Original Article
DOI: https://doi.org/10.70749/ijbr.v3i5.2974

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Diagnostic Utility of p63, Ck5/6 and ER in Papillary Neoplasms of Breast

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ARTICLE INFO

Keywords: Papilloma, Myoepithelial, Encapsulated Papillary Carcinoma.

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Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 01-03-2025 Revised: 07-05-2025
Accepted: 20-05-2025 Published: 30-05-2025

ABSTRACT

Background: Papillary neoplasms of breast consist of broad spectrum of lesions ranging from intraductal papilloma to papilloma with ADH/DCIS, papillary DCIS, encapsulated papillary carcinoma, solid papillary carcinoma(insitu/invasive) and invasive papillary carcinoma. It is very challenging to classify these lesions as benign or malignant as they have similar papillary architecture. The classification as benign or malignant depends on the presence or absence of myoepithelial layer and epithelial atypia. Various myoepithelial and epithelial markers are used for this purpose. However, certain markers are more specific and helpful than others. In this study, we are evaluating the diagnostic utility of myoepithelial markers (p63 and CK5/6) and epithelial marker (ER) in the various papillary lesions, so the timely diagnosis and early management can be done in better way. **Material and Methods:** In this study, we determine the expression of myoepithelial immunohistochemical markers (p63 and ck5/6) and epithelial marker (ER) in benign and malignant papillary lesions of breast (201 cases) constituting of intraductal papilloma (142 cases), papillary DCIS (5 cases), encapsulated papillary carcinoma (15 cases), solid papillary carcinoma in situ (6 cases), invasive solid papillary carcinoma (15 cases) and invasive papillary carcinoma (15 cases). **Results:** Ck5/6 shows 99%, p63 shows 98.51% and ER shows 99% diagnostic accuracy in all the cases of intraductal papillomas. P63 shows 100% diagnostic accuracy in invasive solid papillary carcinoma while in other atypical and malignant lesions it shows 99.50% diagnostic accuracy. Ck5/6 shows 100% diagnostic accuracy in papillary DCIS, invasive solid and invasive papillary carcinoma, 99.50% in encapsulated papillary carcinoma and 99% in solid papillary carcinoma insitu. While ER has 100% diagnostic accuracy in papillary DCIS and solid carcinoma in situ, 99.50% in encapsulated papillary carcinoma, 99% in invasive papillary carcinoma and 94.4% in invasive solid papillary carcinoma. **Conclusion:** Combined panel of myoepithelial and epithelial immunohistochemical markers is required to differentiate various benign and malignant papillary lesions with diagnostic accuracy, timely diagnosis and early treatment.

INTRODUCTION

Papillary lesions of breast are heterogenous group of lesions primarily composed of papillary architecture with true fibrovascular cores. The categorization into benign, atypical or malignant depends on the distribution of myoepithelial cells at the periphery and within the fibrovascular core. In the same way the nature of epithelium determines the benign or malignant potential of the papillary lesion[1]. It is very challenging to diagnose these lesions on microscopy alone. So, the panel of myoepithelial and epithelial markers are applied for confirmation[2]. In this study we are evaluating the efficacy of myoepithelial marker(p63, CK5/6) and epithelial marker(ER)[3].

All the benign lesions of breast including intraductal papilloma and atypical lesion including papilloma with ADH/DCIS are surrounded by myoepithelial layer at periphery and along the papillae and insitu lesions including papillary DCIS have intact peripheral myoepithelial layer and absence of myoepithelial cells along the papillae. Malignant papillary lesions including encapsulated papillary carcinoma, solid papillary carcinoma(invasive) and invasive papillary carcinoma have absented myoepithelial layer. Solid papillary carcinoma insitu lesions may have completely absent or sometimes attenuated myoepithelial layer. However, distinction among these various lesions can be made on the nature of epithelium on morphology and immunohistochemistry[4]. There are various myoepithelial markers such as p63, SMA, calponin and CK5/6. They have intact expression in benign lesions. Insitu lesions can show intact or sometimes patchy positivity of myoepithelial

markers. Similarly, epithelial markers such as ER shows mosaic pattern in benign lesions while show diffuse positivity in insitu and malignant lesions. The aim of this study is to determine the expression of these myoepithelial and epithelial markers in differentiating the various benign papillary lesions from atypical and malignant papillary neoplasms to overcome the diagnostic challenge and accurate diagnosis as they have different treatment modalities[5].

MATERIAL AND METHODS

This is a prospective study carried out in Chughtai Institute of Pathology. Duration of the study ranges from 26th Feb,2024 to 25th Feb,2025. This study is carried out after the institutional review board approval of Chughtai Institute of Pathology.

