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The Potential Diagnostic Utility of TROP-2 & C-Kit in Thyroid Neoplasms

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Authors' contributions

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

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ABSTRACT

Background: Thyroid nodules are common finding, only 5% of nodules are malignant and the vast majority is non-neoplastic lesions or benign neoplasms. Thyroid cancer incidence is increasing faster than any other cancer types, thus representing one of the most common and clinically worrying malignant tumors of the endocrine system. Trophoblast antigen 2 (TROP2) is a transmembrane receptor glycoprotein encoded by the tumor-associated calcium signal transducer 2(Tacstd2) gene, which is located on chromosome 1p32. Although the biological function of TROP2 is unclear, accumulating evidence has demonstrated that its expression is elevated in various malignant tissues, whereas in human normal tissues relatively low or no TROP2 expression is observed. C-Kit is a type III receptor tyrosine kinase. C-Kit expression and signaling have been well characterized in several tumors, including gastrointestinal stromal tumors (GISTs). However, few studies have investigated c-Kit in the thyroid gland or in thyroid malignancies. The aim of this study was to investigate the diagnostic utility of TROP-2 on a large set of neoplastic thyroid lesions & to investigate the utility of TROP-2 & c-Kit markers to distinguish between benign and malignant thyroid neoplasms on Paraffin blocks.
Methods: Immunohistochemistry for TROP2 and c-Kit was carried out on 85 different thyroid lesions (40 benign, 7 borderline and 38 malignant).

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Results: Malignant thyroid lesions were found to have negative expression of c-Kit in contrast to 80% of benign thyroid neoplasms. TROP2 was strong positive in 87.5% of papillary thyroid carcinomas (PTC), but there was no TROP2 expression in benign thyroid neoplasms, non-invasive follicular thyroid neoplasm with papillary like nuclear features, follicular carcinoma, anaplastic and poorly differentiated thyroid carcinoma.
Conclusions: TROP2 is a good diagnostic tool for PTCs to differentiate between PTCs & other lesions with papillary like nuclear features as NIFTP, c-Kit is a good diagnostic tool for follicular adenoma & to differentiate between follicular adenoma & follicular carcinoma.

Keywords: TROP-2; C-Kit; thyroid neoplasms.

1. INTRODUCTION

Thyroid nodules are prevalent in general population. The lifetime risk for developing a clinically palpable thyroid nodule is estimated to be 5-10%, however, high resolution ultrasound has revealed thyroid nodules in 19-68% of randomly selected individuals [1] Thyroid tumors arise either from the follicular epithelium or parafollicular C-cells. They are painless nodules, compressed & displaces the adjacent structures [2]. Thyroid cancer is histologically classified into papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, poorly differentiated thyroid carcinoma, anaplastic thyroid carcinoma, and medullary thyroid carcinoma. Papillary thyroid carcinoma (PTC) is the most frequently encountered subtype, accounting for 80 to 85% of all thyroid cancers [3].

Trophoblast antigen 2 (TROP-2) is a transmembrane glycoprotein encoded by the tumor-associated calcium signal *transducer 2* (Tacstd2) gene. It was originally identified in human trophoblast and choriocarcinoma cell lines [4]. It was subsequently reported to be over-expressed in a variety of human carcinomas and only rarely in normal tissues [5]. Over-expression of TROP-2 in human carcinomas is associated with tumor aggressiveness and poor prognosis [6-8]. The antigen has been reported as a novel immunohistochemical marker of PTC [9, 10]. It has been actively studied as a prognostic marker and an attractive immunotherapeutic target in human cancer treatment [5, 11, 12].

The proto-oncogene c-Kit encodes for the tyrosine kinase receptor (CD117) and is involved in cell signal transduction with different downstream pathways: MAPK, phosphatidylinositol 3-kinases (PI3K), Janus kinase (JAK)/signal transducers and activators of transcription (STAT), SRC family kinases (SFK) and phospholipase Cγ [13]. Furthermore, c-Kit is a mutagenic effective proto-oncogene with a stem-cell factor (SCF) as a ligand, and it leads to tumor growth through impairment of cellular growth regulation [14]. Normally c-Kit is activated (phosphorylated) by binding of its ligand, the stem cell factor. This leads to a phosphorylation cascade ultimately activating various transcription factors in different cell types. Such activation regulates apoptosis, cell differentiation, proliferation, chemotaxis, and cell adhesion [15]. It plays various roles in hematopoiesis, melanogenesis and spermatogenesis, and in the development of the interstitial cells of Cajal [16]. Despite several carcinomas showed activating mutations of c-Kit gene (gastrointestinal stromal tumors (GISTs), melanomas, hematopoietic and lymphoid tumors), they have not been described in highly metastatic melanomas, breast cancer and thyroid carcinoma, the progression into a malignant phenotype correlates mostly with loss of c-Kit expression [17].

The aim of this work was to investigate the diagnostic utility of TROP-2 on a large set of neoplastic thyroid neoplasms and utility of TROP-2 & c-Kit markers to distinguish between benign and malignant thyroid neoplasms on Paraffin blocks.

2. PATIENTS AND METHODS

This retrospective study was carried out on 85 paraffin blocks of tissues from patients with different thyroid neoplasms. The cases were collected from archive of Pathology department, Faculty of Medicine, Tanta University & Tanta Cancer Center (Labeled by code numbers) instead of name of the patient to maintain privacy of participants &confidentiality of the data, during the period from August 2018 to May 2020. They were chosen for the research depending on the quality of the blocks. The first group included forty cases which were diagnosed as benign thyroid neoplasms categorized as: Twenty-six benign cases (65%) were diagnosed as follicular adenoma, eleven benign cases (27.5%) were

diagnosed as Hurthle cell adenoma and three benign cases (7.5%) were diagnosed as hyalinizing trabecular adenoma. The second group included seven cases which were diagnosed as borderline thyroid neoplasms categorized as: all seven cases (100%) were diagnosed as Noninvasive Follicular Thyroid neoplasm with Papillary-like nuclear features (NIFTP).

