. TROP-2 H score mean $\pm$ SD was 108.20 ± 107.19 with a median of 67.5 and ranged from 0 to 290. Details of immunohistochemical expression of TROP-2 in classic and follicular variants of PTC cases are presented in Table 3.
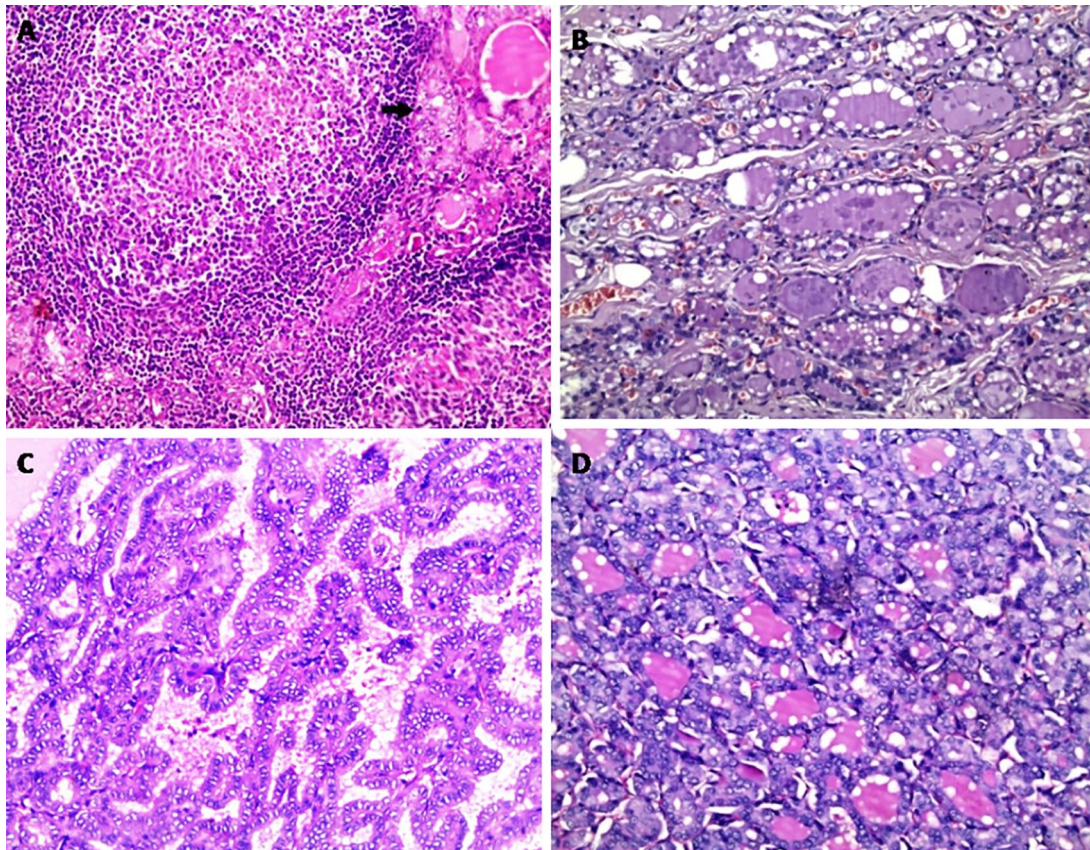
Figure 2. A case of Hashimoto thyroiditis with arrow refers to Hurthle cell with pale nuclei (A), Graves' disease showing follicles with pale scalloped colloid (B), and PTC classic variant (C) and PTC follicular variant (D) (hematoxylin and eosin staining ×200). PTC indicates papillary thyroid carcinoma.

Table 1. The clinicopathological characteristics of non-malignant (suspicious) groups.

VARIABLES		N = 21	%
Age	Mean ± SD	36.80 ± 17.45	
	Median	31	
	Range	19–75	
Gender	Female	18	85.7
	Male	3	14.3
Type of operation	Total thyroidectomy	12	57.1
	Hemithyroidectomy	9	42.9
Histopathologic types	Follicular adenoma	6	28.57
	Multinodular goiter	8	38.09
	Hashimoto thyroiditis	5	23.80
	Graves' disease	2	9.52

Differences between PTC and non-malignant (suspicious) cases with respect to TROP-2 expression

Positivity of TROP-2 was significantly higher in PTC compared with non-malignant (suspicious) groups with a significant difference (P = .001). In the same line, malignant cases showed higher mean and median values of TROP-2 TES

score, percentage of expression together with H score in comparison with non-malignant cases (P < .001) (Table 4).

The diagnostic validity of TROP-2 expression in diagnosing PTC versus non-malignant (suspicious) cases

1. Total PTC versus non-malignant cases

Table 2. The clinicopathological characteristics of papillary thyroid carcinoma group.

VARIABLES		N = 56	%
Age/year	Mean ± SD	42.23 ± 16.82	
	Median	38.50	
	Range	10-75	
Gender	Female	40	71.4
	Male	16	28.6
Type of operation	Total thyroidectomy	37	66.1
	Hemithyroidectomy	19	33.90
Size/cm	Mean ± SD	2.72 ± 2.17	
	Median	1.59	
	Range	0.2–10	
Histopathologic types	CVPTC	35	62.5
	FVPTC	21	37.5
Background	MNG	47	83.9
	Thyroiditis	9	16.1
T stage	T1	29	51.8
	T2	15	26.8
	T3	12	21.4
Lymph node status[a]	Positive lymph node (N1)	18	78.3
	Negative lymph node (N0)	5	21.7
Focality	Single	38	67.9
	Multifocal	18	32.1

Abbreviations: CVPTC, classic variant papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; MNG, multinodular goiter.
[a]Cases with available lymph node were 23.

The diagnostic power of TROP-2 positivity in all studied PTC cases (positive vs negative) revealed 71% sensitivity, 81% specificity, 91% PPV, and 52% NPV. The diagnostic accuracy (DA) was 74% with area under the curve (AUC) for the ROC curve = 0.76 (Table 5).

2. Classic variant of PTC (CVPTC) versus non-malignant cases

Regarding CVPTC, the diagnostic validity of TROP-2 expression (positive vs negative) revealed 100% sensitivity, 81% specificity, 90% PPV, and 100% NPV. The DA was 93% with AUC = 0.90.

3. Follicular variant PTC versus non-malignant cases

The sensitivity of TROP-2 reaction (positive vs negative) in FVPTC was very low (23%), while the specificity was 81%.

The PPV was 56%, the NPV was 52%, and the DA was 52% with AUC = 0.52.

Note. Application of ROC curve to determine the best cut-off point of TROP-2 TES, percentage of expression, and H score in total PTC and CVPTC cases was not effective because their values in negative cases were zero, so a value of 1% or 1 score was considered a positive reaction. TROP-2 cutoff points' sensitivity, specificity, PPV, NPV, and DA do not differ from those of TROP-2 positivity; therefore, only TROP-2 positivity was considered in PTC diagnosis.

