Section: Pathology

Original Research Article

COMPARATIVE STUDY OF DIAGNOSTIC EFFICACY OF MYOEPITHELIAL CELL MARKERS SUCH AS SMA, P63 AND CALPONIN IN DIFFERENTIATING BENIGN AND MALIGNANT LESIONS OF BREAST

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ABSTRACT

Background: Distinguishing benign, in situ, and invasive breast lesions is a common diagnostic challenge. The presence of an intact myoepithelial cell (MEC) layer characterizes benign and DCIS lesions, while its absence indicates invasion. Immunohistochemistry for MEC markers aids in this distinction. **Aims and Objectives:** To compare the diagnostic efficacy of p63, Smooth Muscle Actin (SMA) and Calponin in differentiating benign, DCIS, and invasive breast lesions.

Materials and Methods: A retrospective study of 100 operated breast specimens was conducted at Department of Pathology, Victoria Hospital & Bowring & Lady Curzon Hospitals, Bangalore from Jan 2017 to June 2017. Cases included 45 benign proliferative lesions, 20 DCIS, and 35 Infiltrating Duct Carcinoma-NOS. IHC was performed for p63, SMA, and Calponin. Staining was scored as: 0/Negative, 1+ <25%, 2+ 26-90%, 3+ 91-100%. Data was analyzed using SPSS v26. Chi-square/Fisher's Exact test was used for comparison.

Results: All 3 markers showed 100% concordance. All 45 benign and 20 DCIS cases showed intact MEC layer with 3+ staining for p63, SMA, and Calponin. All 35 IDC-NOS cases showed complete loss of MEC layer with negative staining for all 3 markers. p63 showed crisp nuclear staining without background. SMA and Calponin showed cytoplasmic staining and also highlighted myofibroblasts and vascular smooth muscle.

Conclusion: p63, SMA, and Calponin are all effective in demonstrating MEC layer. p63 offers the highest specificity due to nuclear staining and lack of background, while SMA and Calponin are sensitive but require careful interpretation. A panel of nuclear + cytoplasmic MEC markers is recommended for difficult cases to differentiate in situ from invasive carcinoma.

Keywords: Myoepithelial cells, p63, SMA, Calponin, Breast, DCIS, Invasive carcinoma, Immunohistochemistry.

INTRODUCTION

Breast cancer is the most common malignancy affecting women worldwide. Breast lesions has always been a diagnostic challenge for a pathologist. The presence of an intact peripheral myoepithelial cell layer characterizes all normal and benign breast lesions as well as ductal carcinoma in situ (DCIS).[1] Loss of this outer myoepithelial layer is the hallmark of invasive carcinoma and demonstration of this loss has been documented by immunohistochemical techniques.[2] Myoepithelial cells (MECs) are contractile elements in salivary, sweat, and mammary glands with features of both smooth muscle and epithelial cells.[3] The normal breast gland consists of three cell types with distinct

protein expression: luminal, basal, and myoepithelial.[3,4] While luminal and basal cells express different cytokeratins (CKs), MECs express basal-type CKs and specific markers including smooth muscle actin, calponin, and p63. Preservation of the MEC layer characterizes benign and in situ lesions; its absence is the diagnostic hallmark of invasive cancer.[4]

Identification of myoepithelial cells using antibodies such as p63, smooth muscle actin (SMA) and calponin, can play an important role in distinguishing invasive carcinoma.[3,4]

Aims and objectives

To compare diagnostic efficacy of myoepithelial cell markers such as SMA, p63 and calponin in differentiating benign, insitu and malignant lesions of breast.

MATERIALS AND METHODS

Study design: Retrospective study.

B) Period of study: Six months (Jan 2017 to June 2017).

Place of study: Department of Pathology, Victoria Hospital & Bowring & Lady Curzon Hospitals, Bengalore.

Sample size: 100 operated cases of breast which includes excision biopsy, breast conserving surgery and mastectomy specimen received during study period at Victoria hospital & Bowring & Lady Curzon Hospitals.

Inclusion Criteria: Diagnosed case of benign proliferative lesion, DCIS and Infiltrating duct carcinoma.

Exclusion Criteria: Cases with inadequate tissue, necrotic tissue, or poor fixation.

Methodology: Paraffin-embedded tissue blocks of already diagnosed cases meeting the inclusion criteria were retrieved. 4µm sections were cut and subjected to Immunohistochemistry using antibodies against p63, SMA, and Calponin as per standard protocol.

p63, a p53 homologue, detects MECs in breast with a dotlike nuclear staining pattern.

Smooth muscle myosin actin(SMA) shows a continuous cytoplasmic staining pattern.

Calponin is one of the MEC markers demonstrating a continuous cytoplasmic linear staining pattern.

