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RESEARCH ARTICLE

TROP-2: a unique immunohistochemical marker for diagnosis of papillary thyroid carcinoma

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ABSTRACT

Background: Histomorphology remains the gold standard in the diagnosis of thyroid tumors; specifically papillary thyroid carcinoma (PTC). However, equivocal cytohistologic features may be encountered, and warrant special criteria for distinction. **Aim:** This study aimed to determine the potential use of TROP-2 immunoreactivity in the diagnosis of PTC (in comparison to CK19 immunoreactivity) and its role in the segregation of PTC from non-neoplastic, benign as well as malignant thyroid lesions, and to predict the diagnostic significance of both markers in combination. **Material and Methods:** The current work was carried out on 249 cases of thyroid lesions, retrieved as paraffin blocks; from the Department of Pathology, Faculty of Medicine, Tanta University. The study was conducted during the period from March 2019 to May 2020. H&E staining, as well as immunohistochemical staining using TROP-2 and CK19, was performed for each case. **Results:** For discriminating PTC from non-neoplastic/benign thyroid lesions; TROP-2 showed 100% specificity, 87.78% sensitivity, 100% PPV and 94.47% accuracy, CK19 showed 100% sensitivity, 25.69% specificity. For segregation of PTC, from other (non-neoplastic, benign or malignant) thyroid lesions; TROP-2 showed 87.78% sensitivity, 98.11% specificity, 96.34% PPV, 93.41% NPV and 94.38% accuracy. CK19 showed 100% sensitivity and 100% NPV, yet it had low specificity of 40.25%, a low PPV of 48.65% and low accuracy of 61.85%. The combined usage of both markers didn't show better results than TROP-2 alone. **Conclusions:** CK19 exhibits high sensitivity over TROP-2 in PTC diagnosis, however, TROP-2 shows high specificity and better accuracy. The combination of both markers doesn't show remarkable findings than TROP-2 alone.

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INTRODUCTION

Thyroid malignancies rank the top among other endocrine tumors; the incidence of which has been doubled since 1990; owing to the widespread application of special diagnostic procedures and introduction of the interventional technique of Sonar guided fine needle aspiration cytology (FNAC) (Raman and Koenig, 2014). Thyroid malignancies are mostly of well-differentiated histogenesis and include a spectrum of papillary thyroid carcinoma (PTC) *(which accounts for 80% of thyroid cancers),* follicular thyroid carcinoma (FTC), C-cell derived medullary thyroid carcinoma (MTC), poorly differentiated thyroid carcinoma (PDTC), and anaplastic thyroid carcinoma (ATC) (Yang *et al.,* 2018). Furthermore, a long list of benign as well as suspicious thyroid lesions does also exist.

Histopathological examination using routine Hematoxylin and Eosin (H&E) staining represents the cornerstone for diagnosing various thyroid lesions. Such a matter would not always go straight, and pathologists often face the dilemma of morphologically challenging and ambiguous cases. Among which stands out the problem of differentiating PTC from its mimickers; especially benign and suspicious cases that show nuclear cytologic features or architectural growth patterns of PTC, Moreover, some variants of PTC do show nuclear features in a focal or subtle manner (Bose *et al.,* 2012). Hence, the immunohistochemical (IHC) application may be a must in many cases and still plays a major role in establishing the appropriate diagnosis and excluding purposed differential diagnosis (Liu and Lin, 2015). To

date, controversial reports exist regarding both old as well as recently introduced immunohistochemical markers for confirming the diagnosis of PTC, with conflicting and unreliable results.

Trophoblast cell-surface antigen 2 (TROP-2) or tumor-associated calcium signal transducer 2 (TAC-STD2), *a type 1 transmembrane glycoprotein of about 35-kD weight,* was originally detected in trophoblastic cell lineage and choriocarcinomatous tissue. Biologically, it behaves as a cell surface receptor, which recognizes and encodes special types of ligands; leading to a large number of vital cellular events (eg. Subsequent elevation of calcium level, intracellularly). Various reports claimed its potential role in several malignant human tumors, whereas, in normal human tissues, the expression of TROP-2 is generally undetectable. TROP-2 regulates neoplastic growth, local infiltration and regional as well as distant metastasis by controlling several signaling pathways. An argued relation to stem cell biology and integration through several human diseases has been found, as well (Guan *et al.,* 2017).

