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Original Research Paper

Statistically evaluate the role of CD56 and p63 in the diagnosis of PTC and differentiating them from PTC mimickers.

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ABSTRACT:

Introduction: The coastal belt of Kerala is known for its radiation hazard and the increased incidence of thyroid disorders including PTC. PTC, if it shows classical histology is easy to diagnose. However, a lot of being or non neoplastic lesions mimic PTC and then the diagnostic dilemma arises, hence, the need for a specific marker to diagnose PTC. **Aim and objective:** With this background in mind this study was conducted to evaluate the usefulness of CD56 and p63 IHC markers as diagnostic tools for PTC. **Materials and method:** This was a descriptive study conducted over a period of 18 months in the Dept of Pathology at GTDMC, Alappuzha. 100 consequetive thyroidectomy specimens received in the Dept were included in the study. The exclusion criterion was all pure colloid goiters which never pose as mimics of PTC. The specimens were studied for morphology and IHC done as per protocol with CD56 and p63 markers. A positive membranous staining without a cytoplasmic staining in 10% or more neoplastic cell is considered as positive staining for CD56 and any nuclear staining is counted as positive for p63. The findings were analyzed and sensitivity, specificity, PPV and NPV were calculated for these markers. **Results:** There were 34 cases of PTC and 33 showed negative staining with CD56, giving a Sensitivity of 97.1%, Specificity of 88.9%, Positive predictive value of 82.5% and a Negative predictive value of 98.2%. With p63, Sensitivity was 37.1%, Specificity 92.2%, positive predictive value 72.2% And Negative predictive value was 72.8%.other malignancies obtained include, Hurthle cell Carcinoma and Follicular Carcinoma.
Conclusion: Hence to conclude, a combined panel of CD56 and p63 is very accurate in diagnosing PTC and differentiating it from its mimickers.

Key words: PTC mimickers, CD56, p63, thyroid neoplasms

INTRODUCTION:

Thyroid disorders are very common in Kerala, most common being multi nodular goiter. Papillary thyroid carcinoma [PTC**]** is the most common type of malignant neoplasm of thyroid gland.[1]It is very common in Kerala, due to terrestrial radiation along its shoreline along with regular consumption of iodised salt.[2,3] It is more common in females especially middle age group with a female to male ratio of 2:1-4:1. Mean age of presentation is 40 years. The diagnosis of PTC is based on the characteristic nuclear features seen in H & E stained sections, considered the gold standard.[1] Appreciation of the nuclear features of PTC is mainly dependent on tissue processing. Morphologic similarities between non neoplastic lesions, benign and malignant lesions are frequent; papillary and follicular architectures and nuclear irregularity may be seen in both benign and malignant lesions (PTC mimickers) for e.g., severe chronic lymphocytic thyroiditis, Hashimoto thyroiditis, follicular adenoma, follicular carcinoma and hyperplastic nodule. CD56 - a neural cell adhesion molecule (NCAM) is a homophilic glycoprotein of the

immunoglobulin superfamily which is expressed on normal and IL-2-activated thyroid follicular cells. It is observed as membrane positivity. Reduced expression or loss of CD56 is implicated in tumour progression in patient with thyroid cancer, mainly PTC and its variants in particular the follicular variant.[4] p63 is a p53-homologue nuclear transcription factor present in the basal layer of squamous epithelium, in breast and prostate myoepithelial cells and in the transitional epithelium of the bladder. Any nuclear p63 staining is accounted as positive expression of p63. It is known to show positivity in PTC cases.[5]

AIM AND OBJECTIVE:

With this background the aim of the present study is to statistically evaluate the role of IHC markers CD56 and p63 in the diagnosis of PTC.