The relationship between TROP-2 positivity and H score versus the studied clinicopathological parameters of PTC cases

Positive TROP-2 expression was significantly associated with histopathological type ($P < .001$) and lymph node status ($P < .001$), because positive expression was in favor of classic variant and positive lymph node status compared with follicular variant and cases lacking lymph node metastasis.
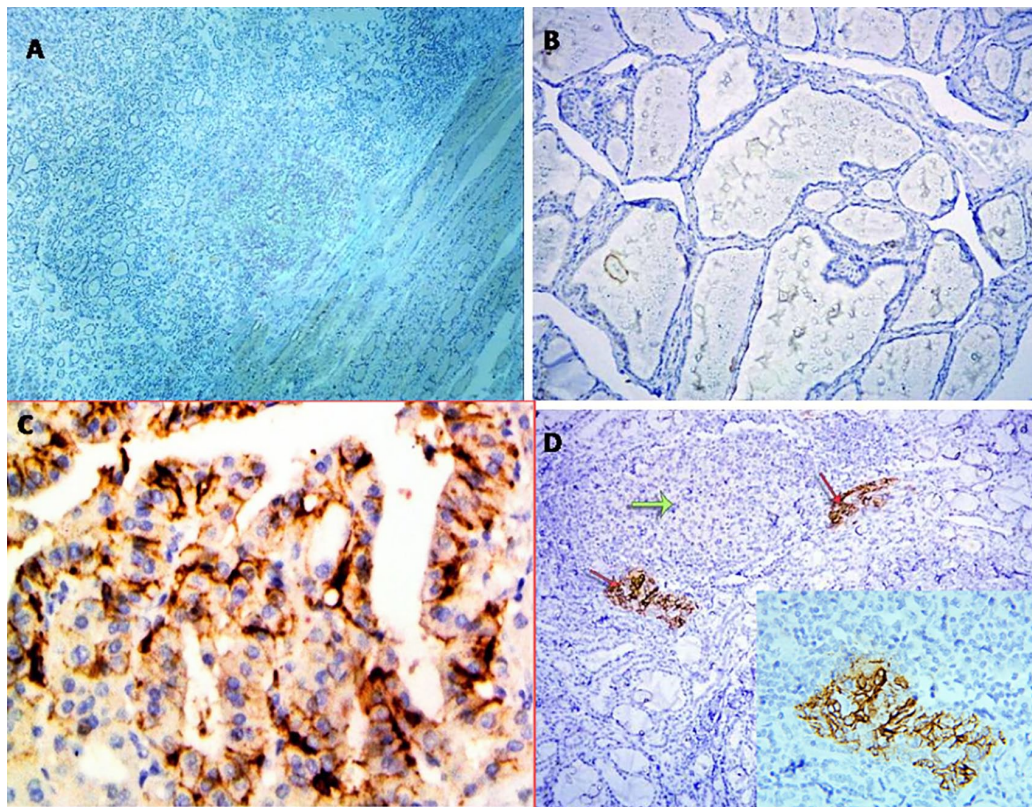
Figure 3. Negative TROP-2 expression in follicular adenoma (A) and Graves' disease (B). Positive TROP-2 expression in hyperplastic papillae (C) and Hashimoto thyroiditis (D) (IHC ×100 for A, B, and D and ×400 for C) (green arrows referred to lymphoid follicles, red arrows referred to positivity in follicles, inset [IHC ×200] highlights the positivity).
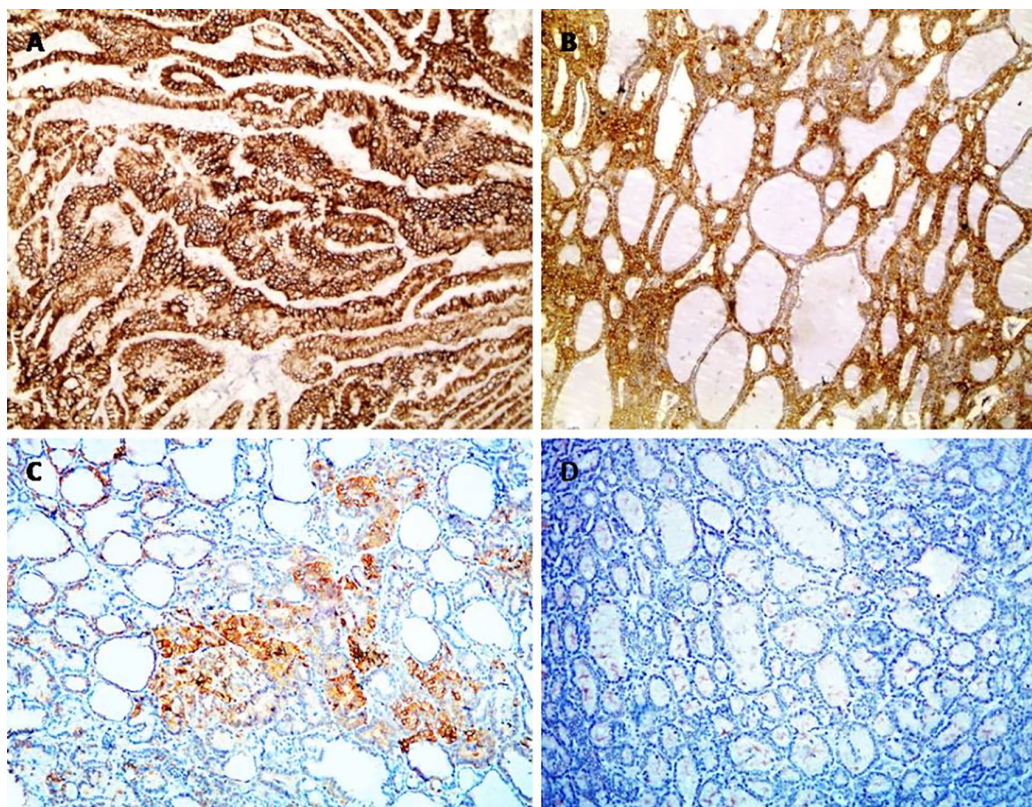

Figure 4. Positive and diffuse TROP-2 membranous expression in a case of classic PTC (A) and FVPTC (B). FVPTC showing focal TROP-2 positivity (C) and negative expression (D) (IHC ×100). FVPTC indicates follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma.

Table 3. TROP-2 and CK19 expression in total malignant, CVPTC, and FVPTC cases.

VARIABLES		TOTAL PTC (N=56)		CVPTC (N=35)		FVPTC (N=21)	
		N	%	N	%	N	%
TROP-2 positivity	Negative	16	28.6	0	0	16	76.2
	Positive	40	71.4	35	100	5	23.8
TROP-2 TES	Mean ± SD	5.93 ± 4.56		8.66 ± 2.93		1.38 ± 2.82	
	Median	7		10		0	
	Range	0–12		2–12		0–10	
TROP-2 % of expression	Mean ± SD	45.07 ± 41.64		67.26 ± 33.92		8.1 ± 22.67	
	Median	30		80		0	
	Range	0–100		2–100		0–100	
TROP-2 H score	Mean ± SD	108.20 ± 107.19		160 ± 93.17		21.19 ± 64.19	
	Median	67.5		170		0	
	Range	0–290		2–290		0–290	
CK19 positivity	Negative	4	7.1	0	0	4	19.05
	Positive	52	92.9	35	100	17	80.95
CK19 TES	Mean ± SD	7.27 ± 3.16		8.86 ± 1.97		4.62 ± 3	
	Median	7.5		10		4	
	Range	0–12		4–12		0–10	
CK19 percentage of expression	Mean ± SD	63 ± 37.05		81.86 ± 25.4		39 ± 31.1	
	Median	70		95		20	
	Range	0–100		20–100		10–100	
CK19 H score	Mean ± SD	164.02 ± 107.88		209.29 ± 89.93		109.4 ± 91.89	
	Median	170		240		60	
	Range	0–300		25–300		20–300	

Abbreviations: CK19, cytokeratin 19; CVPTC, classic variant papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; TES, total estimated score.