Cytokeratins (CKs) are a well-known family of intermediate cytoskeletal filaments of epithelial cells; they are located in the cellular cytoplasm showing an integrated network providing a connection between the cellular membrane and the nucleus. CKs participate in many vital cellular processes such as mitosis, post-mitotic period, cell migration, differentiation, tissue specialization, and programmed cell death (apoptosis). CK19 is considered an established specific and unique biomarker for a range of malignant tumors namely, PTC, hepatocellular carcinoma, squamous cell carcinoma and colonic adenocarcinoma (Kaliszewski *et al.,* 2016). The current work aimed to determine the diagnostic utility of TROP-2 immunohistochemical expression (in comparison to CK19) in differentiating PTC from various non-neoplastic, benign and malignant thyroid lesions; and to predict the diagnostic significance of both markers in combination.

MATERIAL AND METHODS

This retrospective study was carried out on 249 formalin-fixed paraffin-embedded tissue blocks of various non-neoplastic, benign and malignant thyroid lesions (Table 1). 189 cases were females, and 60 cases were males, age ranged between (17 – 65 years), collected from the archive of Pathology Department, Faculty of Medicine, Tanta University. The study was conducted during the period from March 2019 to May 2020. Approval from the research ethics committee (REC), Faculty of Medicine, was taken antecedent to conduct the study. Serial sections were prepared from each block. Histological sections, 4-µm thick, were stained by Hematoxylin and Eosin (H&E) for histopathological assessment of different thyroid lesions. Examined H&E sections were reviewed and representative areas of the tumor were selected for further immunohistochemical evaluation.

Immunohistochemistry

From each paraffin block, 4-µm thick sections mounted onto positively charged slides were performed for immunohistochemical staining according to recommendations of the manufacturers using a DAKO automated immune-stainer system (Auto-stainer Link 48).

The primary antibodies used were TROP-2 (a monoclonal mouse antibody sc-376181; (clone (F-5), Santa Cruz Biotechnology, Inc, California, USA, dilution 1:100). The positive control was urothelial tissue and the negative control was non-neoplastic thyroid, and CK19 (a monoclonal mouse antibody sc-53258; Santa Cruz Biotechnology, California, USA, dilution 1:50). The positive control was pancreatic tissue. Negative controls replaced the primary antibody with buffer). The secondary antibody used was Biotinylated Goat (Anti-Mouse IgG (H+L) (Lab vision, USA, Cat.# TM-060-BN), diluted, ready to use). Incubation of the slides with previous primary antibodies was done (for 20–30 min), after the addition of a peroxidase-blocking reagent (for 5 min); followed by treatment using horseradish per-oxidase (HRP) reagent (for 20 min) and di-amino-benzidine (DAB) chromogen solution (for 10 min). Counterstaining using Hematoxylin stain was applied finally.

Assessment of immunostaining

For TROP-2 evaluation: strong continuous membranous staining in more than 5% of the

cells was considered positive. Cytoplasmic or nuclear staining was ignored for TROP-2 (Simms *et al.,* 2016). The distribution was recorded as negative (no stain or < 5% of tumor cells stained), 1+ (5% to 25%), 2+ (26% to 50%), 3+ (51% to 75%), or 4+ (>75%) (Murtezaoglu and Gucer, 2017).

For CK19 evaluation: membranous and/or cytoplasmic staining qualified the case as positive (Xin *et al.,* 2017). The ratio of positively stained cells was scored as 1+ (< 5% of cells), 2+ (5%–25% of cells), 3+ (25%–75% of cells) or 4+ (>75% of cells) (Bose *et al.,* 2012).

Statistical analysis

The collected data were statistically analyzed using the SPSS software statistical computer package (version 23). Data were expressed in terms of frequencies (number of cases) and percentages for categorical variables and range as well as a median for continuous variables.

To confirm the diagnostic role of TROP-2 and CK19, agreement analysis was performed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy as follows:

- Sensitivity: True positive / (True positive + False negative) × 100
- Specificity: True negative / (True negative + False positive) × 100
- PPV: True positive / (True positive + False positive) × 100
- NPV: True negative / (True negative + False negative) × 100
- Accuracy: (True positive + True negative) / (True positive + True negative + False positive + False negative) × 100

RESULTS

The current study included 249 histological thyroid specimens of various thyroid lesions (summarized in Table 1), ages ranged from 17 – 65 years; (median 30 years). One hundred and eighty-nine cases (75.9%) were females and 60 cases (24.1%) were males. One hundred and forty cases were malignant (with PTC representing the predominant subtype; 64.3%) and one hundred and nine cases were non-neoplastic/benign.

