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Research Article

EVALUATION OF THE DIAGNOSTIC ROLE OF P63 IMMUNOSTAINING IN ASPIRATION CYTOLOGY BY SMEAR OR CELL BLOCK PREPARATION FOR THE CHARACTERISATION OF BREAST LESIONS

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ABSTRACT: A study was conducted to determine the diagnostic role of p63 in breast FNAC sample for proper identification of myoepithelial cells. The sample comprised of 51 patients with breast lesions who have undergone FNAC and subsequently biopsy. Following conventional smear preparations, the entire materials fixed and processed for histopathology staining. The cell blocks were embedded in paraffin, sectioned and processed for Immunostaining. As p63 is a reliable nuclear marker of myoepithelial cells in the breast, it was used to distinguish these cells from their mimics in FNABs. Benign lesions usually contained p63 positive myoepithelial cells, and it was demonstrated that it may be a useful marker for highlighting these cells. p63 positive cells were also observed frequently in samples of DCIS and IDC, although, based on careful cytomorphologic evaluation, they may have been classified correctly as malignant cells. Hence, based on previously published data and on our findings, we advocate that anti-p63 antibodies may be used to identify myoepithelial cells as well as to overcome the cytomorphologic distortion of myoepithelial cells in FNABs of the breast.

Key words: P63, Myoepithelial cell, FNAC, Breast Cancer.

INTRODUCTION

Breast carcinoma is the most prevalent cancer among Indian women and the most common cancer diagnosed in women worldwide with over 1.3 million new cases per year. There is a wide variation in the geographical burden of the disease with the highest incidences seen in the more developed regions of the world and the lowest incidences observed in the least developed regions.

India is undergoing a period of dramatic social and economic change. Cancer is now the second leading cause of death in Indians after cardiovascular disease. Amongst women, cervical cancer is still the most frequently diagnosed cancer but breast cancer is now the

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most commonly diagnosed cancer in urban Indian women. The reasons for the recent observed increase in incidence of breast cancer in the Indian population are not clearly understood but thought to be largely explained by 'westernization' of lifestyles and changes in reproductive behaviour (Gathani *et al.*, 2011).

Recent Indian Council of Medical Research (ICMR) data shows that the incidence of breast cancer is high among Indian females in the metropolitan cities of Mumbai, Chennai, and Delhi. It is estimated that one in 22 Indian females is likely to develop breast cancer during her lifetime in contrast to one in eight in America. The incidence varies between urban and rural women. The incidence in Chennai is about 29.3 new cases per 100,000 women per year while in rural Maharashtra it is only 9.7 per 100,000. Currently, India reports roughly 100,000 new cases annually. Widespread use of mammography has resulted in a marked increase in early detection of this carcinoma, when it is still localized and small in size.

The risk factors influencing breast cancer risk are broadly classified into modifiable and non -modifiable factors. The non modifiable risk factors are age, gender, number of first degree relatives suffering from breast cancer, menstrual history, age at menarche and age at menopause. While the modifiable risk factors are BMI, age at first child birth, number of children, duration of breast feeding, alcohol, diet and number of unsuccessful pregnancies (abortions). Having a 1st-degree relative (mother, sister, and daughter) with breast cancer doubles or triples the risk of developing the cancer. About 5% of women with breast cancer carry a mutation in one of the 2 known breast cancer gene, BRCA1 or BRCA2. If relatives of such a woman also carry the gene, they have a 50 to 85% lifetime risk of developing breast cancer (Wang *et al.*, 2001).

Women with a higher than average risk of developing breast cancer may be offered screening and genetic testing for the condition. NHS (National Health Service, England) Breast Screening Programme recommends that women between 50-70 years of age of should be screened once every three years. Heightened awareness of breast cancer risk in the past decades has led to an increase in the number of women undergoing mammography for screening, leading to detection of cancers in earlier stages and an improvement in survival rates.

FNA is a widely used method for evaluating palpable and radiologically directed nonpalpable breast abnormalities. The main advantage of FNA is immediate sample assessment for adequacy. Although a touch imprint of a CNB can be done for rapid diagnosis, the usefulness of this approach is controversial. Compared with CNB, the nondiagnostic rate for FNA is higher, and FNA has a lower negative predictive value (Edmund *et al.*, 2009).

The human breast epithelium is a branching ductal system composed of an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells that terminate in distally located terminal duct lobular units (TDLUs). While the luminal epithelial cell has received the most attention as the functionally active milk-producing cell and as the most likely target cell for carcinogenesis, attention on myoepithelial cells has begun to evolve with the recognition that these cells play an active part in branching morphogenesis and tumor suppression (Wiseman *et al.*, 2002).

Mammary myoepithelial cells have been a

neglected facet of breast cancer biology, largely ignored since they have been considered to be less important for tumorigenesis than luminal epithelial cells from which most of breast carcinomas are thought to arise. Emerging data raise the hypothesis whether myoepithelial cells play a key role in breast tumor progression by regulating the in situ to invasive carcinoma transition and that myoepithelial cells are part of the mammary stem cell niche. Paracrine interactions between myoepithelial and luminal epithelial cells are known to be important for regulation of cell cycle progression, establishing epithelial cell polarity, and inhibiting cell migration and invasion. Based on these functions, normal mammary myoepithelial cells have been called "natural tumor suppressors." However, during tumor progression myoepithelial cells seem to loose these properties, and eventually this cell population diminishes as tumors become invasive. Better understanding of myoepithelial cell function and their role in tumor progression may lead to their exploitation for cancer therapeutic and preventative measures (Sirvent *et al.*, 2001).

The myoepithelial cells in TDLUs are discontinuous, stellate-shaped, and form a basket-like network around acini, allowing some luminal epithelial cells to directly contact the basement membrane (BM) (Gudjonsson *et al.*, 2005).

One of the hallmarks of breast cancer is loss of polarity and organization of epithelial cells.The myoepithelial cells express cytokeratins (CK) characteristic for the basal layer of stratified epithelia, such as CK 5, CK 14, and CK 17. The CK 5 and 14 have an important role in the cytoarchitecture of myoepithelial cells, as they are connected to desmosomes and hemidesmosomes, which mediate the connection of myoepithelial cells to adjacent cells and the underlying basement membrane, respectively.

The myoepithelial cell markers smooth muscle actin (SMA) and p63 are most commonly used since their specificity and sensitivity are well established. However, recent studies have indicated that some morphologically distinct myoepithelial cells fail to stain for SMA and that p63 positivity can be rarely expressed by a subset of malignant epithelial cells (Ribeiro-Silva *et al.*,2005).

