Significance of Combined Immuno-histochemical Expression of P63 & CD56 in Papillary and Follicular Thyroid Carcinoma

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Abstract

Background: Thyroid neoplasm is the commonest endocrine neoplasm. More than 80% of thyroid malignancies are papillary thyroid carcinoma (PTC) followed by follicular carcinoma (FC). The diagnosis of papillary thyroid carcinoma is based on characteristic nuclear morphology of a thyroid neoplasm. In contrast, follicular variant of PTC may cause, if the nuclear features of PTC are insufficiently appreciated, severe problems in differentiating from follicular thyroid carcinoma. Several immunohistochemical (IHC) markers such as P63 and CD56 have been recommended to differentiate between this two thyroid malignancies with overlapping histomorphology.

Objectives: Our aim was to identify the possible diagnostic role of P63 and CD56 immunoexpression that distinguish PTC, including the follicular variant from follicular thyroid carcinoma.

Methods: This cross-sectional descriptive study was conducted in the Department of Pathology, Rajshahi Medical College from March 2020 to February 2022. A total of 44 cases, histologically confirmed as papillary and follicular thyroid carcinoma were included in the study. Immunohistochemistry was done for P63 and CD56 from selected paraffin blocks.

Results: Histologically, 39 cases were diagnosed as papillary thyroid carcinoma (27 were classical PTC & 12 were FVPTC) and the rest 5 were follicular thyroid carcinoma. In this study, mean age of the patients was 40.86 ± 6.57 years (SD) and male to female ratio was 1:2.4. P63 showed 74.4% positivity with papillary thyroid carcinoma. Positive immunoreactivity of P63 was highly significant in distinguishing papillary from follicular thyroid carcinoma (P=0.003). Combined P63 positive CD56 negative (P=0.048) and P63 negative CD56 negative immunoexpression (P=0.039) were statistically significant in differentiating papillary from follicular thyroid carcinoma.

Conclusion: This study suggested that the use of P63 and CD56 may be helpful in the diagnosis of papillary and follicular thyroid carcinoma along with histopathological examination and their combination may also help in this purpose.

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Introduction

According to Globocan (Global Cancer Observatory) 2022, thyroid malignancy bears the 17th position among the carcinomas in Bangladesh regarding the incidence rate and 24th position regarding the mortality rate.[1] Papillary thyroid carcinoma (PTC) is the commonest type of thyroid cancer comprising approximately 80% of all thyroid epithelial malignancies.[2,3] Through the recent decades a marked increase in its incidence has occurred. Such increase reflects not only true increase in incidence of PTC but also a minor component of over diagnosis of PTC.

The gold standard in the diagnosis of thyroid lesions particularly PTC light microscopic evaluation of histopathologic slide using routine haematoxylin and eosin (H & E) staining. PTC is diagnosed by the presence of nuclear characteristics including nuclear overlapping, nuclear enlargement, chromatin clearing, intranuclear pseudo-inclusions and grooves. There is no battle for the pathologists in the diagnosis of classical variant of PTC with the papillary structures and complete nuclear characteristics. On the contrary, invasive encapsulated follicular variant papillary thyroid carcinoma (FVPTC) throws diagnostic challenge while differentiating from follicular thyroid carcinoma (FC).

Definitive diagnosis is sometimes difficult even in histopathology specimens and requires ancillary techniques. A growing number of some promising immunohistochemical markers for the differential diagnosis of thyroid lesions have emerged, including P63 and CD56 but till now none of them are conclusive.[4,5]

P63, a member of the tumor suppressor gene family, is the homologous nuclear transcription factor of p53 and is essential for normal development.[6,7,8] P63 plays a key role in epithelial proliferation and differentiation and causes defects in epithelial differentiation.[8] Focal staining is expected in PTC while some reports state no staining in other thyroid tumors.[5,9,10]

CD56 is a neural cell adhesion molecule and its migration influences the migratory characteristics of tumor cells. Various studies have reported that release of CD56 is reduced in malignant thyroid tumors specially in papillary thyroid carcinoma while it is released in normal thyroid epithelial cells.[5,11,12]

Most studies have evaluated the single expression of markers in various thyroid lesions and a few reports have studied the combined expression of markers. Therefore, the aim of this study was to identify the possible diagnostic role of P63 and CD56 in the papillary and follicular thyroid carcinoma.

