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The Role of P63 Immunocytochemistry for Myoepithelial Cells in the Diagnosis of Atypical and Suspicious Cases in Breast Fine Needle Aspiration Cytology (FNAC)

NESREEN H. HAFEZ, M.D. and NEVEEN S. TAHOUN, M.D.

The Department of Pathology, Cytopathology Unit, National Cancer Institute (NCI), Cairo University

ABSTRACT

Purpose: Evaluation of the usefulness of p63 immunocytochemical marker for myoepithelial cells in the diagnosis of atypical and suspicious lesions in breast cytology.

Patients and Methods: This is a retrospective study on 122 selected patients presented at Cytology Unit, Pathology Department, NCI, Cairo University, in three years interval from 2007 to 2009, with breast lumps who underwent preoperative FNAC and diagnosed cytologically as 'atypical or suspicious breast lesion for biopsy' then they were followed by excisional biopsy for histopathologic assessment that was considered as the golden standard diagnosis against which FNAC diagnoses were compared. Paucicellular cytologic slides as well as cases with no corresponding final histopathological diagnosis were excluded. The destained cytologic slides were subjected to p63 immunocytochemical staining. Only the nuclear immunoreactivity for p63 was considered specific, cytoplasmic and membranous staining was considered nonspecific. The stained slides with p63 marker were quantified according to the percentages of positive epithelial cell clusters and positive single bare nuclei in the background. The immunocytochemical results were compared with histopathologic diagnoses.

Results: Of the 122 studied breast aspirates, 84 cases with atypical findings and 38 cases with suspicious findings were included. The two categories yielded malignant diagnoses in 53 cases (63.1%) and 31 cases (81.6%), respectively. Invasive duct carcinoma was the most common malignant diagnosis in both categories. The most common benign diagnosis in the atypical group was fibrocystic changes (48.4%), while atypical ductal hyperplasia was the most common non-malignant diagnosis in the suspicious group (42.8%). P63 consistently stained the nuclei of myoepithelial cells, either overlying clusters and/or single bare nuclei. Of the histologically confirmed malignant cases 69% and 91.7% showed no p63 nuclear staining in cell clusters or bare nuclei, respectively; while 8 cases showed staining pattern similar to that of benign lesions. On the other hand, 84.2% and 57.9% of the benign cases showed staining in more than 75% of the clusters and bare nuclei, respectively. The staining pattern of p63 was significantly different between malignant and benign lesions (p-value <0.005). The p63 sensitivity, specificity, positive, and negative predictive value were 90.5%, 84.2%, 92.7%, and 80%, respectively. Scattered p63 positive ductal cells (<10% of duct cells) were detected in 6% of all malignant cases.

Conclusion: The p63 was a reliable nuclear marker of myoepithelial cells in breast cytology. Benign and malignant breast lesions showed significantly different staining pattern for p63 on inconclusive breast cytology. The diagnostic sensitivity, specificity, positive and negative predictive value of p63 marker were 90.5%, 84.2%, 92.7%, and 80% respectively. The p63 immunostaining may be used as a diagnostic adjunct to the routine fine needle aspiration cytology in cases of breast lesions with atypical and suspicious results.

Key Words: *P63 immunocytochemistry – Breast FNAC – Inconclusive diagnoses.*

INTRODUCTION

Fine needle aspiration cytology (FNAC) is an important, cheap, simple, and acceptable tool for rapid and accurate diagnosis of various benign and malignant breast lesions with high sensitivity and specificity **[1]**. Breast is one of the organs that are readily accessible for FNAC. Regardless of the growing popularity of stereotactic core needle biopsy of the breast because it has similar negative predictive value when compared with FNAC, FNAC remains one of the methods included in the triple-test approach to breast lesions and cytologic diagnoses are used to tailor the best management for a given patient **[2]**.

Correspondence: Dr Nesreen Hassan Hafez, Cytopathology Unit, NCI, Cairo University, nesreennci@hotmail.com

FNAC is also used to assess prognostically significant pathologic parameters of the breast carcinoma, including nuclear grade, histologic type, proliferative index, and immunocytochemical expression of hormone receptors and p53. Moreover, FNA smears are used for follow-up and monitoring of diseases especially cancers and for early recognition of relapse **[3]**.

However, in certain occasion it is difficult to provide a definitive diagnosis in breast neoplasm with help of FNAC. This is particularly true in proliferative breast disease, papillary breast tumors, and certain types of well differentiated carcinoma such as tubular carcinoma. Also it is difficult to differentiate ductal carcinoma in situ from invasive carcinoma in cytologic aspirates **[4]**.

The "more information with less material" is the challenge that faces the cytologist and pathologist. This challenge is the result of the current treatment strategies and surgical approaches that provide a conservative treatment for the benign and pre-invasive breast lesions and more aggressive surgical treatment or even submission of neoadjuvant chemotherapy for malignant lesions **[5]**.

The presence of myoepithelial cells has long been recognized as a prominent feature of benign lesions and they considered the key to distinguish benign from malignant neoplasms. Thus, the identification of them in cytologic smears is of particular diagnostic value because they are retained in most benign breast lesions while being lost in malignancy **[6]**. Myoepithelial cells are identified in the cytologic smears as small, oval, and sometimes curved bipolar cells with smudged chromatin and stripped cytoplasm that may be either adherent to epithelial cell clusters or appear singly. However, the correct identification of myoepithelial cells in cytology is sometimes difficult because they might be confused with apoptotic cells, stromal cells, epithelioid histiocytes, or even cancer cells with spindle phenotype **[7]**.