A total of 201 cases of papillary lesions of breast are included in this study. Out of which 142 cases are benign, all of them are intraductal papillomas. 59 cases are malignant, comprising of papillary DCIS(5 cases), encapsulated papillary carcinoma(15 cases), solid papillary carcinoma in situ(6 cases), invasive solid papillary carcinoma(15 cases) and invasive papillary carcinoma(15 cases).

Two myoepthelial immunohistochemical markers p63, ck5/6 and one epithelial marker ER is applied on all the cases and there expression is noted.

For immunohistochemistry, we use autostainer link 48(Dako) by using monoclonal rabbit anti-human estrogen receptor α clone EP1, monoclonal anti-human p63 protein clone DAK-p63 and monoclonal mouse anti-human cytokeratin5/6 clone D516 B4.

Inclusion Criteria

We have included all the cases of papillary neoplasms of the breast irrespective of the age or the nature of the biopsy.

Exclusion Criteria

All the benign and malignant lesions of breast other than papillary lesions are excluded from the study.

RESULTS

This study comprises of all the benign, atypical and malignant papillary lesions of breast irrespective of age and nature of biopsy. Total of 201 cases of papillary lesions are taken. Out of which 142 are benign and 59 are malignant. Expression of both myoepithelial and epithelial markers is studied in these cases(shown in table 1).

Table 1

Expression of immunohistochemical markers(p63, ck5/6 and ER).

	Total no of cases	P63	Ck5/6	ER
Intraductal papilloma	142(70.6%)	Positive(within and at periphery of papillae) in 139(97.8%)	Positive in 140(98%)	Positive in 141(99 %)
Papillary DCIS	5(2.5%)	Positive(at periphery of papillae) in 4(80%)	Positive in 5(100%)	Positive in 5(100%)
Encapsulated papillary carcinoma	15(7.5%)	Negative (within and at periphery of papillae) in 14(93.3%)	Negative in 14(93.3%)	Positive in 14(93.3 %)
Solid papillary carcinoma in situ	6(3%)	Negative(within and periphery of papillae) in 5(83.3%)	Negative in 5(83.3%)	Positive in 6(100%)
Invasive solid papillary carcinoma	18(8.9%)	Negative(within and at periphery of papillae) in 18(100%)	Negative in 18(100%)	Positive in 16(88.8 %)
Invasive papillary carcinoma	15(7.5%)	Negative(within and at periphery of papillae) in 14(93.3%)	Negative in 15(100%)	Positive in 13(86.6 %)
Total	201			

All the benign cases are intraductal papillomas. P63 is positive in 97.8% cases. The p63 has characteristeric expression in intraductal papillomas. It is expressed within papillae and at the periphery of lesion(figure 1). While in 2.2% it is either focal positive or negative. The results show 97.93% sensitivity, 100% specificity with diagnostic accuracy of 98.51% and statistical significance($p<0.01$).

We have studied 5 cases of papillary DCIS. Papillary DCIS is atypical lesion with positivity of p63 at periphery of lesion and absence within papillae(figure 2). The results show positive p63 expression in 80% cases while 20% cases show patchy p63 positivity. The results are 83.33% sensitive, 100% sensitive with diagnostic accuracy of 99.50%.

The study includes 15 cases of encapsulated papillary carcinoma. P63 is absent within papillae and at periphery of lesion. In our study, 93.3% cases are negative while 6.7% cases are patchy positive. The results have 93.75% sensitivity, 100% specificity, 99.50% diagnostic accuracy and significant p-value(0.0001).

There are 24 cases of solid papillary carcinoma. Out of which 6 are insitu lesions and 18 are invasive. P63 is negative in both of these lesions. The results show 100% sensitivity and specificity in invasive lesions while 85.71% sensitivity, 100% specificity in insitu lesions.

We have studied 15 cases of invasive papillary carcinoma. P63 shows negative expression in these lesions. The results are 93.75% sensitive and 100% specific.

The sensitivity, specificity, diagnostic accuracy and p-value of p63 in all these lesions in shown in table 2.

