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Significance of TROP-2 and P63 Expression in Diagnosis of Papillary Thyroid Lesions: An immunohistopathological Study

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ABSTRACT

Background: Thyroid cancer is the most common endocrine malignancy, accounting for 2.1% of all cases of cancer worldwide. About 77% of these cases occurring in women**[1].** Approximately 90% of all thyroid cancers are differentiated; meaning that they arise from thyroid follicular cells**[2].** Papillary thyroid carcinoma (PTC) is the most common histopathological type of thyroid cancer**[3].** TROP-2 is a trophoblastic transmembrane glycoprotein, also known as tumour-associated calcium signal transducer 2 (TACSTD2) that signals cells for self-renewal, proliferation, invasion, and survival. It gives the cell stem cell-like qualities**[4].**P63 is a transcription factor belonging to the p53 family and shares structural and sequence homology with p53.Different studies support the hypothesis that p63 can function as a tumour suppressor, especially TAp63 isoform**[5].**

The aim: This study was conducted to evaluate the significance of TROP-2- and p63 expression in differentiation malignant from benign papillary thyroid lesions in thyroid histopathological biopsy specimens.

Methods: Forty two cases (21 cases of papillary hyperplasia associated lesions included (7 cases nontoxic colloid goiter, 7 cases toxic colloid goiter and 7 cases follicular adenoma) and 21 of papillary thyroid carcinoma(classic variant) were examined immunohistochemically using antibodies against TROP-2 and p63.

Results: TROP-2 expression was observed in 90.5 % of cases of papillary thyroid carcinoma, while none of papillary hyperplasia associated lesions cases showed TROP-2 positivity.P63 expression was observed in 19.1% of cases of PTC, while none of papillary hyperplasia associated lesions cases showed p63 positivity. There is a statistically significant difference in TROP-2 expression between PTC and papillary hyperplasia associated lesions ($P<0.001$). There is no statistically significant difference in p63 expression between PTC and papillary hyperplasia associated lesions ($p=0.11$).

Conclusion:TROP-2 was overexpressed in 90.5% of cases of PTC classic variant with specificity 100% and sensitivity 90.5% . Our results suggest that TROP-2may be considered as a useful marker in diagnosis of papillary thyroid carcinoma(classic variant). P63 was expressed in 19.1% of PTC cases with specificity 100%% and sensitivity 19%, this result suggests that p63 is less effective in diagnosis of PTC, TROP-2 and p63together can be used as diagnostic markers for papillary thyroid carcinoma.

Key words: TROP-2, p63, papillary thyroid carcinoma, papillary hyperplasia, Immunohistochemistry.

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, accounting for 2.1% of all cases of cancer worldwide, with 77% of these cases occurring in women**[1]**.Approximately 90% of all thyroid cancers are differentiated, meaning that they arise from thyroid follicular cells**[2].**In Egypt, National Cancer Institute Pathology Registry indicated that thyroid cancer constitutes 1.97% of all malignancies**[6].**Papillary thyroid carcinoma(PTC) is the most common histological type of thyroid cancer**[3].**

Although a majority of papillary cancers can be diagnosed and classified on the basis of pathologic criteria, there exists a group of benign thyroid lesions such as multinodular goiter or follicular adenoma which may mimic the architecture of PTC if they contain papillary areas, posing diagnostic problems **[7].**

TROP-2 is an intracellular calcium signal transducer that signals cells for self-renewal, proliferation, invasion, and survival. It gives the cell stem cell-like qualities. TROP-2 expression in cancer cells has been correlated with drug resistance[4].

TROP-2 gene may also be considered as a potential marker for lymphatic metastasis in PTC **[8].** Several authors have claimed its involvement in tumour invasion and metastasis, as it is correlated with a poor prognosis, probably

increasing tumour cell resistance to apoptosis. TROP-2 overexpression is associated with decreased patient survival as well as increased tumor aggressiveness and metastasis. Its overexpression in metastatic tissue makes it a very attractive and potential therapeutic target for late stage disease **[9]**.

P63 a p53-homologue, has been demonstrated in the basal cells of epidermis, ectocervix, urothelium ,prostate acini and ducts. The p63 gene encodes transcriptionally active TAp63 isoforms and N-terminally truncated dominant negative isoforms; both may play a role in thyroid tumor progression **[5]**. P63 is expressed in a proportion of papillary thyroid carcinoma**[10]**. It is usually negative in normal follicular epithelium and follicular neoplasms**[11]**.Positive P63 expression has also been recently observed in thyroid carcinoma with thymus like elements **[12].**

MATERIALS AND METHODS

This work is a retrospective study carried out on forty two thyroid paraffin blocks that were previously diagnosed as 21 cases of papillary hyperplasia associated lesions divided as (7 cases of nontoxic colloid goiter), (7 cases of toxic colloid goiter) and (7 cases of follicular adenoma), 21 cases of PTC classic variant, collected from the Pathology Department, Faculty of Medicine, Zagazig University and Omar EL-Mokhtar university in the period from March 2016 to March 2017. The selected specimens were obtained by surgical excision.