Several myoepithelial immunocytochemical markers are currently available to demonstrate the presence of myoepithelial cells in cytologic aspirates. Smooth muscle myosin heavy chain (SMMHC), calponin, and h-caldesmone are utilized to highlight myoepithelium. S-100 protein and specific cytokeratins (keratin 5, 7, 14, and 17) also stain myoepithelial cells but the staining is not specific and is not optimally sensitive. Maspin and CD10 are also considered as markers for myoepithelial cells **[8]**.

Recently p63, a p53 homologue, has been characterized as a reliable marker of myoepithelial cells of the breast **[5]**. Preliminary studies indicated that p63 might be better than other conventional myoepithelial cell markers because it decorates the nuclei of myoepithelial cells, thereby overcoming the cytoplasmic fragility of myoepithelial cells in fine needle aspirate. This means that p63 overcomes the problem of the other myoepithelial cell markers that decorate either the cytoplasm or cytoplasm and nucleus of the myoepithelial cells **[9]**.

The current study has been assigned to demonstrate the usefulness of myoepithelial cells overlying the atypical or suspicious epithelial cell clusters in the breast fine needle aspiration smears to differentiate the benign from malignant breast lesions using a nuclear myoepithelial cell marker, p63, a p53 homologue nuclear transcription factor. Based on the fact that the cytomorphologic identification of myoepithelial cells in the breast aspirate may be difficult.

PATIENTS AND METHODS

In the current study, histologically confirmed 122 selected cases of modified Papanicolaou stained smears of breast lumps aspirates, that were diagnosed cytologically as 'atypical or suspicious breast lesion for biopsy', were retrieved retrospectively from Cytopathology Unit, Pathology Department, National Cancer Institute, Cairo University in the three year interval from 2007 to 2009.

The slides were revised to determine the cytomorphological criteria. These criteria included cellularity, background, pattern, and nuclear features. The most representative Papanicolaou-stained cytology slides were chosen for the immunocytochemical staining. Paucicellular slides as well as cases with no corresponding final histopathological diagnosis were excluded from the current study. The slides were destained using the technique described by Miller and Kubier **[10]**. The destained slides were subjected to p63 immunocytochemical staining according to the streptavidin-biotin-peroxidase technique using the mouse mono-

clonal antibody 4A4 raised against p63 (Clone 63P02; Neomarkers, Freemont, CA).

Histologic section of a sclerosing adenosis with myoepithelial hyperplasia is used as positive control, and negative control used by substituting phosphate buffer saline (PBS) for the primary and secondary antibodies. Both were included in each slide run. All controls yielded appropriate results.

Only the nuclear immunoreactivity for p63 was considered specific, cytoplasmic and membranous staining was considered nonspecific. The two pathologists independently evaluated the presence of p63 positive cells overlaying the atypical or the suspicious cell clusters as well as the presence of the positive cells in the background (naked nuclei). The evaluation of p63 stained cells was done blindly, without knowledge of the final histopathological diagnoses of the excised specimens.

All the epithelial clusters were examined on each slides for the presence of nuclear staining for p63 (Myoepithelial cells) and the percentage of cell clusters containing p63 nuclei was scored. The positive p63 single bare nuclei were also detected in the background and the percentage of these positive cells was scored. Then the final histopathological diagnoses of the included cases were reviewed. The immunocytochemical results were compared with these final histologic diagnoses.

Sensitivity for the presence of malignancy (true positive/true positive + false negative), specificity for absence of malignancy (true negative/true negative + false positive), positive predictive value (PPV) for the probability that the patient with positive test has, in fact, the disease in question (true positive/true positive + false positive), and negative predictive value (NPV) for the probability of a patient with a negative test not having the disease in question (true negative/true negative + false negative) were calculated and compared with other studies.

RESULTS

One hundred twenty two selected breast aspirates were reviewed from the years 2007-2009 that were reported as being 'atypical or suspicious breast lesions for biopsy' and for which histologic follow-up data were available. Of those, 84 with atypical findings and 38 with suspicious findings were included in the study (Table 1).

Among the atypical and suspicious FNA diagnoses, 53 (63.1%) and 31 (81.6%) samples, respectively, yielded malignant diagnoses on histologic examination. In both groups, invasive duct carcinoma was the most common malignancy (Table 2 & Fig. 1). On the other hand, 31 (36.9%) atypical aspirates and 7 (18.4%) suspicious aspirates yielded benign findings on histologic examination. Fibrocystic change was the most common benign diagnosis in the atypical group (Table 2). While atypical ductal hyperplasia was the most common one in the suspicious group (Table 2).

Cytological analysis of p63 stained slides showed a distinctive nuclear staining pattern in the myoepithelial cells, a faint to moderate background staining was seen in 23 cases, although this did not impair the evaluation of the slides. A remarkable feature seen in the present study was the presence of scattered p63 positive duct cells in 5 included cases (<10% of duct cells). These cases were two invasive duct carcinoma, two duct carcinoma in situ (Fig. 6), and one invasive lobular carcinoma.

