



COMPARATIVE EVALUATION OF CALPONIN AND P63 IN DIFFERENTIATING BENIGN AND MALIGNANT BREAST LESIONS: AN IMMUNOHISTOCHEMICAL STUDY

Pathology

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ABSTRACT

Background & objectives Accurate differentiation between benign and malignant breast lesions is crucial for appropriate patient management. The presence of an intact myoepithelial cell layer is a key histopathological feature that distinguishes benign and in situ lesions from invasive carcinoma. However, this distinction may be difficult on routine hematoxylin and eosin staining, particularly in small biopsies and borderline lesions. Immunohistochemical markers such as p63 and calponin have been widely used to identify myoepithelial cells. The present study aimed to compare the diagnostic utility of p63 and calponin in differentiating benign and malignant breast lesions. **Methods** This cross-sectional comparative study was conducted over a period of two years at a tertiary care hospital. A total of 60 breast lesion specimens obtained from trucut biopsies and modified radical mastectomies were included in the study. Tissue sections were processed routinely and stained with hematoxylin and eosin for histopathological diagnosis. Immunohistochemistry was performed using antibodies against p63 and calponin on formalin-fixed paraffin-embedded sections. Staining intensity was graded from 0 to 3 based on the degree of immunoreactivity. The expression patterns of both markers were evaluated and compared across benign, in situ, and invasive lesions. **Results** Among the 60 cases studied, invasive ductal carcinoma constituted the largest group (33.3%), followed by ductal carcinoma in situ (16.7%), fibroadenoma (16.7%), intraductal papilloma (16.7%), fibrocystic disease (10%), and phyllodes tumour (6.7%). The majority of patients belonged to the 40–49 yr age group (30%). Breast lump was the most common presenting symptom (53.3%). p63 demonstrated nuclear staining predominantly in benign and in situ lesions, while most invasive carcinomas showed absence of staining. Calponin showed cytoplasmic staining in myoepithelial cells but occasional stromal and myofibroblastic positivity was observed. **Interpretation & conclusions** Both p63 and calponin are useful markers for identifying myoepithelial cells in breast lesions. p63 demonstrated higher specificity owing to its nuclear staining pattern, while calponin showed higher sensitivity but occasional stromal cross-reactivity. The combined use of both markers may improve diagnostic accuracy in differentiating benign and malignant breast lesions.

KEYWORDS

Breast lesions; calponin; immunohistochemistry; myoepithelial cells; p63

INTRODUCTION

Breast carcinoma is one of the most common malignancies affecting women worldwide and is associated with significant morbidity and mortality. Histopathological evaluation remains the cornerstone for diagnosing breast lesions; however, certain lesions may pose diagnostic challenges, particularly in small biopsy samples or in borderline proliferative lesions.

A critical histological feature that helps differentiate benign and in situ lesions from invasive carcinoma is the presence of the myoepithelial cell layer. Loss of this layer is considered a defining characteristic of invasive breast carcinoma. Conventional hematoxylin and eosin staining may not always reliably demonstrate this layer, especially in limited biopsy material.

Immunohistochemical markers that highlight myoepithelial cells have therefore become an essential diagnostic adjunct. Among these markers, p63, a nuclear transcription factor belonging to the p53 gene family, and calponin, a smooth muscle-associated contractile protein, are widely used. p63 demonstrates nuclear staining in myoepithelial cells and has minimal cross-reactivity with stromal cells. In contrast, calponin is a cytoplasmic marker with high sensitivity but may occasionally stain myofibroblasts and vascular smooth muscle cells.⁽¹⁾

Despite their widespread use, variations in sensitivity and specificity have been reported in different studies. Therefore, comparative evaluation of these markers is important to determine their diagnostic utility.

The present study was conducted to evaluate and compare the expression of p63 and calponin in benign and malignant breast lesions using immunohistochemistry.

Material & Methods

Study design

Cross-sectional comparative study.

Study setting

Department of Pathology, Osmania General Hospital.

Study duration

Two years from the date of ethical approval.

Study population

Patients presenting with breast lesions who underwent biopsy or surgical excision.

Sample size

A total of 60 breast lesion specimens were included in the study.

Inclusion criteria

- Female patients with breast lesions
- Histologically diagnosed benign proliferative lesions, ductal carcinoma in situ, or invasive carcinoma
- Patients who provided informed consent

Exclusion criteria

- Patients who did not provide consent
- Inflammatory breast lesions
- Metastatic tumours
- Inadequate biopsy specimens

Histopathological examination

Specimens obtained from trucut biopsies and modified radical mastectomies were fixed in 10 per cent neutral buffered formalin. Following gross examination, representative sections were processed and embedded in paraffin. Sections of 4 µm thickness were cut and stained with hematoxylin and eosin.

Tumours were classified and graded according to the Nottingham modification of the Scarff–Bloom–Richardson grading system.

Immunohistochemistry

Immunohistochemical staining for p63 and calponin was performed on formalin-fixed paraffin-embedded tissue sections using a horseradish peroxidase polymer detection system.

Primary antibodies

p63: Mouse monoclonal antibody (Clone A4)

- Calponin: Rabbit monoclonal antibody (Clone EP63)

Antigen retrieval was performed using microwave heating with appropriate buffers.