Among all studied cases; TROP-2 immunoreactivity showed positive results in 32.9% (82/249) of cases; all of which were malignant, TROP-2 immunohistochemical expression was significantly higher in PTC in comparison to other thyroid lesions (non-neoplastic, benign and malignant). Of the included 90 PTC cases; 79 cases (87.8%) demonstrated TROP-2 immunoreactivity. In contrast, the entire nodular goiter (NG), Grave's disease (GD), Hashimoto thyroiditis (HT) and follicular adenoma (FA) lesions were TROP-2 negative. All cases (100%) of MTC, ATC and the majority of FTC (86.67%) and PDTC (80%) showed a negative reaction for TROP-2 (Table 2, Figure 1).

As regard CK19 immunoreactivity in non-neoplastic and benign cases; 74.3% of cases showed positive reaction for CK19; 88.9%, 85% and 69% of FA, HT and NG were positive, respectively, meanwhile 73.3% of GD cases showed negative reaction for CK19. Positive CK19 immunoreactivity showed predominantly intermediate scores among NG, GD, HT and FA lesions (score 2 or 3). Among malignant cases; 74.4% were CK19 positive; 100% and 60% of PTC and PDTC were CK19 positive (score 3 or 4), respectively. However, 85%, 70%, and 66.7% of MTC, ATC and FTC were negative for CK19, respectively (Table 2, Figure 2).

In discriminating PTC from other non-neoplastic and benign thyroid lesions; diagnostic significance of TROP-2 and CK19 immunoreactivity, as well as their combinations, was performed in terms of sensitivity, specificity, PPV, NPV, and diagnostic accuracy. TROP-2 showed the highest value of specificity of 100%, PPV of 100% and an accuracy of 94.47% for PTC diagnosis. With the combined usage of TROP-2 and CK19 *(which showed 100% sensitivity for PTC diagnosis; and much lower figures among other data),* no difference was noticed as regards the assessment of sensitivity, specificity, PPV, NPV and accuracy. CK19 is a highly sensitive marker for PTC diagnosis, but its low specificity warrants its use with caution. TROP-2 expression had overall better results (Table 3).

Table 1. Distribution of the studied cases

Clinicopathological characteristics	Cases (No.) 249	%
Sex		
Female	189	75.9%
Male	60	24.1%
Diagnosis		
Benign/non neoplastic (n = 109)		
NG	29	26.6%
GD	15	13.8%
HT	20	18.3%
FA	45	41.3%
Malignant (n = 140)		
FTC	15	10.7%
PTC	90	64.3%
MTC	20	14.3%
PDTC	5	3.6%
ATC	10	7.1%

Abbreviations: NG (nodular goiter), GD (Grave's disease), HT (Hashimoto thyroiditis), FA (follicular adenoma), FTC (Follicular thyroid carcinoma), PTC (papillary thyroid carcinoma), MTC (medullary thyroid carcinoma), PDTC (poorly differentiated thyroid carcinoma), ATC (anaplastic thyroid carcinoma)

Table 2. Immunoreactivity of TROP-2 and CK19 in various thyroid lesions

Clinicopathological characteristics	Cases (No.)	Positive expression for TROP-2 %		Negative expression for TROP-2 %		Positive expression for CK19 %		Negative expression for CK19 %	
Diagnosis		**82**		**167**		**185**		**64**	
Benign/non-neoplastic	**109**	**0**	**0.0%**	**109**	**100%**	**81**	**74.3%**	**28**	**25.7%**
NG	**29**	0	0.0%	29	100%	20	69%	9	31%
GD	**15**	0	0.0%	15	100%	4	26.7%	11	73.3%
HT	**20**	0	0.0%	20	100%	17	85%	3	15%
FA	**45**	0	0.0%	45	100%	40	88.9%	5	11.1%
Malignant	**140**	**82**	**58.6%**	**58**	**41.4%**	**104**	**74.4%**	**36**	**25.6%**
FTC	**15**	2	13.33%	13	86.67%	5	33.3%	10	66.7%
PTC	**90**	79	87.8%	11	12.2%	90	100%	0	0.0%
MTC	**20**	0	0.0%	20	100%	3	15%	17	85%
PDTC	**5**	1	20%	4	80%	3	60%	2	40%
ATC	**10**	0	0.0%	10	100%	3	30%	7	70%