MATERIALS AND METHODS

The study was conducted in the Command Hospital (EC), Alipore Road, Kolkata-700027 from February 2012 to October 2013. Patients attended the Pathology Department of Command Hospital(EC), Alipore, Kolkata during the study period. The sample comprised of 51 patients with breast lesions, who have undergone FNAC and subsequently biopsy. Pilot study comprising of 5 patients was carried out in the department for understanding intricacies of study and understanding Immunostaining procedures and limitations better.

Following conventional smear preparations, the entire material was centrifuged at 4000 rpm to create one or more cell pellets and the supernatant fluid was decanted and the deposit fixed in freshly prepared alcohol formalin substitute consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. The cell pellets were wrapped in crayon paper or Millipore filters, placed in a cassette, and processed as other tissue were processed for histopathology reporting. The cell blocks were embedded in paraffin and sectioned at 3 µm thickness (Istvanic *et al.*, 2007).

Table 1: Different diagnosis according to FNAC, Histopathology & p63.

Diagnosis	FNAC	Histopathology	P63
Fibroadenoma	07	13	07
Benign breast disease	09	12	06
Atypical ductal hyperplasia	05	1	05
Suspicious Malignancy	06	0	03
Ductal carcinoma	21	25	01
No Diagnosis	03	0	0
Total	51	51	22

Immunostaining would be performed within 24 Hrs of smear rehydration.

For performing IHC the following reagents were used:

EDTA
TRIS Salt
Sodium Hydroxide (NaOH)
Hydrochloric acid (HCl)
Xylene and absolute alcohol
Acetone
Poly-L-Lysine (Sigma-USA)
Distilled water
Primary antibodies (p63)
Secondary antibody detection kit (super sensitive polymer- HRP detection system -A Biotin –free detection system from Biogenex)
DPX
Mayer's hematoxylin.

For cytological smears Immuno histo-chemistry (IHC) procedure is similar to other IHC protocol except :

1. Cytology smears are not contain wax so there was no need for overnight backing in oven and no need for putting the slides in xylol**.**

2. Cytology smears are not subjected to formalin so the epitope retrival technique is unnecessary for immunocytochemical staining of these specimens.

Fig. 1: FNAC diagnosis.

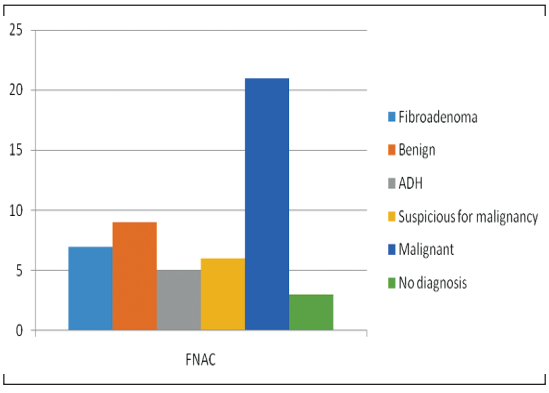


Fig. 2: Histopathology diagnosis.

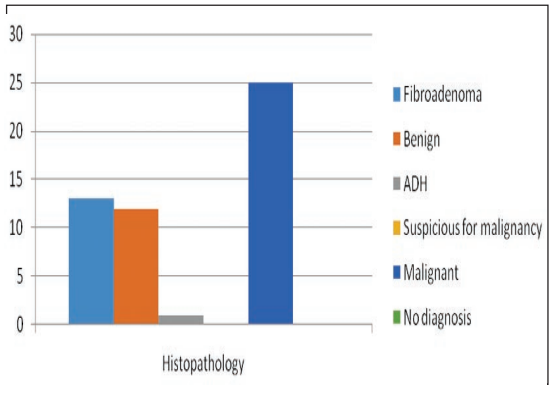


Fig. 3: FNAC diagnosis in percentage.

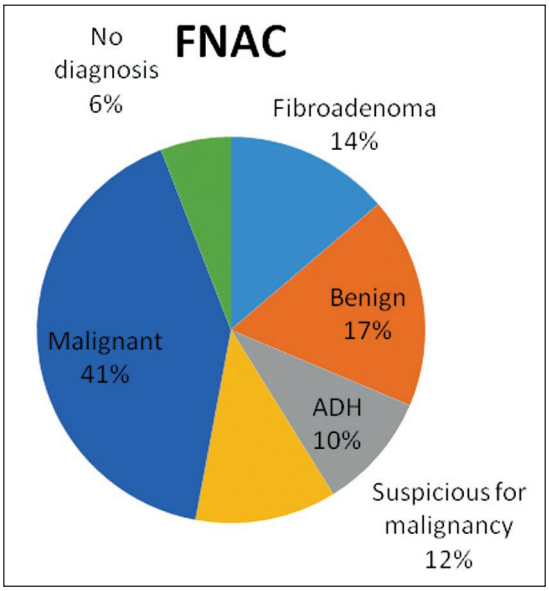


Fig. 4: Histopathology diagnosis in percentage.

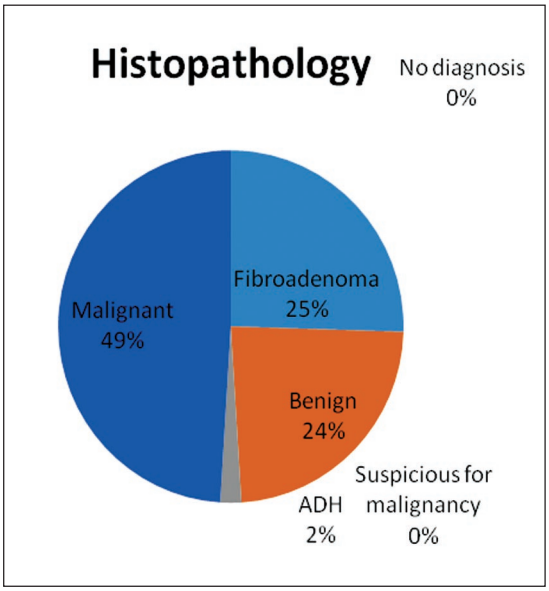
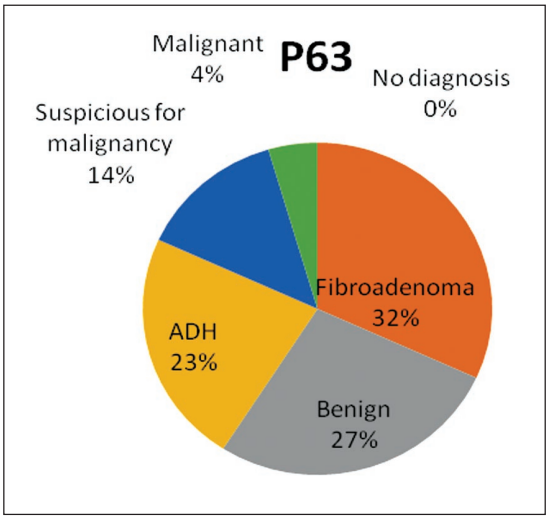


Fig. 5: p63 positivity in percentage according to FNAC diagnosis.