Methods

This was a cross-sectional descriptive study carried outin thedepartment of Pathology, Rajshahi Medical College and Armed Forces Institute of Pathology (AFIP). The duration of the study was two years, fromMarch 2020 to February 2022.All patients admitted in ENT and Surgery Department of Rajshahi Medical College Hospital, Rajshahi diagnosed clinically and later on histopathologically as papillary and follicular thyroid carcinoma were enrolled in this study.

Representative slide from each specimen was examined under an Olympus multi-headed microscope (Model u-MDO10R3, Tδ, SN 5J42483, 201509, Olympus corporation toxyo-163-0914, Japan). According to WHO classification, slides of all cases were examined and interpreted by experienced pathologists. Decision in controversial cases was made by consensus meeting at the multi-

head microscope. Immunohistochemistry was done in Armed Forces Institute of Pathology (AFIP), Dhaka. Sections (4-micrometer) of formalin fixed, paraffin-embedded tissues block containing representative area of the lesion were used. Immunohistochemical analysis was done with the commercially available antibody against P63 antigens (Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-P63, source Dako) and CD56 antigen (CD56/NCAM-1 Ab-4 Mouse Monoclonal Antibody, Clone 56C04, source Epredia)in appropriate dilutions. Immunostaining was performed with an automated immunostainer (VENTANA BENCHMARK XT) according to the manufacturer's recommended procedure. Proper positive controls were prepared for each antibody i.e. normal tonsil tissue for P63 and follicular adenoma for CD56. The immunohistochemical stained slides were also evaluated separately by two pathologists.

Results

Forty four cases of papillary and follicular thyroid carcinoma, proven by histopathology, were included in this study. The mean age of the patients was 40.86 ± 6.57 years (SD) and the male female ratio was 1: 2.4. Among the total of 44 patients that were included in this study, 39 cases were diagnosed as papillary thyroid carcinoma (27 classical PTC & 12 Follicular variant PTC) and 5 cases were diagnosed as Follicular thyroid carcinoma (Table I). P63 was expressed in 74.1% of classical PTC and 75% of FVPTC. All cases of follicular thyroid carcinoma were negative for P63. Only 4 cases (14.8%) of classical variant and 2 cases (16.7%) of FVPTC showed positive expression of CD56. Only single case (20%) of follicular thyroid carcinoma showed positive expression of CD56. (Table II). In the present study, 29 cases (74.4%) of papillary thyroid carcinoma showed positive expression of P63 and all cases of follicular thyroid carcinoma were negative for P63. The result was statistically highly significant. Only 6 cases (15.4%) of papillary thyroid carcinoma and single case (20%) of follicular thyroid carcinoma showed positive expression of CD56 (Table III). Out of 27 classical PTC, most of the cases 16 (59.3%) showed P63 positive CD56 negative expression followed by 7 cases (25.9%) showed P63 negative CD56 negative expression. Among FVPTC, 6 cases (50%) showed P63 positive CD56 negative expression and 4 cases (33.3%) showed P63 negative CD56 negative expression. Among 5 cases of follicular thyroid carcinoma, 4 cases (80%) showed P63 negative CD56 negative expression (Table IV). In present study, 22 cases (56.4%) of papillary thyroid carcinoma showed P63 positive CD56 negative expression but no follicular thyroid carcinoma showed this pattern of expression. The result was statistically significant. 4 cases (10.3%) of papillary thyroid carcinoma and single case (20%) of follicular thyroid carcinoma showed P63 negative CD56 positive expression. Only 2 cases (5.1%) of papillary thyroid carcinoma and no cases of follicular thyroid carcinoma showed P63 positive CD56 positive expression. P63 negative CD56 negative pattern expression was shown in 11 cases (28.2%) of papillary thyroid carcinoma and 4 cases (80%) of follicular thyroid carcinoma. This result was also statistically significant (Table V).