The clinical data concerning age and sex were obtained from the patients files.

Paraffin blocks of all cases were sectioned at 3-4 micron thickness and stained with routine hematoxylin and eosin stain to re-evaluate and confirm the diagnosis.

The inclusion criteria used in the current study were: 1) Multinodular colloid goiter (toxic and nontoxic) with papillary hyperplasia. 2) Follicular adenoma with papillary hyperplasia .3) Papillary thyroid carcinoma(classic variant)

The exclusion criteria were: 1) Thyroiditis. 2) follicular variant of PTC 3) Follicular carcinoma. 4) Medullary carcinoma.

Immunohistochemical staining:

Immunohistochemical staining was carried out using the streptavidin–biotin immunoperoxidase technique (Dako- Cytomation, Glostrup, Denmark). Sections of 3–4 mm thickness were cut from formalin-fixed, paraffin-embedded blocks, mounted on positively charged slides, deparaffinized in xylene, and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH 6.0) for 20 minutes and then washed in PBS (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 6% H2O2 in methanol was carried out. The slides were then incubated overnight with monoclonal antibodies: TROP-2: rabbitpolyoclonal antibody, Dilution 1:50; (CloneTACSTD2, lab vision corporation, GeneTex,USA).

P63: mouse monoclonal antibody, Dilution1:100; (Clone*4A4*, lab vision corporation, Santa Cruz, California, USA). Incubation with a secondary antibody and product visualization were performed (Dako-Cytomation) with diaminobenzidine substrate (Research Genetics, Huntsville, Alabama, USA) as the chromogen. The slides were finally counterstained with Mayer's hematoxylin (BioGenex Laboratories, San Ramon, California, USA) and washed once each with distilled water and PBS. Positive and negative controls were stained at the same staining setting with the studied cases: Samples of skin tissue and placental tissue were used as positive control for p63 and TROP-2 respectively. Negative controls were obtained by eliminating the primary antibody.

Evaluation of the results of immunohistochemical staining:

1- TROP-2 immunostaining:

The TROP-2 staining was considered positive when the (uniform and complete) brown reaction was found in the membranes. Immunoreactivity was determined semiquantitatively by examining fields (magnification, x200). Using a (0–3+) scale, the staining was described as 0 staining (negative), 1+ staining (5%-25%), 2+staining (26%–50% of cells), or 3+staining (>50% of cells).

2 – P63 immunostaining:

The p63 staining was considered positive when the granular brown reaction was found in the nucleus. Semi-quantitation of nuclear p63 immunoreactivity was scored as follows: Negative: less than 10% of tumor nuclei stained, weakly positive: 10-25%, moderately positive: 26-75%, and strongly positive: 76-100% of tumor nuclei stained. Only nuclear reactivity was considered positive.

The slides were evaluated by three different pathologists working separately. All discrepancies were discussed and consensus reached.

Statistical analysis:

Statistical analysis was performed using SPSS software (SPSS, Chicago, IL, USA). Data were expressed as mean ±SD for quantitative variables. For categorical variables Fisher's exact test or chi-square was used. P-value less than 0.05 was considered significant.

RESULTS

The age of the studied cases ranged from (12-65) and the mean was 37.55± 11.04 years. The age of the studied cases of PTC ranged from (23-46) and the mean was 35.48± 7.61. The majority of studied cases were female 88.1% and female to male ratio was 7:1.

None of papillary hyperplasia associated lesions cases showed TROP-2 positivity(Table I,Fig.1).TROP-2 expression was observed in 90.5% of cases of PTC classic variant (Table II, Fig.2). There is a statistically significant difference in TROP-2 expression between papillary hyperplasia associated lesions and PTC (P<0.001)(Table III).

None of papillary hyperplasia associated lesions cases showed p63 positivity (Table IV, Fig.3). P63 expression was observed in 19% of PTC cases (Table V,Fig.4). There is no statistically significant difference in p63 expression among studied cases (p=0.11)(Table VI).

Sensitivity of TROP-2 in differentiating papillary thyroid carcinoma 90.5%, specificity 100%, positive predictive value 100% and accuracy 95.2 (Table VII). Sensitivity of p63 19%, specificity 100%, positive predictive value 100%, negative predictive value 55.3% and accuracy 59.5% (Table VII).