Of the 84 histologically confirmed malignant lesions, 58 cases (69.8%) showed no p63 nuclear staining in the epithelial cell clusters, whereas 32 cases (84.2%) of the 38 histologically proven benign cases showed p63 nuclear staining in more than 75% of the epithelial cell clusters (Table 3). Eighteen malignant cases (21.4%) showed focal p63 staining in 25% or less of the examined cell clusters, while the 8 malignant lesions that showed p63 staining in 25-75% of the cell clusters proved histologically to be 7 cases of duct carcinoma in situ and one case of invasive duct carcinoma. This case of invasive duct carcinoma showed 65% positivity for p63 in the examined epithelial cell clusters. This case also showed positivity in scattered duct cells. The only benign lesion that showed no staining for p63 in any of the epithelial cell clusters proved histologically to be atypical ductal hyperplasia (Table 3). The difference in the percentage of p63 positive epithelial cell clusters between benign and malignant lesions was statistically significant (p-value <0.005).

Of the 84 histologically confirmed malignant lesions, 77 cases (91.7%) did not have any p63 positive single cells in the background (Table 4). The 7 cases (8.3%) that showed fewer than 25% p63 positive single cells were proved to be ductal carcinoma in situ. The invasive duct carcinoma that stained for p63 in 65% of the examined epithelial cell clusters showed no p63 staining in the single bare nuclei. The histologically proven benign lesions showed the whole spectrum of p63 single cell staining ranging from no staining to more than 75%. The 4 cases (10.5%) that showed no p63 positive single cell staining included 3 cases of chronic non specific inflammation and one case of atypical ductal hyperplasia. The 2 cases that showed 1-25% p63 positive staining included one case of fibroadenoma and one case of fibrocystic change (Table 4). The difference in the percentage of p63 positive single cells in the background between the benign and the malignant lesions was statistically significant (p-value <0.005).

Table (1): Relation between histopathologic and cytologic diagnoses of 122 cases of atypical and suspicious breast lesions.

Cytology / Pathology	Atypical (n = 84)	Suspicious (n = 38)	Total (n = 122)
Malignant	53 (63.1%)	31 (81.6)	84
Benign	31 (36.9%)	7 (18.4%)	38

Table (2): The corresponding histopathologic and cytologic diagnoses.

Pathology diagnoses / Cytology diagnoses	Atypical	Suspicious	Total
Malignant lesions:	53	31	84
IDC	44 (83%)	26 (83.9%)	70
DCIS	3 (5.7%)	4 (12.9%)	7
ILC	3 (5.7%)	0	3
IPC	2 (3.8%)	0	2
Tubular carcinoma	1 (1.9%)	0	1
Mucinous carcinoma	0	1 (3.2%)	1
Benign lesions:	31	7	38
FCC	15 (48.4%)	1 (14.3%)	16
FA	8 (25.8%)	1 (14.3%)	9
ADH	4 (12.9%)	3 (42.8%)	7
FDH	2 (6.5%)	0	2
Chronic inflamation	2 (6.5%)	2 (28.6%)	4

IDC : Invasive duct carcinoma.
DCIS : Duct carcinoma in situ.
ILC : Invasive lobular carcinoma.
IPC : Invasive papillary carcinoma.
FCC : Fibrocystic change.
FA : Fibroadenoma.
ADH : Atypical ductal hyperplasia.
FDH : Florid ductal hyperplasia.

Table (3): The percentage of p63+ cell cluster correlated with the histopathologic diagnoses.

Pathologic Diagnoses	% of p63+ cell clusters				
	0%	1-25%	25-50%	50-75%	>75%
Malignant lesions n = 84	58 (69%)	18 (21.4%)	6 (7.1%)	2 (2.4%)	0
Benign lesions n = 38	1 (2.6%)	0	2 (5.3%)	3 (7.9%)	32 (84.2%)

Table (4): The percentage of p63+ single cells in the background (naked nuclei) correlated with the histopathologic diagnoses.

Pathologic Diagnoses	% of p63+ single cells				
	0%	1-25%	25-50%	50-75%	>75%
Malignant lesions n = 84	77 (91.7%)	7 (8.3%)	0	0	0
Benign lesions n = 38	4 (10.5%)	2 (5.3%)	6 (15.8%)	4 (10.5%)	22 (57.9%)

Table (5): Relation between final histopathologic diagnoses and re-classification of the inconclusive cytologic diagnoses according to p63 staining.

Pathologic Diagnoses	Diagnoses according to p63 staining	
	Malignant	Benign
Malignant n = 84	76 (TP) (90.5%)	8 (FN) (9.5%)
Benign n = 38	6 (FP) (15.8%)	32 (TN) (84.2%)

TP: True positive cases. FP: False positive cases.
FN: False negative cases. TN: True negative cases.

Sensitivity (true positive / true positive + false negative), specificity (true negative / true negative + false positive), positive predictive value (true positive / true positive + false positive), negative predictive value (true negative / true negative + false negative).

Table (6): Diagnostic efficacy of p63 staining results on the inconclusive breast cytology.

Statistics	Percentage
Sensitivity	90.5%
Specificity	84.2%
PPV	92.7%
NPV	80%

PPV : Positive predictive value.
NPV: Negative predictive value.