Abbreviations: NG (nodular goiter), GD (Grave's disease), HT (Hashimoto thyroiditis), FA (follicular adenoma), FTC (Follicular thyroid carcinoma), PTC (papillary thyroid carcinoma), MTC (medullary thyroid carcinoma), PDTC (poorly differentiated thyroid carcinoma), ATC (anaplastic thyroid carcinoma)

For differential diagnosis of PTC from other malignant thyroid lesions; CK19 showed 100% sensitivity vs. 87.78% sensitivity of TROP-2. On the other hand, TROP-2 showed higher specificity and PPV; yet, both markers had the same accuracy (Table 4). For segregation of PTC, from other (non-neoplastic, benign or malignant) thyroid lesions; TROP-2 showed 87.78% sensitivity, 98.11% specificity, 96.34% PPV, 93.41% NPV and 94.38% accuracy. CK19 showed 100% sensitivity and 100% NPV, yet it had low specificity of 40.25%, low PPV of 48.65% and low accuracy 61.85% (Table 5). The combined usage of both markers didn't show better results than TROP-2 alone.

Table 3. Sensitivity, specificity, PPV, NPV and accuracy for TROP-2 and CK19 in differentiating papillary thyroid carcinoma (PTC) from benign/non-neoplastic thyroid lesions

	Benign/non-neoplastic (n = 109)		PTC (n = 90)		Sensitivity	Specificity	PPV*	NPV**	Accuracy
	No.	%	No.	%					
TROP-2									
Negative	109	100.0	11	12.2	87.78	100.0	100.0	90.83	94.47
Positive	0	0.0	79	87.8					
CK19									
Negative	28	25.7	0	0.0	100.0	25.69	52.63	100.0	59.30
Positive	81	74.3	90	100.0					
Combination									
Negative	109	100.0	11	12.2	87.78	100.0	100.0	90.83	94.47
Positive	0	0.0	79	87.8					

*PPV: positive predictive value, **NPV: negative predictive value

Table 4. Sensitivity, specificity, PPV, NPV and accuracy for TROP-2 and CK19 in differentiating papillary thyroid carcinoma (PTC) from other malignant thyroid lesions

	Malignant (n = 50)		PTC (n = 90)		Sensitivity	Specificity	PPV*	NPV**	Accuracy
	No.	%	No.	%					
TROP-2									
Negative	47	94.0	11	12.2	87.78	94.0	96.34	81.03	90.0
Positive	3	6.0	79	87.8					
CK19									
Negative	36	72.0	0	0.0	100.0	72.0	86.54	100.0	90.0
Positive	14	28.0	90	100.0					
Combination									
Negative	36	72.0	0	0.0	100.0	72.0	86.54	100.0	90.0
Positive	14	28.0	90	100.0					

*PPV: positive predictive value, **NPV: negative predictive value

Table 5. Sensitivity, specificity, PPV, NPV and accuracy for TROP-2 and CK19 in differentiating papillary thyroid carcinoma (PTC) from other thyroid lesions

	Other (n = 159)		PTC (n = 90)		Sensitivity	Specificity	PPV*	NPV**	Accuracy
	No.	%	No.	%					
TROP-2									
Negative	156	98.1	11	12.2	87.78	98.11	96.34	93.41	94.38
Positive	3	1.9	79	87.8					
CK19									
Negative	64	40.3	0	0.0	100.0	40.25	48.65	100.0	61.85
Positive	95	59.7	90	100.0					
Combination									
Negative	156	98.1	11	12.2	87.78	98.11	96.34	93.41	94.38
Positive	3	1.9	79	87.8					

DISCUSSION

Previous studies have investigated dozens of immunohistochemical markers to help in the diagnosis of PTC, but their results have yielded a varying degree of sensitivity and specificity. CK19 is considered, by far, the most sensitive marker for PTC. However, CK19 expression is non-specific; as it has also been expressed in benign and non-neoplastic thyroid lesions. CK19 may, also, exhibit focal expression in equivocal cases, aberrant staining in oncocytic neoplasms, and false positivity in Hashimoto's thyroiditis (Bychkov *et al.,* 2016). TROP-2 is a transmembrane glycoprotein that is highly expressed in various human malignancies. It is considered by many authors as an oncogene (Murtezaoglu and Gucer, 2017). In the present