Table 2

Statistical values of p63 in papillary lesions.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Accuracy	P-value
Intraductal papilloma	97.93%	100%	100%	94.92%	98.51%	0.0001
Papillary DCIS	83.33%	100%	100%	99.49%	99.50%	
Encapsulated papillary carcinoma	93.75%	100%	100%	99.46%	99.50%	
Solid papillary carcinoma insitu	85.71	100%	100%	99.49%	99.50%	
Invasive solid papillary carcinoma	100%	100%	100%	100%	100%	
Invasive papillary carcinoma	93.75%	100%	100%	99.46%	99.50%	

Similarly, ck5/6 has 100% specificity in all lesions while 99.46% specificity in encapsulated papillary carcinoma. It shows 98.61% sensitivity in intraductal papillomas, 75% sensitivity in solid papillary carcinoma insitu and 100% sensitivity in all other lesions. The statistical significance of ck5/6 in all these lesions is 0.0001. The statistical values are shown in table 3.

Table 3

Statistical values of Ck5/6 in papillary lesions.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Accuracy	p-value
Intraductal papilloma	98.61%	100%	100%	96.61%	99%	0.0001
Papillary DCIS	100%	100%	100%	100%	100%	
Encapsulated papillary carcinoma	100%	99.46%	93.75%	100%	99.50%	
Solid papillary carcinoma insitu	75%	100%	100%	98.97%	99%	
Invasive solid papillary carcinoma	100%	100%	100%	100%	100%	
Invasive papillary carcinoma	100%	100%	100%	100%	100%	

ER has shown 100% specificity in all lesions. The sensitivity is 100% in papillary DCIS and solid papillary carcinoma insitu, 93.75% in encapsulated papillary carcinoma, 94.12% in invasive solid papillary carcinoma 88.24% in invasive papillary carcinoma. The p-value is 0.0001. The statistical values are shown in table 4.

Table 4

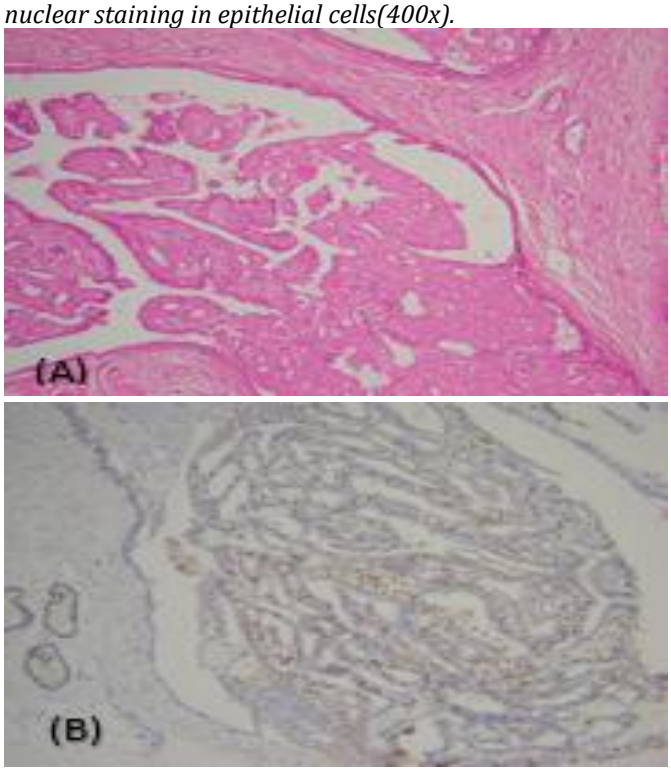
Statistical values of ER in papillary lesions.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic accuracy	P-value
Intraductal papilloma	98.61%	100%	100%	96.61%	99%	
Papillary DCIS	100%	100%	100%	100%	100%	
Encapsulated papillary carcinoma	93.75%	100%	100%	99.46%	99.50%	0.0001
Solid papillary carcinoma insitu	100%	100%	100%	100%	100%	
Invasive solid papillary carcinoma	94.12%	100%	100%	98.73%	94.44%	
Invasive papillary carcinoma	88.24%	100%	100%	98.89%	99%	

The results show that p63 and ER has 100% specificity in all lesions while ck5/6 is more sensitive. However, ER is more sensitive immunohistochemical marker for diagnosing of malignant papillary lesions.

Figure (1)

(A) H&E of intraductal papilloma(100x). (B) p63 IHC showing nuclear positivity in myoepithelial cells within papillae and at the periphery(100x). (C) ck5/6 IHC showing cytoplasmic expression in myoepithelial cells within papillae and at periphery(100x). (D) ER showing mosaic pattern of nuclear staining in epithelial cells(400x).