Furthermore, mean H score values of TROP-2 expression were higher in classic PTC compared with FVPTC ($P < .001$) and in cases with lymph node metastasis compared with cases without lymph node metastasis ($P = .01$) (Figure 5).

Immunohistochemical expression of CK19 in non–malignant (suspicious) group

Cytokeratin 19 showed positive expression in 18 out of 21 non-malignant cases (85.7%) (Figure 6) and only 3 cases showed negative expression (14.3%). Cytokeratin 19 TES ranged from 0 to 6 with a mean ± SD of 3.81 ± 1.86 and a median of 4. Cytokeratin 19 percentage of expression ranged from 0 to 70 with a mean ± SD of 27.48 ± 25 and a median of

20. The calculated CK19 H score mean ± SD was 76.48 ± 71.66 and ranged from 0 to 210 with a median of 45.

Immunohistochemical expression of CK19 in total PTC cases

Most of the studied PTC cases showed CK19 positivity (52/56 cases [92.9%], 35 classic [100%]) (Figures 6D and 7A) and 17 FVPTC (80.95%) (Figure 7B and C) and only 4 cases (7.1%) were negative (Figure 7D). The TES of CK19 in the malignant cases ranged from 0 to 12 with a mean ± SD of 7.27 ± 3.16 and a median of 7.5. The percentage of CK19 expression ranged from 0% to 100% with a mean ± SD of 63 ± 37.05 and a median of 70. Cytokeratin 19 H score mean ± SD was

Table 4. Comparison between PTC and non-malignant (suspicious) cases as regards TROP-2 and CK19 expression.

VARIABLES		SUSPICIOUS (N=21)		MALIGNANT (N=56)		TEST	*P*
		N	%	N	%		
TROP-2 positivity	Negative Positive	17 4	81.0 19.0	16 40	28.6% 71.4%	$\chi^2=17.11$	<.001 HS
TROP-2 TES	Mean ± SD Median Range	0.81 ± 1.89 0 0-7		5.93 ± 4.56 7 0-12		$U=4.34$	<.001 HS
TROP-2 percentage of expression	Mean ± SD Median Range	3.10 ± 7.66 0 0-30		45.07 ± 41.64 30 0-100		$U=4.31$	<.001 HS
TROP-2 H score	Mean ± SD Median Range	7.86 ± 19.59 0 0-70		108.20 ± 107.19 67.5 0-290		$U=4.24$	<.001 HS
CK19 positivity	Negative Positive	3 18	14.3 85.7	4 52	7.1 92.9	FE=0.28	.59
CK19 TES	Mean ± SD Median Range	3.81 ± 1.86 4 0-6		7.27 ± 3.16 7.5 0-12		$U=4.42$	<.001 HS
CK19 % of expression	Mean ± SD Median Range	27.48 ± 25 20 0-70		63 ± 37.05 70 0-100		$U=3.74$	<.001 HS
CK19 H score	Man ± SD Median Range	76.48 ± 71.66 45 0-210		164.02 ± 107.88 170 0-300		$U=3.29$	.001 HS

Abbreviations: CK19, cytokeratin 19; FE, Fisher's exact test; HS, highly significant; PTC, papillary thyroid carcinoma; TES, total estimated score; *U*, Mann-Whitney test.

Table 5. The diagnostic validity of TROP-2 and CK19 positivity in diagnosing PTC and its variants versus non-malignant (suspicious) cases.

VARIABLES		CUTOFF	AUC	SENSITIVITY	SPECIFICITY	PPV	NPV	DA
I-TROP-2 positivity (+ve vs −ve) in total PTC			0.76	71.0%	81.0%	91.0%	52.0%	74.0%
II-TROP-2 positivity (+ve vs −ve) in CVPTC			0.90	100%	81%	90%	100%	93%
III-TROP-2 positivity (+ve vs −ve) in FVPTC			0.52	23.0%	81.0%	56%	52%	52%
Total PTC	CK TES	4.50	0.826	78.6%	66.7%	86.0%	54%	75%
	CK19 %	17.5	0.759	83.9%	47.6%	81%	50%	73%
	CK H score	57.5	0.744	76.8%	57.1%	83%	48%	71%
CVPTC	CK TES	5.50	0.96	91.4%	81%	89%	85%	88%
	CK19 %	52.5	0.89	80%	81%	88%	71%	80%
	CK H score	70	0.87	91.4%	67%	82%	82%	82%
FVPTC	CK TES	1.5	0.59	81%	14.3%	49%	43%	48%
	CK19 %	8.5	0.52	81%	23.8%	52%	56%	52%
	CK H score	25.5	0.53	76.2%	23.8%	50%	50%	50%

Abbreviations: AUC, area under the curve; CVPTC, classic variant of papillary thyroid carcinoma; DA, diagnostic accuracy; FVPTC, follicular variant papillary thyroid carcinoma; NPV, negative predictive value; PPV, positive predictive value; PTC, papillary thyroid carcinoma; TES, total estimated score.
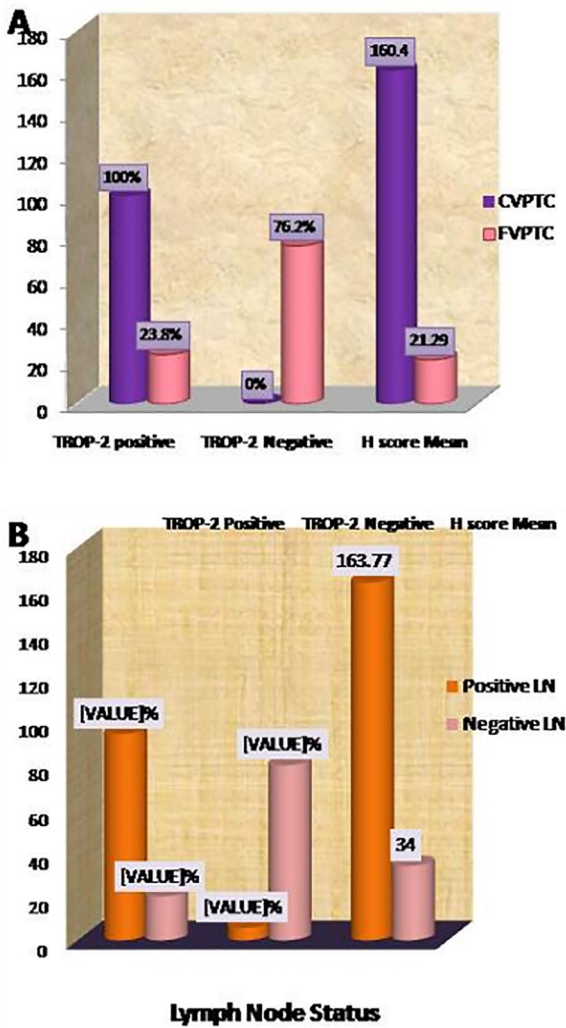
Figure 5. TROP-2 positivity (*P* < .001) and high score mean values were significantly in favor of classic PTC (A) and positive lymph node metastasis (B) compared with FVPTC and negative nodal cases. FVPTC indicates follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma.