MATERIAL AND METHODS:

This is a descriptive study consisting of 100 thyroidectomy specimens (after exclusion criteria was met) received in the Dept of Pathology, Govt TD Medical College, Alappuzha, Kerala, over an 18 months period. It is a prospective study from the period of attaining IEC clearance. Since pure colloid goiters do not come as differential to PTC, they were excluded from this study. Sections were stained with H&E and IHC with CD56 and p63 was done on these cases according to the protocol suggested by the manufacturer, Pathnsitu Biotechnology Pvt Ltd. HRP-DAB system was used. Antigen retrieval was by Tris buffer EDTA by heat using Multi Epitope Retrieval System. Mouse monoclonal antibodies were used. Normal thyroid was the positive control for CD56 and skin was the positive control for p63. Negative controls were created by omitting primary antibody and using buffer alone. Membrane staining without cytoplasmic staining in 10% or more of cells is considered positive for CD56 and any nuclear staining is considered positive for p63

DECLARATION:

It is declared that this article has not been published or submitted elsewhere.

RESULTS:

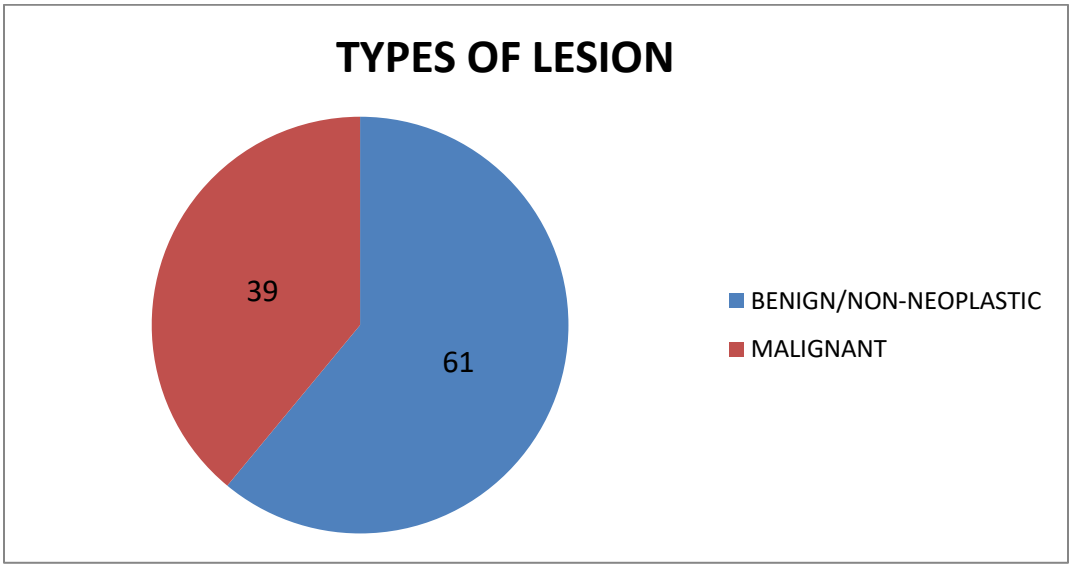

Figure 1: Incidence of benign/non-neoplastic vs malignant lesion. Out of 100 specimens, 61were non neoplastic/benign and 39 were malignant

Non neoplastic/Benign lesion	Number	Percentage
Lymphocytic thyroiditis	39	64
Hashimoto thyroiditis	11	18
Cellular nodule	8	13
Follicular adenoma	3	5
Total	**61**	**100**
Malignant lesion	**Number**	**Percentage**

Papillary thyroid carcinoma	35	89.7
Hurthle cell carcinoma	2	5.1
Medullary carcinoma	1	2.6
Follicular carcinoma	1	2.6
Total	**39**	**100**

