

Original research article

Expression of p63 in Breast Lesions – A Cross Sectional Study

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Abstract

Background: Breast lesions are not a single entity rather they represent heterogenous group of disease with marked clinic morphological diversity, varied disease aggressiveness and response to treatment. Myoepithelial markers help to distinguish invasive carcinomas from benign neoplasms with similar morphological appearance. In breast, p63 antibody is a myoepithelial marker that exclusively stains the nuclei of myoepithelial cells and is not expressed in vascular smooth muscles, stromal myofibroblasts and in surrounding adipocytes. This makes p63 more specific and superior to other myoepithelial markers.

Materials and Methods: The present study was done on total of 53 breast specimens received in the Department of Pathology. All specimens were routinely processed and stained with H & E and then subjected to p63immunohistochemical staining using normal breast tissue as positive control.

Results: Out of 53 cases, 30 cases were benign, 05 cases were premalignant and 18 cases were malignant. All malignant cases were negative for p63 expression, all benign and premalignant cases were positive for p63 with varied pattern of expression. Benign non proliferative lesions were continuous positive (score-3), proliferative lesions showed less continuous positive (score-2) and premalignant lesions like ductal carcinoma in situ were discontinuously positive (score-1) with well-defined two or more nuclear spaces between two positive p63 nuclei.

Conclusion: There is considerable interobserver disagreement in the interpretation of difficult breast lesions based on histopathology alone. Myoepithelial markers like p63 are useful in helping to distinguish invasive carcinomas from benign proliferative lesions and most premalignant lesions with an intact myoepithelium.

Keywords: Myoepithelial marker p63, Non-proliferative lesions, Proliferative lesions, Breast carcinoma.

Introduction

Breast lesions represent heterogenous group of diseases with marked clinical and morphological diversity.¹ They are the most commonly encountered lesions in women that require prompt histopathological diagnosis and immunohistochemical analysis (IHC).² Breast cancer accounts for 22% of all female cancers worldwide and is the most common cancer in women.^{2,3} Ductal carcinomas considered to be derived from cells of the terminal ductal lobular units are reported to be the most common among all tumors.⁴

The glandular tree of the breast is invested by a peripheral layer of myoepithelial cells (MCs) which lie between the luminal epithelial cells and the identifiable on routine hematoxylin and eosin (H&E) stained sections, immunohistochemical methods have been used to highlight the presence of intact MEC layer.^{5,6} The precise identification of myoepithelial cells is of diagnostic clue to differentiate benign lesions, benign proliferative lesions with similar morphological appearance, in situ neoplasms from invasive carcinoma of breast.⁷

Myoepithelial marker p63, a member of p53 gene family is expressed in the nuclei of myoepithelial cells of normal breast.⁶ It is also expressed in epithelial cells of stratified epithelia such as skin, esophagus, ectocervix, transitional epithelia of bladder, basal cells of glandular structures of the prostate, salivary glands and in bronchi.^{5,6} p63 is more sensitive marker as it stains exclusively nuclei in the myoepithelial cells of the breast and does not cross react with stromal myofibroblasts, vascular smooth muscles and adipose tissue like other myoepithelial cell markers like smooth muscle actin (SMA), calponin, caldesmon, smooth muscle myosin heavy chain (SMMHC), cytokeratins 5/6 and CD 10.^{7,8} This makes p63 more sensitive and superior over other myoepithelial markers and can be included in IHC panels to identify myoepithelial cells in problematic breast lesions.⁹

In normal breast, p63 is demonstrated as continuous intense staining pattern.¹⁰ In the benign non-proliferative lesions it is continuously positive, in proliferative lesions it is discontinuously positive and in situ lesions show focal positivity.^{11,12} Invasive carcinomas lack the myoepithelial cell layer and hence negative for p63 staining.^{13,14} Thus p63 expression is of diagnostic clue to differentiate benign lesions, benign proliferative lesions with similar morphological appearance, in situ neoplasms and invasive carcinoma of the breast.^{15,16}

Materials and Methods:

The present study was done on total of 53 cases of breast specimens received in the Histopathology unit, Department of Pathology, Belgaum Institute of Medical Sciences and Hospital, irrespective of age and gender during the period from November 2019 to May 2021. Clinical history and examination findings of the patients were collected in all the cases. All specimens were routinely processed and stained with hematoxylin and eosin (H&E) and detailed histopathological examination (HPE) was done. Then unstained sections subjected to p63 antibody staining using Standard non-biotin polymerized horse radish peroxidase (HRP) technique to localise p63 antigen using normal breast tissue as positive control.