Table (I): Immunoreactivity pattern of TROP-2 in papillary hyperplasia associated lesions:

Papillary hyperplasia associated lesions	TROP-2 immunoreactivity										
	No	Negative		Positive						Total Positive	
		(0)		(+)		(++)		(+++)			
		No	%	No	%	No	%	No	%	No	%
Nodular colloid goiter	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Toxic nodular goiter	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Follicular adenoma	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Total	21	21	100	0	0.0	0	0.0	0	0.0	**0**	**0.0**

Table (II) : Immunoreactivity pattern of TROP-2 in papillary thyroid carcinoma :

Papillarythyroidcarcinoma	TROP-2 immunoreactivity										
	No	Negative		Positive						Total Positive	
		(0)		(+)		(++)		(+++)			
		No	%	No	%	No	%	No	%	No	%
Classic variant	21	2	9.5	4	19.1	4	19.1	11	52.3	19	**90.5**

Table (III): Comparison of TROP-2 immunoreactivity among the papillary thyroid lesions:

Variable	Papillary hyperplasia associated lesions (n=21)		Papillary thyroid carcinoma (PTC) (n=21)		χ^2	P value
	No	%	No	%		
TROP-2						
Positive	**0**	**00.0**	**19**	**90.5**	**36.62**	**<0.001**
+1	0	00.0	4	19.1		**(HS)**
+2	0	00.0	4	19.1		
+3	0	00.0	11	52.3		
Negative	**21**	**100**	**2**	**9.5**		

Table (IV): Immunoreactivity pattern of p63 inpapillary hyperplasia associated lesions:

Papillary hyperplasia associated lesions	P 63 immunoreactivity										
	No	Negative (0)		Positive						Total Positive	
				Weak		moderate		Strong			
		No	%	No	%	No	%	No	%	No	%
Nodular colloid goiter	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Toxic nodular goiter	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Follicular adenoma	7	7	100	0	0.0	0	0.0	0	0.0	0	**0.0**
Total	21	21	100	0	0.0	0	0.0	0	0.0	**0**	**0.0**

Table (V):Immunoreactivity pattern of p63 in papillary thyroid carcinoma :

Papillary thyroid carcinoma	P 63 immunoreactivity										
	No	Negative (0)		Positive						Total Positive	
				Weak		Moderate		Strong			
		No	%	No	%	No	%	No	%	No	%
Classic variant	21	17	80.9	1	4.8	1	4.8	2	9.5	4	**19.1**

Table (VI):Comparison of p63 immunoreactivity among the papillary thyroid lesions:

Variable	Papillary hyperplasia associated lesions (n=21)		Papillary thyroid carcinoma (PTC) (n=21)		χ^2	P value
	No	%	No	%		
P 63						
Positive	**0**	**0.0**	**4**	**19.1**	**4.43**	**0.11**
Weak	0	0.0	1	4.8		**(NS)**
Moderate	0	0.0	1	4.8		
Strong	0	0.0	2	9.5		
Negative	**21**	**100**	**17**	**80.9**		

Table (VII):Sensitivity,Specificity,PPV,NPV ,Accuracy of TROP-2 and p63

	Sensitivity	Specificity	PPV	NPV	Accuracy
TROP-2	**90.5%**	**100%**	**100%**	**91.3%**	**95.2%**
P63	**19%**	**100%**	**100%**	**55.3%**	**59.5%**