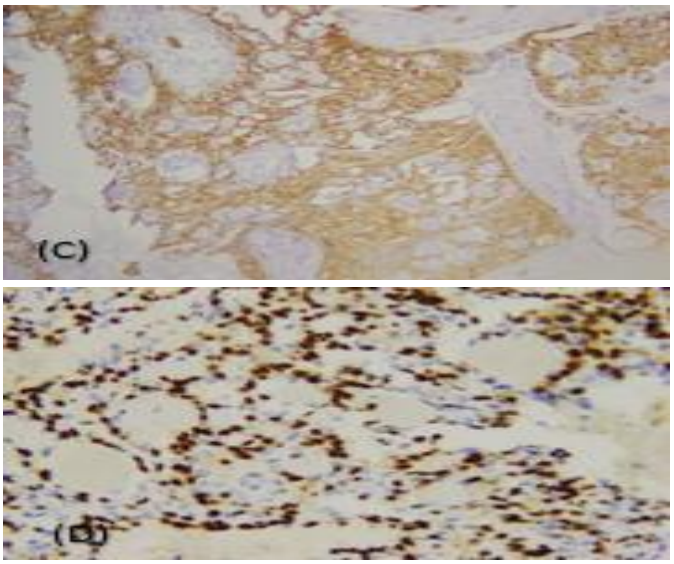
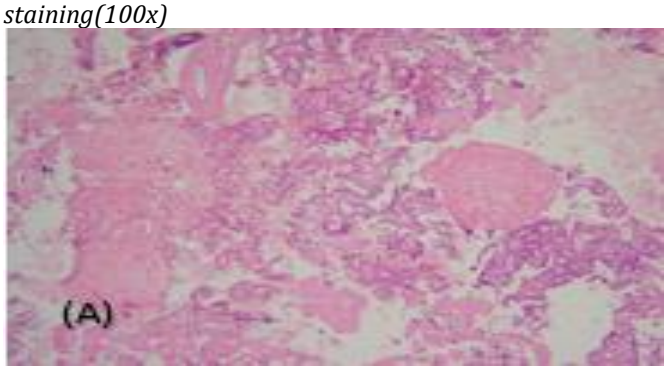


Figure (2)

(A) H&E of papillary DCIS(100x). (B) Image showing focal p63 staining at periphery and negative staining within papillae(400x). (C) Image showing ck5/6 absence within papillae(100x). (D) ER showing diffuse strong nuclear staining(100x)



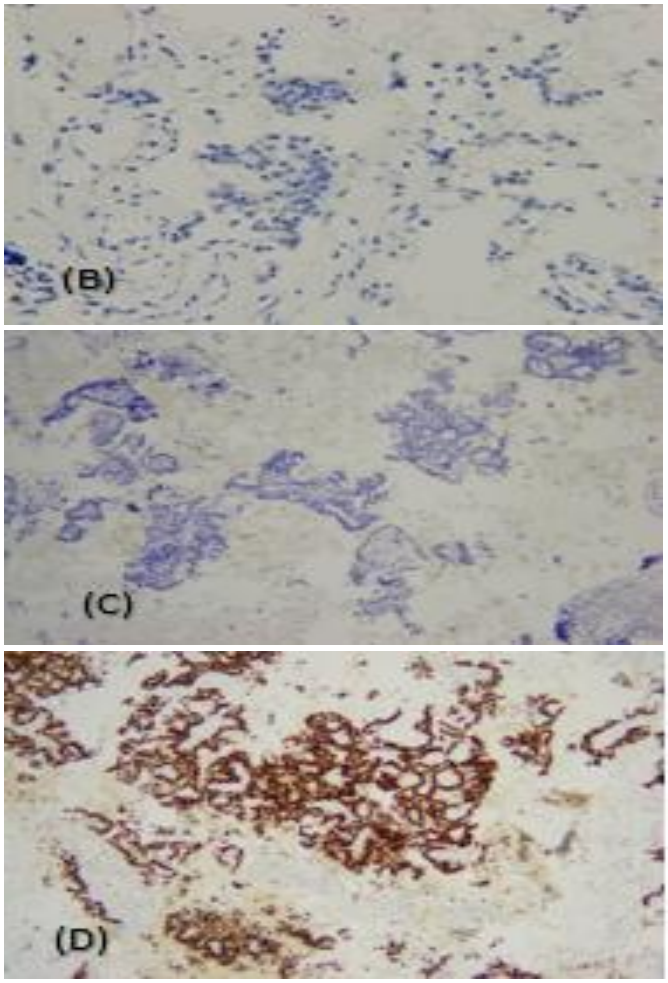
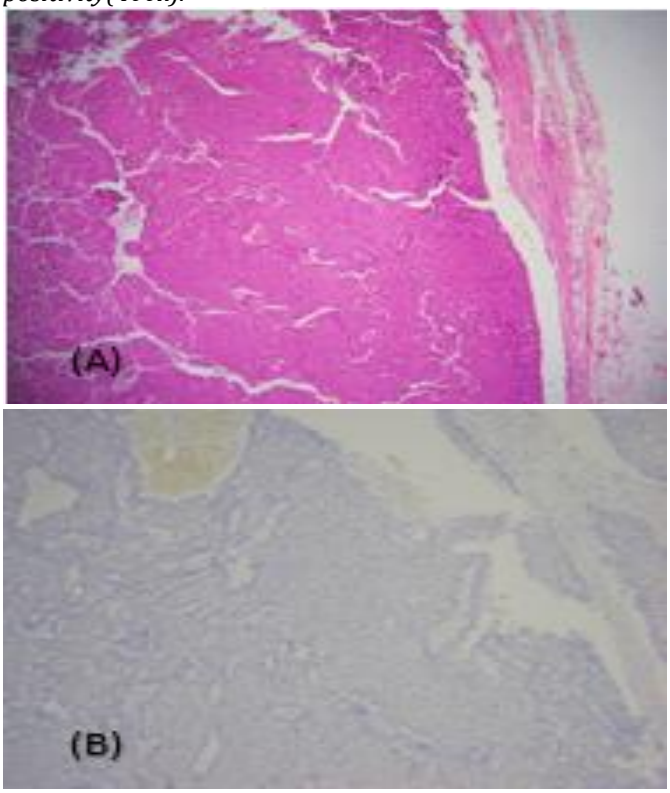