164.02 ± 107.88 with a median of 170 and ranged from 0 to 300. Details of immunohistochemical expression of CK19 in CVPTC and FVPTC are presented in Table 3.

Differences in CK19 expression between total PTC and non-malignant cases

Cytokeratin 19 positivity failed to show any significant difference between the studied PTC and non-malignant cases because most of the cases in both groups showed positive expression for CK19 (*P* = .59). In contrast, the mean and median values of CK19 TES and percentage of expression together with H score were significantly higher in PTC cases in comparison with suspicious ones (*P* < .001) (Table 4).

The diagnostic validity of CK19 expression in diagnosing total PTC versus non-malignant (suspicious) cases

The cutoff points of CK19 TES, percentage of expression, and H score were 4.5, 17.5%, and 57.5, respectively. This means that a CK19 value at or above these points is required to diagnose a case as PTC. At or above these points, the diagnostic power of CK19 showed 78.6% sensitivity, 66.7% specificity, 86% PPV, 54% NPV, and 75% DA for TES. For CK19 percentage of expression, it showed 83.9% sensitivity, 47.6% specificity, 81% PPV, 50% NPV, and 73% DA. For CK19 H score, there was 76.8% sensitivity, 57.1% specificity, 83% PPV, 48% NPV, and 71% DA. Area under the curve of the ROC curve for CK19 TES was the highest of all (0.826) providing the highest DA (75%). The diagnostic validity of CK19 expression in diagnosing CVPTC versus non-malignant (suspicious) cases as well as FVPTC versus non-malignant cases is presented in Table 5.

The relationship between CK19 H score and the studied clinicopathological parameters in PTC

High H score mean value of CK19 was significantly associated with CVPTC (*P* < .001) and cases with lymph node metastasis (*P* = .01) in comparison with FVPTC and cases lacking nodal metastasis (Figure 8).

The diagnostic power of combined TROP-2 and CK19 expression in identification of PTC

If both TROP-2 and CK19 were positive (tests in series). In total PTC cases, when TROP-2 positivity was combined with CK19 TES cutoff point at or above 4.5, the AUC of the ROC curve became the highest (AUC = 0.783), yielding 66.1% sensitivity, 90.5% specificity, 94.9% PPV, 50% NPV, and 72.7% DA. Whereas by combining TROP-2 positivity and CK19 percentage of expression at 17.5%, the AUC of the ROC curve became 0.768, yielding 67.9% sensitivity, 85.7% specificity, 92.7% PPV, 50% NPV, and 72.7% DA. On the contrary, combined TROP-2 positivity and CK19 H score at 57.5 showed 0.759 as AUC providing 66.1% sensitivity, 85.7% specificity, 92.5 PPV, 48.6% NPV, and 71.4% DA. The diagnostic validity of the combined markers in diagnosis of CVPTC and FVPTC is presented in Table 6.

If either TROP-2 or CK19 was positive (tests in parallel). In total PTC cases, when PTC exhibited TROP-2 positivity or CK19 TES at or above 4.5, the AUC = 0.682 resulted in 83.9% sensitivity, 52.4% specificity, 82.5% PPV, 55% NPV, and 75.3% DA. On the contrary, when PTC exhibited TROP-2 positivity
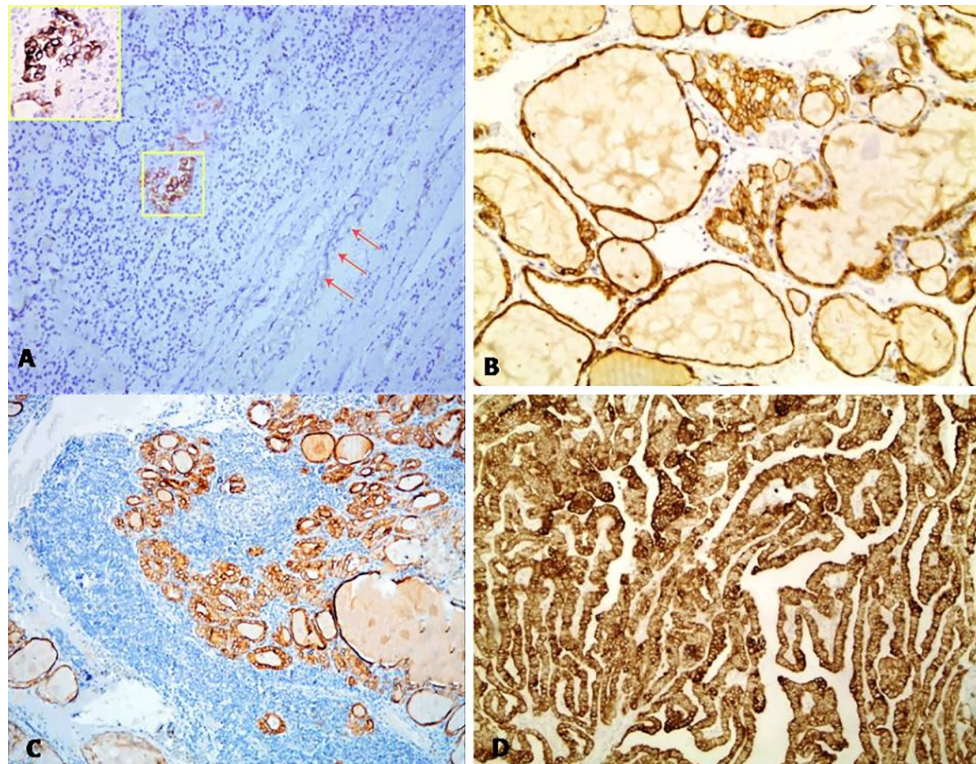
Figure 6. Focal positive CK19 expression in a case of follicular adenoma (IHC ×40). Inset highlighting membranous staining (IHC ×400), arrows pointing to the capsule (A). Positive CK19 membranous expression in a case of multinodular goiter (B) (IHC ×100), Hashimoto thyroiditis (C) (IHC ×40), and classic variant of papillary thyroid carcinoma (D) (IHC ×100).
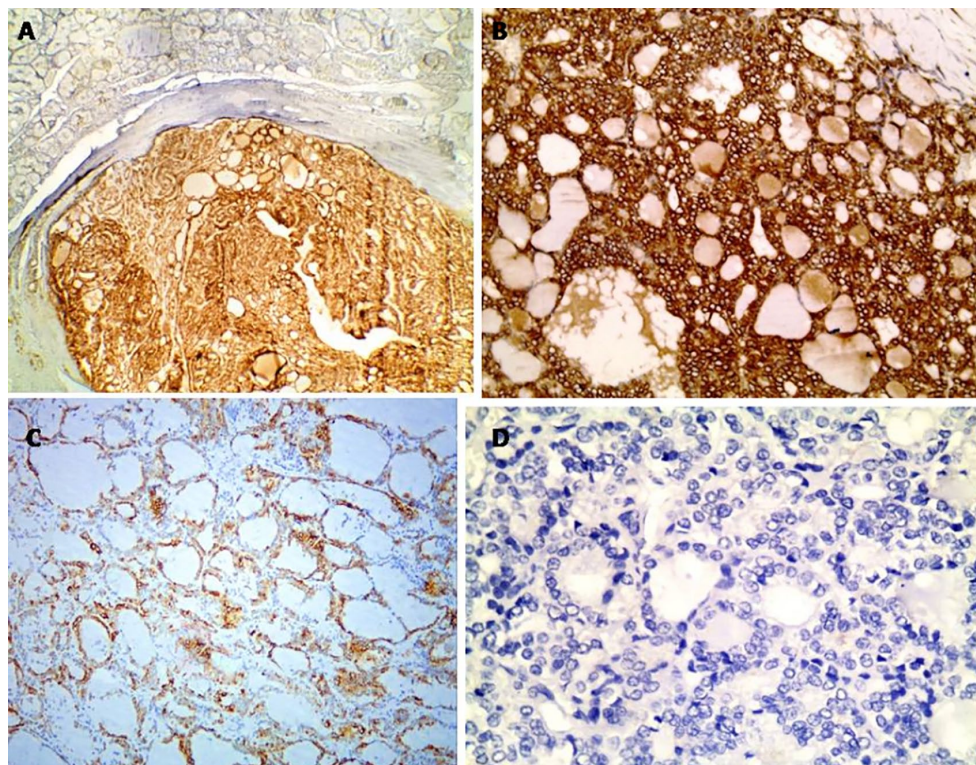