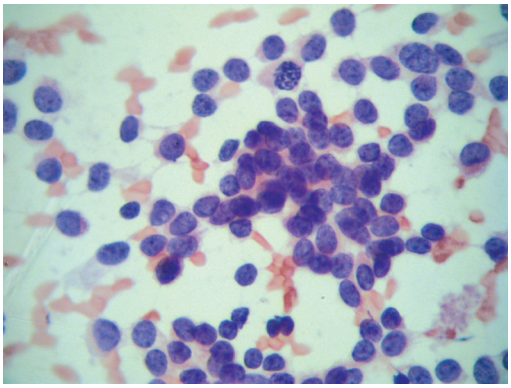

Fig. (1): A case of 65 years old females, presented by breast lump, cytologically diagnosed as atypical and proved histologically as well differentiated duct carcinoma. Smear shows monotonous but discohesive epithelial cells with derranged orientation and attempt to form acini (Papanicolaou stain x 400).

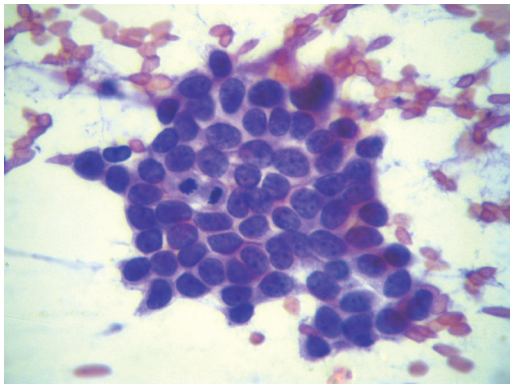

Fig. (2): A case of 48 years old female, presented by breast lump, cytologically diagnosed as suspicious and proved histologically as atypical ductal hyperplasia. Smear shows cohesive monolayered sheet of monotonous epithelial cells with rather high N/C ratio, Mitotic figure is seen in this sheet (Papanicolaou stain x 400).

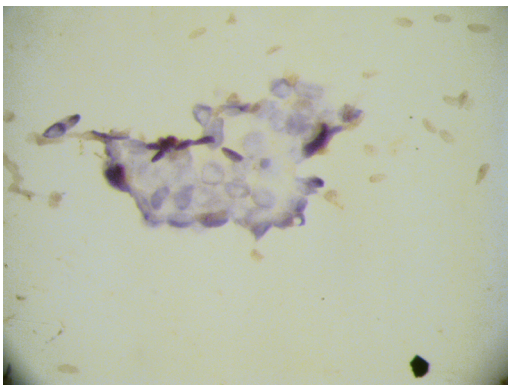

Fig. (3): The p63 immunocytochemical stain highlighting the nuclei of the few myoepithelial cells at the edge of a suspicious epithelial cluster (original magnification x 400).

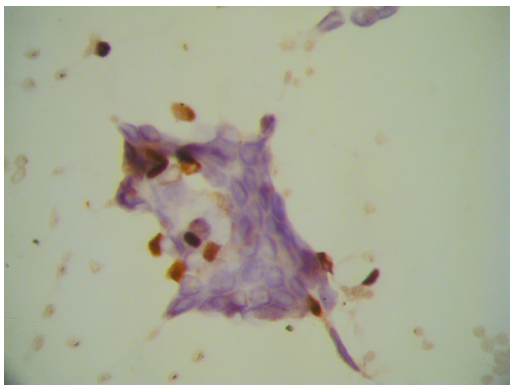

Fig. (4): The p63 immunocytochemical stain decorated the nuclei of the myoepithelial cells overlying a highly suspicious epithelial cluster (original magnification x 400).

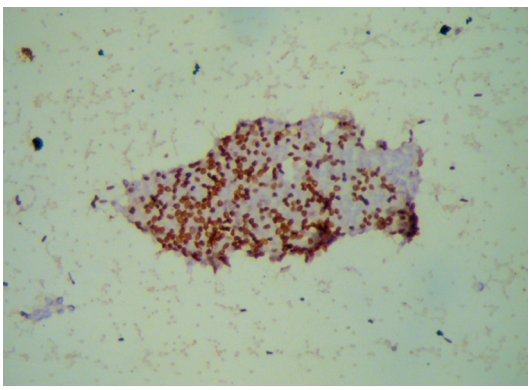

Fig. (5): The p63 immunocytochemical stain decorated the nuclei of many myoepithelial cells overlying a cohesive atypical epithelial cell cluster as well as the naked nuclei in the background (original magnification x 100).

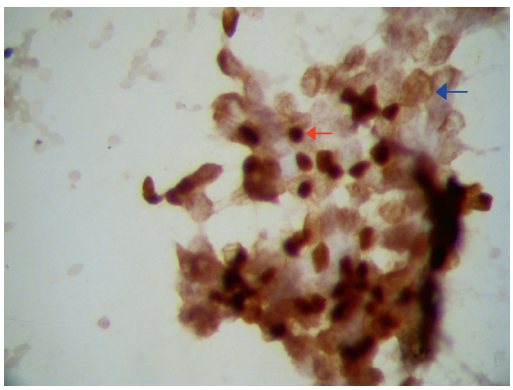

Fig. (6): The p63 immunocytochemical stain decorated the nuclei of the myoepithelial cells overlying a suspicious epithelial cell cluster (red arrow). Note the presence of p63 positive nuclei of ductal cells (blue arrow) (original magnification x 400).