Figure (3)
(A) H&E of encapsulated papillary carcinoma(100x). (B) Negative expression of p63(100x). (C) Negative expression of ck5/6(100x). (D) ER is showing diffuse nuclear positivity(400x).



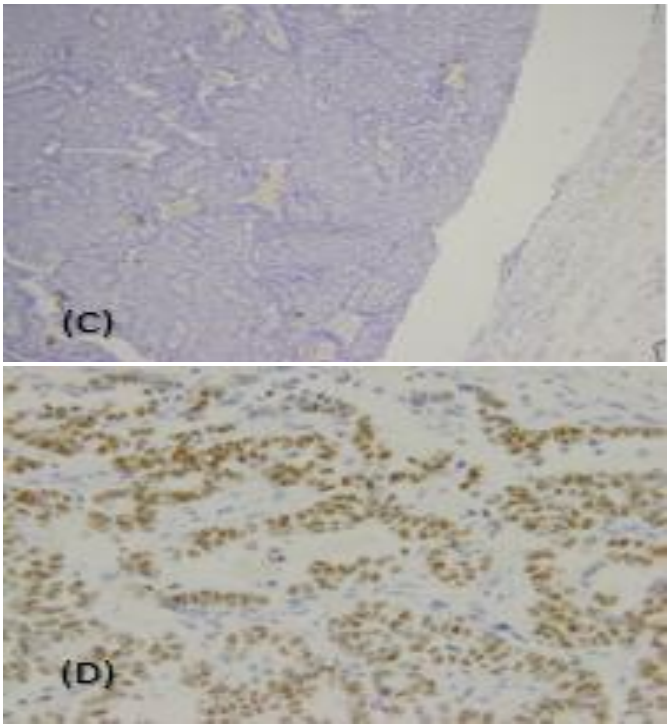
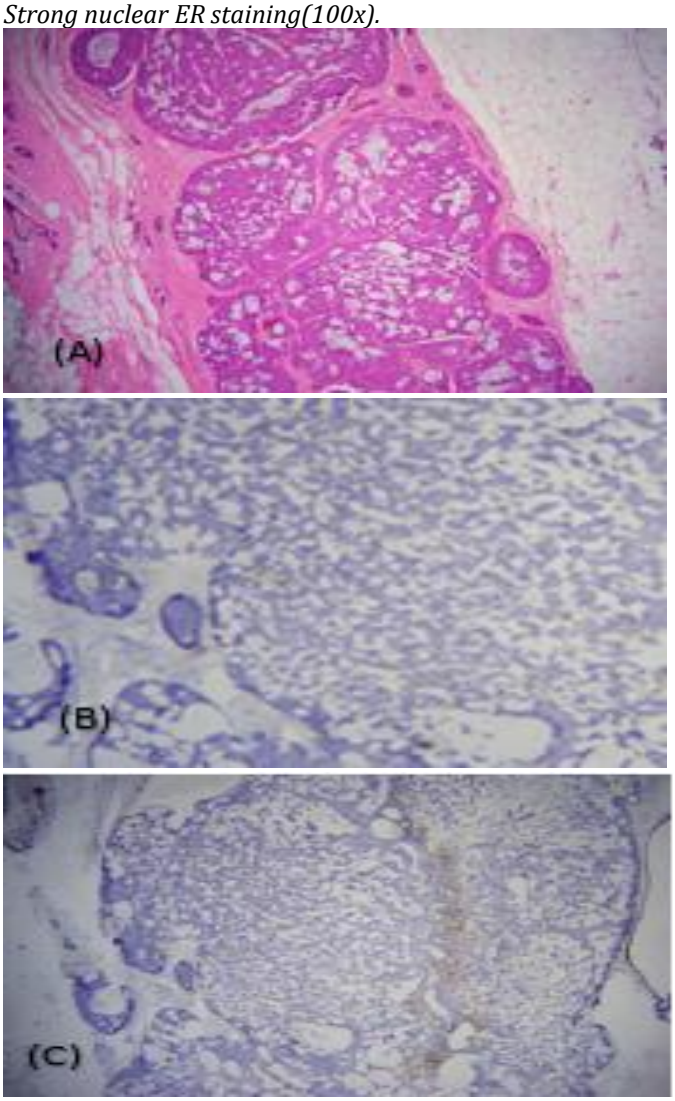