RELATED STEPS

Deparaffinization: This step required for cell block only. Warm xylene is used for this purpose. Two changes required. Each change required 5 minutes.

Rehydration through descending alcohol series (Fresh absolute ethanol, 95% ethanol, 70% ethanol, 30% ethanol and distilled water) for 5 minutes for each step.

Peroxidases block*:* Peroxidase blocking reagent was placed onto the slides and incubated for 20 minutes in the humid chamber; then washing in phosphate buffer saline. Slides are drained and blotted.

Power block*:* Power blocking reagent was then placed to slides and incubated for 10 min. Slides werecleaned by blotting. No wash by PBS buffer.

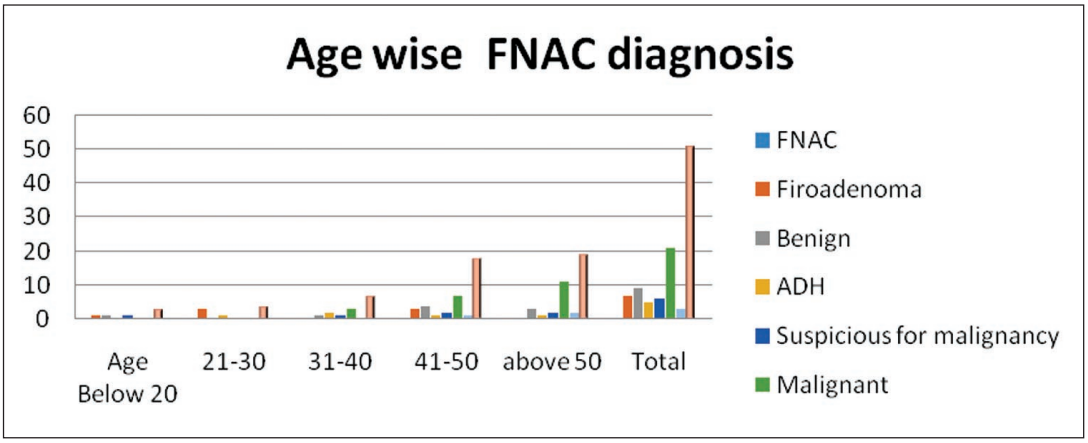
Primary antibody: Primary antibody was placed onto the sections and incubated in the humid chamber at 37^0C for 15 minutes then we leave the humid chamber for one hour. Then we place the slides in fresh buffer bath for 5 minutes.Drain and blot gently.

Super enhancer/ amplifier were applied on to the slides and were incubated for 30 mins. Slides were rinsed with PBS buffer.

Table 2: Age wise FNAC diagnosis.

Age	Below 20	21-30	31-40	41-50	Above 50	Total
Firoadenoma	01	03	0	03	00	07
Benign	01	00	01	04	03	09
ADH	00	01	02	01	01	05
Suspicious for malignancy	01	00	01	02	02	06
Malignant	0	00	03	07	11	21
No diagnosis	00	00	00	01	02	03
Total	03	04	07	18	19	51

Fig. 6: Age wise FNAC diagnosis



Secondary (biotinylated link) antibody were applied to cover the specimen and slides were incubated for 1 hour at 37^{0}C in humid chamber. The slides were rinsed with tris phosphate buffer solution and then drained and blotted gently.

Substrate-chromogen solution*:* we applied enough drops of diamino benzedine (DAB) substrate chromogen solution in dark field. Substrate chromogen solution was prepared freshly in each run by adding the substrate drops in graduated tube until 1ml then we add one drop of chromogen. The slides then are put in the humid chamber for 10 minutes at 37^{0}C. Rinse gently with distilled water.

Counter staining:

Slides are immersed in the Mayer's haematoxylin for about 10 seconds then slides are rinsed with slowly running tap water. Slides are then immersed in distilled water for 3 minutes. The slides are drained and blotted and left to dry in air. Then mount the slides with DPX.

Retrospective cases :

FNAC cases were selected and

Fig. 7: Age wise distribution of benign lesions in FNAC.

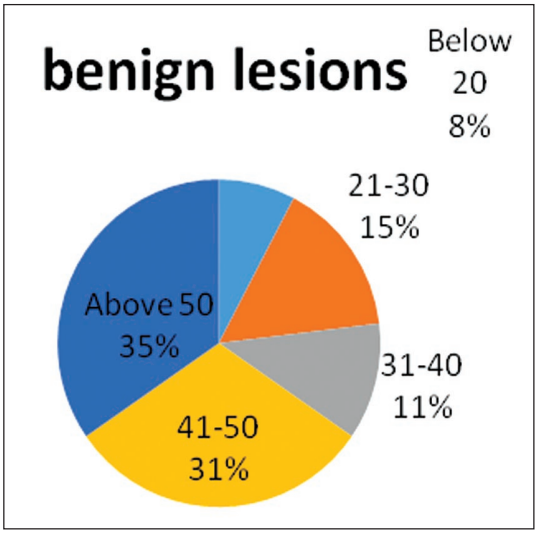
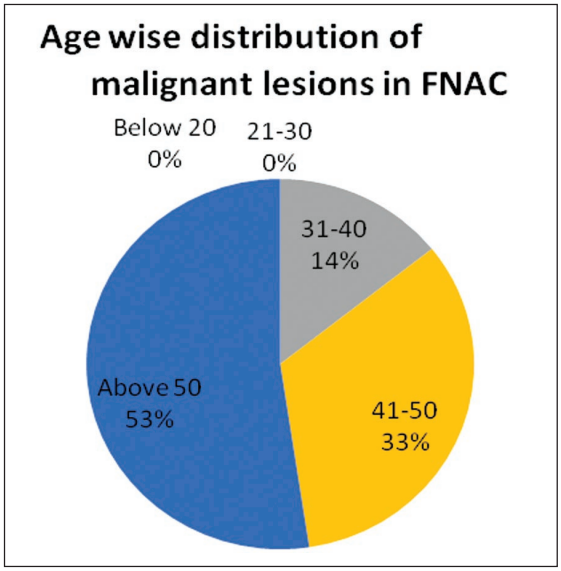


Fig. 8: Age wise distribution of malignant lesions in FNAC.