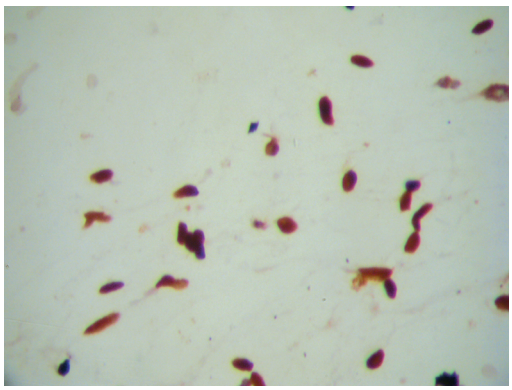

Fig. (7): The p63 immunocytochemical stain decorated all naked nuclei in the background (original magnification x 400).

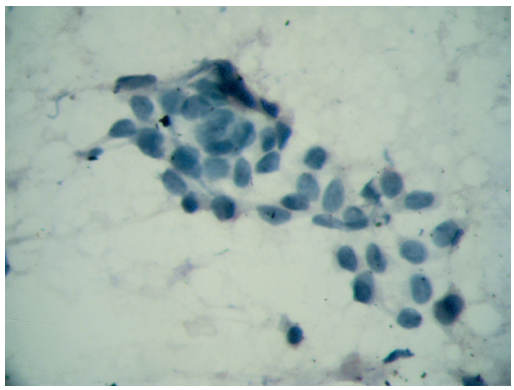

Fig. (8): The p63 immunocytochemical stain showed discohesive epithelial cluster with no p63 positive cells (original magnification x 400).

DISCUSSION

A lump in the breast is a common complaint presenting in the surgery out-patient clinic of all major hospital, with anxiety regarding a possible malignancy being extremely common. Hence a quick diagnosis of a lump in the breast is essential **[11]**. 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 **[12]**. There is no limit to the number of passes; an unsatisfactory aspirate can be easily repeated. It could help in avoiding the diagnostic excisional/incisional biopsy in most patients **[13]**.

The major clinical problem facing the clinician in the surgical management of the breast lump is how extensive a resection should be performed. Currently, the treatment of breast lumps is tailored according to a constellation of clinical, radiological, and cytopathological findings (Triple test). Some studies have demonstrated 100% diagnostic accuracy using this approach **[14]**. Although the majority of breast aspirates can be readily classified as benign or malignant, some aspirates yield equivocal cytologic findings (the gray zone), the cases in which an unequivocal diagnosis of benignity or malignancy cannot be reached based on the cytologic findings due to overlap of their criteria. This is reflected in part by the existence of similar, though smaller, gray zone in the histopathology of breast lesions and wide spectrum of premalignant lesions of the breast **[15]**.

In an effort to standardize the diagnostic terminology for reporting breast fine needle aspiration cytology, the National Cancer Institute (NCI) met in Bethesda, Maryland in 1996 **[16]** and recommended the use of five distinct categories for breast fine needle aspiration cytology diagnosis: Benign, atypical (probably, but not definitively, benign), suspicious (probably malignant), malignant, and unsatisfactory. However, others concluded that the distinction between the atypical and suspicious categories, as recommended by the NCI, is not warranted and would not lower the biopsy rate. Therefore, they suggested the use of single term such as "equivocal" to describe the inconclusive diagnoses on breast fine needle aspiration cytology **[17,18]**. Diagnosing the equivocal lesions in cytology causes no delay in treatment as excisional biopsy is recommended **[19]**. In our study, we followed categories defined by NCI as it is the most popular one.

This retrospective study included one hundred twenty two selected cases of breast lump aspirates that gave inconclusive cytologic diagnoses and then followed by excisional biopsy. The aspiration cytology findings were then matched with the final pathologic reports. None of our cases was subjected to a core biopsy and its correlation with fine needle aspiration was not a part of our study. Our study also did not attempt to draw any conclusion as to whether one diagnostic modality could replace the other.

The included cases were diagnosed cytologically as atypical, 84 cases (68.9%), and suspicious cytologic diagnosis, 38 cases (31.1%). These results concluded that the atypical cytologic diagnoses represented the majority of our studied cases. However, this result cannot be considered as a good representative index of the true frequency of the two categories as the cases were selected. For the same reason, we could not report the combined incidence of the inconclusive cytologic diagnosis among the total breast aspirates during the studied time interval, so, we could not detect whether these categories are being underused or overused in our institute.

Most cytologically suspicious aspirates in the current study, 31 cases (81.6%) were found to be malignant on histologic examination. Although the likelihood of an underlying malignancy decreased in the atypical cytologic aspirate (63.1%), it remained considerably high. These results were comparable to those previously reported by others **[17,20]** who recorded that the atypical cytologic diagnoses were associated with subsequent considerable diagnosis of malignant disease (52% in both studies), whereas higher percentages (ranged from 76% to 83%) of the suspicious aspirates yielded malignant finding on histologic examination in their series. Ozkara et al. **[19]** in their study found that 50% of the included atypical cases were benign on histologic examination and 50% were malignant.

However, our results are different from the results reported in most previous studies in which they concluded that the benign final histologic diagnosis constituted the majority of the atypical cytologic diagnosis, 64%, 84%, and 56% respectively **[4,21,22]** compared to lower percentage in our study which results in (36.9%). These differences in the results might be attributed to the differences in the evaluating data between our institute and the others, as some cytopathologists consider that some atypical changes may be acceptable in some lesions where others may deal more seriously with such atypical features and so this lead to increase the sensitivity of the breast cytology and avoid discharging any patients with hidden malignant lesions.