The third group included thirty-eight cases which were diagnosed as malignant thyroid neoplasms categorized as: Seven malignant cases (14.8%) were diagnosed as follicular carcinoma. Three malignant cases (7.9%) were diagnosed as poorly differentiated carcinoma. Four malignant cases (10.5%) were diagnosed as undifferentiated thyroid carcinoma*.* Twenty-one malignant cases (55.2%) were diagnosed as papillary thyroid carcinoma. Three malignant cases (7.9%) were diagnosed as metastatic PTC.

Specimens were received as paraffin blocks. Sections were prepared for: Routine hematoxylin & eosin (H&E) staining (for reevaluation) and immunohistochemical staining by TROP2 & C-kit antibodies.

The primary antibody used was **TROP2** rabbit monoclonal antibody, ready to use, Cat. No. 2-TU022-13. It was diluted antibody in TRIS, pH 7.4, with <0.1% sodium azide. Quartett, Berlin CA 12307, Germany.

C-Kit rabbit polyclonal antibody, was ready to use for immunohistochemical staining with <0.1% sodium azide. Cat. No. D5-0054-A. Diagnostic Biosystems, Pleasanton, CA 94588, USA.

3. METHODOLOGY OF IMMUNO-HISTOCHEMICAL STAINING [18]

I. Sectioning: Formalin-fixed, paraffin-embedded tissues were sectioned into thin slices (4-5µm) with a microtome. Thicker sections may cause difficulty during staining& problems in interpretation due to the multi-layering of cells. II. Mounting: The sections were then mounted onto adhesive-coated glass slides. After sections are cut, they were usually floated on the water and picked up onto glass slides. Sections must lay flat against the glass to prevent lifting during staining or bubble formation, which may trap staining reagents. III. Antigen Retrieval and Blocking: Firstly, Dewaxing was done by immersing the sections into a dewaxing solution (fresh xylene bath). Due to the fixation process, antigenicity of the antigens on the tissue were affected. An antigen retrieval treatment was applied to unmask the epitopes, by heat (heat-induced epitope retrieval; **HIER**). IV. primary antibody reaction: for **TROP2** immunostaining: two to three drops of TROP2 rabbit monoclonal IgG antibody were placed on each slide. The antibody was ready to use, with an overnight incubation at room temperature in the humidity chamber. For **c-Kit** immunostaining: Two to three drops of c-Kit rabbit polyclonal antibody were placed on each slide. The polyclonal antibody was ready to use, with an overnight incubation at room temperature in the humidity chamber.

The excess reagent was tapped off and the slides were washed for 5 minutes in phosphate buffer solution (PBS). Then the slides were dried around the tissue sections without drying the sections itself. V. Labeled Antibody Reaction:

3.1 Exposure to Secondary Biotinylated Antibody

Two to three drops of the secondary biotinylated antibody were placed on the slides, so that the tissue sections were covered completely. The slides were incubated in the humidity chamber at room temperature for 45 minutes. Excess reagent was tapped off and the slides were washed for 5 minutes in PBS, then the slides were dried.

3.2 Exposure to Labeled Streptavidin-biotin (LSAB) Enzyme

Two to three drops of streptavidin enzyme label were placed on each slide. The slides were incubated for 45 minutes at room temperature in the humidity chamber. Excess reagent was tapped off and the slides were washed for 5 minutes in PBS.

3.3 Staining & Counterstaining

1- Preparation of working color reagent: Diamino-Benzedine (DAB) chromogen was prepared by addition of one drop of DAB chromogen per one ml of buffered substrate using the provided graduated test tube to measure the amount of buffered substrate needed. The components were mixed well. 2- Color development: Several drops of the working color reagent were placed on each slide using the provided transfer pipette.

The slides were incubated for 5-10 minutes at room temperature in the humidity chamber.

The slides were washed in tap water & Counter stain with Mayer's hematoxylin for one minute was done& then the slides were washed in tap water. Sections were dehydrated in alcohol.

3.3.1 Interpretation of TROP2 positivity (Hiayan et al., 2017)

The validated PTC tissue was used as positive control and the normal thyroid tissue as negative control. Only membranous staining was considered positive. The staining considered positive, 1+ (if 5% to 25% of tumor cells stained), 2+ (if 26% to 50% of tumor cells stained), 3+ (if 51% to 75% of tumor cells stained), or 4+ (if >75% of tumor cells stained) & recorded as negative (if no stain or <5% of tumor cells stained).

3.3.2 Interpretation of c-Kit positivity (Bizzarro et al., 2015)

C-Kit staining showed membranous positivity. Immunoreactivities staining considered positive if >10% of the epithelial follicular cells were stained and graded as 1+, 2+ or 3+ according to the percentage of stained cells (respectively, 10-25%, 26-50% and 51-100%) & recorded as negative (if no stain or <10% of tumor cells stained).

3.4 Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range, mean, standard deviation, median and interquartile range (IQR). Chi-square test was used to test the difference between two groups. Sensitivity and specificity of TROP2 &c-Kit were calculated. P values of <0.05 were considered statistically significant and P values of <0.001 were considered statistically highly significant.

4. RESULTS

Classification of the thyroid neoplasms is shown in Table 1.