Immunocytochemical studies were performed following destaining of prevously stained slides by immersion in 1% acid alcohol (prepared by adding 1 ml of HCL to every 100 ml of 70%of alcohol) for 10-30 min. then we proceed in the same above protocol.

All the slides were examined under light microscope. A positive immunocytochemical reaction will appear as a brownish discoloration of myoepithelial cells**.**

Only cells those show distinctive nuclear immunoreactivity for p63 will be considered positive. Cytoplasmic and membranous staining will be considered as non specific. Comparative analysis between conventional stained smear and p63 stained smear and other diagnostic tools like core biopsy, surgically excised specimen would be performed. The strength of agreement with final histological diagnosis will be measured by appropriate statistical analysis.

A valid consent was obtained from all patient/ guardian before enrolling them into the study.

RESULTS AND DISCUSSION

Final histological diagnosis includes 25 benign, 01 ADH and 25 malignant lesions. In FNAC diagnosis, there were 16 benign, 05 ADH, 06 suspicious for malignancy, 03 with no definite

Table 3: Age wise histopathological diagnosis.

Age	Below 20	21-30	31-40	41-50	Above 50	Total
Benign	03	04	04	06	09	26
Malignant	00	00	04	09	12	25
Total	03	04	08	15	21	51

Fig. 9: Age wise histopathological diagnosis.

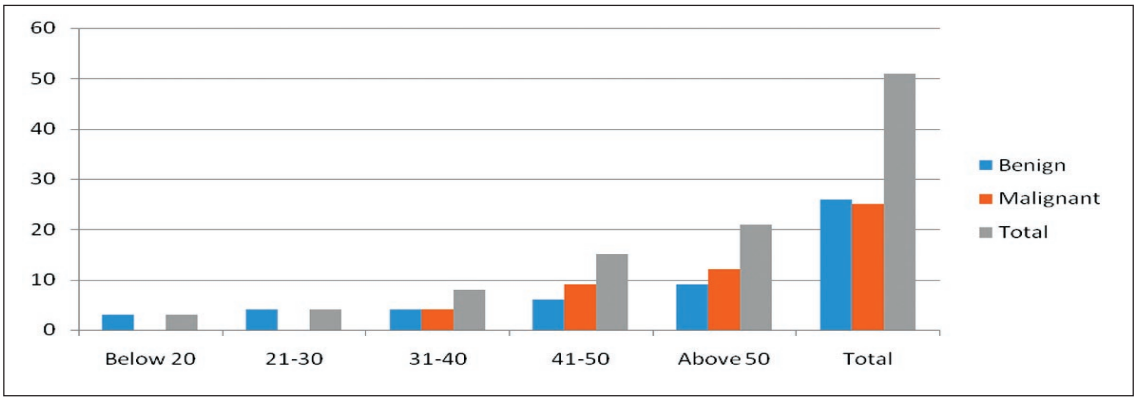


Fig. 10: Age wise distributions of benign lesions in histopathology.

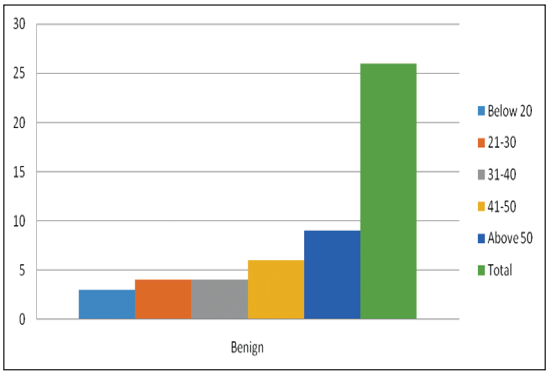
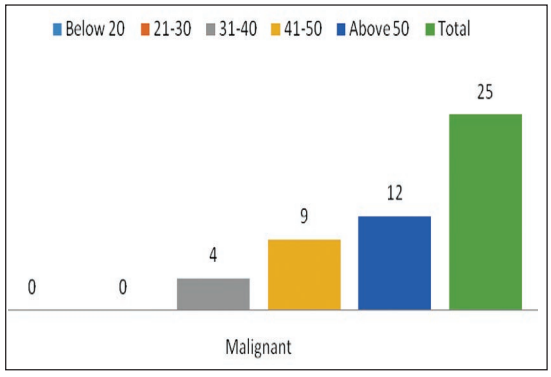


Fig. 11: Age wise distributions of malignant lesions in histopathology.



diagnosis and 21 malignant lesions. In IHC 22 cases were found positive for p63 (Table 1).

Fig 1. shows FNAC with different diagnosis and without diagnosis. Malignant cases are more than benign in this study population.

The Fig.2 shows every cases with a diagnosis. Malignant cases were the highest among different diagnosis. The pie chart shows there were 6% cases without diagnosis (Fig.3).

Fig.4 shows malignant cases were 49% of total samples.25% cases were fibroadenoma. Only 02% patient had atypical ductal hyperplasia. Others were benign lesions with different types of diagnosis.

The pie chart shows fibroadenoma and benign lesions were mostly positive in p63 immuno-staining (Fig.5).

Fig.6 shows breast lesions are increasing with advancing age.

It was observed that most of the benign lesions(66%) found above 40 yrs. (Fig.7).

The age of 53% cases with malignant lesion were above 50 yrs. Above 40 yrs, malignant cases were 86%. No malignant cases were found below 30 yrs (Table 2 and Fig. 6,7 and 8).

The table no. 3 revealed Chi-Sq = 4.249, DF = 1, P-Value = 0.039.

Table 4: Sex wise distribution.

Final Diagnosis	Male	Female	Total
Benign	04	22	26
Malignant	03	22	25
Total	07	44	51

In Fig. 9, it appars that most of benign and malignant lesions occur above 40 yrs. No malignant case found below 30 yrs of age.

It appears that benign lesions increases with advancing age (Table 10.).