Table I: Baseline characteristics of study population (n=44)

Characteristics	
Age [mean±SD]	40.86± 6.57
Male:female ratio	1: 2.4
Histopathological diagnosis	
a) Papillary Thyroid Carcinoma (n=39)	
1. Classical PTC	27(61.4%)
2. FVPTC	12(27.3%)
b) Follicular Thyroid Carcinoma (n=5)	05(11.4%)
Total	(n=44)

Table II: Frequency of expression of P63 and CD56 among classical variant papillary, follicular variant papillary and follicular thyroid carcinoma (n=44)

		Papillary carcinoma		Follicular carcinoma N (%)	Total
		Classical N (%)	Follicular variant N (%)		
P63	Positive	20 (74.1)	9 (75.0)	0 (0)	29 (65.9)
	Negative	7 (25.9)	3 (25.0)	5 (100)	15 (34.1)
CD56	Positive	4 (14.8)	2 (16.7)	1 (20)	7 (15.9)
	Negative	23 (85.2)	10 (83.3)	4 (80.0)	37 (84.1)
Total		27 (100)	12 (100)	5 (100)	44

Table III: Significance of P63 and CD56 expression in papillary and follicular thyroid carcinoma (n=44)

		Papillary carcinoma N (%)	Follicular carcinoma N (%)	Total	p
P63	Positive	29 (74.4)	0 (0)	29 (65.9)	0.003**
	Negative	10 (25.6)	5 (100)	15 (34.1)	
CD56	Positive	6 (15.4)	1 (20.0)	7 (15.9)	1.00
	Negative	33 (84.6)	4 (80.0)	37 (84.1)	
Total		39 (100)	5 (100)	44	

** Highly significant.
P value was obtained from Fisher's Exact Test

Table IV: Frequency of combined immunoexpression of P63 and CD56 among classical variant papillary, follicular variant papillary and follicular thyroid carcinoma (n=44)

P63 and CD56	Papillary carcinoma		Follicular carcinoma N (%)	Total
	Classical N (%)	Follicular variant N (%)		
P63 positive CD56 negative	16 (59.3)	6 (50.0)	0 (0.0)	22 (48.8)
P63 negative CD56 positive	3 (11.1)	1 (8.3)	1 (20.0)	5 (11.6)
P63 positive CD56 positive	1 (3.7)	1 (8.3)	0 (0.0)	2 (4.7)
P63 negative CD56 negative	7 (25.9)	4 (33.3)	4 (80.0)	15 (34.9)
Total	27 (100)	12 (100)	5 (100)	44 (100)

Table V: Significance of combined immunoexpression of P63 and CD56 in papillary and follicular thyroid carcinoma (n=44)

P63 and CD56	Papillary carcinoma N (%)	Follicular carcinoma N (%)	Total	p
P63 positive CD56 negative	22 (56.4)	0 (0.0)	22 (48.8)	0.048*
P63 negative CD56 positive	4 (10.3)	1 (20.0)	5 (11.6)	0.47
P63 positive CD56 positive	2 (5.1)	0 (0.0)	2 (4.7)	1.00
P63 negative CD56 negative	11 (28.2)	4 (80.0)	15 (34.9)	0.039*
Total	39 (100)	5 (100)	44 (100)	

* Significant. P value was obtained from Fisher's Exact Test

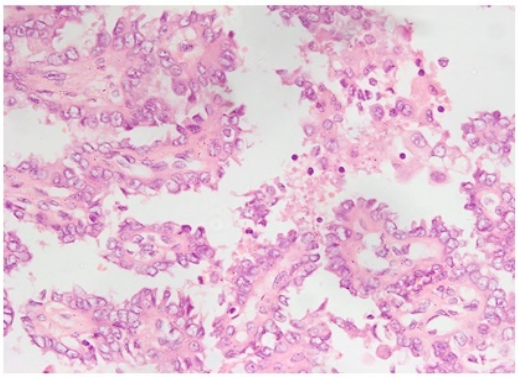

Figure 1. Photomicrograph shows classical papillary thyroid carcinoma (Case no. 24, H&E stain, x400)

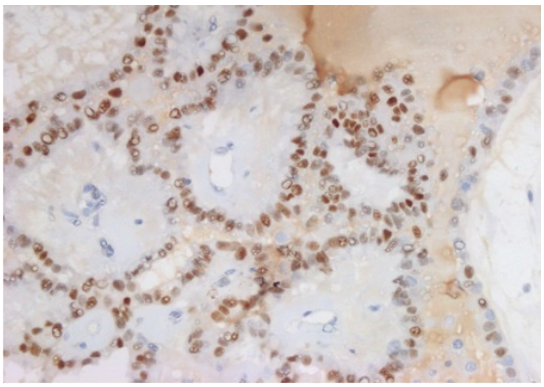

Figure 2. Photomicrograph shows P63 immunostaining in classical PTC (Case no. 24, x400)