Table 1. Classification of the thyroid neoplasms

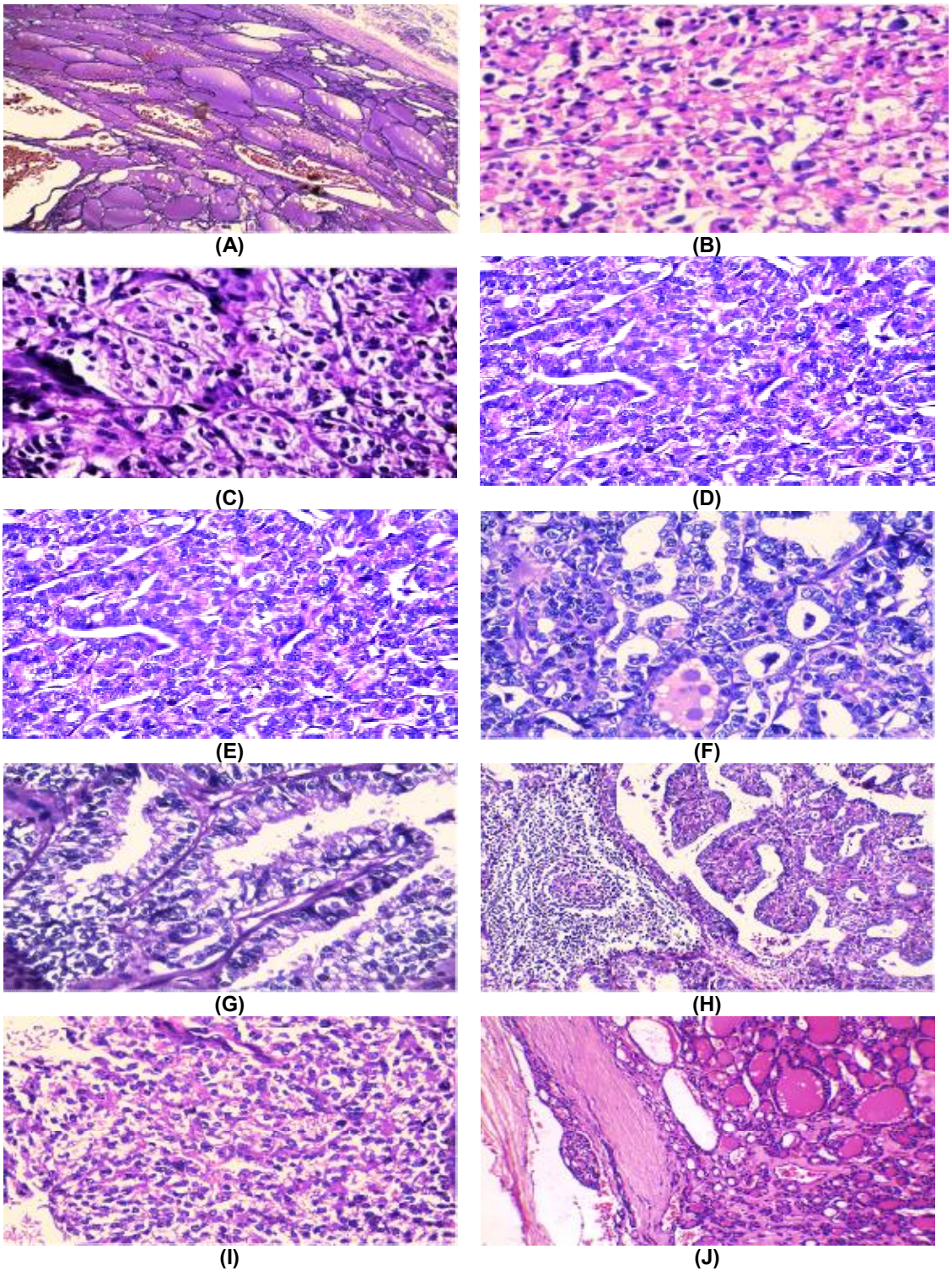
Tumor	No.	%
Benign (n = 40)		
Follicular adenoma	26	65.0
Hurthle cell adenoma	11	27.5
Hyalinizing trabecular adenoma	3	7.5
Borderline (n = 7)		
NIFTP	7	100
Malignant (n = 38)		
Follicular carcinoma	7	18.4
Poorly differentiated thyroid carcinoma	3	7.9
Anaplastic thyroid carcinoma	4	10.5
PTC	21	55.2
Classic variant	6	28.6
Microcarcinoma	4	19.0
Follicular variant	6	28.6
Warthin like variant	2	9.5
Tall cell variant	2	9.5
Solid variant	1	4.8
Metastatic PTC	3	7.9

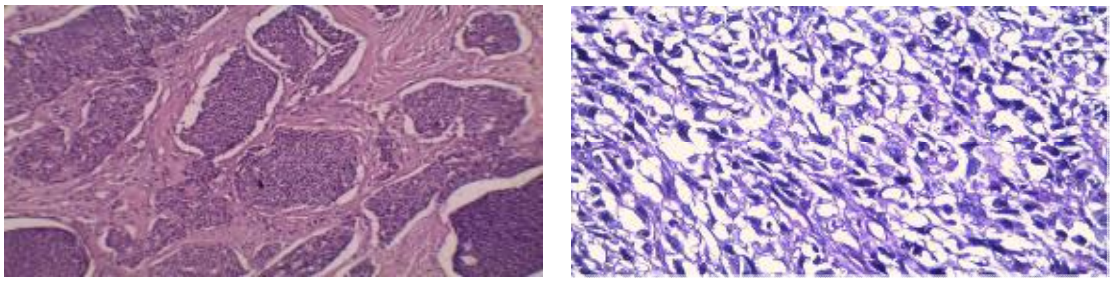
Age and sex distribution among benign, borderline and malignant studied thyroid neoplasms are shown in Table 2

Table 2. Age and sex distribution among benign, borderline and malignant studied thyroid neoplasms

	Total (n = 85)		Tumor			
			Benign (n = 40)		Borderline & malignant (n = 45)	
	No.	%	No.	%	No.	%
Gender						
Male	37	43.5	17	42.5	20	44.4
Female	48	56.5	23	57.5	25	55.6
Age (years)						
<45	51	60.0	23	57.5	28	62.2
≥45	34	40.0	17	42.5	17	37.8
Min. – Max.	22.0 – 72.0		22.0 – 65.0		22.0 – 72.0	
Mean ± SD.	40.66 ±11.97		40.25 ±12.18		41.02 ±11.91	

Histopathological examination of the studied cases was done to reevaluate the pathological diagnosis. It revealed that; forty cases were benign thyroid neoplasms; seven cases were borderline thyroid neoplasms & thirty-eight cases were malignant thyroid neoplasms. Examples are shown in Fig. 1.