In the current study, invasive duct carcinoma was the most commonly encountered malignant diagnosis in both atypical and suspicious categories, 83% and 83.9% respectively (Fig. 1). This finding agreed with what others have been recorded in their series **[4,17]**. Duct carcinomas in situ were identified among the atypical and the suspicious categories in 5.7% and 12.9% respectively. These results are also in line with what reported by others **[17]**. It is a matter of considerable controversy whether ductal carcinoma in situ can be distinguished from ductal hyperplasia of various grades including atypical form and even from invasive carcinoma in cytologic smears **[23]**. It is very important to know that the histologic analysis of such lesions may also be controversial **[18]**. Reis-Filho et al. **[23]** noted that in aspiration smears, only the presence of fat and/or stromal fragments infiltrated by cancer cells favors a diagnosis of invasive carcinoma; however this criterion is not present in all aspirates. In contrast, the presence of myoepithelial cells overlying tumor cell clusters points towards a diagnosis of ductal carcinoma in situ.

In the current study, invasive lobular carcinoma was identified among the atypical group in 5.7%. It is well known that invasive lobular carcinoma is often underdiagnosed in breast aspirates. According to literature reports, 7-20% of all inconclusive diagnoses correspond to invasive lobular carcinoma **[17]**. This finding is understandable given the bland morphology of lobular carcinoma cells in general and on cytologic smear **[24]**. One case of tubular carcinoma, in our series, was underdiagnosed as "atypical breast lesion" with a recommendation for removal of the breast lump for detailed tissue examination. A review of the smears revealed that the case was characterized by bland and orderly appearance of cells in sheet, focal cell atypia, tubular structures and somewhat angular epithelial clusters. The rarity of occurrence of tubular carcinoma in the breast with limited experience of cytopathologists regarding the diagnosis of this neoplasm, the bland morphology, and the mostly orderly appearance of cells in the FNAC sample, as in tissue, are considered to cause the difficulty in the diagnosis of this tumor; also, these are features found in other lesions of the breast like atypical ductal hyperplasia, microglandular adenosis, or tubular adenoma **[25]**.

Invasive papillary carcinoma was observed in 3.8% of the atypical studied cases. Papillary

tumor is another group of breast tumors that may cause significant diagnostic problem in cytologic smears. Duct papillomas and low grade papillary carcinoma, which may occur in the lining of breast cysts or in ducts, have similar cytomorphologic features. Malignant papillary tumors contain an occasional nuclear enlargement in the epithelial cells, and sometimes evidence of mitotic activity in addition to increased cellularity and absence of apocrine cells and myoepithelial cells. However, a reliable cytologic diagnosis of papillary carcinoma cannot be made on cytologic basis and the papillary breast lesions observed in cytologic material should be excised for histologic examination. A firm diagnosis of invasive papillary carcinoma can be rendered only on the basis of histologic material showing invasion of the parenchyma of the breast beyond the confines of the duct of origin, or the presence of carcinoma in the adjacent ducts **[8]**. One case (3.8%) of mucinous carcinoma was detected among the suspicious cytologic cases in the current series. The smear showed well circumscribed epithelial clusters with high intercellular cohesion and focal mucin in the background with no intracellular mucin. However, there was a considerable cytologic atypia. Regarding the young age of the patient, excisional biopsy is recommended. Lesions of the breast containing extravasated mucin span a continum from benign mucocele to invasive mucinous (colloid) carcinoma. It is well known that distinguishing benign from malignant mucinous lesions is difficult in fine-needle aspiration material **[15]**.

Like others **[17,26]** we found that fibrocystic changes and fibroadenoma were the most common benign lesions in the atypical cytologic category (48.4% and 25.8% respectively). It is well known that both lesions may exhibit cellular smears, marked discohesiveness, and occasional nuclear atypia that would warrant histologic evaluation to exclude the possibilities of malignant disease **[14]**. Atypical ductal hyperplasia was the most common non malignant lesion encountered in the suspicious category (42.8%) (Fig. 2), while it was encountered in 12.9% of the atypical group. The other less common benign lesions found in the cytology/pathology correlation included florid usual ductal hyperplasia, accounted 6.5% of the included atypical cases, and chronic non specific inflammation, recorded in 6.5% and 28.6% of the studied atypical and suspicious categories, respectively. These results were nearly similar to what has been reported by others **[17]**. It was found that it is not possible to differentiate cytologically the various subgroups of benign ductal hyperplasia from one another because the smears does not necessarily reflect all of the several types of abnormalities present side by side **[27]**. In about 95% of these aspirates, it is relatively simple to recognize that process as benign based on the arrangement of epithelial cells in flat cohesive sheets of uniform small cells and with the presence of spindle shaped "bipolar" myoepithelial cells. In a relatively small number of these cases, diagnostic problems may be encountered. Such dilemmas occur when the epithelial cells in the smears show enlarged nuclei and visible nucleoli. Depending on the proportion of such cells, the smears are considered "atypical" and sometimes as "suspicious" **[28]** Reactive duct epithelial cells from inflammatory breast lesions may demonstrate considerable nuclear atypia. Their differentiation from carcinoma undergoing necrosis may be difficult **[11]**.