Inclusion criteria:

- Patients of all age and both gender will be included in the study.
- Core needle biopsy, trucut biopsy of breast, lumpectomy and mastectomy specimens.

Immunohistochemistry(ihc) analysis/ scoring for breast lesions:¹⁰

p63 expression was evaluated as continuous positive/ less continuous positive/ discontinuous positive/ Negative and scoring is done with reference to verma et.al.

RESULTS:

Among total 53 cases in our study, 30 cases were benign which included 17 cases fibroadenoma (Figure 1A), 6 fibrocystic disease, 3 usual ductal hyperplasia, 2 benign phyllodes, 1 tubular adenoma and 1 benign papilloma. Premalignant lesions include 05 cases of ductal carcinoma in situ (DCIS) and malignant cases include 15 cases of Infiltrating ductal carcinoma not otherwise specified (NOS) (Figure 1B) and 03 cases of papillary carcinoma (Table-1).

TABLE 1 – Distribution of different breast lesions

DIAGNOSIS	No of cases	Percentage (%)
BENIGN		
Fibroadenoma	17	32.07%
Fibrocystic disease	06	11.32%
Benign phyllodes tumor	02	3.77%
Tubular adenoma	01	1.88%
Usual ductal hyperplasia	03	5.66%
Benign papilloma	01	1.88%
PREMALIGNANT		
Ductal carcinoma insitu	05	9.44%
MALIGNANT		
Infiltrating ductal carcinoma, not otherwise specified	15	28.30%
Papillary carcinoma	03	5.66%
TOTAL	53	100%

Age & Size wise distribution

The age of patients ranged from 14 to 75 years and majority of cases 21 (39.62%) were between age group 31-50 years.

In our present study majority, 38 cases (71.70%) were of size 2-5 cm. The mean size of the benign tumor was 3.5 cm and malignant tumor was 7.5 cm.(Table-2).

TABLE 2 - Distribution of cases on the basis of size of lump (n=53)

Size (cm)	Benign		Malignant		Total	
	No	%	No	%	No	%
< 2cm	01	1.89%	-	-	01	1.89%
2-5 cm	30	56.60%	08	15.09%	38	71.69 %

> 5 cm	02	3.77%	12	22.65%	14	26.42%
Total	33	62.26%	20	37.74%	53	100%

p63 expression

Among total of 53 cases, 30 cases (56.60%) were benign lesions and all were positive for p63 expression. 05 cases were premalignant and were least positive for p63 expression. All malignant cases 18 cases (33.96%) were negative for p63 expression. Among the benign cases, fibroadenoma was the most common and showed continuous p63 expression with score 3 (Figure-2A), Benign papilloma also showed continuous p63 expression with score 3. Other benign lesions like fibrocystic disease, Usual ductal hyperplasia and tubular adenoma (Figure-2B) showed less continuous positive with score 2. Premalignant lesions like DCIS showed least positivity with score 1 (Figure-3) and all malignant lesions, invasive ductal carcinoma (Figure-4) and papillary carcinoma were negative for p63 expression. (Table-3)

TABLE 3 – p63 scoring in different spectrum of Breast lesions

Type of breast lesions	No of cases	p63 scoring			
		Score-0	Score-1	Score-2	Score-3
BENIGN					
Fibroadenoma	17				✓
Fibrocystic disease	06			✓	
Benign phyllodes tumor	02		✓		
Tubular adenoma	01			✓	
Usual ductal hyperplasia	03			✓	
Benign papilloma	01				✓
PREMALIGNANT					
Ductal carcinoma in situ	05		✓		
MALIGNANT					
Invasive ductal carcinoma, not otherwise specified	15	✓			
Papillary carcinoma of breast	03	✓			
TOTAL	53				

