TAJ December 2023; Volume 36 Number-2

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Significance of P63 Expression in Papillary and Follicular Thyroid Carcinoma

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Abstract

Background: Papillary thyroid carcinoma (PTC) is the most frequent histologic type of all thyroid malignancies followed by follicular thyroid carcinoma (FC). Follicular variant of papillary thyroid carcinoma (FVPTC) is the second most common variant of PTC. A diagnostic dilemma may arise, when histologically the distinctive nuclear features are either not well developed or present focally within an encapsulated thyroid lesion. A panel of immunomarkers has been tested to overcome the limitation of histopathology. P63 is commonly used immunomarkers among them.

Objectives: The aim of the present study was to see the expression of P63 in PTC including FVPTC and FC in order to evaluate their possible roles in the differential diagnosis between these two mimics.

Patients and methods: This cross-sectional descriptive study was conducted over 44 cases with histologically confirmed papillary and follicular thyroid carcinoma. Immunohistochemistry was done for P63.

Results: Out of the 44 cases, 39 were histologically diagnosed as papillary thyroid carcinoma (27 were classical PTC & 12 were FVPTC) and the rest 5 were follicular thyroid carcinoma. In the present study, P63 showed 74.4% positivity with varying intensity and extent of staining in papillary thyroid carcinoma. Positive immunoreactivity of P63 was highly significant in distinguishing papillary from follicular thyroid carcinoma (P=0.003).

Conclusion: This study suggested that the use of P63 may be helpful in the diagnosis of papillary and follicular thyroid carcinoma along with histopathological examination.

Key words: Immunohistochemistry (IHC), PTC, FVPTC, FC, P63

TAJ 2023; 36: No-2: 121-128

Introduction

Thyroid carcinoma is the most common malignancy of the endocrine system. Due to the sophisticated imaging modalities, detection of thyroid carcinoma has been steadily increased in the past few decades. Papillary thyroid carcinoma is the most frequent histologic type, comprising 70% to 85% of all thyroid malignancies.[1]

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Papillary thyroid carcinoma is characterized by distinctive nuclear features such as enlarged ovoid nucleus, irregular nuclear membrane, powdery chromatin, longitudinal grooves and intranuclear cytoplasmic inclusion. The most common variants of papillary thyroid carcinoma include the classical subtypes with papillary architecture and the follicular variant PTC that lacks papillae but retains classical nuclear atypia. Follicular thyroid carcinoma is usually defined as a form of thyroid malignancy arising from follicular cells in which the diagnostic nuclear features of papillary carcinoma are absent. It is the second most common cancer of the thyroid, after papillary thyroid carcinoma.[2]

Still now the gold standard for diagnosis of thyroid lesions particularly PTC is pathologic evaluation using routine haematoxylin and eosin (H & E) staining.[3] Characteristic nuclear changes are necessary for the diagnosis of PTC, however when present focally they cause diagnostic dilemma in distinguishing it from other thyroid lesions.[4] In such cases, an objective consistent diagnosis based merely on morphologic assessment is sometimes impossible.[5]

Some of the ancillary studies as immunohistochemistry and molecular techniques were investigated to aid in the diagnosis of these problematic cases[6,7] P63 is a member of p53 family nuclear transcription factor. It is consistently expressed in basal, squamous and myoepithelial cells such as in basal cells of the prostate acini and ducts, myoepithelial cells of the breast and squamous cell carcinoma.[8] Studies on the involvement of P63 protein in thyroid tumor processes are few and have different results.[5,9,10]

If appropriately diagnosed and treated, papillary carcinoma has an excellent long term prognosis. Therefore, the aim of this study was to investigate the expression of P63 immunohistochemistry expected to be of value in the diagnosis of follicular and papillary thyroid carcinomas which include the follicular variant.

Materials and Methods

This study was conducted in the department of Pathology, Rajshahi Medical College during the period of March 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 thyroid carcinomas (Papillary and follicular thyroid carcinoma) were enrolled in this study.