It appears from the study that most of the malignant lesions occur in the age group of above 40 years and cases were mostly above 50 yrs. No case was found below 30 yrs of age (Fig.11).

As expected, our study result showed that breast lesions are commonly found in female (Table 4 and Fig.12) and only 14% cases were male (Fig. 13) .

Fig. 13: Sex wise distribution of different lesions.

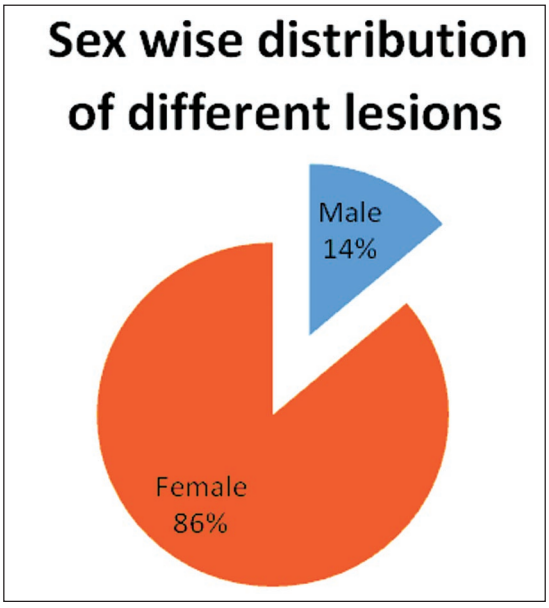
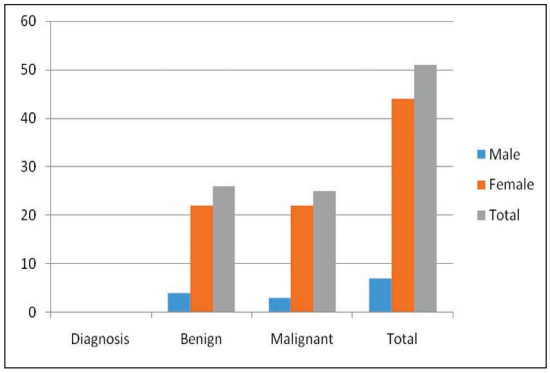


Fig. 12: Sex wise distribution benign and malignant lesions.



To observe percentage of p63+ clusters is associated FNAC diagnosis, we found that the p-value of the test 0.000 (Table 5)

Most of the benign lesions are positive for p63. There were 69% cases found positive (Fig. 14) and 84% malignant cases were p63 negative (Fig. 15).

A total of 24 cases with myoepithelial cells were seen in FNAC out of 51 cases. 22 cases were positive for p63. 29 cases were found p63 negative.91.67% cases with myoepithelial cells were positive for p63. In benign cases, p63 positivity were 94.74%. In malignant cases with myoepithelial cells, the p63 positivity were 80%. Positive predictive value of p63 in benign lesions was 69.2% and in malignant lesions was 16%. No significant difference was observed between p63 and myoepithelial cells

Table 5: Percentage of p63+ Clusters in FNAC according to final Diagnosis

Final	P63+ clusters(%) according to FNAC				
Diagnosis	0	1-24	25-49	50-74	75-100
Benign	08	04	06	08	00
Malignant	21	02	01	01	00

Fig 14: p63+ Clusters According to FNAC diagnosis.

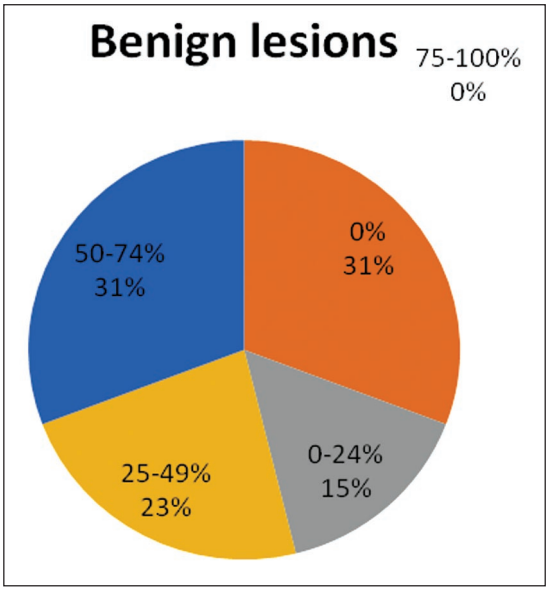


Fig. 15: p63+ Clusters According to FNAC diagnosis.

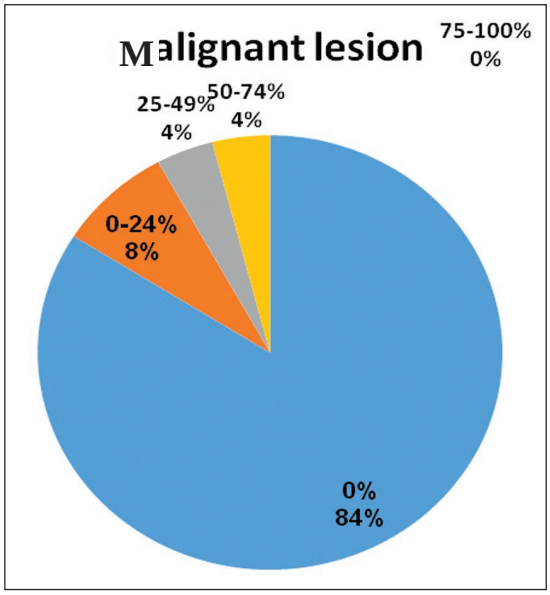


Table 6: Myoepithelial cells & p63.

FNAC Diagnosis	Myoepithelial cells present	P63 positive	P63 negative	Total
Benign	19	18 (94.73%)	6	24
Malignant	5	4 (80%)	23	27
Total	2 4	22 (91.67%)	2 9	51

identification in FNAC (p value 0.844, Z=0.20, Standerd error of difference =0.0985). The sensitivity of p63 in our study was 80.77% and specificity was 96% (Table 6).

Bar diagram of Fig.16 shows p63 was informative in benign as well as malignant lesions.

A lump in the breast is a common complaint presenting in the surgery out-patient clinic of all major hospital, with anxiety regarding possible malignancy being extremely common.