Figure (4)
(A) H&E of solid papillary carcinoma insitu(100x). (B) Negative p63 IHC(100x). (C) Negative ck5/6 IHC(100x). (D) Strong nuclear ER staining(100x).



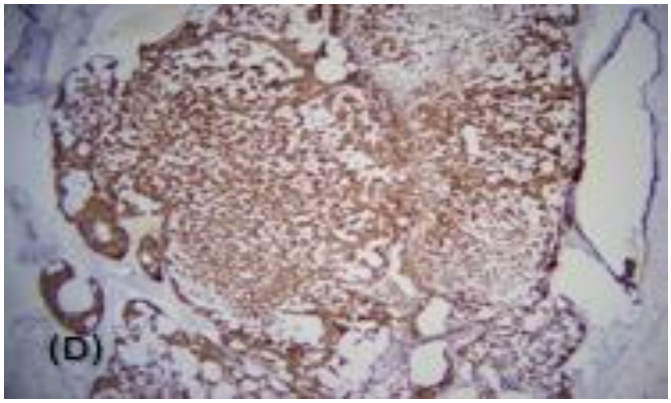
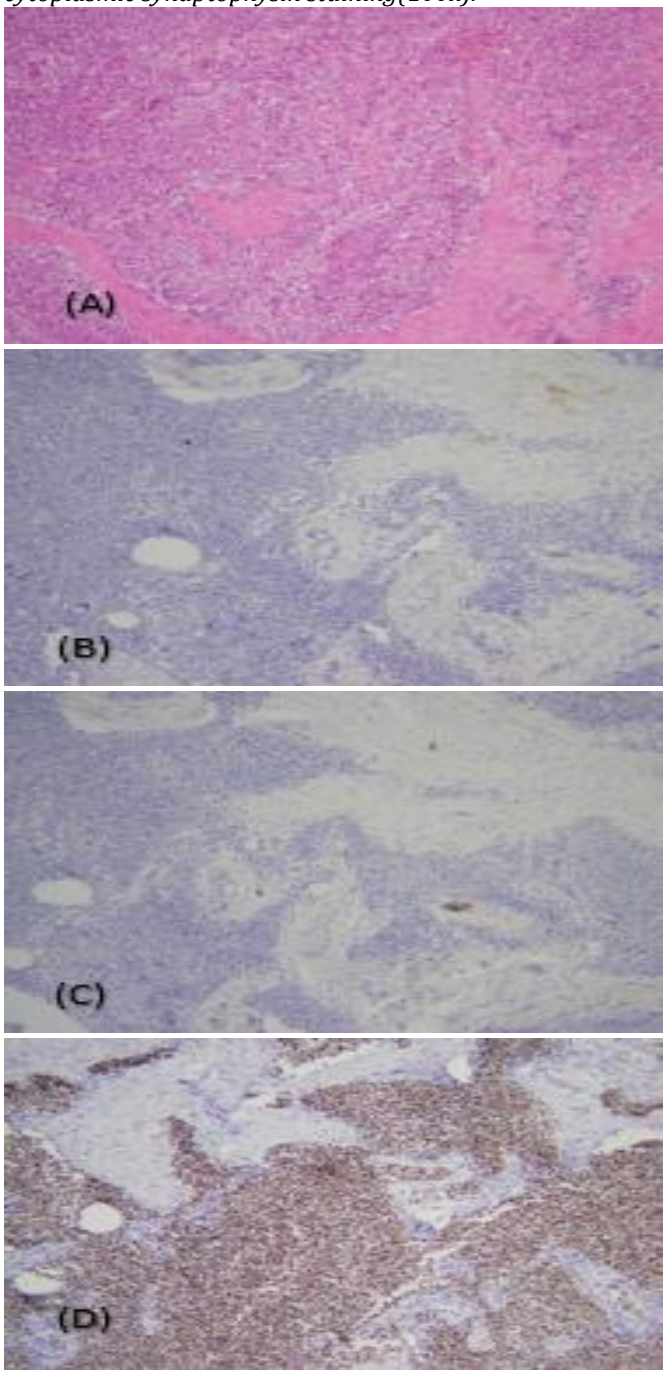