(K) (L)

Fig. 1. (A) A case of macrofollicular adenoma surrounded by thin fibrous capsule and composed of large follicles without capsular or vascular invasion [H&E, x40]. (B) A case of Hurthle cell adenoma showing the characteristic large cells with eosinophilic cytoplasm & central rounded nuclei [H&E, x400] (C) A case of hyalinizing trabecular adenoma showing polygonal cells with light eosinophilic cytoplasm containing hyaline material [H&E, x400]. (D) A Case of NIFTP showed neoplasm of thyroid follicular cells with follicular pattern & nuclear features of PTC [H&E, x400]. (E) A case of PTC high power view showing papillae with fibro vascular cores and lined by cubical cells with the characteristic nuclear features (overcrowding, grooving, nuclear pseudo-inclusion and ground glass appearance) [H&E, x400] (F) A case of FVPTC showing predominant follicular growth pattern with overcrowded nuclei, grooving & ground glass appearance (H&E, x400). (G) A case of tall cell variant PTC showing papillary growth pattern with tall cells showing papillary nuclear features (H&E, x400). (H) A case of warthin like variant PTC showing papillae covered by oncocytic cells with fibrovascular cores associated with lymphoid cells [H&E, x200]. (I) A case of solid variant of PTC showed solid sheets of tumor cells with typical nuclear features of PTC (H&E, x400). (J) A case of follicular carcinoma with vascular invasion showing large vessel invasion and RBCs inside the lumen (H&E, x200). (K) A case of insular carcinoma showed nests formed of small cells without papillary nuclear features and with associated mitosis (H&E, x400). (L) A case of anaplastic thyroid carcinoma showing malignant cells with large bizarre nuclei (H&E, x400)

4.1 Immunohistochemical Results of TROP2 Expressions: Fig. 2

TROP2 expression was localized in membranes of follicular cells.

a) TROP2 expression in benign studied cases:

Follicular adenoma cases: All 26 cases of follicular adenoma were negative for TROP2 expression score 0. Hurthle cell adenoma cases: All 11 cases of hurthle cell adenoma showed negative expression score 0. Hyalinizing trabecular adenoma cases: All 3 cases of hyalinizing trabecular adenoma were negative for TROP2 expression score 0.

b) TROP2 expression in borderline thyroid cases:

NIFTP: All 7 cases showed TROP2 negative expression score 0.

c) TROP2 expression in malignant thyroid cases:

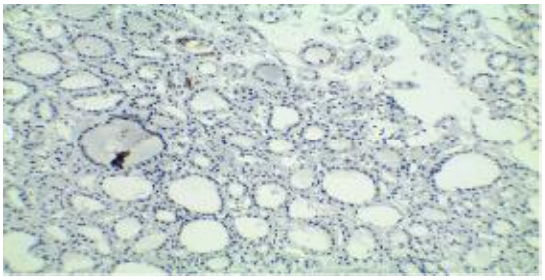
Papillary carcinoma: 18 cases (5 classic, 4 microcarcinoma, 2 TCV-PTC, 6 FVPTC & 1 warthin like variant) showed positive expression for TROP2 & 3 cases were negative for trop2 (1 classic, 1 solid & 1 warthin like variant). Metastatic PTC: All 3 cases were positive for TROP2 expression. Follicular carcinoma: All 7 cases showed TROP2 negative expression score 0. Poorly differentiated thyroid carcinoma: All 3 cases showed TROP2 negative expression score 0.

Anaplastic thyroid carcinoma: All 4 cases of anaplastic carcinoma showed TROP2 negative expression score 0.

Regarding expression of TROP2 between benign, borderline and malignant thyroid cases, it was statistically highly significant. Table 3.

Table 3. Comparison between TROP2 immuno-expression in benign, borderline and malignant studied thyroid cases

	Benign (n = 40)		Tumor				Sensitivity	Specificity	PPV	NPV	Accuracy
			Borderline (n=7)		Malignant (n = 38)						
	No.	%	No.	%	No.	%					
Trop2											
Negative	40	100.0	7	100.0	17	44.7	55.26	100.0	100.0	62.50	71.76
Positive	0	0.0	0	0.0	21	55.2					
x^2(p)			24.792* (<0.001*)				Highly	Significant P Value			



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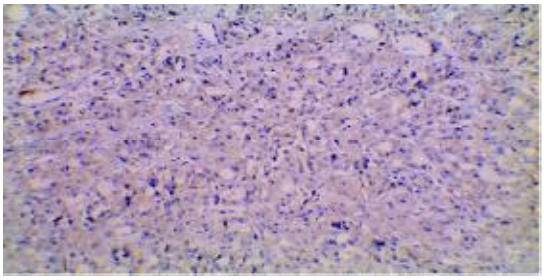

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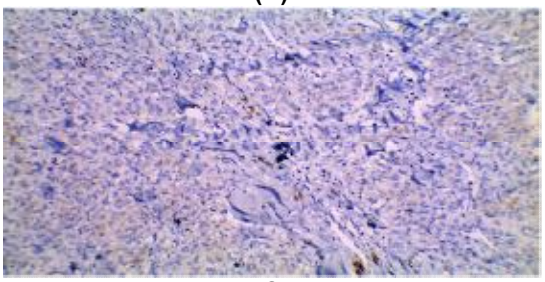

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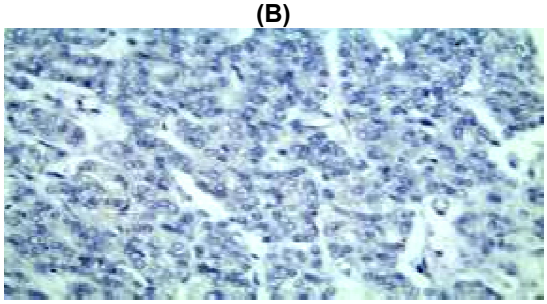