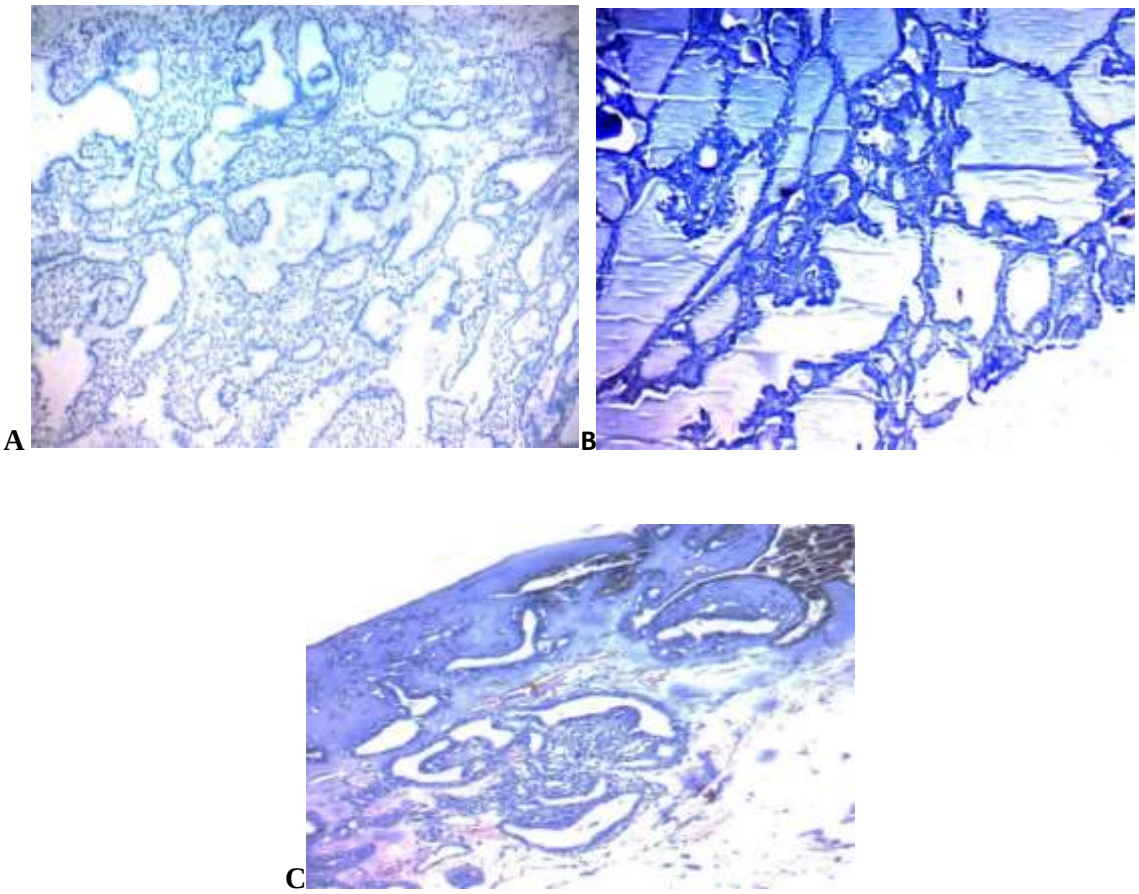

Fig. 1: TROP-2 immunoreactivity in papillary hyperplasia associated lesions:

A. Toxic colloid goiter with papillary hyperplasia showing negative TROP-2 immunoreactivity (Immunoperoxidase stain,X200).

B. Nontoxic colloid goiter with papillary hyperplasia showing negative TROP-2-immunoreactivity (Immunoperoxidase stain,X200).

C.Follicular adenoma with papillary hyperplasia showing negative TROP-2 immunoreactivity (Immunoperoxidase stain,X200).

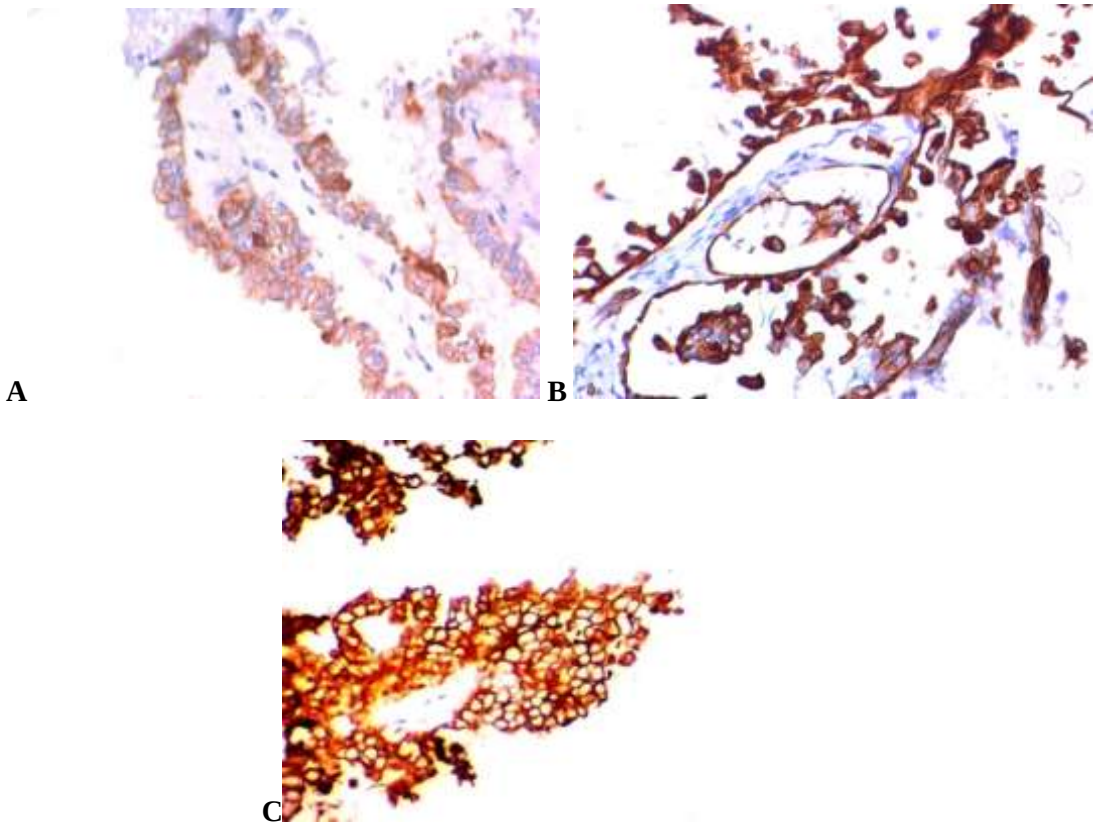

Fig. 2: TROP-2 immunoreactivity in papillary thyroid carcinoma:

A. Papillary thyroid carcinoma (classic variant) showing weak positivity of TROP-2 immunoreactivity (Immunoperoxidase stain,X400).