Examination of representative slide from each specimen was done on an Olympus multi-headed microscope and interpreted by experienced pathologists according to its WHO classification. Immunohistochemistry was done in Armed Forces Institute of Pathology (AFIP), Dhaka. Immunohistochemical analysis was done with the commercially available antibody against P63 antigens (Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-P63, source Dako) in appropriate dilutions. The immunohistochemical stained slides were also evaluated by two pathologists and independently. Intensity of P63 nuclear staining was evaluated in each case and assigned an incremental 0, 1+, 2+, 3+ score. Extent of staining was categorized as focal (positive in less 25% of the tumor cells), non-focal (positive in 25% to 75% of cells) or diffuse (positive in more than 75% of cells).[11]

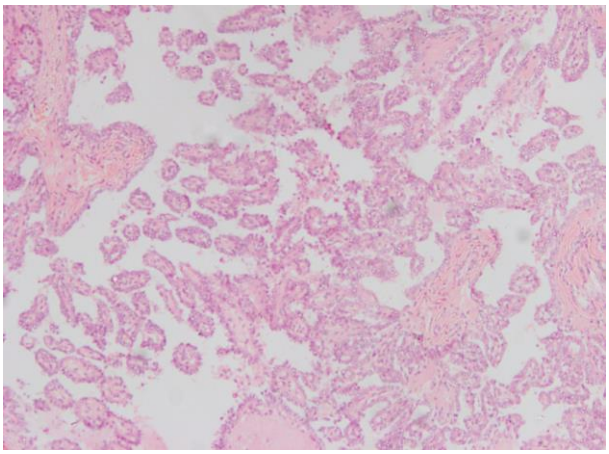

Figure 1: Photomicrograph showing classical papillary thyroid carcinoma (H&E stain, x400)

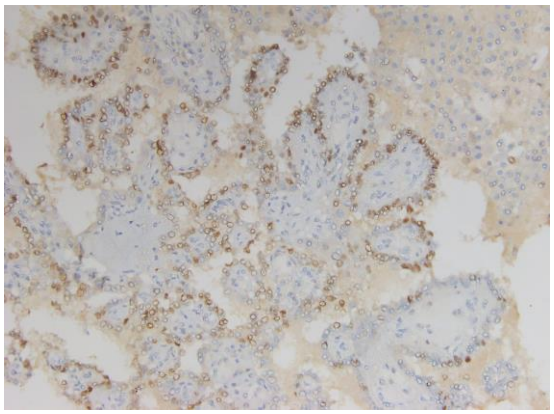

Figure 2: Photomicrograph showing 2+ intensity, diffuse nuclear P63 immunostaining in classical PTC (x400)

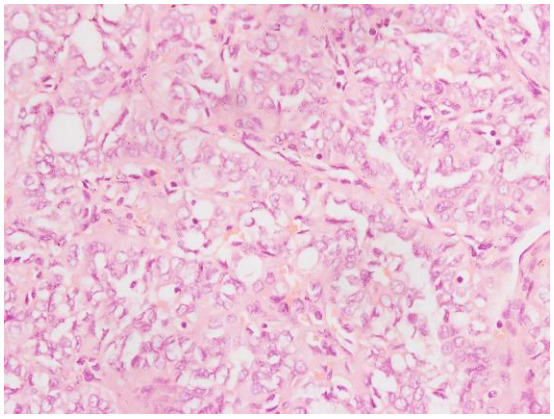

Figure 3: Photomicrograph showing follicular variant of papillary thyroid carcinoma (H&E stain, x400)