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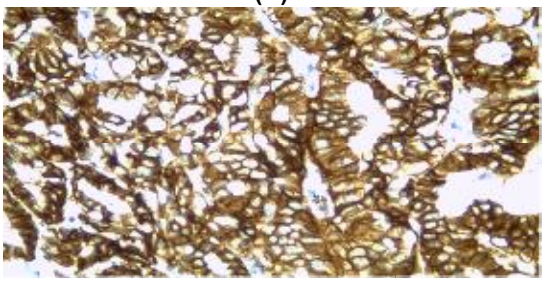

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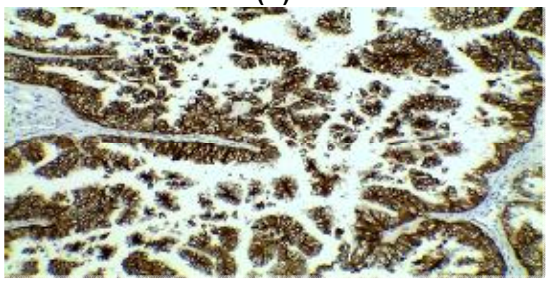

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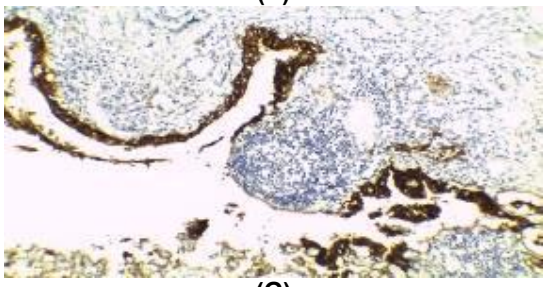

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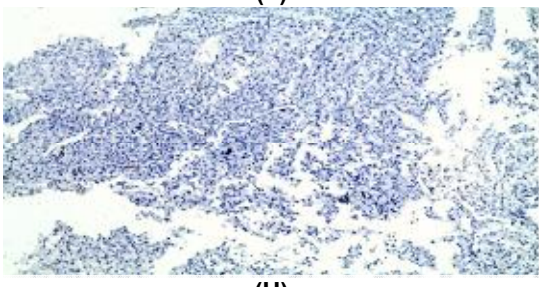

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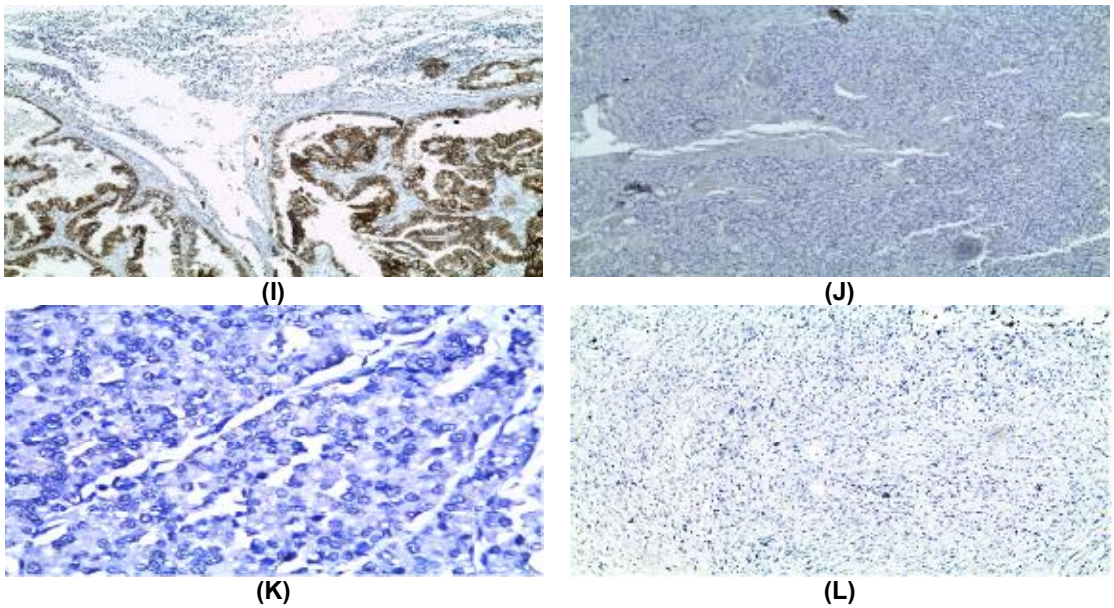


Fig. 2. (A) A case of follicular adenoma showing TROP2 negative expression score 0 [streptavidin biotin, x100] (B) A case of Hurthle cell thyroid adenoma showing TROP2 negative expression score 0 [streptavidin biotin, x200] (C) A case of Hyalinizing trabecular adenoma showing TROP2 negative expression score 0 [streptavidin biotin, x100] (D) A case of NIFTP showing TROP2 negative expression score 0 [streptavidin biotin, x400] (E) A case of papillary thyroid carcinoma follicular variant showing TROP2 positive membranous expression score +4 [streptavidin biotin, x400] (F) A case of Tall cell variant papillary thyroid carcinoma showing TROP2 positive membranous expression score +4 [streptavidin biotin, x200] (G) A case of Warthin like variant papillary thyroid carcinoma showing TROP2 positive membranous expression score +2 [streptavidin biotin, x200] (H)A case of solid variant papillary thyroid carcinoma showing TROP2 negative membranous expression score 0 [streptavidin biotin, x100] (I) A case of nodal metastatic papillary thyroid carcinoma showing TROP2 positive membranous expression score +4 [streptavidin biotin, x100] (J) A case of follicular carcinoma with capsular invasion showing TROP2 negative expression score 0[streptavidin biotin, x100] (K) A case of Insular carcinoma showing TROP2 negative expression score 0 [streptavidin biotin, x400] (L) A case of anaplastic thyroid carcinoma showing TROP2 negative expression score 0[streptavidin biotin, x100]

Regarding to TROP2 immunohistochemical expression, there was a statistically highly significant difference between PTC and both benign thyroid neoplasms and other malignant and borderline studied thyroid cases as shown in Table 4.