B. Papillary thyroid carcinoma (classic variant) showing moderate positivity of TROP-2-immunoreactivity (Immunoperoxidase stain,X400).

C.Papillary thyroid carcinoma (classic variant) showing strong positivity of TROP-2 immunoreactivity (Immunoperoxidase stain,X400).

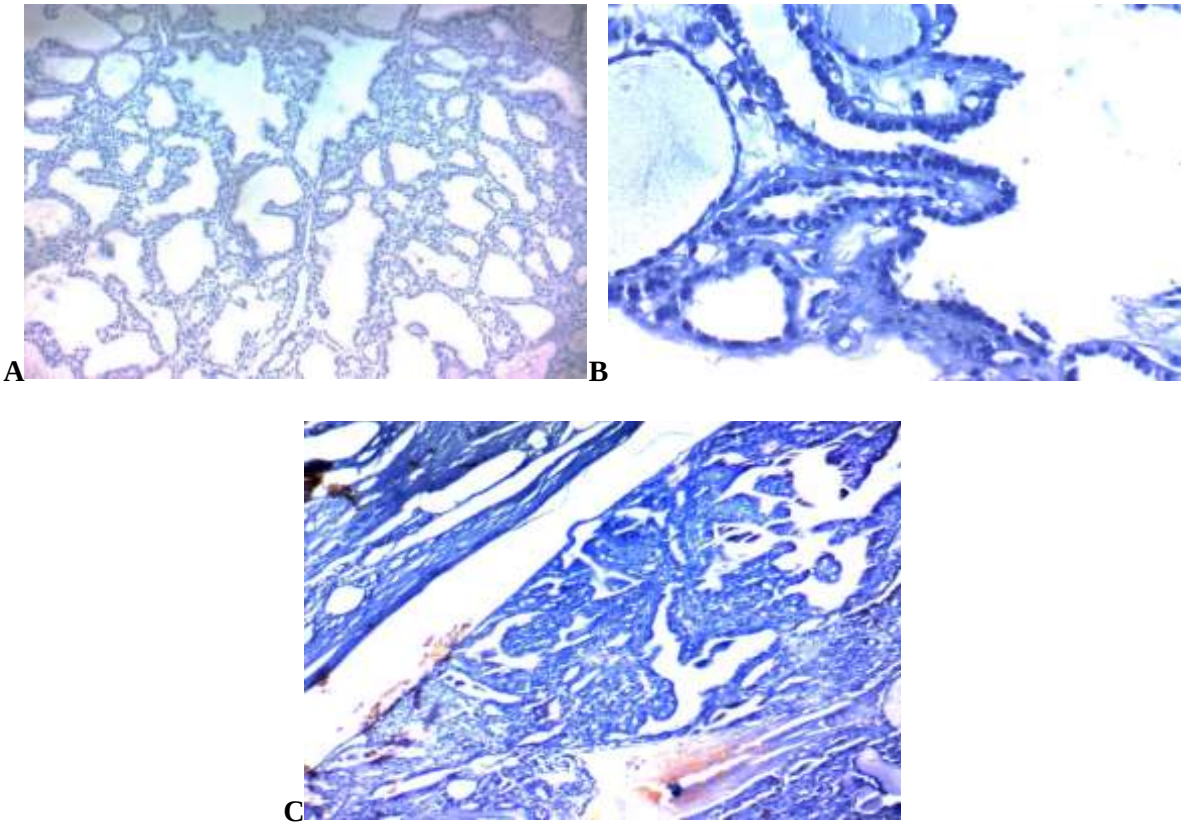

Fig. 3: P63 immunoreactivity in papillary hyperplasia associated lesions:

A. Toxic colloid goiter with papillary hyperplasia showing negative p63immunoreactivity (Immunoperoxidase stain,X200).

B. Nontoxic colloid goiter with papillary hyperplasia showing negative p63 immunoreactivity (Immunoperoxidase stain,X 400).