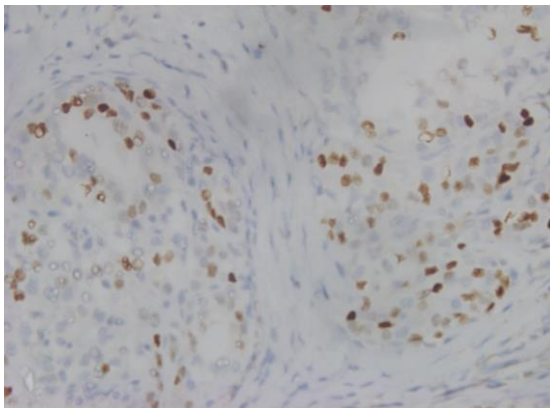

Figure 4: Photomicrograph showing 3+ intensity, non-focal nuclear P63 immunostaining in follicular variant of PTC (x400)

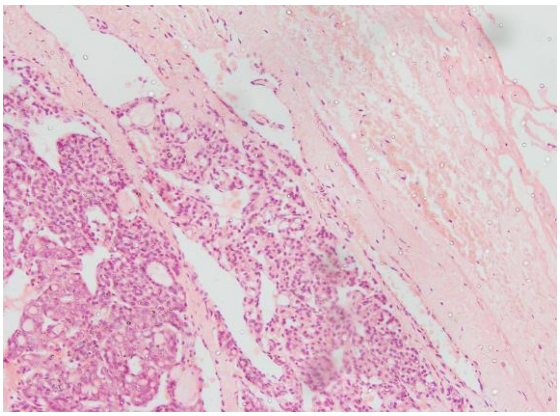

Figure 5: Photomicrograph showing of follicular thyroid carcinoma (H&E stain, x400)

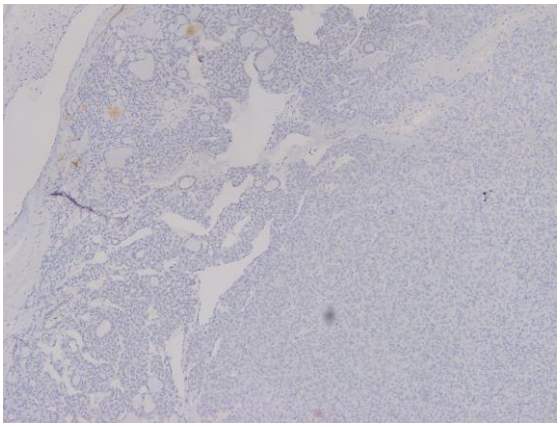

Figure 6: Photomicrograph showing negative P63 immunostaining in follicular thyroid carcinoma (x400)

Results

A total number of 44 histopathologically diagnosed cases of papillary and follicular thyroid carcinomas were included in this study.

Table 1: Baseline characteristics of study population (n=44)

	Frequency (%)	Mean±SD	Minimum	Maximum
Age groups				
<30 years	1(2.3 %)			
30-39 years	27(61.4%)			
40-49 years	10(22.7%)			
>50 years	6(13.6%)			
Age		40.86± 6.57	29 year	58 year
Sex				
Male		13(29.5%)		
Female		31(70.5%)		

In this study, mean age of the patients was 40.86 ± 6.57 years (SD) with age range from 29 to 58 years. Most (61.4%) of the patients belonged to 30-39 years age group. There was no case below 29 years or above 58 years. The male female ratio was 1: 2.4.

Table 2: Frequency of different histologic type of studied thyroid lesions (n=44):

Histopathological diagnosis	Frequency	%
a. Papillary thyroid carcinoma	39	88.6
1. Classical PTC	27	61.4
2. FVPTC	12	27.3
b. Follicular thyroid carcinoma	5	11.4
Total	44	100

Among the 44 cases, the highest number were diagnosed as papillary thyroid carcinoma (39 cases, 88.6 %).Among them 27 cases were classical variant (61.4%) and 12 cases were follicular variant of papillary thyroid carcinoma (27.3%). The number of follicular thyroid carcinomas were 5 cases (11.4%).