The reactivity of each antibody was scored separately on the myoepithelium, myofibroblasts, vascular smooth muscle cells and tumour cells in each case and assigned a score:

0 /Negative (−): No staining,

1+: <25% of target cells positive,

2+: 26–90% of target cells positive,

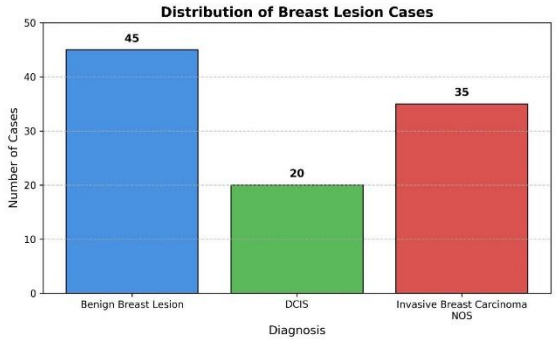
3+: 91–100% of target cells positive.

Statistical Method: Descriptive analysis was done. Data was entered into Microsoft Excel and analyzed using SPSS software version 26.0. Comparison of staining patterns between the 3 groups was done using Chi-square test or Fisher's Exact test.

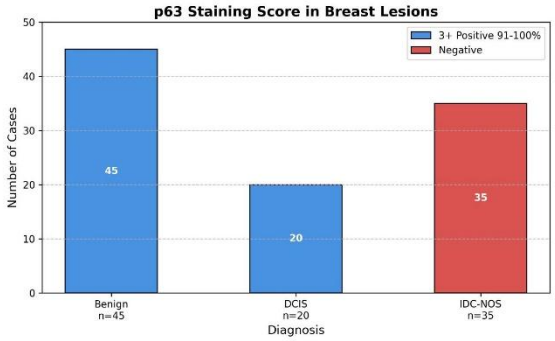
RESULTS

Table 1: Distribution of histopathological diagnosis of breast lesions

Sl. no	Histopathological diagnosis	Number
1	Benign proliferative lesion of breast	45
2	Ductal carcinoma insitu	20
3	Invasive carcinoma of no special type	35



Graph 1: Distribution of Breast lesion cases



Graph 2: p63 staining score in breast lesions

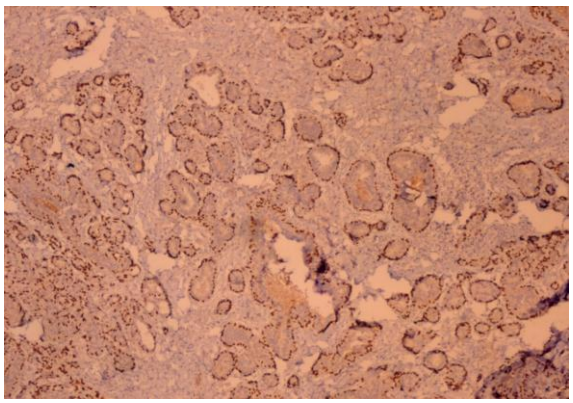
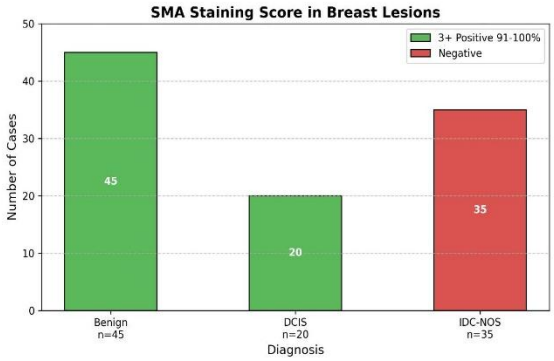


Figure 1: p63 Staining in benign proliferative lesion of breast



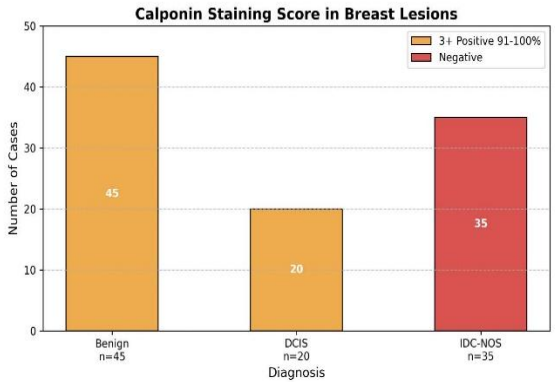
Graph 3: SMA staining score in breast lesions