Figure 7. Diffuse and strong CK19 positivity in a case of papillary microcarcinoma (A) (IHC ×100) and FVPTC (B) (IHC ×400). Follicular variant papillary thyroid carcinoma showed focal CK19 (C) and negative expression (D) (IHC ×100). CK19 indicates cytokeratin 19; FVPTC, follicular variant papillary thyroid carcinoma.
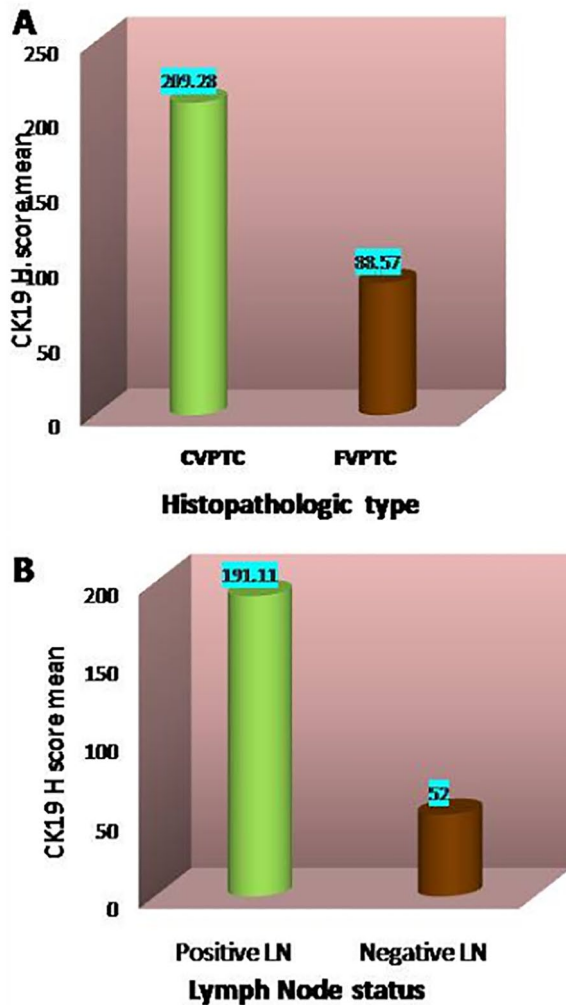
Figure 8. CK19 H score mean values were higher in classic variant of PTC (A) and in cases with lymph node metastasis (B) compared with FVPTC and negative nodal cases. FVPTC indicates follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma.

or CK19 expression percentage cutoff point ≥17.5%, the sensitivity increased to 87.5%, the specificity was 42.9%, the PPV was 80.3%, the NPV was 56.3%, and the DA was 75.3%. With positive TROP-2 or CK19 H score at or above the cutoff point of 57.5, the sensitivity was 82.1%, the specificity was 52.4%, the PPV was 82.1%, the NPV was 52.4%, and the DA was 74%. The diagnostic validity of the combined markers in diagnosis of CVPTC and FVPTC is presented in Table 7.

Cytological specimens

Four cases were diagnosed as Thy2 (33.3%), 6 cases as Thy4 (50%), and the remaining 2 cases were Thy5 (16.7%). All Thy2 cases were diagnosed as MNG (33.3%) and the 8 cases that were diagnosed cytologically as Thy4 and Thy5 were CVPTC (66.7%).

TROP-2 and CK19 expression in cytological specimens

TROP-2 was negative in the 4 studied Thy2 cases that were diagnosed as MNG (4/4[100%]) and positive in all Thy4 and Thy5 cases, which proved to be CVPTC (8/8[100%]) (Figure 9). Cytokeratin 19 showed negative expression in 3/4 (75%) of Thy2 cases with focal positivity in 1 Thy2 case (25%), while it was positive in all studied Thy4 and Thy5 cases (8/8[100%]) (Figure 10).

Diagnostic validity of TROP-2 and CK19 in diagnosing PTC versus non–neoplastic cases in cytological specimens

TROP-2 diagnostic validity in cytological specimens exhibited 100% sensitivity, 100% specificity, 100% PPV, 100% NPV, and

Table 6. The diagnostic power of combined TROP-2 and CK19 expression in identification of total PTC (if both of them were positive).

TESTS IN SERIES		AUC	SENSITIVITY	SPECIFICITY	PPV	NPV	DA
Total PTC	TROP-2 positivity + CK19 TES cutoff point (4.5)	0.783	66.1%	90.5%	94.9%	50%	72.7%
	TROP-2 positivity + CK19 percentage cutoff point (17.5%)	0.768	67.9%	85.7%	92.7%	50%	72.7%
	TROP-2 positivity + CK19 H score cutoff point (57.5)	0.759	66.1%	85.7%	92.5%	48.6%	71.4%
CVPTC	TROP-2 positivity + CK19 TES cutoff point (5.5)	0.938	97.1%	90.5%	94.4%	95%	94.6%
	TROP-2 positivity + CK19 percentage cutoff point (52.5%)	0.929	100%	85.7%	92.1%	100%	94.6%
	TROP-2 positivity + CK19 H score cutoff point (70)	0.914	97.1%	85.7%	91.9%	94.7%	92.9%
FVPTC	TROP-2 positivity + CK19 TES cutoff point (1.5)	0.524	14.3%	90.5%	60%	51.4%	52.4%
	TROP-2 positivity + CK19 percentage cutoff point (8.5%)	0.5	14.3%	85.7%	50%	50%	50%
	TROP-2 positivity + CK19 H score cutoff point (25.5)	0.5	14.3%	85.7%	50%	50%	50%

Abbreviations: AUC, area under the curve; CK19, cytokeratin 19; CVPTC, classic variant of papillary thyroid carcinoma; DA, diagnostic accuracy; FVPTC, follicular variant papillary thyroid carcinoma; NPV, negative predictive value; PPV, positive predictive value; PTC, papillary thyroid carcinoma; TES, total estimated score.