Figure (5)
(A) H&E of invasive solid papillary carcinoma(100x). (B) Absent p63 staining(100x). (C) Absent ck5/6 staining(100x). (D) Diffuse nuclear ER staining(100x). (E) Positive cytoplasmic synaptophysin staining(100x).



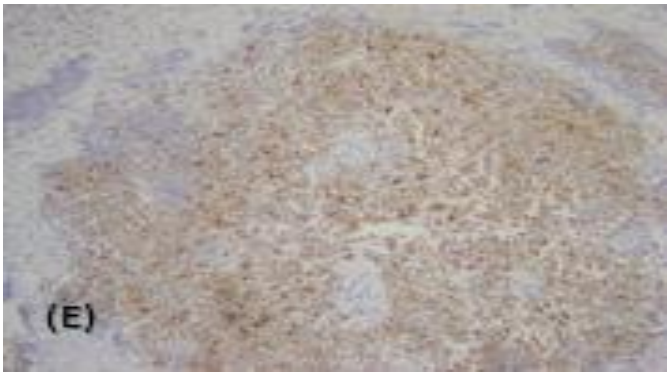
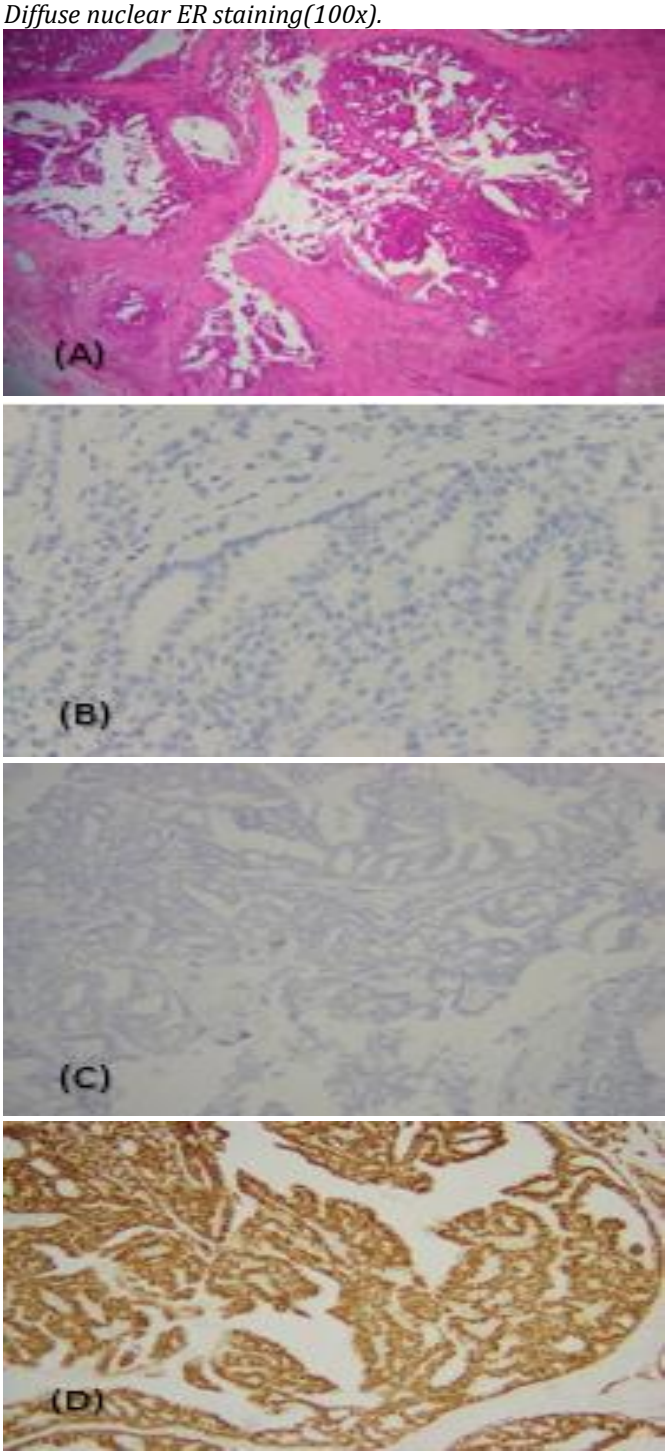


Figure (6)
(A) H&E of invasive papillary carcinoma(40x). (B) Negative p63 staining(400x). (C) Negative ck5/6 staining(100x). (D) Diffuse nuclear ER staining(100x).