Table 2: SMA Expression and Scoring

Diagnosis	Total(n)	SMA 3+(91-100%)	SMA -Negative	MEC Layer Status
Benign	45	45 (100%)	0	Intact, Continuous
DCIS	20	20 (100%)	0	Intact, Continuous
IDC-NOS	35	0	35(100%)	Absent

Table 3: Calponin Expression and Scoring

Diagnosis	Total(n)	Calponin 3+(91-100%)	Calponin -Negative	MEC Layer Status
Benign	45	45 (100%)	0	Intact, Continuous
DCIS	20	20 (100%)	0	Intact, Continuous
IDC-NOS	35	0	35(100%)	Absent



Graph 3: Calponin staining score in breast lesions

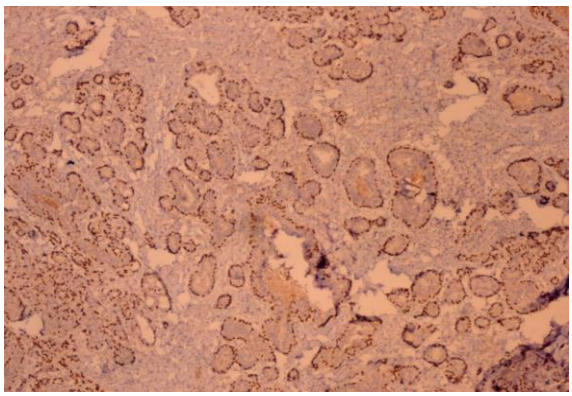
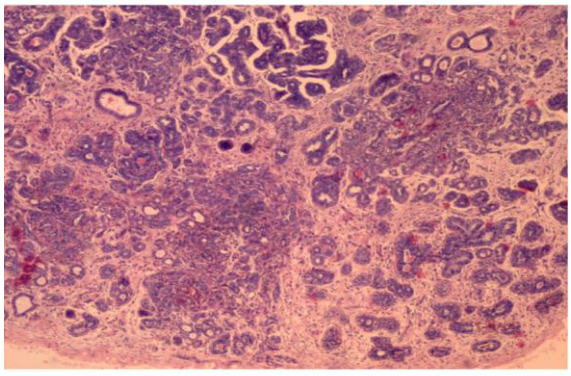


Figure 2: p63 Staining in Benign proliferative lesion of breast



H & E: Benign proliferative lesion of breast

Figure 1: H&E of Benign proliferative lesion of breast

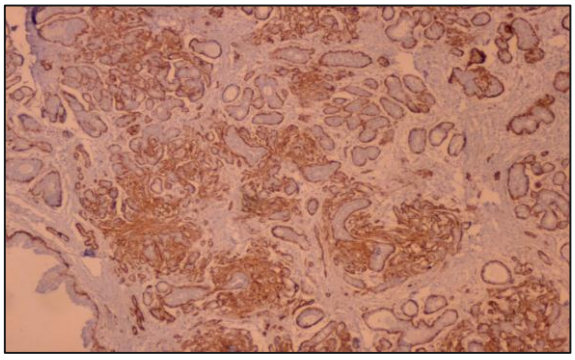


Figure 3: SMA & Calponin staining in benign breast lesions. (scoring 3)

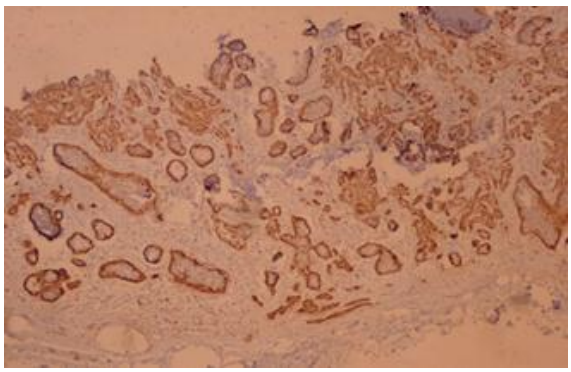


Figure 3: SMA & Calponin staining in benign breast lesions. (scoring 3)

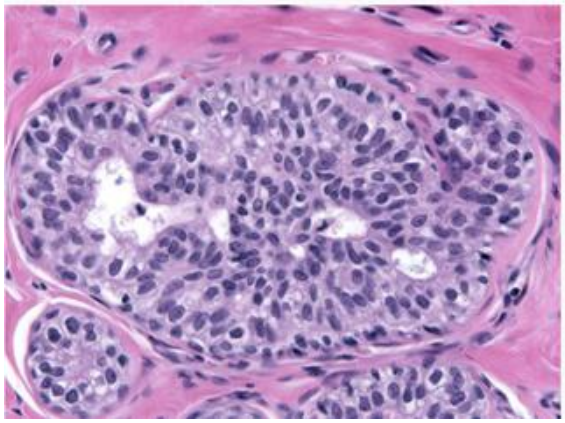


Figure 4: H & E of Ductal carcinoma insitu (DCIS)