C. Follicular adenoma with papillary hyperplasia showing negative p63 immunoreactivity (Immunoperoxidase stain,X 200).

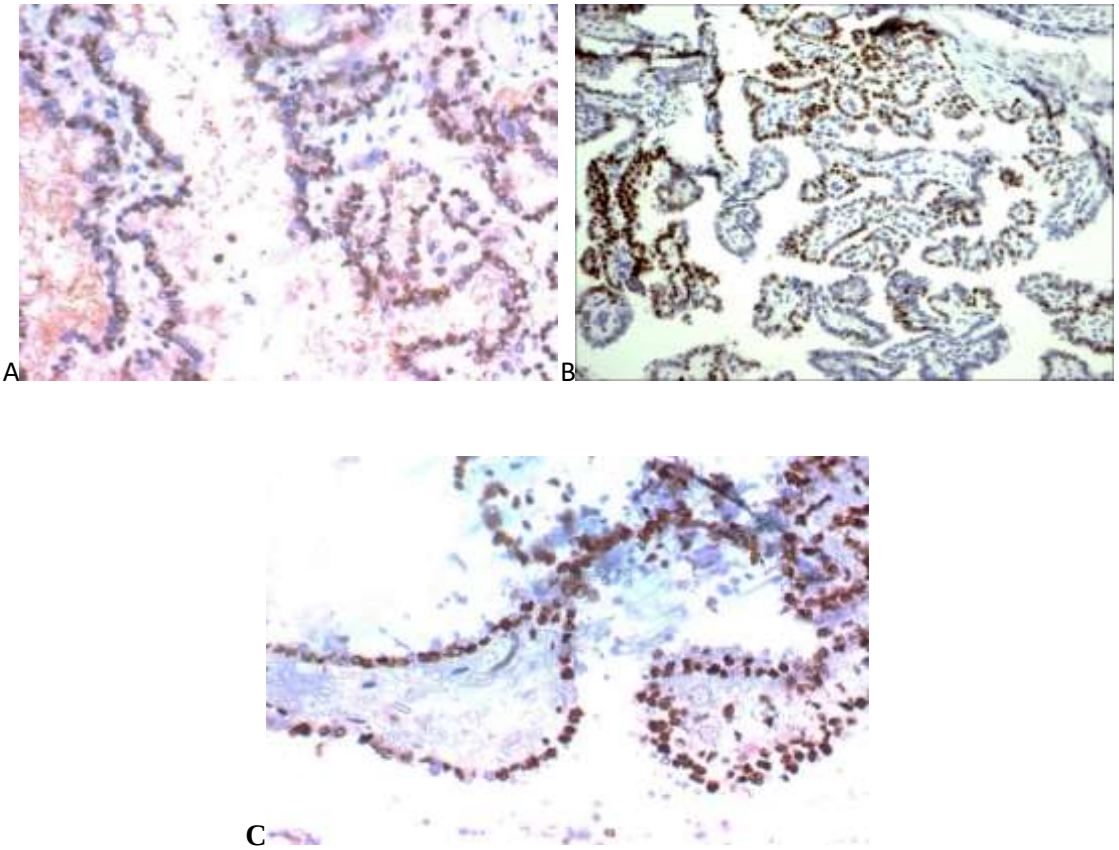

Fig. 4: P63immunoreactivity in papillary thyroid carcinoma:

A. Papillarythyroid carcinoma(classic variant) showing weak positivity of p63 immunoreactivity (Immunoperoxidase stain,X 400).

B.Papillary thyroid carcinoma (classic variant)showing moderate positivity of p63 immunoreactivity (Immunoperoxidase stain,X 200).

C. Papillary thyroid carcinoma (classic variant)showing strong positivity of p63 immunoreactivity (Immunoperoxidase stain,X 400).

DISCUSSION

TROP-2 was expressed in 90.5% of papillary carcinoma cases. There is a statistically significant difference between TROP-2 expression in PTC and papillary hyperplasia associated lesions (P<0.001). This was consistent with **Haiyan et al [13]** who studied TROP-2 expression in 136 cases of thyroid neoplasm, 15 cases of normal thyroid tissue and 20 cases of non neoplastic thyroid lesions . They found that 43 (90%) of 48 cases of PTC showed positive staining for TROP-2, all cases of follicular adenoma and non neoplastic thyroid lesions showed negative expression. This contradicts the data of **Addati et al [8]** who detected TROP-2 immunoreactivity in two of 24 follicular adenomas. This can be partially explained by the different clone of antibody employed for IHC. Our result was consistent also with **Anthony et al [14]**who studied TROP-2 expression in 331 cases of thyroid lesions ,60 of them were a classic variant of papillary carcinoma , 180 cases were benign thyroid lesions.54/60(90%) of papillary carcinoma(classic variant) showed positive staining for TROP-2, but all the benign lesions were negative for TROP-2 immunostaining. Our result was close to **Andrey et al [15]** who studied TROP-2 expression in 226 cases of thyroid tumors and 207 control cases . They found that 94 (82.5%) of 114 cases of papillary thyroid carcinoma showed positive staining for TROP-2, all the biopsies of benign and non neoplastic thyroid lesions group were negative for TROP-2 immunostaining. They found that high TROP-2 expression was associated with the presence of BRAFV600E mutation. TROP-2 utility was its uniform negativity in benign thyroid nodules (BTN) on both fine needle aspiration cytology sample (FNAC) and histological tissue samples, this result may prove to be very helpful in separating benign lesions from classic PTC **[14].**In our study a strong membranous staining with TROP-2 was seen in 11 cases of papillary thyroid carcinoma 11/19(60%) with the majority

being diffuse this was consistent with **Haiyan et al [13],** .**Andery et al[15]** considered that diffuse TROP-2 staining of a thyroid lesion favours a diagnosis of classic variant of PTC. Negative staining rules out classic variant of PTC, but cannot exclude follicular thyroid carcinoma and PTC follicular variant. In our study, we found that diffuse and strong TROP-2 expression is characteristic of classic variant of PTC, the negative stain in multinodular goiter and follicular adenoma that associated with papillary hyperplasia is a good evidence against PTC. Based on our findings and finding of other previous authers, TROP-2 has the potential for being a useful marker, with an ability to accurately and reliably detect the classic variant of PTC.Our study showed that TROP-2 has several advantages over CK19, galectin-3, or HBME-1 regarding diagnostic performance ,it doesnot give false positivity, no colloid or blood staining in addition to its strong diffuse staining and therefore may be recommended in the majority of situations where these three markers are deemed suitable.