DISCUSSION

Papillary neoplasms comprises of various benign and malignant lesions. The one thing common in all these lesions is papillae formation. Papillae comprises of true fibrovascular cores. The difference occurs in the presence or absence of myoepithelial cells within the papillae and at the periphery of lesion and the nature of epithelial cells in the lesion. It becomes very challenging to diagnose these lesions on morphology alone due to overlapping features[6]. There are various myopeithelial markers such as p63, ck5/6, calponin, SMA and CD 10 and epithelial markers such as ER, CK7, CK8, CK14 and CK18. In our study we are evaluating the use of p63, ck5/6 and ER[7]. Our study comprises of all the papillary lesions that are included in WHO 5th edition[8]. They are intraductal papillomas, papillary DCIS, encapsulated papillary carcinoma, insitu and invasive solid papillary carcinoma and invasive papillary[9]. However, no case of intraductal papilloma with ADH/DCIS is included in this study.

P63 is a nuclear marker that highlights benign myoepithelial cells in benign lesions and absent or reduced in invasive lesions[10]. It is superior to cytoplasmic markers such as SMA, CD10 because it doesnot cross react with stroma and show intact myoepithelial stain. Intraductal papillomas show p63 staining within papillae and at the periphery. Papillary DCIS has intact peripheral p63 staining while absence of staining within papillae. Similary, insitu solid papillary carcinoma have absent or reduced p63 expression. While, all other malignant papillary lesions show negative staining[11].

Ck5/6 is a basal/ myoepithethelial marker that highlights intact myoepithelial cell layer in benign lesions and is absent in malignant lesions. It also shows heterogenous staining pattern in hyperplastic cells in case of hyperplasia in benign ductal papillomas[12]. So, the combination of both p63 and ck5/6 markers is more effective in differentiating benign vs malignant lesions. Similarly, ck5/6 is more effectively used in core biopsies[13].

ER is a nuclear epithelial marker that shows heterogenous expression in benign lesions while in papillary DCIS and other malignant lesions with monotonous cell population, it shows strong and diffuse staining pattern.

In our study, we have studied 201 cases of papillary lesions and we have applied p63, ck5/6 and ER on all the cases and have noted there expression in all the cases. The study shows 100% specificity of p63 and ck5/6 in benign lesions and 97.93% and 98.61% sensitivity, respectively with statistical significance of 0.0001. ER shows 100% specificity in benign and malignant lesions with sensitivity of 98.61% in benign lesions , 100% in papillary DCIS, solid papillary carcinoma insitu and 93.75%, 94.12% and 88.24% in encapsulated papillary carcinoma, invasive solid papillary carcinoma and invasive papillary carcinoma respectively. Ck5/6 has more sensitivity in malignant lesions than p63.

So, the results are consistent with the previous studies that p63 and ck5/6 have high sensitivity and specificity[14]. They are of great significance in differentiating benign vs invasive lesions. Similarly, the expression of ER is very helpful to determine the nature of epithelial cells.

Benign and atypical papillomas(showing clonal proliferation) have activating PIK3CA mutation with 5 to 7.5 fold increase in risk of malignancy associated with atypia. Other insitu and invasive malignancies also show mutations including PIK3CA, LOH on16q23 and increase in copy number alterations(CNA)[15].

Diagnosing the papillary lesions on morphology alone is very challenging due to the overlapping features[16]. So the IHC is required for definitive diagnosis and to prevent overtreatment[17]. Benign, insitu and low grade lesion undergo complete excision while invasive and high grade lesions are treated aggressively.

CONCLUSION

The combination of myoepithelial and epithelial markers is used in differentiating benign, atypical and malignant papillary lesions because of their complex architecture. P63, ck5/6 and ER are more sensitive and specific than other myoepithelial and epithelial markers. Papillary lesions of breast have a spectrum of lesions. As these lesions have different interventional modalities, the combination of clinicoradiological findings, along with morphology and immunohistochemical markers is required for definitive diagnosis. So, the early management can be done effectively.

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