In routine cytologic preparation, the precise identification of myoepithelial cells plays a major role in the diagnostic assessment of several types of breast lesions. These cells constitute the basal cell layer of normal mammary duct and lobular system that lie between the epithelium of the glands and their basement membrane and usually are lost during malignant progression **[29]**. Although the cells' auxiliary role in the lactational physiology is well recognized, they control many aspects of the luminal function. They could regulate polarity, electrolyte, fluid flow, and control signals of endocrine or paracrine nature. One school of thoughts has indeed invested the myoepithelial cells with great significance as paracrine inhibitor of invasion and thus an inhibitor of tumor progression. Other workers have proposed that, in the absence of fully differentiated myoepithelial cells, a failure to sequester local growth factor such as B-fibroblast growth factor may contribute to, uncontrolled growth of malignant breast cells **[6]**.

The myoepithelial cells in fine needle aspiration cytology are identified as oval to bipolar cells with scanty cytoplasm and elongated densely stained nuclei. However, identification

of myoepithelial cells in breast biopsies and fine needle aspiration smears sometimes is difficult using Papanicolaou-stained or Giemsa-stained preparations **[29]**.

Based on their biphenotypic (epithelial and smooth muscle-like) properties, several antibodies directed against myoepithelial cells have been recognized. These target either smooth muscle-related antigens (smooth muscle actin, smooth muscle myosin heavy chain 'SMMHC', calponin, and h-caldesmone), however, most of them can cross react with breast stromal cells and myofibroblasts as well as with neoplastic cells; or cytokeratins that are expressed specifically by basal/myoepithelial cells (cytokeratin 5/6, 7, 14, and 17). They have a low sensitivity for myoepithelial cells, mainly for those located in the lobules, and also stain a variable proportion of breast carcinomas. S-100 protein has a high sensitivity but a very low specificity for myoepithelial cells **[8]**. Maspin and CD10 are also considered as markers for the myoepithelial cells **[30]**.

Recently, p63 has been characterized as a reliable marker of myoepithelial cells of breast lobules and ducts. Thus within the benign group, p63 nuclear staining is identified in all cases; whereas invasive carcinomas are devoided of p63 staining **[5]**. Many authors consider p63 to be the modern golden standard for myoepithelial cell staining in breast lesions. The advantage of using p63 as a myoepithelial marker is that the staining is nuclear; hence the interpretation of positivity is easier due to overcoming the cytoplasmic fragility of myoepithelial cells in fine needle aspirates **[30]**.

The p63 is one of p53 homologues and related genes. Its gene is located on chromosome 3q27-28 and encodes at least six different proteins that play a crucial part in the regulation of epithelial proliferation and differentiation **[8]**.

On the basis of the premise that myoepithelial cells are absent or far fewer in the malignant breast lesions compared with benign lesions, we evaluated p63 immunocytochemical marker in the detection of myoepithelial cells in the inconclusive cytologic breast lesions aiming to separate benign from malignant cases.

As regard the presence of p63 positive myoepithelial cells overlying the epithelial cell clusters, 58 cases (69%) of the 84 histologically confirmed malignant cases showed no p63 staining of the myoepithelial cells (Fig. 8), while 18 cases (21.4%) showed p63 staining of myoepithelial cells overlying 25% or less of the examined epithelial clusters (Fig. 3). The presence of p63 positive single myoepithelial cells in the background, on the other hand, was not detected at all or detected in 25% or less of the examined background single naked cells in 91.7% and 8.3%, respectively. This result was in line with early report that suggesting that the malignant breast lesions contain no or few p63 staining of myoepithelial cells either in the cell clusters or in the background bare nuclei **[5]**. In a preliminary report, authors concluded that there was no invasive carcinoma when many p63 positive cells are observed in the tumor cell clusters or in the background but they did not quantify the term 'many' **[31]**. Our result was also in accordance with previous report where 65% and 24% of their included malignant cases showed no staining or focal staining for p63 in the 15 examined epithelial clusters, respectively; and 88.2% and 11.8% of their studied malignant cases showed no or focal (<25%) nuclear positivity for p63 in the examined single background cell, respectively **[32]**.

In our study, an unexpected finding was the presence of two malignant cases that showed p63 nuclear staining of myoepithelial cells overlying 50%-75% of the examined cell clusters. These cases were proved histologically to be one case of ductal carcinoma in situ and one cases of invasive duct carcinoma. Whereas the 6 studied malignant cases that showed p63 staining of myoepithelial cells overlying 25%-50% of the examined clusters, were proved to be ductal carcinoma in situ (Fig. 4). From these results we found that 100% of the included ductal carcinoma in situ cases and one case (1.3%) out of 77 invasive carcinoma cases showed p63 staining pattern similar to that of benign lesions, in more than 25% of the examined epithelial cell clusters. However the p63 staining in the invasive carcinoma case was less conspicuous compared with that observed in the in situ and benign cases. This case of invasive duct carcinoma showed 65% positivity in the epithelial cell clusters but it showed no staining in the single cells in the background.