Andrey et al.[15]found that all micropapillary thyroid carcinoma (mPTCs) had strong immunoreactivity, this indicates that the TROP-2 antigen is expressed in PTC at the very early stage of thyroid carcinogenesis, immediately after transformation. Tumour progression to poorly differentiated and further undifferentiated thyroid carcinoma was accompanied by loss of TROP-2 immunoreactivity. Some papillary remnants in poorly differentiated cancer retained weak staining, while the others were negative.

In our study, p63 was expressed in 4/21 (19.1 %) of papillary thyroid carcinoma cases, and none 0/21(0.0%) in papillary hyperplasia associated lesions. The staining pattern was exclusively nuclear . When comparing papillary thyroid carcinoma with papillary hyperplasia associated lesions, the expression of p63 (P=0.11) this means that no significant difference in expression of p63 between benign and malignant papillary thyroid lesions. Our result was close to **Ji Yun et al[16]** who studied p63 expression in 129 cases of papillary thyroid carcinoma, 80 cases of follicular adenoma and 40 cases of nodular hyperplasia. They found that 19(14.7%) of papillary thyroid carcinoma showed positive staining for p63, one case of follicular adenoma showed focal weak positivity and no case of nodular hyperplasia was positive for p63, p value <0.001. Our result is better than **Adela et al[17]** who studied p63 expression in 204 cases of papillary carcinoma . They found that 14 (6.9%) of PTC showed positive staining for p63 with the majority of p63-positive cases being classic variant. Our results revealed very low p63 expression, which is in contrast to what **Demellawy et al.[11]** have found, that is focal p63 positivity in 70% of PTC cases (from a total of 72 cases) and no p63 staining in normal thyroid tissue and non-PTC lesions also they found that p63 is specific but less sensitive for diagnosing PTC. One possible explanation of high positivity could be the higher positive cut-off value for p63 in our study (at least 5% of tumor cells staining positively) as compared with **Demellawy's** study, in which any nuclear p63 staining was counted as a positive result. However, this method of assessment seems to be difficult to apply in daily practice, as positivity in only one nucleus can sometimes be very challenging and difficult to evaluate**. Kim et al [18]** described that p63 was detected in 12.5% of papillary thyroid carcinomas, 11.1% of poorly differentiated carcinomas, and 71.4% of anaplastic carcinomas, while normal thyroid follicles, hyperplastic thyroid follicles, follicular carcinomas, and medullary carcinomas were all negative for p63**. Kim et al [18]** and **Ji Yun et al [16**] suggest that p63 is associated with poor prognosis factors in thyroid tumors or thyroid tumor progression Studies on p63 protein reaction in thyroid cancers show that this positivity can go from 6.9% **Adela et al[17]** to 81.8% **Unger et al [19]**. In our study, p63 appeared positive only in four cases, accounting for only 19% of classic variant of papillary carcinoma .

In our study there is a significant difference in expression of the two immunohistochemical markers in papillary thyroid carcinoma, TROP-2 posititivity was in 90.5% of PTC cases with strong and diffuse staining in majority of cases, while p63 positivity was in 19.1% of PTC cases with focal staining. TROP-2 and p63 were negative in the lesions that associated with papillary hyperplasia. In our study the sensitivity of TROP-2 in differentiating papillary thyroid carcinoma was 90.5%, the Specificity was 100%, positive predictive value was 100% and accuracy was 95.2, this means that TROP-2 is highly sensitive and specific for PTC with high PPV and high percentage of accuracy in differentiating benign from malignant papillary thyroid lesions, this result is consistent with **Anthony et al[14]** that showed TROP-2 sensitivity 90%, specificity 95.2% and high PPV 97.7%, also our result is close to **Addati et al [8]** that showed TROP-2 sensitivity of 87% and specificity of 89%.In our study the sensitivity of p63 was 19%, specificity was 100%, positive

predictive value was 100%, negative predictive value was 55.3%, this means that p63 is specific but less sensitive for PTC with high PPV and accuracy 59.5% in diagnosis of papillary thyroid carcinoma, this result is close to **Ji Yun et al [16]** that showed p63 sensitivity 14.7%, specificity 99.2%, PPV 99.2%, NPV 52% and accuracy 55.4%.