Table 1: Distribution of benign/non-neoplastic and malignant lesions

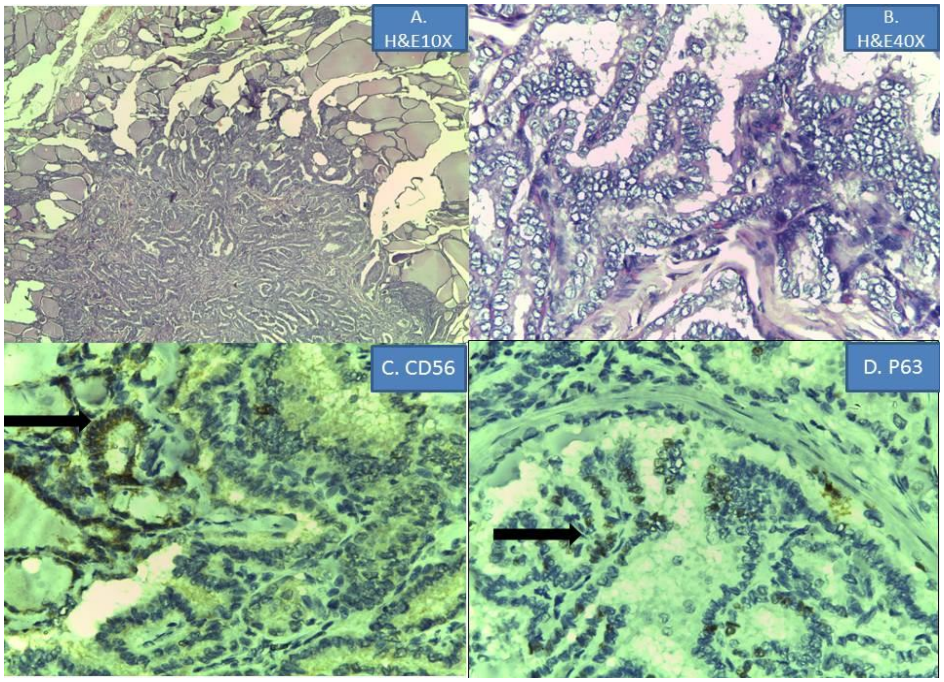

Figure 2: (A, B) PTC at 10x and 40x. (C) CD56 membrane positivity in the normal thyroid follicle cells (arrow). Adjacent focus of PTC shows negative expression. (D) p63 showing nuclear positivity in PTC(arrow). Adjacent normal tissue shows negative expression. (40x)

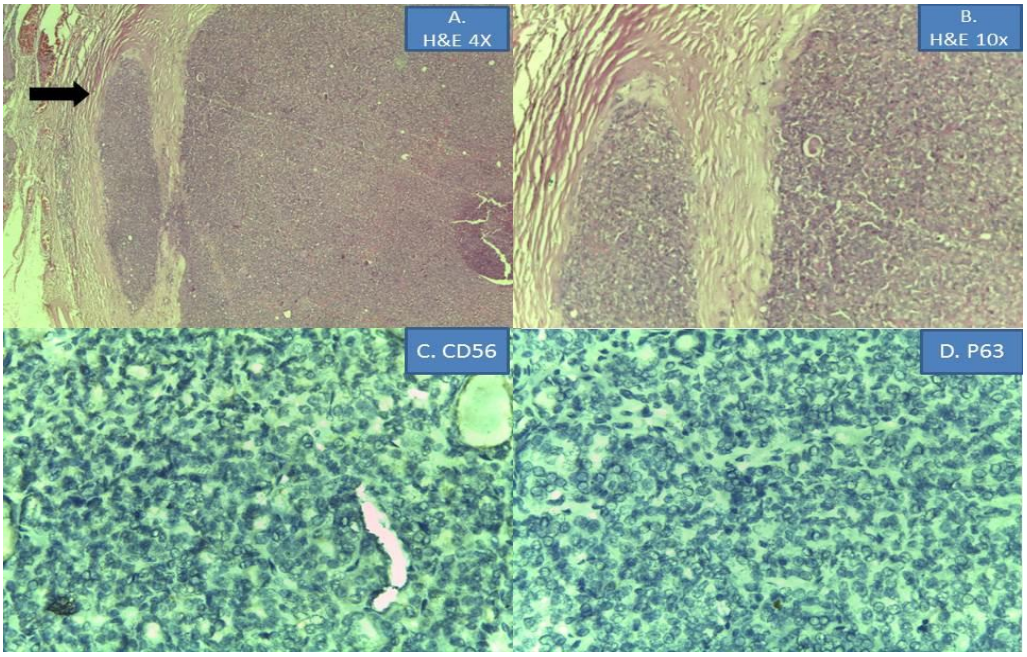

Figure 3: A&B Follicular carcinoma at 4x and 10x; C&D showing absent expression for CD56 and p63 respectively(40x)