Table 3: Frequency of expression of P63 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)
Total		27 (100)	12 (100)	5 (100)	44

In this study, all cases of follicular thyroid carcinoma were negative for P63.In classical variant of papillary thyroid carcinoma, 20 cases (74.1%) showed positive expression and 7 cases (25.9%) showed no expression of P63. Among 12 cases of follicular variant of papillary thyroid carcinoma, 9 cases (75%) showed positive expression and 3 cases (25%) showed no expression of P63.

Table 4: Distribution of P63 positive cases according to intensity and grading (n=29) in studied thyroid lesions

	1. Classical PTC				2. FVPTC			
Grade	Focal	Non focal	Diffuse	Total	Focal	Non focal	Diffuse	Total
Intensity	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
1+	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (11.1)
2+	1 (25.0)	2 (25.0)	2 (25.0)	5 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
3+	3 (75.0)	6 (75.0)	6 (75.0)	15 (75.0)	3 (100)	2 (66.7)	3 (100)	8 (88.9)
Total	**4 (100)**	**8 (100)**	**8 (100)**	**20 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**9 (100)**

No P63 expression in Follicular thyroid carcinoma.

Out of 39 cases of papillary thyroid carcinoma, total 29 cases showed positivity for P63 and none of follicular thyroid carcinoma showed this expression. In classical variant of papillary thyroid carcinoma, 20 cases (out of 27 cases) showed positivity. Staining intensity of P63 showed 2+ positivity in 5 cases

(25%) and 3+ positivity in 15 cases (75%). Grading of P63 expression showed focal positive in 4 cases (2+ intensity in 1 case & 3+ intensity in 3 cases), non- focal positive in 8 cases (2+ intensity in 2 cases & 3+ intensity in 6 cases) and diffuse positive in 8 cases (2+ intensity in 2 cases & 3+ intensity in 6 cases).In follicular variant of papillary thyroid carcinoma, 9 cases (out of 12 cases) showed positivity. Staining intensity of P63 showed 1+ positivity in 1 case (11.1%) and 3+ positivity in 8 cases (88.9%). Grading of P63 expression showed focal positive in 3 cases (all were 3+ in intensity), non- focal positive in 3 cases (1+ intensity in 1 case & 3+ intensity in 2 cases) and diffuse positive in 3 cases (all were 3+ in intensity). In follicular thyroid carcinoma, all cases were negative for P63.

In this study, both classical PTC (75%) and FVPTC (88.9%) showed 3+ intensity for P63 and in context of grading, both non-focal& diffuse PTC belonged 8 cases (out of 20) equally but FVPTC belonged 3 cases (out of 9) equally in focal, non-focal & diffuse group.

Table 5: Significance of P63 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)	
Total		39 (100)	5 (100)	44	

** Highly significant.

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

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.

Discussion

The maximum number of patients (61.4%) in this study was in between 30-39 years age group. Similar pattern of age distribution was observed by Hossain et al.[12], Islam et al.[13] and Hussain et al.[14] The mean age of patients of this study was 40.86 ± 6.57 years. Different studies by Akhtar et al.[15], Mokhtari et al.[16], Merchant D[17], Hussain et al.[14] and Islam et al.[13] showed different mean age like 45, 42.4, 41.8, 38 and 32.7. Age range in our study was 29 to 58 years where as Mokhtari et al.[16] showed 8-85 years and Hussain et al.[14] showed 17-70 years.

In this study, female (70.5%) predominance was observed over male (29.5%) making a male & female ratio 1:2.4 which is comparable to the study conducted by Hossain et al.[18], Merchant D[17] and Islam et al.[13] with male to female ratio of 1:3,1:2.2 and 1:2.3. This was also in agreement with studies of Mokhtari et al.[16], Sarfraz et al.[19], Rahman et al.[20] and Akhtar et al.[15]

In the present study, papillary thyroid carcinoma was the most frequent histologic type among studied thyroid lesions and it comprises 88.6% which is comparable to the local study done by Islam et al.[13] where 68% of all thyroid carcinomas were papillary thyroid carcinoma. Two another studies by Merchant D[17] and Sarfraz et al.[19]

showed their frequency of papillary thyroid carcinoma as 80% & 52.3%. Among the 44 cases of our study, there were 12 cases (27.3%) of follicular variant of papillary thyroid carcinoma but the study by Islam et al.[13] showed 4 cases (8%) out of 50. The frequency of follicular thyroid carcinoma in our study was 11.4% which was close to the study by Merchant D [17] (11%). Studies by Sarfraz et al.[19] and Islam et al.[13] showed 23.8% and 16% cases of follicular thyroid carcinoma.