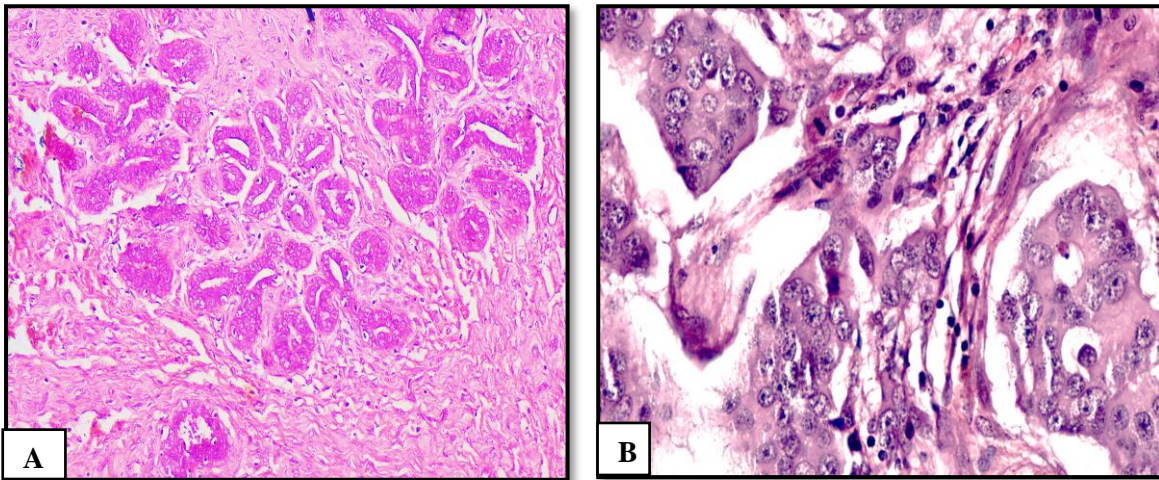


Figure-1: Microphotograph of : A) Fibroadenoma (H&E, 200x); B) Invasive papillary carcinoma (H&E, 400x)

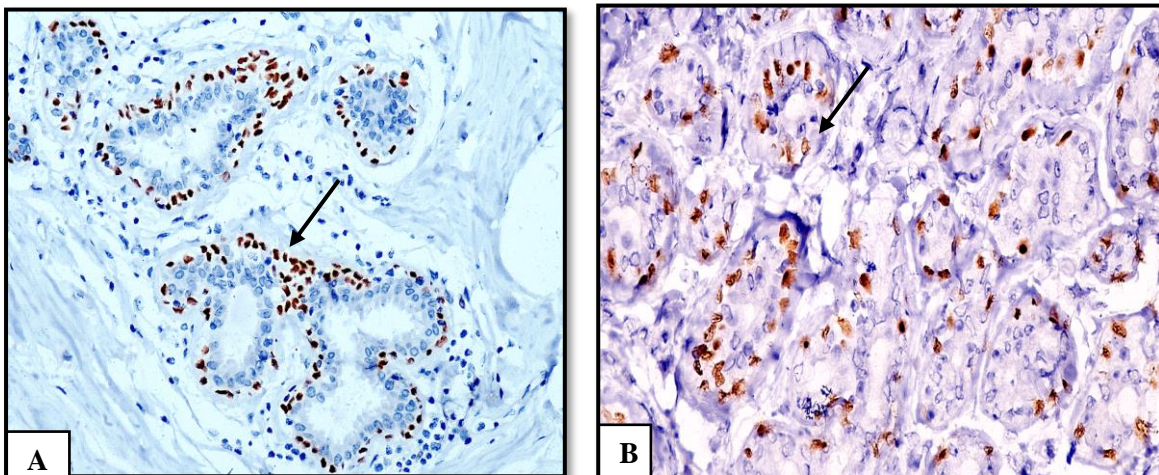


Figure-2: IHC: A- p63 expression in Fibroadenoma (Score-3) (200x)

B- p63 expression in Tubular adenoma (Score-2) (400x)

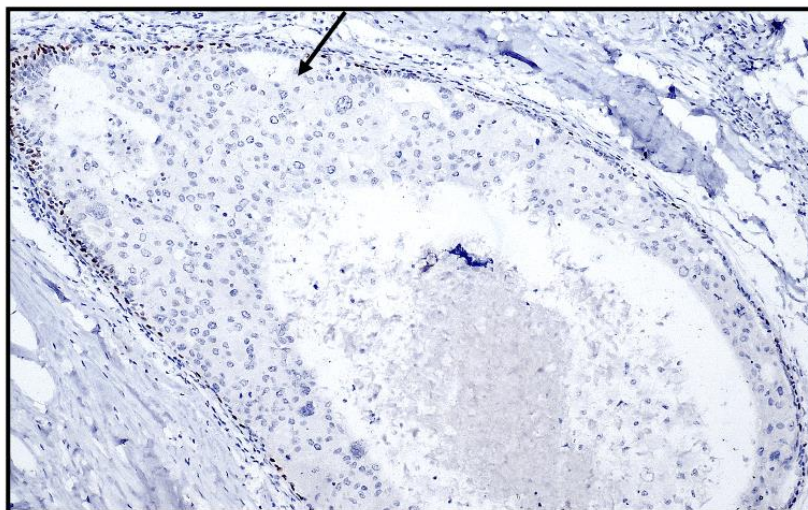


Figure-3: IHC - p63 expression in Ductal carcinoma in situ (Score – 1) (200x)