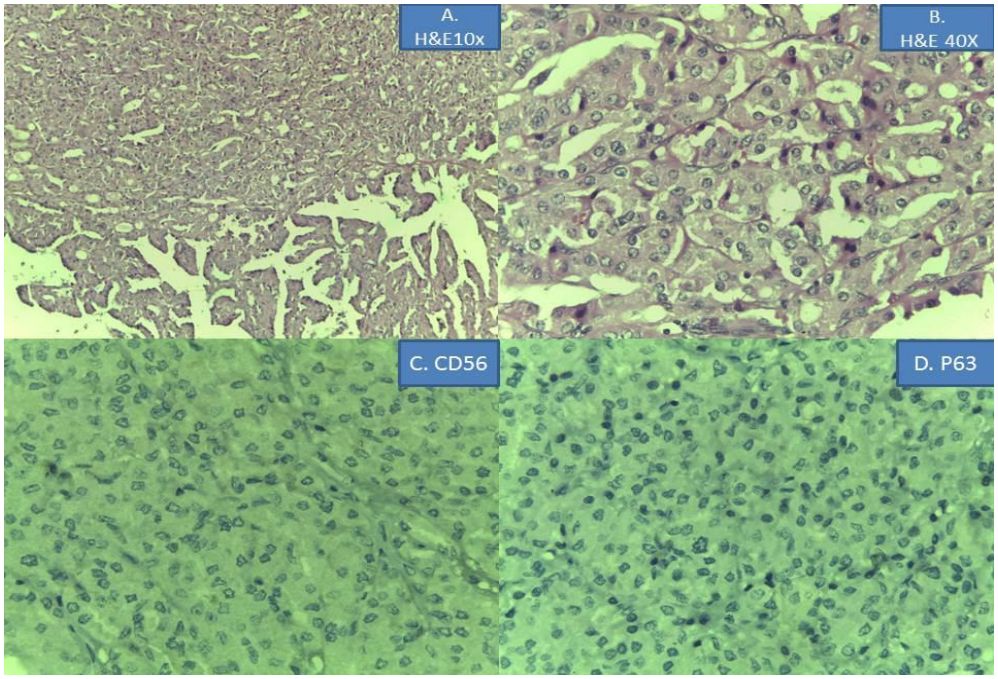

Figure 4: A&B Hurthle cell carcinoma H&E at10x and 40x; C&D showing absent expression for CD56 and p63 respectively (40x)

HP / IHC	PTC	NON PTC	TOTAL
NEGATIVE CD56	**33**	**7**	**40**
POSITIVE CD56	**1**	**56**	**57**
TOTAL	**34**	**63**	**97**

Table 2: Statistical analysis of CD56 expression in PTC and non PTC lesion

Sensitivity : 97.1%
Specificity : 88.9%
Positive predictive value : 82.5%
Negative predictive value :98.2%

HP / IHC	PTC	NON PTC	TOTAL
POSITIVE p63	13	5	18
NEGATIVE p63	22	59	81
TOTAL	35	64	99

Table 3: Statistical analysis of p63expression in PTC and non PTC lesion

Sensitivity :37.1%
Specificity :92.2%
Positive predictive value :72.2%
Negative predictive value :72.8%

HP / **IHC**	**PTC**	**NON PTC**	TOTAL

CD56 –ve p63+ve	**13**	**1**	**14**
CD56 +ve p63--ve	**0**	**51**	**51**
TOTAL	**13**	**52**	**65**

Table 4: Combination of p63 and CD56 in Papillary carcinoma thyroid

For Combined panel of CD56 and p63 study we have excluded 35 cases
Sensitivity :100%
Specificity : 98.1%
Positive predictive value :92.8%
Negative predictive value :100%

DISCUSSION:

Of the total thyroidectomy specimens received in the Dept of Pathology between only 100 specimens satisfying the inclusion and exclusion criteria were included in the present study. The most common lesion in the thyroid gland was colloid goitre, and this was not a differential to PTC. Hence, these were excluded from our study. Next most common lesion observed was lymphocytic thyroiditis which constituted 39% of all cases. This was followed by PTC being 35% of all cases studied. Apart from PTC, there were 2 cases of Hurthle cell carcinoma, and 1 case each of Follicular carcinoma and Medullary carcinoma.