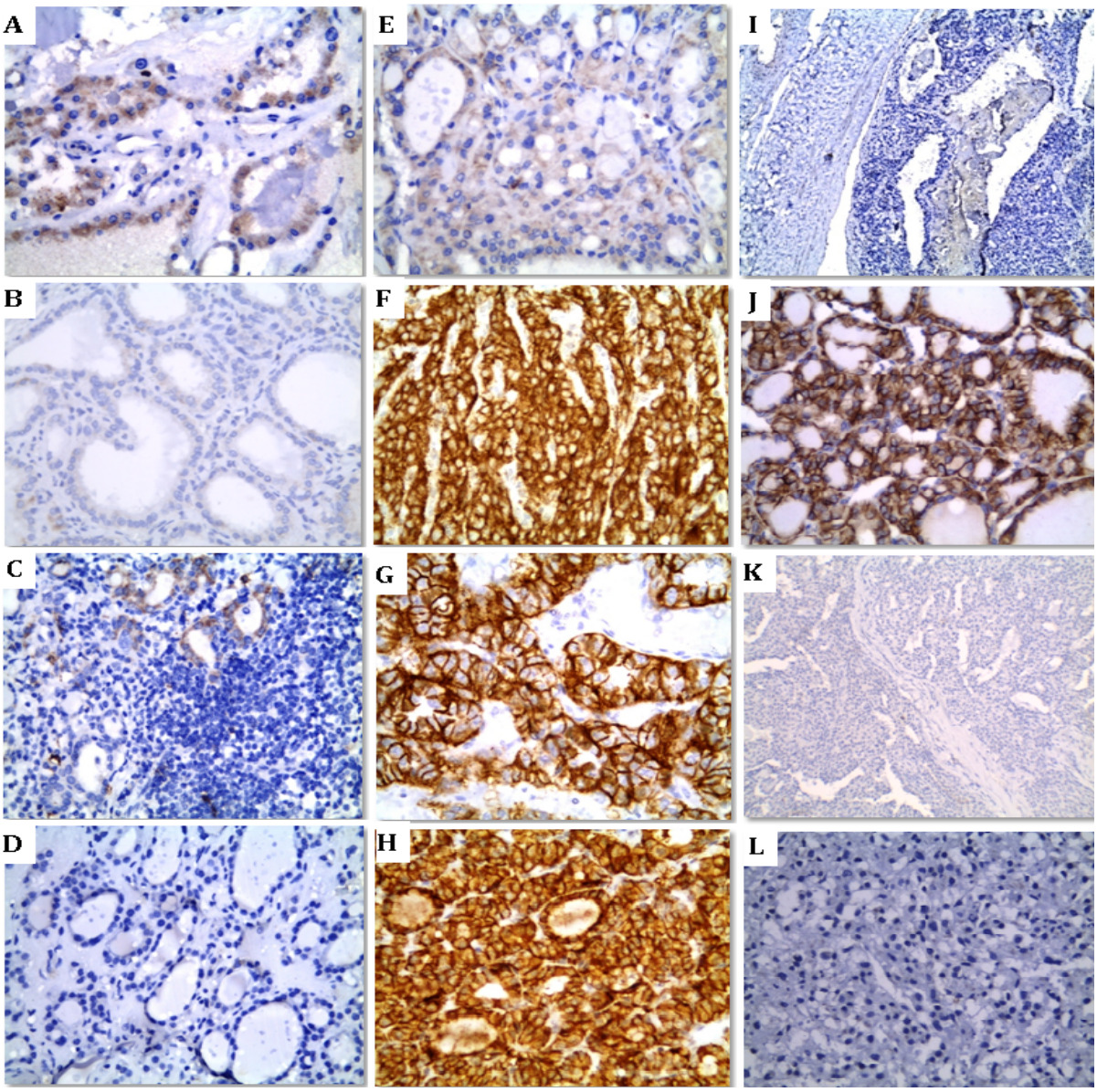

Figure 1. immunohistochemical expression of TROP-2 in various thyroid lesions. (A) cytoplasmic (negative) staining in nodular goiter (x400), (B) negative staining in Grave's disease (x400), (C) focal weak cytoplasmic (negative) staining in Hashimoto thyroiditis (x400), (D) negative staining in follicular adenoma (x400), (E) negative staining in follicular adenoma, Hurthle cell variant (x400), (F) positive membranous staining (score +4) in a classic variant of papillary thyroid carcinoma (x400), (G) positive membranous staining (score +4) in papillary thyroid microcarcinoma (x400), (H) positive membranous staining (score +4) in follicular variant of papillary thyroid carcinoma (x400), (I) negative staining in minimally invasive follicular carcinoma, with vascular invasion (x100), (J) positive membranous staining (score +3) in follicular carcinoma (x400), (K) negative staining in medullary thyroid carcinoma (x100), (L) negative staining in anaplastic thyroid carcinoma (x400).