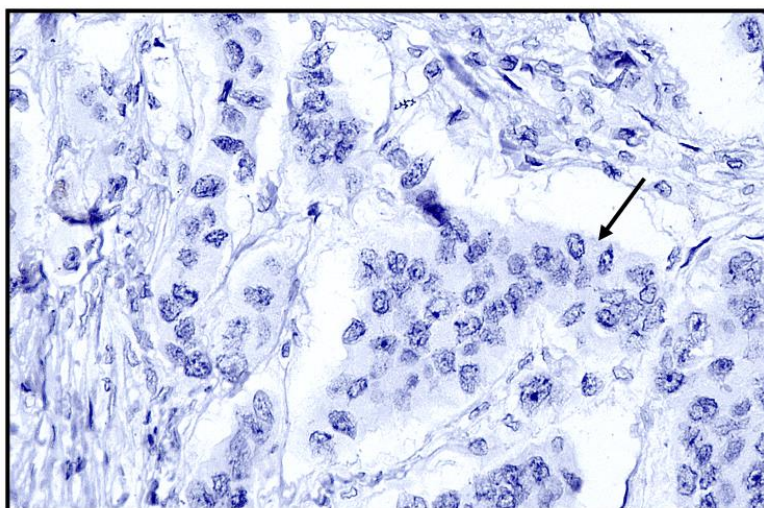


Figure-4: IHC - p63 expression in Invasive ductal carcinoma (NOS) (Score – 0) (400x)

DISCUSSION:

In our study, 56.6% cases were benign which is roughly close to the findings of Verma et al¹ with 67.6% and stefanaou et.al with 52.63% and is higher than Werling et.al¹⁰ who found percentage of benign cases to be 12.8% respectively.

In the present study, 33.9% cases were malignant which is slightly higher than the findings of Verma et al¹ with 32.4% and much lower than Stefanaou et al⁷ and Werling et al¹⁰ as their results were 36.09% and 41.17% respectively. Fibroadenoma accounted for 49.3% of all the breast lumps which was in agreement with most of the available literature on benign breast lumps, where the frequency ranged from 46.6%-667.6%. Invasive ductal carcinoma was the commonest malignant lesion in our study (28.3%), which was similar to findings of Verma et al with 27.5% cases of invasive ductal carcinoma and Stefanaou et al with 23.3% cases respectively.

Size and Age distribution

In our study, all the palpable breast lumps were in range of 0.5-8cm and majority of cases (71.69%) had tumour size 2-5cm. Only 3.77% of benign cases had tumour size more than 5cm

while 22.6% of malignant cases had tumour size >5cm. Verma et al, studied 151 cases of the lumps, with a size range of 0.5- 13cm and reported that 28.30% of the lumps with a size >2cm and 10.9% with a size <2cm were malignant.

In our study, majority of cases 21 cases (39.62%) were in the age group of 30-50 yrs (39.62%) which was similar to study done by Verma et. Al¹, Stefanaou et.al⁷ and Werling et.al¹⁰. Reibero-A et.al have taken only breast carcinoma cases in their study & common age group in majority of cases was between 50-70 yrs.

p63 expression:

In our study, all benign and premalignant tumors were positive for p63 expression while 100% of malignant tumors were devoid of p63 positivity.

In 2000, Barbareschi M et al⁵, investigated 384 samples of normal and diseased human breast, including 300 invasive carcinomas, noted p63 positivity in all benign lesions while Invasive breast carcinomas were consistently devoid of nuclear p63 staining. In 2002, Xiaojuan Wang et al⁶ investigated 40 cases, which all contained normal breast tissue, ductal hyperplasia, ductal carcinoma in situ and invasive ductal carcinoma, p63 was exclusively expressed in the myoepithelial cells of normal breast, partially expressed in ductal hyperplasia, rarely expressed in carcinoma in situ and not expressed in invasive carcinomas.

CONCLUSION:

The pattern of p63 expression was studied on total of 53 cases in our study. Positive correlation was seen between histomorphological features and p63 scoring in all lesions. Majority of the cases were presented in age group 30-50 yrs. Overall mean size of tumor was 4. No correlation was seen between age of patient, size of lesions, lymph node status, histologic grading and staging with the p63 expression in our study. Among the benign category, non-proliferative lesions were continuous positive, proliferative showed less continuous positivity for p63, premalignant lesions showed least positivity and all malignant lesions were devoid of p63 staining. Thus our study suggests that p63 expression has helped us to find the existence of myoepithelial cells in breast lesions and its pattern of expression has helped us in differentiating many complex epithelial lesions of the breast.

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