Original Research Article

Role of p63 expression in non-proliferative and proliferative lesions of breast

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ABSTRACT

Background: Breast lump among females, is a fairly common complaint. Breast lump have a variety of etiologies ranging from inflammatory to benign to malignant lesions. Myoepithelial markers are useful in helping to distinguish invasive carcinoma from benign proliferations with a similar morphological appearance, benign proliferative lesions and most pre-invasive lesions with an intact myoepithelium. Invasive carcinomas lack the myoepithelial cell layer that normally surrounds benign breast glands. p63 antibody is a myoepithelial cell marker that selectively stains nuclei. Also, it is negative in stromal, myofibroblastic and adipocytic cells. This makes p63 more specific and superior to other myoepithelial markers.

Methods: The present study was done on a total of 151 cases of breast diseases, received in the form of core biopsy, tru cut biopsy, lumpectomy, and mastectomy specimens. Clinical history and examination findings of the patients were collected in all the cases. All specimens were routinely processed and stained with haematoxylin and Eosin (H and E) stain and only 50 cases were subjected to immunohistochemical staining for p63.

Results: Out of total 151 cases, 09 were inadequate for evaluation, 96 cases benign and 46 malignant. In benign category, fibroadenoma was most common and infiltrating ductal carcinoma (NOS) was the most common in malignant category. Mean size of benign tumors was found to be less than that of malignant tumors. All malignant cases were negative for p63 expression. In the benign category, 88.6% cases showed positive expression for p63 while 11.4% were negative. Among the benign category, non-proliferative lesions were continuous positive, proliferative showed discontinuous positivity for p63.

Conclusions: Myoepithelial markers are useful in helping to distinguish invasive carcinoma from benign proliferations with a similar morphological appearance, benign proliferative lesions and most pre-invasive lesions with an intact myoepithelium. Invasive carcinomas lack the myoepithelial cell layer while in the benign category, non-proliferative lesions are continuous positive, proliferative lesions show discontinuous positivity for p63.

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

INTRODUCTION

Breast lesions are not a single entity, rather they represent a heterogeneous group of diseases with marked clinical and morphological diversity.¹ Breast tumors with similar histopathological appearance can exhibit divergent clinical presentations, disease aggressiveness and response to treatment. Benign breast diseases continue to dominate in early age group whereas malignancy is more prominent in older ages. The entire ductal-lobular epithelial system of the breast is covered by specialized two cell type epithelial lining: the inner epithelium with secretory and absorptive functions and the outer myoepithelial cells.²,³ Myoepithelial markers are useful in helping to distinguish invasive carcinoma from benign proliferations with a similar morphological appearance, benign proliferative lesions and most pre-invasive lesions with an intact myoepithelium. Invasive carcinomas lack
the myoepithelial cell layer that normally surrounds benign breast glands. Several antibodies directed against myoepithelial cells have been raised. These targets either smooth muscle antigens like alpha smooth muscle actin, calponin, caldesmon, smooth muscle myosin heavy chain, cytokeratin 5/6 or CD10 p63 (a p53 homologue) is expressed in the stratified epithelial and basal cells of prostate and salivary glands. In the mammary epithelium it is expressed in only the myoepithelial cell layer. p63 antibody is a myoepithelial cell marker that selectively stains nuclei. Also, it is negative in stromal, myofibroblastic and adipocytic cells. This makes p63 more specific and superior to other myoepithelial markers.

The aim of present study is to do clinicopathological correlation of histological subtypes of benign breast lesions and malignant tumors of breast and also to establish role of p63 expression in distinguishing benign breast lesions, premalignant lesions and malignant tumours of breast.

**METHODS**

The present study was done in the Department of Pathology on a total of 151 cases of breast diseases, collected over a period from January 2016 to October 2017. The study was done on the core biopsy, tru cut biopsy, lumpectomy, and mastectomy specimens of breast tumors sent by the Department of Surgery of SVBP Hospital attached to LLRM Medical College, Meerut. Clinical history and examination findings of the patients were collected in all the cases. All specimen was routinely processed and stained with Haematoxylin and Eosin (H and E) stain.

**Exclusion criteria**

Tissue sections with inadequate study material were excluded from the study.

**Tissue processing**

The specimen received were fixed in 10% buffered formalin, dehydrated in ascending grades of alcohol, cleared in xylene and finally embedded in paraffin. 2-5micron thick paraffin sections of uniform thickness were cut on a rotary microtome, dewaxed and stained.

**Haematoxylin and eosin staining**

- After dewaxing in xylene, sections were rehydrated to water by passing through descending grades of alcohol;
- Stained with Harris haematoxylin for 10-15 minutes;
- Rinsed in tap water followed by differentiation in 1% acid alcohol;
- Sections were washed in tap water until they become blue;
- Stained with Eosin for 2-3 minutes;
- Dehydrated by passing through ascending grades of alcohol;
- Cleared in xylene and mounted in DPX (Dibutyl Phthalate Xylene).