It was observed in this study that staining intensity of P63 showed 1+ positivity in 1 FVPTC, 2+ positivity in 5 classical PTC and 3+ positivity in 15 classic PTC & 8 FVPTC cases. For grading of P63 expression, classic PTC showed 4 focal positive, 8 non-focal positive & 8 diffuse positive cases and FVPTC showed 3 focal positive, 3 non-focal positive & 3 diffuse positive cases. All cases of follicular carcinoma were negative for P63. A study by Gupta et al.[21] stated that 6 out of 13 PTC cases expressed staining focally for P63 and here also P63 expression was absent in FC.

Various studies of P63 expression in thyroid lesions have been reported to date. Unger et al.[10], Bonzanini et al.[22] and El Demellawy et al.[5] described that P63 was positive in a high percentage of papillary thyroid carcinomas. This study also goes with them in this regard. None of follicular thyroid carcinoma in this study, El Demellawy et al.[5] and Jeong et al.[3] showed positivity for P63. In the study by Abdel-Aziz & Abdallah[23], there was 21.43% positivity of follicular carcinoma for P63.

Comparison of positive P63 immunostaining results with previous studies

Study	Papillary carcinoma	Follicular carcinoma
Unger et al. (2003)[10]	81.8%	--
Bonzanini et al. (2008)[22]	74.1%	--
El Demellawy et al. (2008)[5]	70%	0%
Abdel-Aziz & Abdallah (2019)[23]	54.17%	21.43%
Etem et al. (2010)[24]	30.5%	7.5%
Jeong et al. (2016)[3]	14.7%	0%
Present study	74.4%	0%

Conclusion

Distinguishing the follicular variant of papillary thyroid carcinoma from follicular thyroid carcinoma is an area of controversy. Several immunohistochemical markers may be used to improve diagnostic accuracy. In the present study, P63 showed 74.4% positivity with varying intensity and extent of staining in papillary thyroid carcinoma. Positive immunoreactivity of P63 was highly significant in distinguishing papillary from follicular thyroid carcinoma.

Conflict of interest: None declared

References

1. Pusztaszeri MP, Sadow PM & Faquin WC. CD117: A novel ancillary marker for papillary thyroid carcinoma in fine needle aspiration biopsies. Cancer Cytopathology 2014;122(8):596-603.
2. Santacroce L. Follicular Thyroid Carcinoma. Medscape. 2020. Available from: https://www.google.com/search?q=follicular+thyroid+carcinoma+medscape&oq=follicular+thyroid+carcinoma+medscape&aqs=chrome..69i57j0i22i30.53203j0j15&sourceid=chrome&ie=UTF-8. [cited 2022 March13].
3. Jeong JY, Jung JH and Park JY. Expression and diagnostic availability of p63 and CD56 in papillary thyroid carcinoma. Int J Clin Exp Pathol 2016;9 (7):7402-7410.
4. Baloch ZW and Livolsi VA. Cytologic and architectural mimics of papillary thyroid carcinoma: Diagnostic challenges in fine-needle aspiration and surgical pathology specimens. Am J Clin Pathol 2006;125:135-44.
5. El Demellawy D, Nasr A and Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of thyroid. Diagnostic Pathology 2008;3:5.

6. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacher K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS and Chapelle ADL. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci USA 2001;98:15044-15049.

7. Beesley MF and Mclaren KM. Cytokeratin-19, galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodule. Histopathology 2002;41:236-43.