4.2 C- Kit Expression: Fig. 3

C-Kit expression was localized in membranes of follicular cells.

a) C-Kit expression in benign cases*:*

Follicular adenomas: 23 cases of follicular adenoma showed c-Kit positive expression & 3 cases were negative score 0.

Hurthle cell adenoma: 9 cases were positive & 2 cases were negative expression for c-kit.

Hyalinizing trabecular adenoma cases: All 3 cases of hyalinizing trabecular adenoma were negative for negative expression score 0.

b) C-Kit expression in borderline cases*:*

NIFTP: All 7 cases showed c-Kit negative expression score 0.

c) C-Kit expression in malignant cases*:*

Papillary carcinoma: All 21 cases were negative for c-Kit score 0.

Metastatic PTC: All 3 cases were negative for c-Kit expression score 0.

Follicular carcinoma: All 7 cases showed c-Kit negative expression score 0.

Poorly differentiated thyroid carcinoma: All 3 cases showed c-Kit negative expression score 0.

Anaplastic thyroid carcinoma: All 4 cases of anaplastic carcinoma showed c-Kit negative expression score 0.

Concerning benign, borderline and malignant thyroid neoplasms, there was a statistical highly significant difference between benign, borderline and malignant studied cases. There was statistically highly significant negative (inverse) relation between c-Kit in most benign thyroid cases (positive) especially follicular adenomas and other follicular thyroid neoplasms (negative) include borderline & malignant thyroid neoplasms especially NIFTP & fvPTC as shown in Table 5.

Table 4. Relation between PTC and benign thyroid neoplasms and other malignant and borderline studied thyroid cases regarding TROP2 expression

	Tumor				Sensitivity	Specificity	PPV	NPV	Accuracy
	Benign (n = 40)		PTC (n = 24)						
	No.	%	No.	%					
Trop2									
Negative	40	100.0	3	12.5	87.50	100.0	100.0	93.02	95.31
Positive	0	0.0	21	87.5					
x^2(p)	52.093* (<0.001*)				Highly significant P Value				
	Tumor malignant								
	Other malignant & borderline cases (n = 21)		PTC (n = 24)						
	No.	%	No.	%					
Trop2									
Negative	21	100.0	3	12.5	87.50	100.0	100.0	87.50	93.33
Positive	0	0.0	21	87.5					
x^2(p)	34.453* (<0.001*)				Highly Significant P Value				

Table 5. Relation between the immune-expression of c-Kit in benign and malignant studied thyroid cases

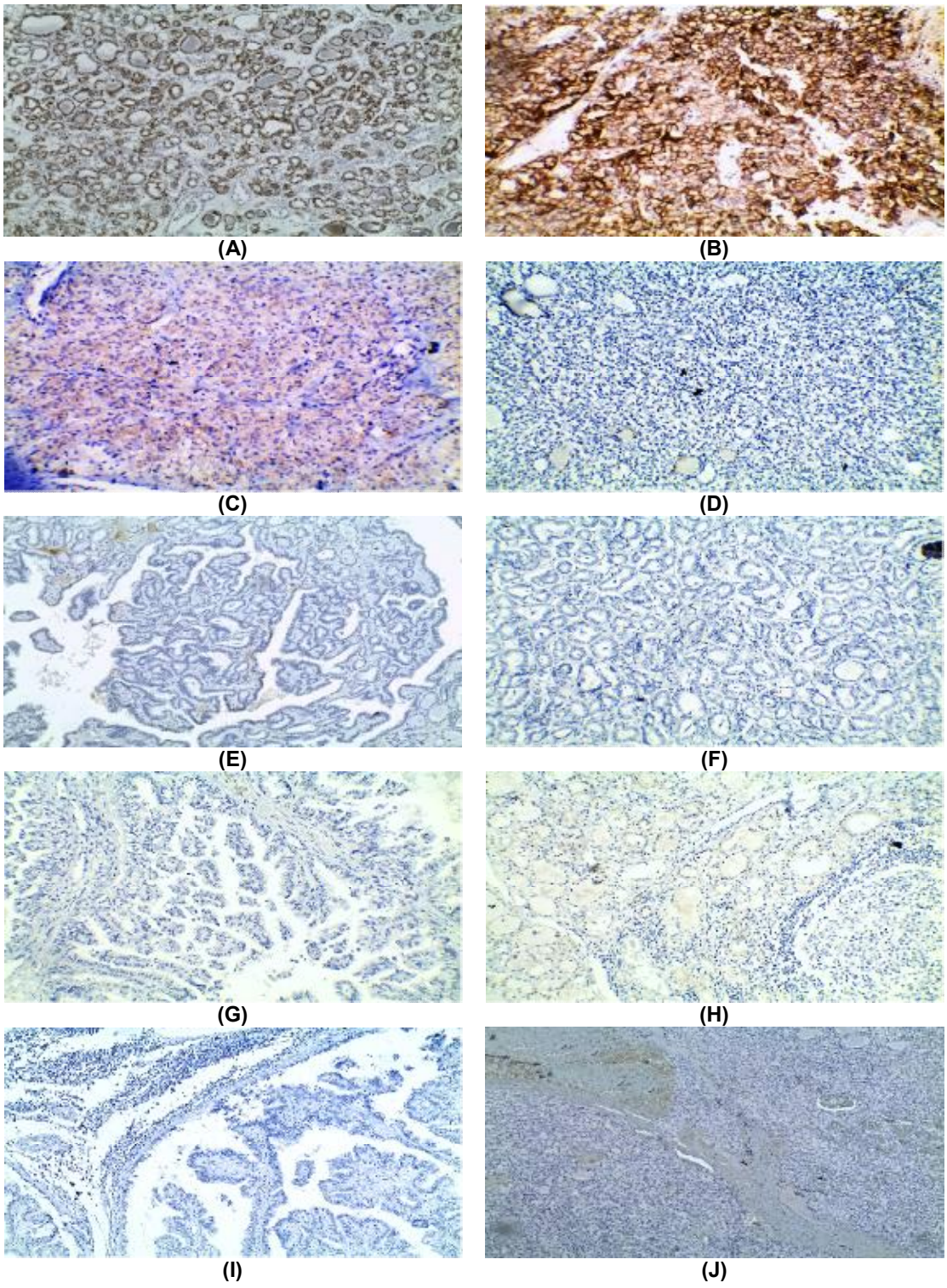
c-kit	Tumor				Sensitivity	Specificity	PPV	NPV	Accuracy
	Benign (n = 40)		Borderline & Malignant (n = 45)						
	No.	%	No.	%					
Positive	32	80.0	0	0.0	100.0	80.0	84.91	100.0	90.59
Negative	8	20.0	45	100.0					
x^2 (p)	57.736* (<0.001*)				Highly Significant P Value				