**Immunostaining method for p63**

Fifty cases out of total 142 were subjected to immunohistochemical staining for p63.

- Four micrometre thin sections were cut and placed on poly L lysine coated slides.
- Sections were incubated, then deparaffinised and rehydrated through graded alcohols followed by distilled water.
- Then the sections were kept in citrate buffer (pH 6.0) for antigen retrieval in pressure cooker for 20 min.
- Endogenous peroxide activity was blocked with 3% hydrogen peroxide.
- Sections were then treated with protein blocking antibody.
- After washing with TBS buffer, p63 immunostaining was performed using Monoclonal Mouse Anti-Human p63 antibody for 1 hr. (Dako Denmark A/S, Glostrup, Denmark).
- Then secondary antibody was put for 30 minutes.
- This was followed by DAB solution (Diaminobenzidine) for 45 minutes.
- The sections were then counter-stained with 10% haematoxylin and mounted in DPX.

**Positive control for p63**

A histological section of skin biopsy was used as positive control with each batch of staining.

**Negative control for p63**

For negative control, 1% non-immune serum was used in place of primary antibody, with rest of the steps being the same as for the positive control.

**Interpretation**

A detailed histopathological examination of haematoxylin and eosin stained slides was carried out. Cases were divided into Benign, carcinoma in situ and malignant.

**Evaluation OF p63 expression**

p63 expression was evaluated as continuous positive/ discontinuous positive/ Negative.
RESULTS

Out of total 151 cases, 96 cases (63.6%) were benign and 46 cases (30.5%) malignant; while 09 cases (5.9%) inadequate for any definite opinion. These 09 (5.9%) inadequate biopsies were excluded from the study and therefore all subsequent data was computed for remaining 142 cases (Table 1). The 142 cases included in the study were between 12-75 years of age and mean age of presentation was 31.3 years.

Table 1: Distribution of total benign and malignant cases (n=151).

| Type of lesion       | No. of cases | %   |
|----------------------|--------------|-----|
| Benign               | 96           | 63.6|
| Malignant            | 46           | 30.5|
| Inadequate           | 09           | 5.9 |
| Total                | 151          | 100 |

Histological diagnosis

Out of 142 cases, 96 cases (67.6 %) were benign and 46 cases (32.4%) malignant. Among the benign category, maximum number of cases were of fibroadenoma 70 cases (49.3%) (Figure 1) while among the malignant, maximum number of cases of Infiltrating ductal carcinoma, NOS, i.e. 39 cases (27.5%) (Figure 2) (Table 2).

Table 2: Distribution of total cases on the basis of histological diagnosis (n=142).

| Diagnosis                             | No. of cases | %   |
|---------------------------------------|--------------|-----|
| Benign                                | 96           | 67.6|
| Fibroadenoma                          | 70           | 49.3|
| Complex fibroadenoma                  | 10           | 07  |
| Fibrocystic disease                   | 04           | 2.8 |
| Gynecomastia                          | 04           | 2.8 |
| Fibroadenomatoid hyperplasia          | 02           | 1.4 |
| Phyllodes tumour                      | 01           | 0.7 |
| Fibroadenosis                         | 01           | 0.7 |
| Adenosis with inflammation            | 01           | 0.7 |
| Duct ectasia                          | 01           | 0.7 |
| Breast abscess                        | 01           | 0.7 |
| Ductal carcinoma in situ              | 01           | 0.7 |
| Malignant                             | 46           | 32.4|
| Infiltrating ductal carcinoma, NOS    | 39           | 27.5|
| Suspicious for malignancy             | 3            | 2.1 |
| Infiltrating ductal carcinoma with Paget’s disease of nipple | 1 | 0.7 |
| Infiltrating ductal carcinoma with adenosis | 1 | 0.7 |
| Stromal sarcoma of breast             | 1            | 0.7 |
| Positive for malignancy               | 1            | 0.7 |
| Total                                 | 142          | 100 |

Figure 1: Fibroadenoma (400x, H and E stain).

Figure 2: Infiltrating ductal carcinoma, NOS (H and E stain, 400x).

Size distribution

The size of lump ranged from 0.5 to 13cm in maximum dimension; majority of cases, 87 cases (61.3%) had tumor size 2-5cm. In 14 cases (9.9%) size could not be assessed, as in 13 of them, only tru cut biopsy was received and in 01 case, mastectomy was performed after chemotherapy and no grossly visible or palpable mass was present. The mean size of tumour was 4.1cm.