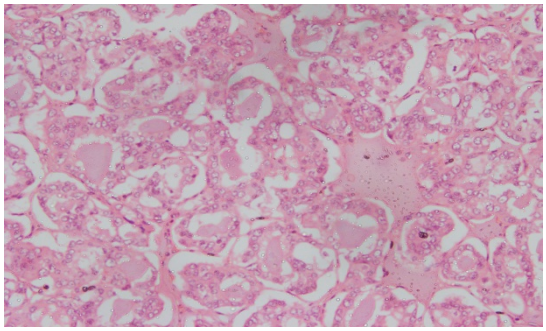

Figure 3. Photomicrograph shows follicular variant of papillary thyroid carcinoma (Case no. 23, H&E stain, x400)

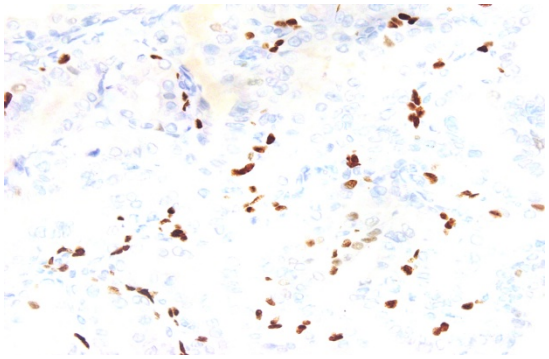

Figure 4. Photomicrograph shows P63 immunostaining in follicular variant of PTC (Case no. 23, x400)

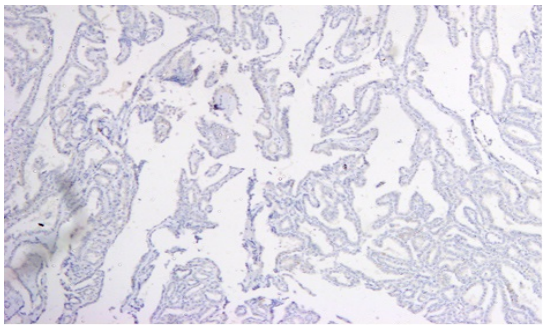

Figure 5. Photomicrograph shows negative CD56 immunostaining in classical PTC (Case no. 2, x400)

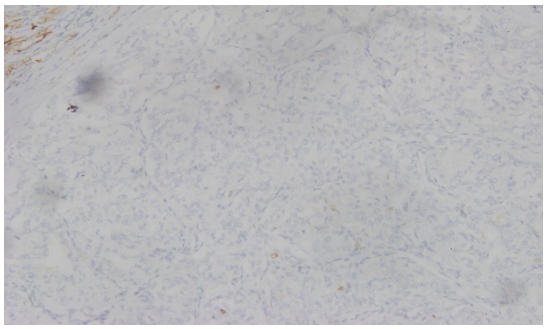

Figure 6. Photomicrograph shows negative CD56 immunostaining in follicular variant of PTC (Case no. 4, x400)

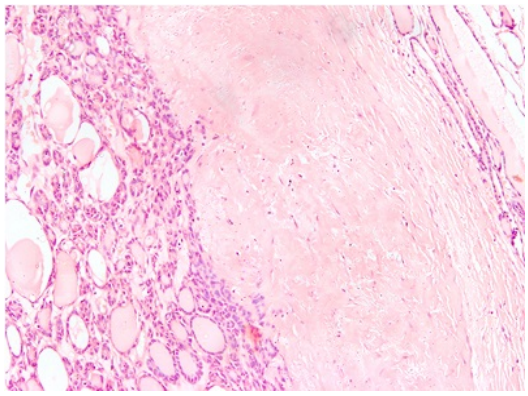

Figure 7. Photomicrograph shows follicular thyroid carcinoma (Case no. 9, H&E stain, x400)

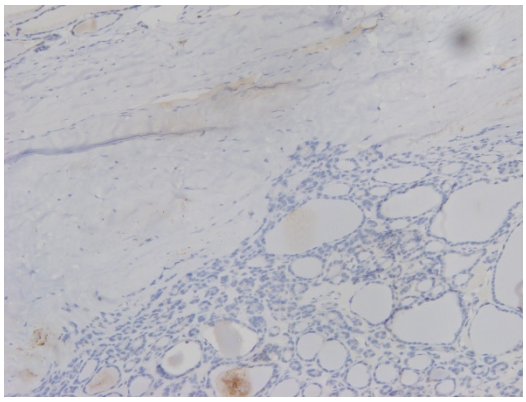

Figure 8. Photomicrograph shows negative P63 immunostaining in follicular thyroid carcinoma (Case no. 9, x400)