Our findings agreed with what was reported by other authors, who found that two cases out

of 17 malignant cases, one invasive and one in situ, showed p63 staining in 50% or more of the examined clusters. However, they reported that the positive p63 stained cells in the invasive case appeared cytomorphologically to be ductal tumor cells rather than myoepithelial cells and explained that p63 marker is not lineage specific and may stain other cell types particularly squamous cells **[32]**. Others reported that 100% of the studied in situ cases and 60% of the invasive cases were positive for p63 marker. They explained this discrepancy, regarding the high percentages of positivity in the invasive cases compared with the other studies, to the small number of included cases in their series or the presence of an associated ductal carcinoma in situ in the diagnosed invasive cases **[23]**. In our study, review of the histopathologic report of the invasive case that showed strong positivity to p63 failed to identify any foci of in situ component. Our results also agreed with what concluded by others who reported that p63 positive myoepithelial cells overlying malignant epithelial clusters were found in all in situ carcinoma cases while it stain few clusters in the invasive cases **[29]**. It should emphasized that in 5 included malignant cases (6%), out of the 84 studied malignant cases, only a minority of neoplastic duct cells (<10% of duct cells) showed p63 nuclear staining (Fig. 6). In previous studies, Mattia et al. **[5]** and Kaufmann et al. **[33]** reported the presence of variable proportion (5-15%) of p63 positive neoplastic cells in up to 4.6% and 11% of breast carcinoma, respectively. However, they suggested that p63 seems to be expressed only in poorly differentiated ductal carcinoma and, in particular, in up to 87% of metaplastic carcinoma and perhaps those with squamous differentiation. A definitive diagnosis of carcinoma of such cases is usually readily rendered on cytology smears and ancillary studies, such as p63 staining, are not called for in this situation. According to other study, the possible explanation of the presence of positive neoplastic cells might reflect a partial myoepithelial/basal differentiation of some breast carcinomas **[23]**.

In the present study, 32 (84.2%) out of the 38 studied benign cases showed p63 nuclear staining of myoepithelial cells overlying more than 75% of the examined epithelial cell clusters (Fig. 5), while 22 cases (57.9%) showed myoepithelial nuclear staining in more than 75% of the examined single cells in the background (Fig. 7).

In the current study, 5 benign cases showed nuclear positivity of the myoepithelial cells overlying 25%-75% of the epithelial clusters, whereas 10 studied cases showed nuclear staining of 25%-75% of the single cells in the background. Only one case showed no staining of the myoepithelial cells overlying the epithelial clusters and this case showed also no staining in the single background cells. Four cases showed no staining for myoepithelial cells in the background bare nuclei (one case showed 0% staining, 2 cases showed 30% staining in the examined clusters and one case showed 55% cluster staining). While 2 cases showed focal staining in less than 25% of the single cells. Harton et al. **[32]** found that 2 benign cases, lactational changes and florid ductal hyperplasia, showed p63 staining in fewer than 25% of the cell clusters. They also found 4 cases that had no p63 positive single cells which included 2 cases of florid ductal hyperplasia and one case each of lactational changes and fibroadenoma.

From our results we found that 37 cases (97.4%) out of 38 studied benign cases showed p63 staining in more than 25% of the examined epithelial clusters while 32 benign cases (84.2%) showed that more than 25% of the single bare nuclei being positive for p63. Others reported that all fine needle aspiration cytology of their studied benign cases (100%) showed consistent distribution of p63 in the nuclei of myoepithelial cells in 3 distinctive patterns; overlying epithelial clusters, on the border of epithelial clusters, and isolated cells in the background. However they did not quantify the presence of p63 positive cells **[29]**.

Our figures approached the figures reported by others who reported that 27 out of the 29 benign studied cases showed positivity for p63 in myoepithelial cells overlying more than 50% of the 15 examined clusters, one cases showed positivity in less than 25% of the clusters and one case showed no staining. In their series, 8 cases showed staining in less than 25% of the examined single cells in the background **[32]**.

In our study, cases that showed fewer than 25% of p63 positive bare nuclei in the background and fewer than 25% of p63 positive cell

clusters were considered as malignant by p63 staining, the rest was considered benign (Table 5). Based on this, 76 out of 84 malignant cases were truely diagnosed as malignant and 8 cases were falsely diagnosed as benign. 32 out of 38 benign cases were truly diagnosed as benign and 6 cases were falsely diagnosed as malignant. When we incorporated the p63 staining results on the inconclusive breast cytology, atypical and suspicious, the sensitivity, specificity, positive predictive value, and negative predictive value were 90.5%, 84.2%, 92.7%, and 80% respectively (Table 6). Thus the p63 staining was relatively accurate in separating benign from malignant breast lesions in the problematic cases in which cytomorphologic features provide no aid in their separation. Harton et al. **[32]** when applied p63 staining on twenty specimens with a less-than-definitive diagnosis (atypical and suspicious), they yielded sensitivity, specificity, positive predictive value, and negative predictive value of 88%, 97%, 94%, and 97%, respectively. But statistically, these twenty cases are considered as unrepresentative sample.

Obviously, "total reliability" in diagnosing breast cancer can not be achieved with any approach. This issue should be openly discussed with women who are led to believe that breast cancer is always diagnosable and that failure to do so is a punishable offense that belongs in a court of law **[34]**.

Conclusion:

1- The p63 was a reliable nuclear marker of myoepithelial cells in breast previously stained Papanicolaou slides, it was a useful marker for highlighting these cells in the atypical and suspicious groups.

2- The application of p63 immunostaining to the inconclusive breast cytology achieved sensitivity, specificity, positive, and negative predictive value of 90.5%, 84.2%, 92.7%, and 80% respectively. Thus it can be use as adjunct in assessing problematic breast lesions in cytology.

3- Low grade ductal carcinoma in situ of the breast remained a diagnostic pitfall in cytology even with the use of p63 marker as it did not seem to be helpful in differentiating low grade ductal carcinoma in situ from atypical or usual ductal hyperplasia.

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