Table 3: Distribution of cases on the basis of size of lump (n=142).

| Size of lump (cm) | Benign cases | Malignant cases |
|-------------------|--------------|-----------------|
|                   | No. | %   | No. | %   |
| <2                | 08  | 8.4 | 05  | 10.9|
| 2-5               | 73  | 76  | 14  | 30.4|
| >5                | 15  | 15.6| 13  | 28.3|
| Can’t be assessed | 00  | 00  | 14  | 30.4|
| Total             | 96  | 100 | 46  | 100 |

The size of benign lump ranged from 01 to 11cm in maximum dimension; majority of cases, i.e. 73 (76%) had tumor size between 2-5cm.
In the benign category, 15 cases (15.6%) had tumor size >5cm. The mean size of benign tumor was 3.8cm. In the malignant category, 13 cases (28.3%) had tumor size >5cm. The mean size of malignant tumor was 4.9cm (Table 3).

**p63 expression**

Out of total 142 cases, only 50 were subjected to p63 immunostaining, of which 35 cases (70%) were benign and 15 (30%) malignant. Out of 35 benign cases, 31 (88.6%) showed positive expression while 04 (11.4%) were negative for p63. All cases of fibroadenoma showed continuous positivity (Figure 3) and fibrocystic disease (Figure 4) showed discontinuous positivity while phyllodes tumor, gynaecomastia and fibroadenosis were negative for p63 expression in benign category. Out of 15 malignant cases, all were devoid of p63 expression (Figure 5) (Table 4).

**Table 4: Distribution of cases according to p63 expression (n=50).**

| Category | Positive for p63 | Negative for p63 | Total |
|----------|-----------------|-----------------|-------|
| Benign   | 31 (88.6%)      | 04 (11.4%)      | 35    |
| Malignant| Nil (0%)        | 15 (100%)       | 15    |
|          | 31 (62%)        | 19 (38%)        | 50    |

**DISCUSSION**

In our study, 67.6% cases were benign which is roughly close to the findings of Modi P et al, who found 72% benign cases and is slightly higher than Bukhari MH et al, and Thakkar et al who found percentage of benign cases to be 60% and 54.16% respectively. In the present study, 32.4% cases were malignant which is much higher than the findings of Modi P et al, Chaudhary S et al and Rahman MZ et al, as their results were 16.7%, 19.23% and 14.7% respectively. Fibroadenoma accounted for 49.3% of all the breast lumps in our study. Our finding was in agreement with most of the available literature on benign breast lumps, where the frequency of fibroadenoma ranged from 46.6%-55.6%. Invasive ductal carcinoma was the commonest malignant lesion in our study (27.5%), which was similar to findings of Modi P et al, who found 28.8% cases of invasive ductal carcinoma. We found single case (0.7%) of stromal sarcoma of breast. Primary sarcomas of the breast are rare and there are only a few hundred cases reported in the literature. Sarcomas represent less than 1% of all primary breast malignancies and less than 5% of all sarcomas.

**Size of breast lump**

Tumour size is one of the most powerful predictor of outcome in breast carcinoma. Tumour size has been shown to affect the survival in node negative breast cancer patients. In the present study, all the palpable breast lumps were in range of 0.5-13cm and majority of
cases (61.3%) had tumour size 2-5cm. Only 15.6 % of benign cases had tumour size more than 5cm while 28.6% of malignant cases had tumour size >5cm. Ballo et al, studied 112 cases of the lumps, with a size range of 1-12cm and reported that 73.8% of the lumps with a size >2cm and 28.38% with a size <2cm were malignant.\textsuperscript{30} While in the study conducted by Munjal et al, on 107 cases, 71 % (76 of 107) had tumor size 2-5cm and 10 % (11 of 107) had tumor size <2cm.\textsuperscript{31}

**p63 expression**

In our study, 88.6% of benign tumors were positive for p63 expression while 11.4% of benign and 100% of malignant tumors were devoid of p63 positivity. In 2000, Barbareschi M et al, 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.\textsuperscript{32} In 2002, Xiaojuan Wang et al 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.\textsuperscript{33}

In a recent report, a few cases (4.6%, 11/238) of p63 expression occurred in invasive ductal carcinoma. However, in our study, virtually no p63 expression was found in the invasive ductal carcinoma components. p63 expression has also been reported in ductal carcinoma with squamous metaplasia.\textsuperscript{32}

**CONCLUSION**

Out of total 142 cases, 96 cases (67.6%) were benign and 46 cases (32.4%) malignant. Among the benign category, fibroadenoma was the most common, 70 cases (49.3%) and infiltrating ductal carcinoma (NOS) was the most common, 39 cases (27.5%) in malignant category. Overall mean size of tumour was 4.1cm. Mean size of benign tumor was 3.8cm and mean size of malignant tumor 4.9cm. In the benign category, 15.6% cases had tumor size >5cm while in the malignant category, 28.3% cases had tumor size >5cm. Mean size of benign tumors was found to be less than that of malignant tumors. All malignant cases were negative for p63 expression. In the benign category, 88.6% cases showed positive expression for p63 while 11.4% were negative. Among the benign category, non-proliferative lesions were continuous positive, proliferative showed discontinuous positivity for p63.

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