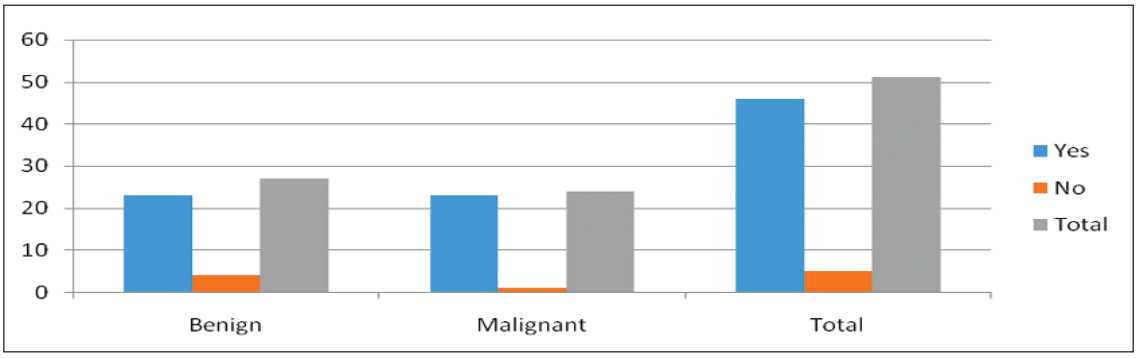
Considering Patients' comfort, low cost, simplicity of the method, lack of requirement of anesthesia, rapid analysis and reporting, and an accurate diagnosis of various benign and malignant breast lesion with high sensitivity and specificity makes fine needle aspiration cytology an ideal initial diagnostic modality in breast lumps.The included cases were diagnosed cytologically as atypical 05 cases (9.8%), and suspicious 06 cases (11.76%) (Table 1).

However, this result cannot be considered as a good representative index of the true

Table 7: Numbers of Specimens for which p63 staining was informative by final diagnosis.

P63 Staining Informative	Benign	Malignant	Total
Yes	23	23	46
No	03	2	5
Total	26	25	51

Fig. 16: Numbers of specimens for which p63 staining was informative by final diagnosis.



frequency of the two categories as the cases were selected. For the same reason, the combined incidence of the inconclusive cytologic diagnosis among the total breast aspirates during the studied time interval can not be reported and so it was not detected whether these categories are being underused or overused in our institute.

In routine cytologic preparations, the precise identification of myoepithelial cells plays a major role in the diagnostic assessment of several types of breast lesions. These cells are a constituent of the normal basal layer of the breast lobules and ducts and usually are lost during malignant progression (Bondeson *et al.*, 1997).

However, identification of myoepithelial cells in breast biopsies and FNAB specimens sometimes is difficult using Papanicolaou-stained or Giemsa-stained preparations (Reis Filho *et al.*, 2002a).

Based on their biphenotypic (epithelial and smooth muscle-like) properties (Lazard *et al.*, 1993) several antibodies directed against myoepithelial cells have been raised.

These target either smooth muscle-related antigens smooth muscle actin,S-100, calponin, h-caldesmon, and smooth muscle myosin heavy chain) or cytokeratins that are expressed specifically by basal/myoepithelial cells (cytokeratin 5/6, cytokeratin 14, and cytokeratins that are recognized by the antibody (34βE12) (Reis Filho *et al.*, 2002b). Currently, several investigations mitigate against using S-100 protein, because it has a high sensitivity but a very low specificity for myoepithelial cells (Dabbs *et al.*, 1999, Mosunjac *et al.*, 2000).

Most of the smooth muscle-related antibodies, such as smooth muscle actin, calponin, h-caldesmon, and smooth muscle myosin heavy chain, lack specificity for myoepithelial cells, because they cross react with breast stromal cells and myofibroblasts as well as with neoplastic cells (Yaziji *et al.*, 2000). Basal layer specific cytokeratins have a low sensitivity for myoepithelial cells (Pattari *et al.*,

Fig. 17: Breast FNAC smear showing epithelial cells as well as myoepithelial cells in LG stain.

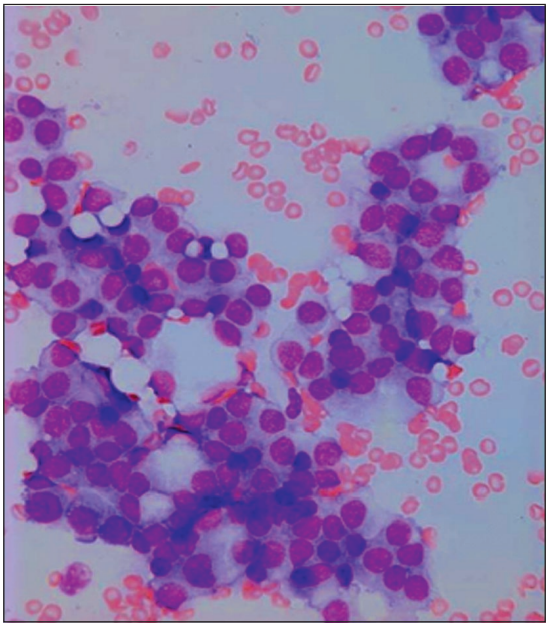
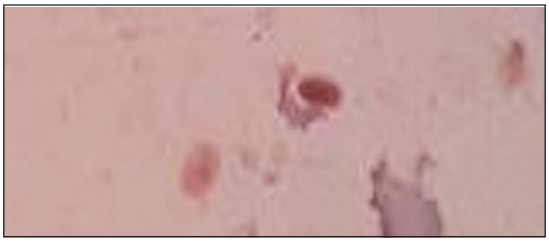


Fig. 19: Myoepithelial cell stained with p63 at centre. Dark nuclear staining is characteristic and faint cytoplasmic staining were considered as negative.



2008) mainly for those located in the lobules, and also stain a variable proportion of breast carcinomas (Raju *et al.*, 1990). The recently characterized p53 homolog, p63, is expressed consistently in the basal cell population of several types of stratified epithelia (Barbareschi *et al.*, 2001).

The p63 gene is located on 3q27 and encodes at least six different isoforms, three with a

Fig. 18: Breast FNAC smear show ductal epithelial cells in Pap stain.