Table 6. Relation between TROP2 and c-Kit expression in studied cases

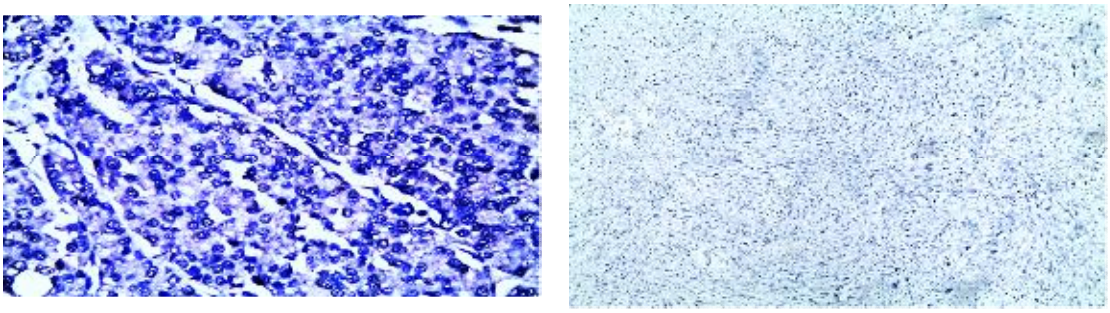
c-kit	Trop2				χ^2	p
	Negative (n = 64)		Positive (n = 21)			
	No.	%	No.	%		
Positive	32	50.0	0	0.0	16.840*	<0.001*
Negative	32	50.0	21	100.0		

Table 7. Sensitivity and specificity of TROP2 and c-Kit in studied cases

	Sensitivity	Specificity	PPV	NPV	Accuracy
TROP2	87.5 %	100%	100%	93.02%	95.31%
C-Kit	100%	80%	84.91%	100%	90.59%



(A) **(B)** **(C)** **(D)** **(E)** **(F)** **(G)** **(H)** **(I)** **(J)**



(K) (L)

Fig. 3. (A) A case of thyroid Follicular adenoma showing c-Kit positive expression score +3 [streptavidin biotin, x100] (B) A case of Hurthle cell thyroid adenoma showing c-Kit positive membranous expression score +3 [streptavidin biotin, x200]. (C) A case of Hyalinizing trabecular adenoma showing c-Kit negative expression score 0 [streptavidin biotin, x100] (D) A case of NIFTP showing c-Kit negative expression score 0 [streptavidin biotin, x100] (D) A case of the papillary thyroid carcinoma classic variant showing negative c-Kit expression score 0 [streptavidin biotin, x100] (E) A case of FVPTC showing negative c-Kit expression score 0 [streptavidin biotin, x200] (F) A case of Tall cell variant papillary thyroid carcinoma showing negative c-Kit expression score 0 [streptavidin biotin, x400] (G) A case of warthin like variant papillary thyroid carcinoma showing negative c-Kit expression score 0 [streptavidin biotin, x200] (H) A case of metastatic papillary thyroid carcinoma showing negative c-Kit expression score 0 [streptavidin biotin, x200] (I) A case of follicular carcinoma with capsular invasion showing c-Kit negative expression score 0 [streptavidin biotin, x100] (J) A case of poorly differentiated thyroid carcinoma showing c-Kit negative expression score 0 [streptavidin biotin, x400] (K) A case of anaplastic thyroid carcinoma showing c-Kit negative expression score 0 [streptavidin biotin, x100]

Regarding relation between TROP2 and c-Kit expression in studied cases, there was a statistical highly significant inverse (negative) relation between positivity of TROP2 & negativity of c-Kit expression in thyroid cases. Table 6.

Sensitivity and specificity of TROP2 and c-Kit in studied cases are shown in Table 7.

5. DISCUSSION

Nodular disorders of the thyroid gland are relatively common among adults, with an overall prevalence of approximately 4-7% in the general population. Most thyroid nodules are benign hyperplastic lesions, but 5-20% of thyroid nodules are true neoplasms [19].

Thyroid carcinoma represents 80.3% of endocrine neoplasms and 2.6% of total malignancies in Egypt. Papillary thyroid carcinoma (PTC) represents the most common histologic type of thyroid carcinoma in Egypt representing 83.3% followed by follicular carcinoma (8.4%) [5].

In agree with Addati et al. [9] findings that age of thyroid neoplasm patients range between 18 and 73 years, but mean age was 53 years. This result is somewhat different from the current study. This could be explained by the fact that this paper worked on a large scale of cases.

In agree with Nabil et al. [5] findings that thyroid neoplasms more common in females than in males with male to female ratio is 1:2. This result was in agreement with the study of Simmis et al. & Murtezaoglu and Gucer, [10, 20], their finding indicated that TROP2 was negatively expressed in 100% of the benign studied thyroid cases.