8. Di Como CJ, Urist MJ, Babayan I, Drobnjak M, Hedvat C, Teruya-Feldstein J, Pohar K, Hoos A and Cordon-Cardo C. p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002;8(2):494-501.

9. Kim YW, Do IG and Park YK. Expression of the GLUT1 glucose transporter, p63 and p53 in thyroid carcinomas. Pathol Res Pract 2006;202(11):759-765.

10. Unger P, Ewart M, Wang BY, Gan L, Kohtz DS and Burstein DE. Expression of p63 in papillary thyroid carcinoma and in Hashimoto's thyroiditis: a pathobiologic link?. Hum Pathol 2003;34(8):764-769.

11. Albadine R, Schultz L, Illei P, Ertoy D, Hicks J, Sharma R, Epstein JI and Netto GJ. PAX8(+)/p63(-) Immunostaining Pattern in Renal Collecting Duct Carcinoma. Am J Surg Pathol 2010;34(7):965-969.

12. Hossain AKMF, Siddiqui MMR & Khatun SF. Socio-demographic and Clinical Characteristics of Patient with Thyroid Cancer. AKMMC J 2020;11(1):54-58.

13. Islam MA, Islam R, Sayeed ANEA, Talukder D, Rumi SNF, Choudhury AA, Fakir MAY & Sitan KN. Pattern of primary thyroid malignancy in a tertiary care hospital. J Dhaka Med Coll. 2018;27(2):161-174.

14. Hussain F, Samra I, Mehmood A, Bazarbashi S, ElHassan T & Chaudhri N. Incidence of thyroid cancer in the Kingdom of Saudi Arabia, 2000–2010'. Hematol Oncol Stem Cell Ther 2013;6(2):58–64.

15. Akhtar S, Khan Z, Ahmed M, Osman L, Ahmad F & Chaudhry AM. Correlation of clinical presentation with investigations and operative findings in solitary nodule thyroid. ANNALS 2001;7(3):158-160.

16. Mokhtari M, Eftekhari M & Tahririan R. Absent CD65 expression in papillary thyroid carcinoma: A finding of potential diagnostic value in problematic cases of thyroid pathology. Journal of Research in Medical Sciences 2013;18(12):1046-50.

17. Merchant D. Demographic review, clinical and histological presentation of patients with primary thyroid carcinoma presenting at tertiary care hospital. the health 2012;3(1):7-9.

18. Hossain AKMF, Siddiqui MMR & Khatun SF. Socio-demographic and Clinical Characteristics of Patient with Thyroid Cancer. AKMMC J 2020;11(1):54-58.

19. Sarfraz T, Ullah K & Muzaffar M. The frequency and histological types of thyroid carcinoma in northern Pakistan. Pak Armed Forces Med J 2000;50(2):98-101.

20. Rahman MA, Biswas MA, Siddika ST, Sikder AM, Talukder SI & Alamgir MH. Histomorphological pattern of thyroid lesion. Dinajpur Med Col J 2013;6(2):134-140.

21. Gupta A, Jain S, Khurana N & Kakar AK. Expression of P63 and Bcl-2 in malignant thyroid tumors and their correlation with other diagnostic immunocytochemical markers. Journal of Clinical and Diagnostic Research 2016;10(7):4-8.

22. Bonzanini M, Amadori PL, Sagramoso C & Palma PD. Expression of cytokeratin 19 and protein P63 in fine needle aspiration biopsy of papillary thyroid carcinoma. Acta Cytol 2008;52(5):541-8.

23. Abdel-Aziz A and Abdallah D. Role of Immunohistochemistry in Diagnosis of Papillary Thyroid Carcinoma: The Use of Ck19, CD56, P63, and CD117. Journal of Cancer and Tumor International 2019;9(1):1-11.

24. Etem H, Özekinci S, Mizrak B & Şentürk S. The role of CD56, HBME-1, and p63 in follicular neoplasms of the thyroid. Cilt 2010;26(3):238-242.

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