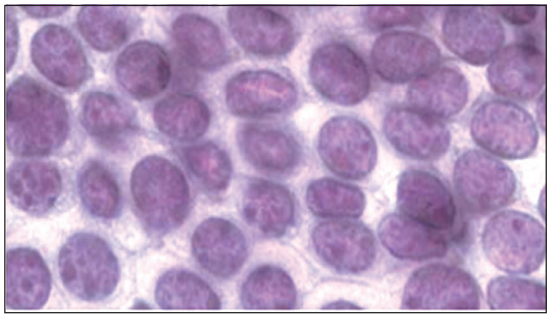
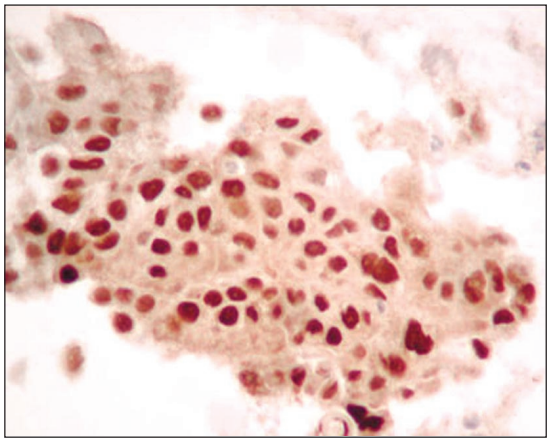


Fig. 20: p63 staining in cell block preparations.



transactivating (TA) N-terminal domain and three dominant negative (∂N) isoforms that lack the N-terminal TA domain (Moinfar *et al.*, 1999). The N-p63 isoforms may participate in an alternative mechanism to overcome p53-related cell cycle arrest and apoptosis, thus constituting an efficient mechanism to maintain a basal cell population **(**Chu and Weiss 2001, Jorge *et al.*, 2003)

In breast FNAB samples, p63 is expressed selectively by myoepithelial cells, for which it is a very reliable marker. It is interesting to note that the consistent expression of p63 in naked nuclei (NN) of benign breast FNAB samples

Fig. 21: Breast FNAC showing fibrocystic changes.

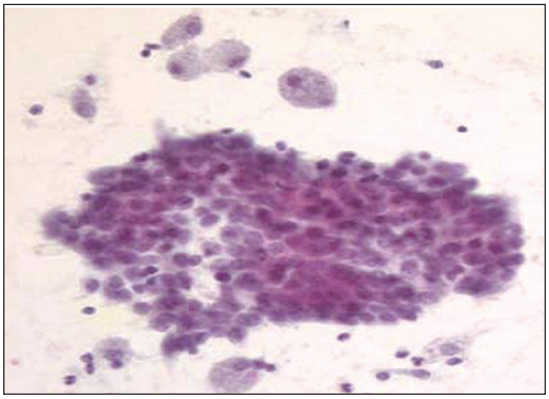


Fig. 22: Breast FNAC of fibroadenoma.

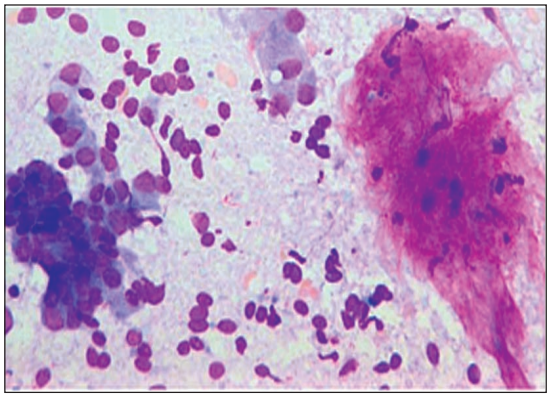


Fig. 23: Breast FNAC of ductal carcinoma.

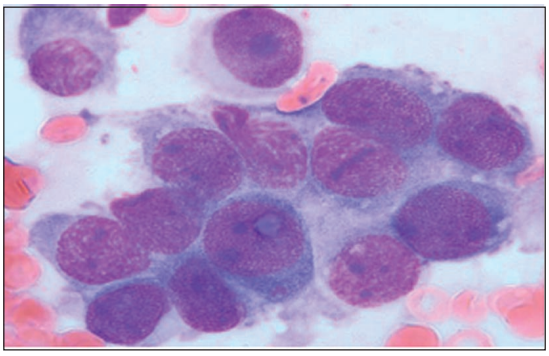


Fig. 24: Breast FNAC showing microcalcification.

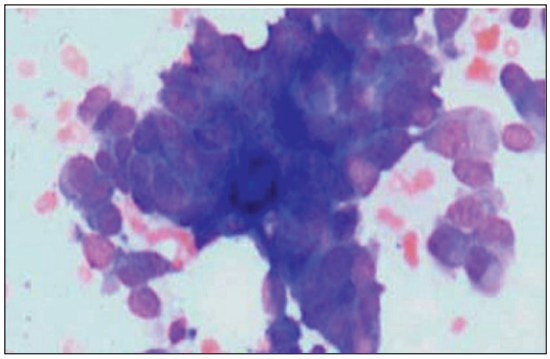
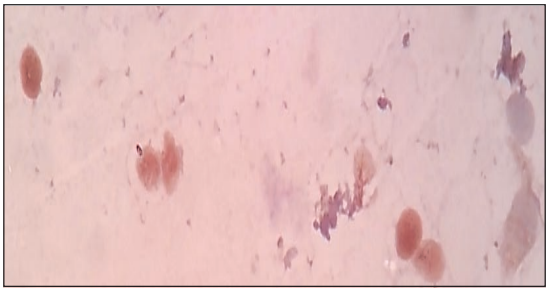


Fig. 25: Breast FNAC shows p63 positive myoepithelial cells (dark brown) and p63 negative ductal epithelial cells(Rt side).



favors a myoepithelial origin for these cells (Barbareschi *et al.*, 2001)

NN have been described in fibroadenomas, Phyllodes tumor, fibrocystic change etc. In all of the fibroadenoma lesions analyzed herein, we observed p63 positive NN admixed with sheets of epithelial cells. The results are in accordance with early reports (Tsuchiya *et al.*, 1987; Zajicek *et al.*, 1970, Mosunjac *et al.*, 2000) suggesting that NN have a myoepithelial origin and with the preliminary results of Barbareschi and colleagues (Barbareschi *et al.*, 2001).

Their designation suggests that NN do not have intact cytoplasm. Hence,we do not expect these nuclei to react with cytoplasmic markers of smooth muscle differentiation (Dabbs *et al.*, 1999).

The presence of myoepithelial cells

Fig. 26: Picture of p63 in core biopsy.