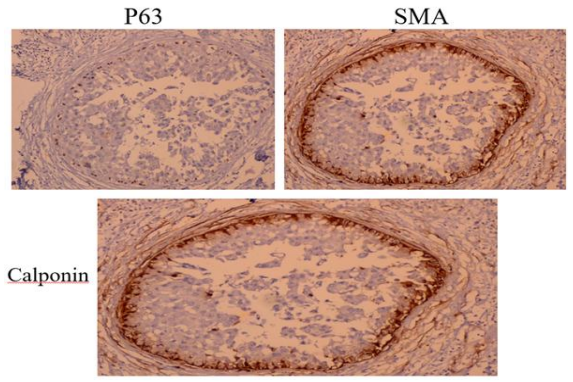


Figure 5: 63, SMA and calponin in ductal carcinoma insitu

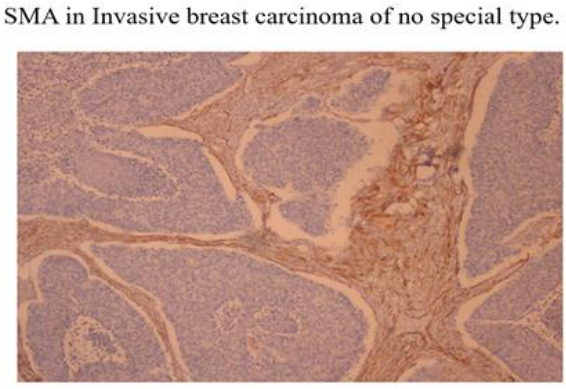


Figure 6: SMA in invasive breast carcinoma of no special type(score-0)

DISCUSSION

The distinction between benign, in situ, and invasive breast lesions is critical for patient management. The presence of an intact myoepithelial cell layer is the defining feature of benign and in situ lesions, while its absence indicates invasion. Immunohistochemistry for MEC markers has become an essential tool in difficult cases. In the present study of 100 cases, we found 100% concordance between p63, SMA, and Calponin in highlighting MECs. All 45 benign and 20 DCIS cases showed intact 3+ staining, while all 35 IDC-NOS cases showed complete loss of MEC layer. This is consistent with the fundamental role of MECs as a barrier to invasion.

Demonstration of an intact myoepithelial cell layer between epithelial cells and basement membrane is essential for distinguishing benign and in situ breast lesions from invasive carcinoma.[5]

Comparison with literature

p63 is a p53 homologue that shows nuclear staining limited to MECs. In our study, p63 showed crisp nuclear staining with no background in stroma, making it the easiest to interpret. Similar findings were reported by Tavassoli and Devilee and Hussain et al. *, who concluded that p63 is the most specific MEC marker due to its nuclear localization and absence of staining in myofibroblasts. Our results support that p63 alone can reliably distinguish DCIS from invasive carcinoma.[6,7]

SMA demonstrated continuous cytoplasmic staining in all benign and DCIS cases. However, like in our study Nielsen et al. noted that SMA also stains myofibroblasts and vascular smooth muscle. This can lead to false-positive interpretation in cases with prominent desmoplasia. Hence, while sensitive, SMA has lower specificity compared to p63.[8]

Calponin showed linear cytoplasmic staining in MECs. Our results align with Werling et al., who found Calponin to be as sensitive as SMA but with similar limitations of background staining.[9]

Several authors including Rakha et al., recommend using a combination of a nuclear marker like p63 and a cytoplasmic marker like SMA/Calponin.[10]

This increases diagnostic confidence, especially in cases with patchy MEC loss or tangential cutting. Our study also supports a panel approach, though p63 alone performed with 100% accuracy in our cohort.

Limitation of p63 is they occasionally demonstrate an apparently discontinuous myoepithelial layer around nests of DCIS.

Calponin is useful as part of a panel but not ideal as a single marker.

Marker	Staining Pattern	Advantages	Limitations
p63	Crisp nuclear staining in MECs	No background staining in stromal cells. Easiest to interpret. Highly specific.	None significant
SMA	Continuous cytoplasmic staining	Good sensitivity	Also stains myofibroblasts and vessel smooth muscle. Can cause interpretation difficulty in desmoplastic stroma
Calponin	Continuous cytoplasmic linear staining	Good sensitivity	Similar limitation as SMA. Background staining in stroma

CONCLUSION

All 3 markers are effective. p63 offers the highest specificity due to nuclear staining and lack of background. SMA and Calponin are good sensitive cytoplasmic markers but must be interpreted with caution. A dual-stain or panel is recommended for difficult cases.

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