work, TROP-2 expression showed a mixed cytoplasmic and membranous pattern of localization in some cases. This result agrees with Shvartsur and Bonavida (2015) who claimed that TROP-2 may be expressed in the cytoplasm when the cells turn malignant, in case of recurrence, and metastasis (Simms *et al.,* 2016). Cytoplasmic positivity of TROP-2 can be explained through cross-reactivity with EpCAM/TROP-1 [epithelial-cell adhesion molecule] that is encoded by another gene of the same family (TACSTD1) and has a very similar sequence with TROP-2. EpCAM is expressed in thyroid carcinomas with the differentiated pattern; including FC, in both cell membrane and cytoplasm. This cross-reactivity with EpCAM may also account for TROP-2 positivity in cases of FC (Stepan *et al.,* 2011).

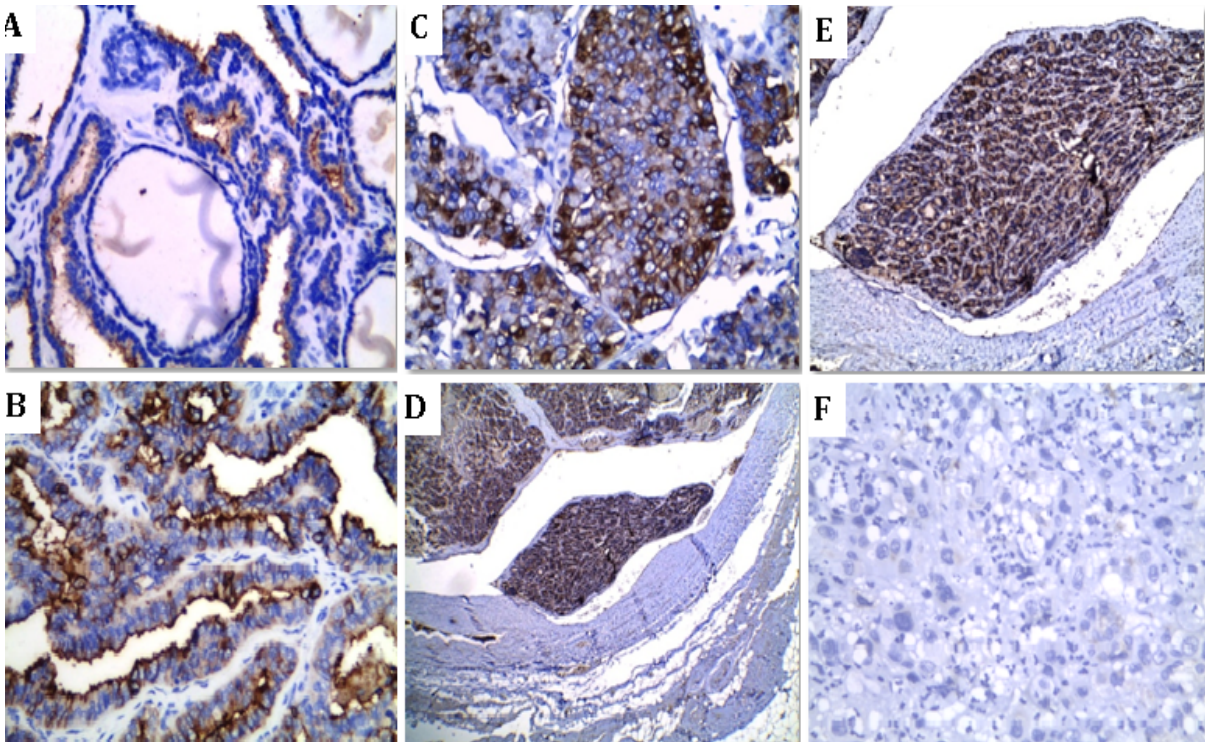

Figure 2. immunohistochemical expression of CK19 in various thyroid lesions. (A) focal positivity in nodular goiter (x400), (B) positive membranous reaction with apical accentuation in classic variant papillary thyroid carcinoma (x400), (C) positive membranous/cytoplasmic reaction in poorly differentiated thyroid carcinoma (x400), (D, E) positive reaction in minimally invasive follicular carcinoma, with vascular infiltration (x100, x200), (F) negative reaction in anaplastic thyroid carcinoma (x400).