Table 7. The diagnostic power of combined TROP-2 and CK19 expression in identification of total PTC cases (if any of them was positive).

TESTS IN PARALLEL		AUC	SENSITIVITY	SPECIFICITY	PPV	NPV	DA
Total PTC	TROP-2 positivity or CK19 TES cutoff point (4.5)	0.682	83.9%	52.4%	82.5%	55%	75.3%
	TROP-2 positivity or CK19 percentage cutoff point (17.5%)	0.652	87.5%	42.9%	80.3%	56.3%	75.3%
	TROP-2 positivity or CK19 H score cutoff point (57.5)	0.673	82.1%	52.4%	82.1%	52.4%	74%
CVPTC	TROP-2 positivity or CK19 TES cutoff point (5.5)	0.762	100%	52.4%	77.8%	100%	82.1%
	TROP-2 positivity or CK19 percentage cutoff point (52.5%)	0.714	100%	42.9%	74.5%	100%	78.6%
	TROP-2 positivity or CK19 H score cutoff point (70)	0.762	100%	52.4%	77.8%	100%	82.1%
FVPTC	TROP-2 positivity or CK19 TES cutoff point (1.5)	0.548	57.1%	52.4%	54.5%	55%	54.8%
	TROP-2 positivity or CK19 percentage cutoff point (8.5%)	0.548	66.7%	42.9%	53.8%	56.3%	54.8%
	TROP-2 positivity or CK19 H score cutoff point (25.5)	0.524	52.4%	52.4%	52.4%	52.4%	52.4%

Abbreviations: AUC, area under the curve; CK19, cytokeratin 19; CVPTC, classic variant of papillary thyroid carcinoma; DA, diagnostic accuracy; FVPTC, follicular variant papillary thyroid carcinoma; NPV, negative predictive value; PPV, positive predictive value; PTC, papillary thyroid carcinoma; TES, total estimated score.
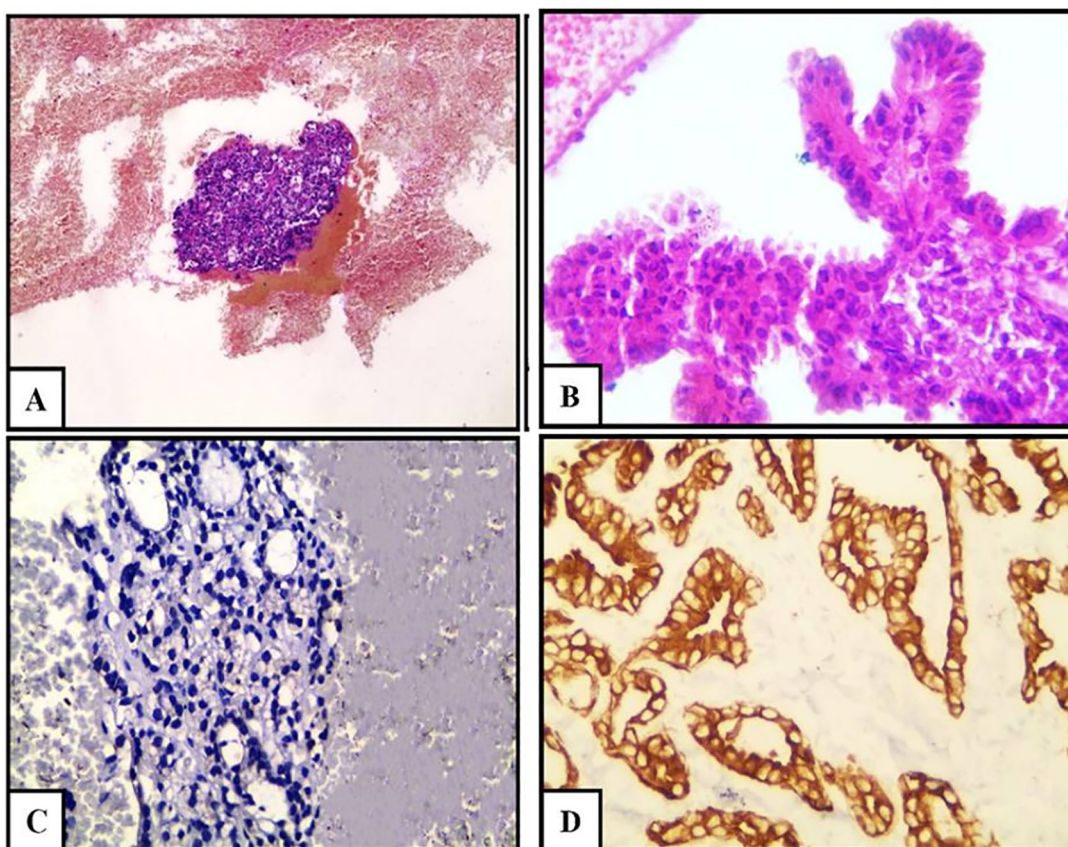

Figure 9. Cell block showing follicular epithelial cells arranged in microfollicles (A) (THY2) (H&E ×100) and papillae with fibrovascular core (THY4) (B) (H&E ×400). TROP-2 showed negative expression in THY2 (C) and positive expression in THY4 (D) (IHC ×400). H&E indicates hematoxylin and eosin.

100% DA. On the contrary, CK19 diagnostic power on cytological specimens had 100% sensitivity, 75% specificity, 88.9% PPV, 100% NPV, and 91.7% DA.