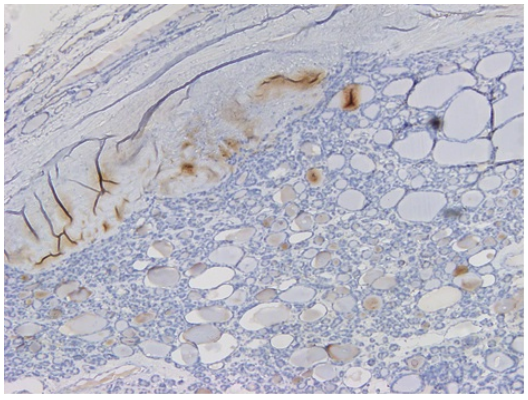

Figure 9. Photomicrograph shows negative CD56 immunostaining in follicular thyroid carcinoma (Case no. 9, x400)

Discussion

The age of papillary and follicular thyroid carcinoma varies in different parts of the world. The mean age of patients of this study was 40.86 ± 6.57 years. Another studies by Akhtar et al.,[13] Mokhtari et al.,[14] Merchant D,[15] Hussain et al.[16] and Islam et al.[17] showed different mean age like 45, 42.4, 41.8, 38 and 32.7.

In this study, female (70.5%) predominance was observed over male (29.5%) and male & female ratio was 1:2.4. Similar pictures are seen in the studies conducted by Hossain et al.,[18] Merchant D[15] and Islam et al.[17] with male to female ratio of 1:3,1:2.2 and 1:2.3.

In the present study, papillary thyroid carcinoma was the most frequent histologic type (88.6%). This is comparable to a local study done by Islam et al.[17] where 68% of thyroid carcinomas were papillary thyroid carcinoma. Studies done by Merchant D[15] and Sarfraz et al.[19] showed their frequency of papillary thyroid carcinoma as 80% & 52.3%. Out of 44 cases of this study, there were 12 cases (27.3%) of follicular variant of papillary thyroid carcinoma but the study by Islam et al.[17] showed 4 cases (8%) out of 50. The frequency of follicular thyroid carcinoma in this study was 11.4% which had similarity to the study by Merchant D [15] (11%). Sarfraz et al.[19] and Islam et al.[17] showed 23.8% and 16% cases of follicular thyroid carcinoma in their studies.

Unger et al. (2003),[20] Bonzanini et al. (2008)[21] and El Demellawy et al. (2008)[5] described that papillary thyroid carcinoma had high percentage of P63 positivity. This study also goes with them in this issue. None of follicular thyroid carcinoma showed positivity for P63 in this study. Studies by El Demellawy et al. (2008)[5] and Jeong et al. (2016)[22] also showed no positivity for P63 of follicular thyroid carcinoma. In the study by Abdel-Aziz & Abdallah (2019),[23] 21.43% of follicular thyroid carcinoma showed positivity for P63.

Only 6 cases (15.4%) of papillary thyroid carcinoma and single case (20%) of follicular thyroid carcinoma showed positive expression of CD56 in this study. Abdel-Aziz &Abdallah (2019)[23] showed high percentage of negativity of CD56 for papillary thyroid carcinoma like this study. But this study observed low negative expression of follicular thyroid carcinoma for CD56 whereas present study showed high negativity (80%). Muthusamy et al. (2018)[24] showed 79.7% negativity of CD56 for follicular thyroid carcinoma which was close to this study.

This study showed positive immunoreactivity of P63 as highly significant in distinguishing papillary from follicular thyroid carcinoma. Studies by Jeong et al. (2016)[22] and Abdel-Aziz & Abdallah (2019)[23] also showed that when comparing papillary with follicular thyroid carcinoma, the expression of P63 was significantly higher in papillary thyroid carcinoma. In this study combined immunoexpression of P63 positive CD56 negative & P63 negative CD56 negative were significant in differentiating papillary from follicular thyroid carcinoma.

Conclusion

It can be concluded that P63 is a useful immunohistochemical marker in distinguishing papillary thyroid carcinoma from follicular thyroid carcinoma. Negative expression of CD56 can also be considered useful in the diagnosis of papillary thyroid carcinoma. Combined P63 positive CD56 negative and P63 negative CD56 negative immunoexpression might be useful in differentiating papillary from follicular thyroid carcinoma.

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