In the current study, TROP-2 showed a positive immunohistochemical expression in 79 out of 90 PTC cases (87.8%), with the majority of positive cases showing diffuse positivity (score 3 or 4). These results were similar to those reported by Bychkov *et al.* (2016) and Liu *et al.* (2017) in which TROP-2 positivity was 81.5% and 81.6%, respectively. Also, Hafez *et al.* (2018) reported that 85.1% (63 of 74) of PTC cases revealed TROP-2 positivity. Regarding TROP-2 expression in other malignant thyroid cases, it was 6% only. A purposed explanation is that TROP-2 may be involved in neoplastic transformation and it may be expressed in early well-differentiated rather than advanced poorly differentiated tumors. A possible association with BRAF mutations may be a matter (Kong *et al.,* 2018).

In the current work; TROP-2 expression was completely negative (0.0%) among non-neoplastic and benign lesions. These results agreed with Simmes *et al.* (2016) and Murtezaoglu and Gucer (2017) who documented that TROP-2 was completely negative among the studied non-neoplastic cases. However, Addati *et al.* (2015) found that TROP-2 expression was positive in 6/56 non-neoplastic cases. The positive expression of TROP-2 in benign lesions encountered in such study might be explained by the presence of Hurthle cells in Hashimoto thyroiditis (which express high endogenous biotin activity), or the possible premalignant nature of such cells.

As regards TROP-2 specificity and sensitivity in the diagnosis of PTC, this study showed 87.78% sensitivity and 100% specificity in differentiating PTC from benign/non-neoplastic thyroid lesions with a diagnostic accuracy of 94.47%. Regarding the usage of TROP-2 in differentiating PTC cases from other studied malignant thyroid tumors, it showed 87.78% sensitivity, 94% specificity and diagnostic accuracy 90%. For differentiation of PTC from other thyroid lesions; TROP-2 showed 87.78% sensitivity, 98.11% specificity and 90% diagnostic accuracy. These results were close to Liao (2016) who documented higher sensitivity and specificity of TROP-2 in detecting PTC (100% for both) as he worked on tissue microarray (TMA) histopathological sections; where TROP-2 was positive in all studied PTC cases and

negative in all other studied thyroid lesions which included neoplastic and non-neoplastic cases. Bychkov *et al.* (2016) reported that TROP-2 sensitivity in the entire types of PTC was 75%, but the specificity was higher (98.4%). This high specificity owed to that all studied benign cases in their work were negative.

Moreover, Liu *et al.* (2017) studied the sensitivity and specificity of TROP-2 for differentiation of PTC from other follicular patterned thyroid lesions. They detected a range of sensitivity differing according to a subtype of PTC (94% for PTC (classic variant) and 81% for a confirmed follicular variant of PTC). This variation might be explained according to the technique of methodology used for the study whether TMA, surgical specimens with a larger size or FNAC preparation. As the overall sensitivity of TROP-2 in PTC on TMA sections was (90%) while that on surgical tissue sections was (70%). The higher sensitivity in the former may be attributed to the fact that these cases were mostly classic variant PTC. Also, the whole slide evaluation can overcome tumor heterogeneity and better assess TROP-2 immunoreactivity.

Liu *et al.* (2017) documented that TROP-2 decorates PTCs, a specifically classic variant with no background stains. In contrast, none of the benign or suspicious thyroid lesions, or normal thyroid tissues expressed TROP-2. The extremely high specificity and reasonable sensitivity of TROP-2 for PTC make TROP-2 an attractive immunomarker for the classification of thyroid tumors, especially for follicular-patterned lesions with equivocal histomorphology.

The discrepancy between studies as regards sensitivity and specificity of TROP-2 may be due to the inclusion of different thyroid lesions; including atypical lesions with diagnostic uncertainty and controversial diagnoses. On the other hand, the usage of different methods, as mentioned earlier, whether TMA, surgical specimens or cell block can affect results among studies. Type of antibody used (monoclonal vs. polyclonal), number of cases encountered and the percentage of lesions in various studies can play a role in this issue. Different variants of PTC can yield different results as classic morphology shows strong positivity, while microcarcinoma and follicular variant of PTC has equivocal histomorphology and lower positivity. Also, different methodological interpretations of the scoring system (exact cut-off value), and different methods for IHC evaluation (automated or visual assessment) may explain the differences among the various reports.