Discussion
TROP-2 was negative in all 21 studied non-neoplastic cases except 4 cases (19%). Three out of the 4 positive cases were Hashimoto thyroiditis and 1 case was MNG with hyperplastic papillae. These results agreed with Addati et al,[24] who found that TROP-2 was positive in 6/56 of non-neoplastic cases, although other studies did not reveal any positivity in non-neoplastic cases.[7,20,22] The positive results encountered in the current benign lesions could be explained by the predominance of Hurthle cells in Hashimoto thyroiditis or by the presence of
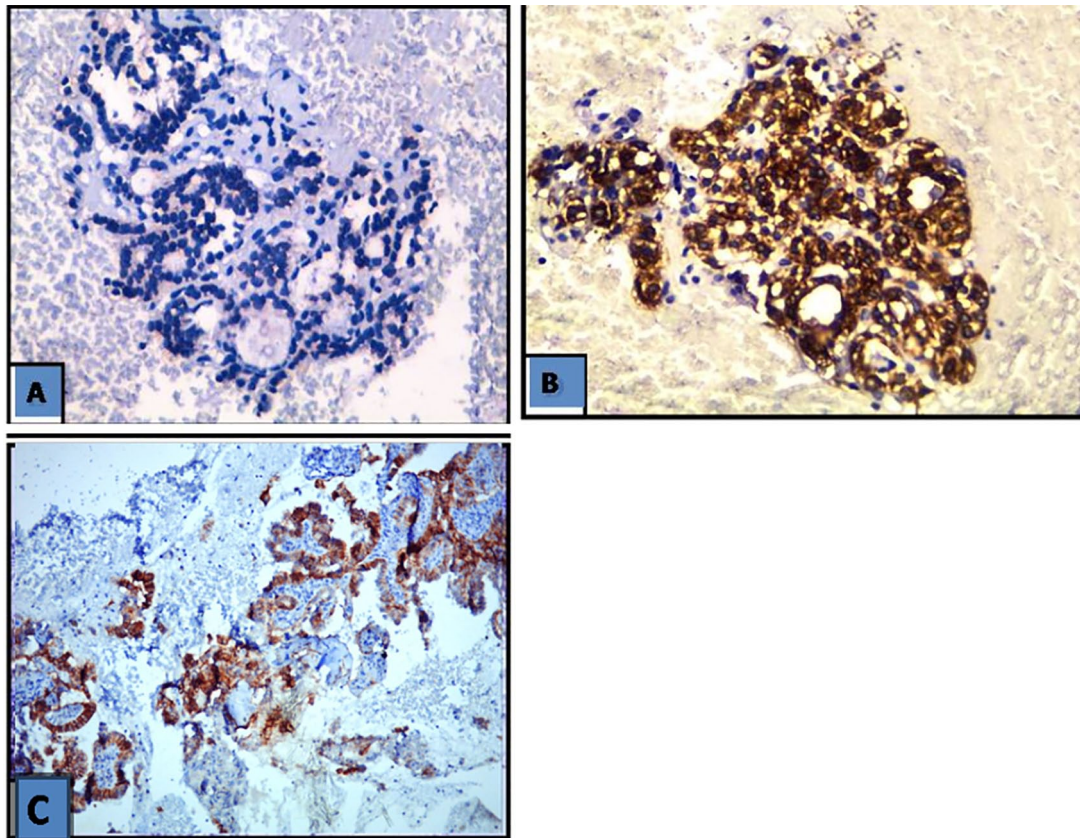
Figure 10. Negative CK19 in benign thyroid follicles (A) and its positive expression in another benign case (B) and PTC case (C) (IHC ×400 for A, ×200 for B, and ×100 for C). PTC indicates papillary thyroid carcinoma.

oncocytic cells lining the hyperplastic papillae. These cells have high endogenous biotin activity and thus may show odd positive staining pattern with different antibodies.[25]

Furthermore, follicular cells showing PTC-like nuclear changes in Hashimoto thyroiditis may carry the same genetic mutation (rearranged during transformation [RET]/PTC rearrangement) as classic PTC and express PTC-associated proteins. This mutation activates the mitogen-activated protein kinases (MAPK) pathway, which is the same pathway through which TROP-2 conducts its signals.[26] Furthermore, follicular epithelial dysplasia are follicular epithelial cells displaying cytological atypia resembling PTC in cases of chronic lymphocytic thyroiditis with a reported immunohistochemical profile similar to PTC, supporting the concept of a premalignant lesion preceding PTC arising in the context of severe chronic inflammation.[27]

The current study showed TROP-2 positivity in 40 out of 56 PTC cases (71.4%). These results were similar to those found in other studies conducted by Bychkov et al,[23] and Liu et al,[28] in which TROP-2 positivity was 81.5% and 81.6%, respectively.

TROP-2 was significantly over-expressed in the current PTC in comparison with non-malignant cases. These results agree with other studies that found TROP-2 as a novel immunostaining marker in differentiating PTC from other non-malignant thyroid lesions [23,24,28]

TROP-2 positivity showed 71% sensitivity and 81% specificity for diagnosis of total PTC cases. Similarly, Bychkov et al,[23] found that TROP-2 sensitivity in all studied types of PTC was 75%, but the specificity was higher (98.4%) because all studied benign cases were negative. Lowered TROP-2 sensitivity in the current study may be due to the presence of a considerable number of FVPTC that showed negative expression, 76.2% of cases, while decreased specificity was due to positive expression in 4 non-neoplastic cases.

Regarding TROP-2 expression in CVPTC, all cases were positive (35/35) including papillary microcarcinoma; thus, TROP-2 diagnostic power had 100% sensitivity for diagnosing CVPTC. In the same line, high TROP-2 sensitivity in the diagnosis of CVPTC was documented by other studies[20,22,23] that reported 90.9%,[20] 90%,[22] and 98.1%[26] TROP-2 sensitivity.

High TROP-2 expression in CVPTC is related to TROP-2 association with the BRAF V600E gene mutation (the most common gene mutation in CVPTC).[29] The TROP-2 molecule contributes to tumorogenesis via activation of MAPK, originally called ERK, extracellular signal-regulated kinases (MAPK/ERK pathway).[30] Similarly, the BRAF V600E gene activates cancer cell proliferation also via sustained MAPK pathway activation. Thus, it is assumed that both the BRAF mutation and TROP-2 could act together either directly or

indirectly to activate the MAPK pathway contributing to PTC tumorogenesis (as PTC is a MAPK-driven cancer).[29]

On the contrary, TROP-2 expression in the current FVPTC was low, causing a decrease in TROP-2 sensitivity to 23% (5/21 cases were TROP-2 positive). Our results agree with Simms et al[20] and Murtezaoglu and Gucer,[22] who found lowered TROP-2 sensitivity in FVPTC with values ranging between 18.8% and 5%, respectively. It is claimed that lowered TROP-2 expression in FVPTC is related again to their genetic profile because RAS mutation is a common event in FVPTC resembling follicular adenoma and follicular carcinoma, which is a rare event in CVPTC.[31] It is reported that tumors driven by RAS mutation usually respond to ERK feedback, resulting in lower MAPK signaling, the pathway involved in TROP-2 signaling.[32]

Multiple evaluation methods of TROP-2 expression were used in the current study to choose the most reliable one, including TROP-2 positivity, TES, H score, and percentage of expression. We found that the most applicable method of TROP-2 assessment was positivity, because statistical evaluation by ROC curve did not reveal any differences between these methods and TROP-2 positivity regarding sensitivity, specificity, and diagnostic validity. Furthermore, it is easier for pathologists to assess positivity than evaluation of intensity and percentage of expression.