	Total cases of PTC	**Absent CD56 expression**	**Percentage**
Current study	35	33	94.2%
Ceyran A B etal.[6]	101	92	91.1%
Mokhtari M et al.[4]	73	72	98.6%
El Demellawy D et al.[7]	72	72	100%
Shin MK et al.[8]	80	76	95%
Jeong JY et al.[5]	129	69	53.5%
Etem H et al.[9]	40	26	65%

Table5: Percentage of CD56 expression in PTC-a comparison with other studies

Except Etem etal [9] and Jeong JY et al[5]. most of the studies correlate with finding in present study. Jeong et al.,[5] studied 60 PTCs which showed positive membrane staining with CD56, leading to a low sensitivity value. They found a decreased expression of CD56 in follicular carcinomas and a complete absence of expression in anaplastic carcinoma. Our study had one case of follicular carcinoma which showed complete absence of CD56 expression. Etem et al.,[9] studied 40 cases of follicular tumours which included 35 follicuar adenoma , 3 follicular carcinoma and 2 cases of tumours of undetermined malignant potential and observed that 14 cases showed CD56 positivity (35%). Their study also included 40 cases of FVPTCs of which 14 cases were positive for CD56 (35%). One of our cases of PTC was inconclusive for CD56 staining, as even the adjacent normal thyroid tissue (internal control) had not stained positive with CD56. Possibility of over fixation, pH imbalance etc might have led to this result. This case was excluded from statistical analysis. In our study, 13 cases of PTC showed p63 nuclear positivity, mainly at the base of the papillary projections. Preto et al.[10] studied 12 cases of PTC and observed that 33.3% cases of PTC showed p63 nuclear positivity in 5-30% of neoplastic cells at the base of papillae. Our study correlates well with their study.

	Total cases of PTC	Positive p63	Percentage
Current study	35	13	37.1%
Preto et al[10]	12	3	33.3%
El Demellawy D et al[7]	72	50	70%
Etem H et al[9]	40	12	30.5%
Jeong JY et al[5]	129	19	14.7%
Kim YW et al[11]	40	5	12.5%
Burstein DE et al.[12]	27	7	25.9%

Table 6: Percentage of p63 expression in PTC- a comparison with others study

	sensitivity	specificity	Positive predictive value	Negative predictive value
Present study	97.1%	88.9%	82.5%	98.2%
El Demellawy D et al[7]	100 %	100 %	-	-
Mokhtari M et al[4]	98.6%	95.8%	-	-
Jeong JY et al[5]	46.5%	60%	55.6%	51.1%
Shin MK et al[8]	95%	72.73%	92.68%	80%
Ceyran A B et al[6]	91.1	91.7%	85.9%	94.8%

Table 7: Diagnostic value of the CD56 in diagnosis of papillary thyroid carcinoma, a comparative analysis

	Sensitivity	specificity	Positive predictive value	Negative predictive value
Current study	37.1%	92.2%	72.2%	72.7%
Jeong JY et al[5]	14.7%	99.2%	95%	52%

Table 8: Diagnostic value of the p63 in diagnosis of papillary thyroid carcinoma, a comparison with another study by Jeong JY et al

A combined IHC panel of CD56 and p63 gave 100% sensitivity and 98.1% specificity with a positive predictive value of 92.8% and negative predictive value of 100%. For combined panel estimation we have excluded 35 cases, both benign and malignant. These cases, showed aberrant expression with the markers. The reasons could be technical factors as have been discussed earlier.

CONCLUSION AND LIMITATION:

Significance of this study lies in the fact that CD56 is a sensitive and specific immune marker for differentiating PTC from its mimickers, thus assisting in picking up micro carcinomas and multi focal lesions. Even though p63 is not sensitive in the diagnosis of PTC, when applied alongside CD56, gives 100% sensitivity and 98% specificity. This is also the limitation of our study that the number of cases was small and quite a few cases had to be excluded from analysis. We propose to continue with the study increasing the number of specimens thus reducing aberrant results.

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Ethical clearance – Institutional Ethics Committee
Conflict of interest - None declared
Funding - None

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