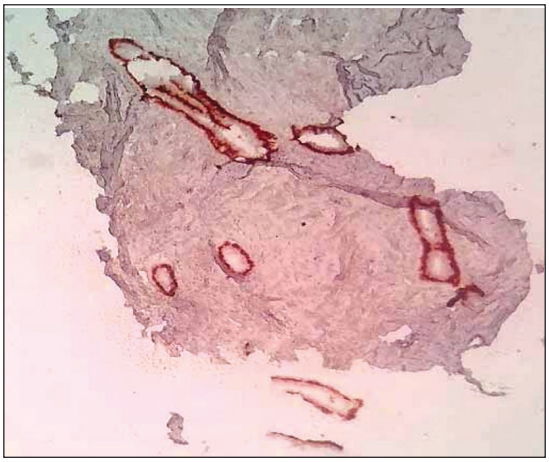
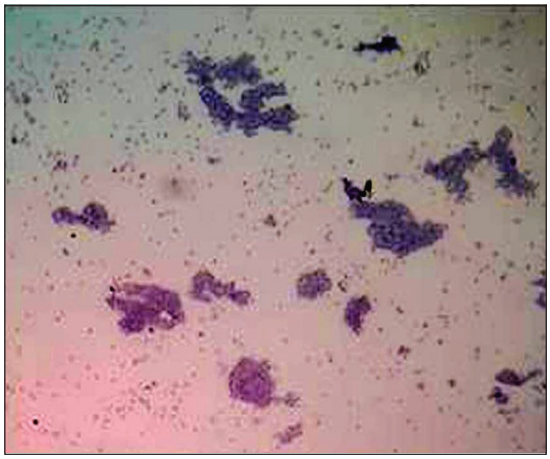


Fig. 27: Picture of p63 negative in FNAC smear.



overlying malignant cell clusters has been suggested as a very specific indicator of an in situ component (McKee *et al.*, 2001). Despite its high prevalence in DCIS samples and DCIS plus IC samples, p63 positive myoepithelial cells also were observed in 56.25% of pure IC samples. Thus, based on our findings, the presence of p63 positive myoepithelial cells should not be used as a specific criterion to rule out the presence of an invasive component.

Another important aspect of p63 immunocytochemistry is that, whereas some markers show strong background staining or even aberrant nuclear immunoreactivity in cytologic samples (Mosunjac *et al.*, 2000). p63 showed strong immunoreactivity that was confined to the nuclei of myoepithelial cells. In only three lesions, the background was so high that it impaired evaluation of the cytologic preparation.

Most importantly, the major surprising and interesting finding of p63 immunoreactivity in cytologic samples is the presence of p63 positive malignant cells.

In the current study, we observed p63 positive malignant cells (1–25%) in 2 specimens (8%) and frequent positive cells (more than 25%) in 2 specimen (8%). Total 24 cases with myoepithelial cells were seen in FNAC out of 51 cases. 22 cases were positive for p63. 29 cases were found p63 negative. Overall 91.67% cases with myoepithelial cells were positive for p63. In benign cases p63 positivity was 94.74%. In malignant cases with myoepithelial cells, the p63 positivity was 80%. It should be noted that, in all but one specimen, the majority of neoplastic cells were p63 negative, and a correct diagnosis of malignancy would obviously be achieved. It is interesting to note that we have demonstrated, along with others, (Barbareschi *et al.*, 2001, Werling *et al.*, 2003) that p63 is expressed consistently in up to 43.13% of breast aspiration samples, independent of their morphologic appearance. In addition, several lines of evidence support the finding that up to 18% of high-grade, invasive ductal carcinomas of the breast show myoepithelial -like or basal-like differentiation (Barbareschi *et al.*, 2001, Jones *et al.*, 2001, Polyak *et al.*, 2005).

Thus, one possible explanation for the expression of p63 in malignant cells may reflect an aberrant or partial myoepithelial-like or basal-like differentiation (Barbareschi *et al.*, 2001, Jones *et al.*, 2001, Polyak *et al.*, 2005).

It was recently reported that the p63 antibody is useful for staining mammary myoepithelial cells due to its selective properties as a nuclear staining antibody which does not stain fibroblasts, the cells of the vascular walls (Douglas-Jones *et al.*, 2005).

A positive predictive value of p63 in benign lesions was found 69.2% and in malignant lesions it was found 14.8%. The sensitivity of p63 was 80.77% and specificity was 96%.

It is also noted that we did not investigate DCIS in this study separately. It was reported that DCIS positively stained for p63 and this said characteristic is useful for distinguishing DCIS from invasive carcinoma. We found only two cases with DCIS component. Thus, future studies must also include p63 staining of DCIS specimens also.

The increasing use of FNAC and core biopsy for non-operative diagnosis and the nature of lesions identified mammographically can result in diagnostic difficulties. Interpretation may be helped by the use of immunohistochemistry used to detect basal/ myoepithelial markers.

The myoepithelial cell has traditionally been distinguished from luminal epithelial cells by the presence of smooth muscle fibres. However, many more proteins have now been identified that are expressed in myoepithelial cells and these fall into three groups: smooth muscle related; cytokeratins; others like S100,CD10,P cadherin, P-63-P53 homologue etc.

The diagnostic areas are radial scar vs tubular carcinoma, papillary lesions, epithelial hyperplasia with nuclear atypia, fibroadenoma, regenerative epithelial atypia, atypia of ductal epithelium incysts, hyperplasia vs atypia vs ductal carcinoma in situ, non-invasive vs invasive carcinoma, lobular carcinoma and small cell, basal-like carcinomas, myoepithelial tumours.

Our study cases included 51 clinical cases and 51 aspirations and 51 surgical excision specimens that fulfilled the inclusion criteria of the study in the time period of February, 2012 to October, 2013.

CONCLUSION

1) p63 is a reliable nuclear marker of myoepithelial cells in the breast and may be used to distinguish these cells from their mimics in FNABs.

2) Benign lesions usually contained p63 positive myoepithelial cells, and we demonstrated it is a useful marker for highlighting these cells.

3) Malignant, p63 positive cells were observed frequently in samples of DCIS and IDC, although, based on careful cytomorphologic evaluation, they may have been classified correctly as malignant cells.

4) Hence, based on previously published data and on our findings, we advocate that anti-p63 antibodies may be used to identify myoepithelial cells as well as to overcome the cytomorphologic distortion of myoepithelial cells in FNABs of the breast. However, by no means may the evaluation of p63 staining preclude a careful search for classic cytopathologic criteria to rule in or rule out a diagnosis of malignancy.

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