Using ROC curve, the AUC that determines the DA was the highest (0.90) for CVPTC when compared with non-malignant cases. Moreover, the former relation achieved the highest sensitivity (100%). Thus, it appeared that TROP-2 has a strong utility in differentiating non-neoplastic cases that express some but not all classic features of PTC, such as hyperplastic nodules of MNG, Graves' disease, or hyperfunctioning adenoma, from classic cases of PTC. This can avoid overdiagnosis of such cases or unnecessary surgery for non-neoplastic lesions, which can be treated medically. On the contrary, TROP-2 showed a limited ability in differentiating FVPTC from other mimickers, which still represented a diagnostic challenge, agreeing with Finley et al.[33]

Furthermore, TROP-2 was significantly associated with lymph node involvement. This result agrees with Guan et al,[34] where TROP-2 was reported to enhance the invasion and migration of thyroid cancer cells via activation of the transcription factor activation protein-1 (AP-1) leading to upregulation of matrix metalloproteinase-2 (MMP2) responsible for degradation of type VI collagen that aids in the spread of tumor through the extracellular matrix.[34] High TROP-2 expression was reported to be used as a potential indicator for lymphatic metastasis in PTC because it is not expressed in follicular carcinoma that spreads hematogenously.[33]

In the current study, CK19 was positive in 18 out of 21 non-malignant cases (85.7%). Variable percentage of CK19 positivity had been reported in non-neoplastic cases in different studies. It was 25.83% in the study of Song et al[35] and 34% in the study of Barroeta et al,[36] and increasing to 79.16% in the study of Scognamiglio et al.[37]

Regarding the current PTC, CK19 was positive in 52 out of 56 (92.9%) cases. All studied CVPTC (35/35) cases and 17/21 (80.95%) of FVPTC cases exhibited CK19 positivity. Many authors have reported high CK19 expression in PTC cases (especially classic variant).[35,38] The expression in FVPTC is controversial, varying from 18%,[15] 83%,[39] to 100%.[14]

The current study showed absence of a significant difference between PTC and non-malignant cases regarding CK19 positivity alone, with a significant difference between them using CK19 TES, percentage of expression, and H score methods, where higher mean and median values were in favor of PTC cases. These results were supported by others[28,40] who reported diffuse and strong expression of CK19 in PTC, unlike focal positivity in benign thyroid lesions.

Similar to TROP-2, different methods were used for assessment of CK19. Among these methods, the highest value of AUC = 0.826 for total PTC cases was achieved with a TES score cutoff point ⩾4.5 with 78.6% sensitivity, 66.7% specificity, and highest DA (75%). Murtezaoglu and Gucer[22] also demonstrated similar results when CK19 TES score was used to differentiate PTC from benign thyroid lesions (83.3% sensitivity and 60% specificity). As regards CK19 percentage of expression using cutoff ⩾17.5% of positive CK19, the sensitivity slightly increased to 83.9% but the specificity dropped to 47.6%. For discrimination of PTC and its mimickers, many studies used ROC curve to set the optimal cutoff points for CK19 percentage. These cutoff points varied from 5% according to Paunovic et al[41] to 9.5% according to Dunđerović et al.[42] High CK19 % cutoff point regarding total PTC cases in the current study may be due to its positivity in a large number of non-malignant cases (18 out of 21), so the large number of false positive cases raised the cutoff point to discriminate between true and false positives.

In the current study, when ROC curve was used to determine the diagnostic validity of CK19 expression in diagnosing CVPTC versus suspicious cases, the AUC increased to 0.96 and 0.89 as the cutoff points of TES and percentage of CK19 expression increased to 5.5% and 52.5%, respectively. Moreover, we noticed improvement in CK19 sensitivity (91.4%) and specificity (81%) with TES. These results appear to be in line with previous studies as all authors have agreed on diffuse CK19 expression as a requirement for PTC diagnosis.[14,40]

Regarding the diagnostic utility of CK19 in differentiating FVPTC from suspicious lesions, the AUC was low using different methods of CK19 assessment together with an obvious decline in DA. This was in concordance with other studies that reported a limited role of CK19 in differentiating follicular patterned lesions with limited sensitivity and specificity.[43,44] The reason for decreased DA together with low specificity may be related to the focal mild staining pattern observed in the

studied both suspicious and FVPTC cases (mean values of H score were 76.48 and 88.57, respectively).

In the current study, it was found that there was a significant positive correlation between CK19 H score and lymph node status in the studied PTC cases. This was in contrast to Song et al[35] who reported that CK19 expression did not differ among PTC cases with lymphatic metastasis and those lacked this type of metastasis. On the contrary, other authors have agreed with us and declared the poor prognostic role of CK19 in other tumors, such as pancreatic[45] and intrahepatic cholangiocarcinoma,[46] and its association with lymphatic metastasis.

No single immunohistochemical marker alone could provide a high sensitivity and specificity in differentiating PTC from its mimickers.[22,47] In the current study, a combination of TROP-2 positivity and CK19 (with different score cutoff points) have been used trying to determine the best one to differentiate PTC (total PTC, CVPTC, and FVPTC) from non-malignant cases. The highest specificity (90.5%) was achieved in all PTC cases when both TROP-2 positivity and CK19 TES ≥4.5 were combined, and both of them should be positive. However, the highest sensitivity was achieved for recognizing total PTC (87.5%) and FVPTC (66.7%) when TROP-2 positivity and CK19 percentage of expression ≥17.5% and 8.5%, respectively, were combined and either of them was positive. For CVPTC, the sensitivity was 100% in all combinations. This high specificity value of this combination observed in our study greatly exceeded that reported by Murtezaoglu and Gucer[22] who demonstrated 60% specificity. The high specificity of TROP-2 in our cases (4/21 false positivity in benign cases) could overcome the high rate of false positive benign cases (18/21) in CK19 when combined together.

Diagnosis of PTC in thyroid FNAC specimens can sometimes pose a difficult and challenging task. In the current study, TROP-2 was a very sensitive (100%) and specific (100%) marker in diagnosing PTC in cytological specimens. This finding highlights the potential and helpful use of TROP-2 in PTC diagnosis in cytological specimens. Many studies have applied TROP-2 in cytological specimens and revealed high sensitivity and specificity.[7,20,24] It seems that in the current study, TROP-2 diagnostic validity in cytological specimens was superior to that of surgical specimens. This may be due to all THY4 and THY5 cases being CVPTC only.

Regarding the diagnostic role of CK19 in thyroid cytology, it is also a good sensitive marker, being positive in all PTC cases, agreeing with others.[47] On the contrary, CK19 was not as specific as TROP-2 because it was positive in 1 out of 4 studied cytologically non-neoplastic thyroid nodules, which was confirmed by histological examination to be MNG. Reduced CK19 specificity in cytological specimens was also reported.[48]

In summary, TROP-2 is a specific rather than sensitive marker in diagnosing PTC with a specificity reaching 81% and 71% sensitivity in surgical specimens, while both reached 100% in assessment of cytological specimens. Unlike TROP-2, CK19

is a sensitive rather than specific marker in differentiating PTC from other mimickers in both surgical and cytological specimens with a sensitivity reaching 78.6% and 100%, respectively. The highest specificity (90.5%) was reached when TROP-2 positivity and CK19 TES score of ≥4.5 were combined and both of them were positive, while the highest sensitivity (87.5%) was accomplished when both TROP-2 positivity and CK19 percentage cutoff point at or above 17.5 were combined and either of them was positive.

Author Contributions

AGA collected, evaluated the cases and shared in writing and publication of article. MS revised the article.

RA evaluated the cases and shared in writting. NN collected, evaluateed the cases and shared in writing as well as statistics of the article.

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