In the current study, CK19 immunoreactivity showed strong positive expression among all studied PTC cases (100%). While in malignant non-PTC cases; CK19 expression was positive in 14/50 (28%) of the studied cases. These findings are close to Song *et al.* (2011) who reported CK19 positivity in 99.20% of papillary thyroid carcinoma without lymphatic metastasis and 92.74% of papillary thyroid carcinoma with lymphatic metastasis. Murtezaoglu and Gucer (2017) reported that CK19 was positively expressed in 95.5% and 70% of cases with classic PTC and follicular variant of PTC, respectively. According to Cheung *et al.* (2001), 80% of classic PTCs and 57% of follicular variants of PTCs were positive for CK19.

Regarding CK19 expression in the studied non-neoplastic thyroid lesions, it was positive in 81/109 (74.3%) and negative in 28/109 (25.7%). These results agreed with Scognamiglio *et al.* (2006) as they reported CK19 positivity in 79.16% of the studied non-malignant cases. Also, Barroeta *et al.* (2006) documented positivity in only 34% of the studied non-malignant cases. Despite this immunoreactivity to CK19, staining was focal and less intense in non-neoplastic cases than that observed in the papillary carcinoma including its variants.

Many other studies have confirmed a variable percentage of CK19 positivity in non-malignant thyroid cases. Interestingly, Murtezaoglu and Gucer (2017) observed stronger and more extensive staining of CK19 in normal thyroid tissues compared to other thyroid lesions, as it was positively stained in about 100% of normal thyroid tissue. This observation would not create a problem to easily recognize normal thyroid tissue during microscopic examination of the thyroidectomy material. However, it may cause misdiagnosis in cytological cell block preparations collected from normal thyroid tissues instead of the lesion.

Regarding CK19 sensitivity and specificity; the current study results showed 100% sensitivity and 25.69% specificity in differentiating PTC from benign thyroid lesions and 59.30% diagnostic accuracy. As regards the usage of CK19 in differentiation from other malignant lesions; CK19 showed 100% sensitivity and 72% specificity with a diagnostic accuracy of 90%. For differentiation of PTC from other thyroid lesions; CK19 showed 100% sensitivity, 40% specificity and diagnostic accuracy of CK19 was 59.30%. The current study results were lower in the specificity of CK19 in comparison to Song *et al.* (2011). They recorded that CK19 was the most sensitive (96.37%) and specific (74.17%) marker in papillary carcinomas. The diagnostic efficiency of CK19 was (90.71%).

According to Dunderovic *et al.* (2015) review, the researchers reported a wide range of figures for the sensitivity and specificity of CK19 in distinguishing malignant and benign lesions, with a median of 80% and 78%, respectively. They concluded that the intensity of CK19 immunoreactivity must be taken into consideration with other diagnostic PTC criteria. In agreement with the literature, the current work suggests that the usage of CK19 by itself had the highest sensitivity as a marker in distinguishing PTC from benign as well as non-neoplastic thyroid lesions (100%). However, its specificity was lower due to its profound positivity in benign as well as non-neoplastic lesions (25.69%).

Regarding the usage of TROP-2 and CK19 as a combination for detection of PTC from benign lesions, the current study found that this panel showed 87.78% sensitivity, 100% specificity and 94.97% diagnostic accuracy. So, the usage of this panel did not add any diagnostic value over the usage of TROP-2 alone. As regards the segregation of PTC from other malignant thyroid tumors; the panel had 100% sensitivity, 72% specificity and 90% accuracy. For differentiation of PTC from all studied benign and malignant cases, the use of combined TROP-2 and CK19 showed 87.78% sensitivity, specificity 98.11% and 94.38% accuracy. In comparison to Abdou *et al.* (2019), who reported the highest specificity (90.5%) in the diagnosis of PTC cases when both TROP-2 and CK19 were combined and positive. The high specificity value of this combination observed in the current study greatly exceeded that reported by Murtezaoglu and Gucer (2017), who demonstrated 60% specificity.

CONCLUSIONS

CK19 showed the highest sensitivity in the diagnosis of PTC, yet with low specificity. TROP-2 immunohistochemistry can be regarded as a unique specific and sensitive marker for the diagnosis of PTC, specifically in morphologically challenging cases.

CONFLICTS OF INTEREST

All authors declared no conflicts of interest.

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