Interpretation of staining

Immunoreactivity was graded based on staining intensity.

Score	Interpretation
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

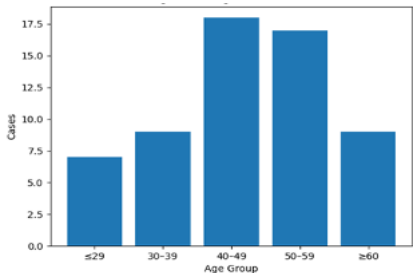
p63 showed nuclear staining, while calponin showed cytoplasmic staining.

RESULTS

Age distribution

The majority of patients belonged to the 40–49 yr age group (30%), followed by 50–59 yr (28.3%). Patients aged ≤29 yr accounted for 11.7 per cent of cases.(chart 1)

Chart 1-Age distribution of study population



Sex distribution

All study participants were female (100%).

Clinical presentation

The most common presenting complaint was breast lump (53.3%), followed by pain associated with lump (20%). Nipple discharge and screen-detected lesions accounted for 6.7 per cent each.

Histopathological spectrum

Table 1: Histopathological Spectrum of Breast Lesions

Histopathological Diagnosis	n	%
Invasive ductal carcinoma	20	33.3
DCIS	10	16.7
Fibroadenoma	10	16.7
Intraductal papilloma	10	16.7
Fibrocystic disease	6	10.0
Phyllodes tumor	4	6.7
Total	60	100

Invasive ductal carcinoma represented the most common malignant lesion.

Immunohistochemical findings

p63 showed nuclear positivity in the myoepithelial layer in benign lesions and ductal carcinoma in situ.

Most invasive carcinomas lacked p63 staining, indicating absence of the myoepithelial layer.

Calponin demonstrated cytoplasmic staining in myoepithelial cells in benign lesions and ductal carcinoma in situ.

Occasional stromal staining was observed with calponin due to cross-reactivity with myofibroblasts.

DISCUSSION

The identification of myoepithelial cells plays a crucial role in distinguishing benign proliferative lesions and carcinoma in situ from invasive carcinoma of the breast. Routine histology may be insufficient in some cases, particularly in small biopsies or lesions with atypical features. Immunohistochemical markers therefore provide an important diagnostic adjunct.

In the present study, most cases occurred in middle-aged women, which is consistent with the typical age distribution of breast lesions reported in previous studies.

p63 demonstrated high specificity because of its nuclear staining pattern and lack of staining in stromal cells. Similar findings have been reported in previous studies where p63 positivity was consistently seen in benign lesions and was absent in most invasive carcinomas.(2)

Calponin showed high sensitivity for myoepithelial cells but also demonstrated staining in myofibroblasts and vascular smooth muscle cells. This cross-reactivity has been documented in earlier studies and may occasionally lead to diagnostic difficulty.(3)

Comparative studies have suggested that p63 is more specific, while calponin is more sensitive, and the combined use of both markers improves diagnostic accuracy.

CONCLUSION

p63 and calponin are valuable immunohistochemical markers for identifying myoepithelial cells in breast lesions.

p63 is highly specific due to its nuclear staining pattern.(4)

Calponin is highly sensitive but may show stromal cross-reactivity.

The combined use of both markers provides better diagnostic accuracy in distinguishing benign and in situ lesions from invasive breast carcinoma.

Figure 1-H & E and IHC images of Fibroadenoma

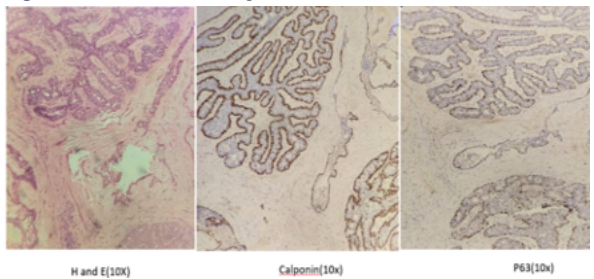


Figure 2-H & E and IHC images of DCIS low-intermediate grade

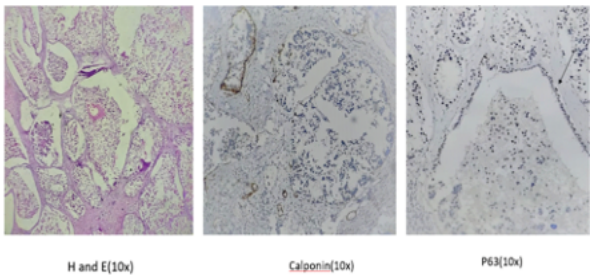
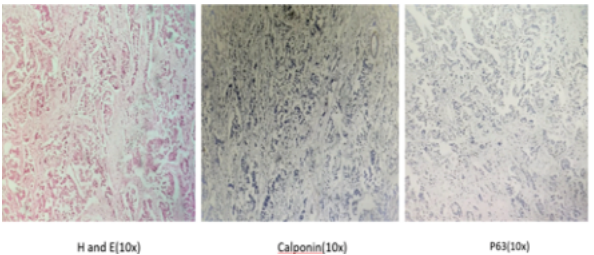


Figure 3-H & E and IHC images of invasive breast carcinoma of no special type



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