CONCLUSION

TROP-2-3 was expressed in 90.5% of cases of papillary carcinoma classic variant. Our results suggest that TROP-2 may be considered as a useful marker in diagnosis of PTC classic variant with sensitivity 90.5%,specificity100% and accuracy 95.2% . P63 was observed in 19.1% of PTC cases suggest that p63 is less significant in diagnosis of PTC with sensitivity 19%,specificity100% and accuracy 59.5%. the two markers together may be considered as diagnostic markers for papillary thyroid carcinoma.

REFERENCES

1.FerlayJ1, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 1:136(5):E359-86.

2. Ron E and Schneider AB :Thyroid cancer. In Cancer Epidemiology and Prevention, Schottenfeld D, FraumeniJr JF (eds).2006. Oxford University Press: 975–994.

3. Cibas ES, Alexander EK, Benson CB et al: Indications for thyroid FNA and pre-FNA requirements: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. DiagnCytopathol 2008 ;36:390–5.

4. Shvartsur A and Bonavida B :Trop2 and its overexpression in cancers: regulation and clinical/ therapeutic implications.Genes& Cancer 2015; Vol. 6 (3-4).

5. Ríos Moreno MJ, Galera-Ruiz H, De Miguel M, Lopez MI, Illanes M, GaleraDavidson H: Immunohistochemical profile of solid cell nests of thyroid gland. EndocrPathol 2011.;22:35-39.

6. Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M,Abdelazim H: Cancer Pathology Registry: 2000–2011, and Time Trend Analysis, National Cancer Institute, Cairo University2016.

7. Baloch Z W, LiVolsi VA : Diagnostic dilemmas in thyroid pathology: follicular variant of papillary thyroid carcinoma and classic papillary thyroid carcinoma arising in lymphocytic thyroiditis. Pathol Case Rev 2003 ;8:47-56.

8. AddatiT, Achille G,Centrone M, Petroni S, Popescu O, Russo S Grammatica L, Simone G: TROP-2 expression in papillary thyroid cancer:apreliminary cyto-histological study Cytopathology 2015, 26, 303–311.

9. Cubas R, Li M, Chen C, Yao Q. : Trop2. a possible therapeutic target for late stage epithelial carcinomas. BiochimBiophysActa 2009;1796:309-314.

10. Radu TG, Mogoanta L, Busuioc CJ, Stanescu C, and Grosu F: Histological and immunohistochemical aspects of papillary thyroid cancer. Rom J MorpholEmbryol 2015.;56(2 Suppl):789-95.

11. Demellawy DE, Nasr AL, Alowami S: Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. DiagnPathol 2008.;3:5. doi: 10.1186/1746-1596-3-5. [PubMed].

12. Wang YF, Liu B, Fan XS, Rao Q, Xu Y, et al: thyroid carcinoma showing thymus like elements: A clinicopathologic, immunohistochemical, ultrastructural and molecular analysis. Am J ClinPathol 2015.;143:223-33.

13. Haiyan Liu, Jianhui Shi, and Fan Lin,(2015): The Potential Diagnostic Utility of TROP-2 in Thyroid Neoplasms; Department of Laboratory Medicine, MC 19-20, Geisinger Medical Center, 100N. Academy Ave., Danville, PA 17822 (e-mail: hliu1@geisinger.edu).

14.Anthony Simms, Reuben P. Jacob, Cynthia Cohen, and Momin T. Siddiqui: TROP-2 Expression in Papillary Thyroid Carcinoma Potential Diagnostic Utility. Diagn. Cytopathol 2015;44:26–31.

15.AndreyBychkov, PichetSampatanukul, ShanopShuangshoti AndSomboonKeelawat: TROP-2 immunohistochemistry: a highly accurate method in the differential diagnosis of papillary thyroid carcinoma . Pathology, org/ / j. pathology.2016.04.002..

16.Ji Yun Jeong, Jin Hyang Jung, Ji Young Park: Expression and diagnostic availability of p63 and CD56 in papillary thyroid carcinoma. Int J ClinExpPathol 2016;9(7):7402-7410.

17. Adela Nechifor-Boilaa, Angela Bordaa, Geneviève Sassolasb, ZakiaHafdi-Nejjarib, Franc¸oiseBorson-Chazotb, Jean Christophe Lifanted, Nathalie Sturme, Marie Hélène Lavérrieree, Nicole Bergerf, MyriamDecaussin-Petruccif: Immunohistochemical markers in the diagnosis of papillary thyroid carcinomas: The promising role of combined immunostaining using HBME-1 and CD56. Pathology – Research and Practice 2013; 209 : 585– 592.

18.Kim YW1, Do IG, Park YK: Expression of the GLUT1 glucose transporter, p63 and p53 in thyroid carcinomas. Pathol Res Pract 2006 ;202:759-65.

19. Unger P, Ewart M, Wang BY, GanL, Kohtz DS, Burstein DE: Expression of p63 in papillary thyroid carcinoma and in Hashimoto's thyroiditis: a pathobiologic link?. HuPathol 2003;34:764-9