In agreement with the study of Hafez et al. [21], their finding indicated that TROP2 was positive in 85.1% (63/74) of PTCs, including 86.9% (53/61) of classic variant, 72.7% (8/11) of follicular variant & 100% (2/2) of tall variant, with the majority of all variants being diffuse (3+ and 4+). In contrast to PTC, only one out of seven FC cases (14.3%) was TROP-2 positive. Hafez et al. [21] reported that TROP2 expression showed a statistically significant difference between papillary carcinoma cases (as a positive marker) when compared to FA and FC & may be useful

as a diagnostic aid to differentiate PTC from other follicular neoplasms.

Liu et al. [7] also reported results that were closely similar to the current findings as they found that TROP2 was positive in 90% (43/48) of PTCs, including 97% (30/31) of cPTCs and 76% (13/17) of FVPTCs, showing a strong membranous staining pattern, with the majority being diffuse with score + 4. Conversely, none of the atypical follicular neoplasm, adenomatoid nodules with focal nuclear atypia & follicular carcinomas expressed TROP-2 in a membranous pattern. Liu et al. [7] reported that TROP-2 staining pattern is highly specific for PTC, which may serve as a potential diagnostic marker aiding in the accurate classification of morphologically equivocal thyroid follicular-patterned neoplasms.

Bychkov et al. [22] results were closely similar to the current findings as they found that TROP2 was positive in 81.6% (93/114) of PTCs, including 98.7% (75/76) of non FVPTCs and 47.8% (18/38) of FVPTCs, demonstrating a strong membranous staining pattern, with the majority being diffuse with score + 4 predominant. In contrast, none of the follicular carcinoma cases expressed TROP-2 in a membranous pattern, 10% (1/10) of ATC expressed TROP2 & 25% (2/8) of PDC expressed TROP2. Bychkov et al. [22] reported that TROP-2 was significantly associated with PTC variant and encapsulated PTC follicular variant ($p<0.001$). Trophoblast antigen 2 membranous staining is a very sensitive and specific marker for PTC, with high overall specificity for PTC.

In somewhat agreement with the study of Hafez et al. [21] which reported that TROP-2 expression showed 85.1% sensitivity and 94.4% specificity for PTC and it was somewhat different from those detected by Bychkov et al. [22], who reported that TROP-2 could identify PTC with 98.1% sensitivity and 97.5% specificity and Liu et al. [7], reported that the overall sensitivity of TROP-2 is 94% for cPTC and 81% for FVPTC with 100% specificity.

The sensitivity in the study conducted by Bychkov et al. [22] and Liu et al. [7] is higher than the current study and this could be explained by the type of PTC variants as they worked on only classic PTC & follicular variant PTC, while the specificity in the study conducted by Bychkov et al. [22] and Hafez et al. [21] is lower than the current study and this could be explained by the few cases which showed positivity for TROP2 other than PTCs.

In the study of Pusztaszeri et al. [23] their findings revealed high expression of c-Kit in benign thyroid nodules (BTNs) (n = 30) including 100% of the studied cases, also normal follicular epithelium was positive for c-Kit (score, 2-3), while c-Kit showed negative expression in most (89%, n = 31) PTCs [lacked immunoreactivity for c-Kit] and 4 samples (11%) had faint reactivity in <2% of tumor cells (score, 0). Pusztaszeri et al. [23] reported that c-Kit expression was mostly absent in tumor cells of thyroid carcinoma especially PTC in contrast to benign lesions, in which, c-Kit expression was present in all specimens ($P < .0001$), both the sensitivity and specificity of c-Kit in PTC were 100% . This is higher than the reported sensitivity and specificity of other commonly used immunochemical markers for PTC. Therefore, the addition of c-Kit as a negative marker in PTC, to one or several positive markers of PTC may be useful in their diagnosis.

In the study of Zamzam et al. [24] found that 100% of benign thyroid tumor (16 FA & 4 HTA) were variably positive for c-Kit & loss of c-Kit expression found in 100% of all malignant studied cases [PTC (5 cases), FC (3 cases) & Hurthle cell carcinoma (2 cases)]. Zamzam et al. [24] reported that c-Kit analysis improves the diagnostic sensitivity and specificity of commonly immunochemical markers like HBME-1, galectin-3, CD56 and CK-19 used for thyroid carcinomas diagnosis to 100% for all.

It is speculated that c-Kit expression values were divided in four defined classes, with class I characterized by the complete silencing of the gene. Class I and IV represented the two most informative groups, with 100% of the samples found malignant or benign respectively. Class III was also very informative including 86% of benign samples and having over all the highest statistical significance. On the other hand, in class II the samples belonging to the malignant group were 66%, which resulted non-significant [17]. .This could explain the maintained elevated c-Kit expression in all benign cases in the study of Pusztaszeri et al. & Zamzam et al. [23, 24] which may represent c-Kit class IV, according to this, the present result represents c-Kit class III.

According to our knowledge no study has used both TROP2 & c-Kit in differentiating benign from malignant thyroid neoplasms. Regarding the relation between the used 2 markers in the current study, TROP2 and c-Kit, 100% (40) of benign cases were negative for TROP2 and 80% were positive for c-Kit immunostaining. In contrast to malignant cases as 87.5% (21) of PTCs were positive to TROP2 and 100% (45) of malignant thyroid neoplasms were negative to c-kit. There was a statistical highly significant inverse (negative) relation between positivity of TROP2 & negativity of c-Kit expression in thyroid cases and combined use of both markers can be of a high diagnostic accuracy in the differentiation between these entities.

Additional studies on a large series of thyroid neoplasms are needed for evaluation of the combined use of TROP2 and c-Kit in immunohistochemical differentiation of follicular thyroid neoplasms of uncertain malignant potential. Further studies to correlate expression of TROP2 & c-Kit to clinic-pathological data and immunotherapeutic outcomes in studied patients. Combined use of TROP2 & c-Kit is useful in the differentiation between FVPTC and follicular adenoma.

6. CONCLUSION

TROP2 is a good diagnostic tool for PTCs to differentiate between PTCs & other lesions with papillary like nuclear features as NIFTP, c-Kit is a good diagnostic tool for follicular adenoma & to differentiate between follicular adenoma & follicular carcinoma.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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