DIAGNOSTIC ROLE OF P63 IMMUNOSTAINING IN FINE-NEEDLE ASPIRATION CYTOLOGY SMEARS OF BREAST LUMPS

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ABSTRACT

Background: Fine-needle aspiration cytology is a simple, non-invasive, reliable and economical procedure for the exact evaluation of lump breast. P63 is a nuclear marker which can be used on FNAC smears for staining of myoepithelial cells that appear as naked nuclei on cytological examination.

Aims and Objectives: To evaluate the expression of p63 in FNAC smears of breast lumps and to determine the predictive value of the p63 expression on FNAC smears for benign and malignant breast tumors.

Material and Methods: This study was conducted in Lahore General Hospital on 50 female patients with a breast lump. Smears were prepared and stained with H&E and p63 immunomarker. Association between histological diagnosis and p63 expression on FNAC was evaluated.

Results: In this study of 50 cases of lump breast, 36% (18) cases showed <25% p63 + staining in their cells, 20% (10) cases showed 25-50% P63 + staining and 44% (22) cases showed >50% P63+ staining concluding 36% (18) cases were malignant (IDC) on P63 staining.

Conclusion: It was concluded that the expression of p63 may be helpful in making a conclusive diagnosis on FNAC smear.

Keywords: P63 expression, Fine- Needle Aspiration Cytology, Breast smears

INTRODUCTION

A breast lump is a common problem in females. It is also a site for different types of benign lesions and may be subjected to acute inflammation and abscess formation[1]. Improper diagnosis of breast malignancy can lead to deficient radical treatment and physical and psychological difficulties. Triple assessment i.e. clinical history, radiographic mammogram, and FNAC smear gives exact diagnosis initially thus decreasing the risk of improper diagnosis[2]. It is seen in one study that FNAC reduces the number of biopsies. After the mammographic study, the lesion is sampled with FNAC smear. Palpable breast lumps are aspirated easily followed by processing for fast diagnosis[3,4]. The myoepithelial cells confirm the diagnosis of various breast lesions. These cells are located at the basal layer of breast ducts and there is loss of myoepithelial cells in malignant tumors. To identify the myoepithelial cells in breast tissue biopsy and FNAC smear, it is difficult to use Pap smear or Giemsa stains[5].

According to studies study, p63 immunostain is useful for myoepithelial cell staining. P63 antibody stains the nucleus without fibroblasts and vascular walls cells staining[6]. p63 staining in the nucleus is useful on FNAC and myoepithelial cells lose the cytoplasm and become naked nuclei by losing the cytoplasm on cytology[7]. In FNAC smear, p63 stains the myoepithelial cells, so p63 is a reliable marker in differentiating the benign and malignant breast lesions[8]. P63 is helping in myoepithelial cells staining i.e. breast lobules and ducts. However, p63 has also stained the squamous epithelial cells of skin, esophagus, cervix and basal cells of prostate and bronchi[9,10].

This study was planned to determine the accuracy of p63 stain in the diagnosis of suspicious cases (class-III and class-IV), regarding benign or malignant cases of the breast lump. This will help in rapid diagnosis as well as planning and starting management without delay.

MATERIAL AND METHODS

FNAC smears of 50 patients were collected from the Histopathology Department of Lahore General Hospital Lahore through non-probability purposive sampling technique. Smear having Class-III, IV (suspicious looking) taken from females of all ages were included

in the study. A patients who was already taking chemotherapy, radiotherapy or hormone therapy and smear having inadequate cellularity were excluded from the study. Smears were stained with H&E according to standard protocols and with p63 using monoclonal antibody i.e. clone 63PO2.

The prepared slides were seen under the microscope by two histopathologists. The histological diagnosis of H&E stained sections was determined and noted in the proforma. FNAC smears were divided into five classes i.e. I = Inadequate/Insufficient, II= Benign, III= Atypical, IV= Suspicious of malignancy, V= Malignant. The results of p63 immunostaining were based on nuclear staining. P63 stained positive cell was scored by the percentage of myoepithelial cells nuclear staining. (Table I).

Table I: Scoring of p63 against the percentage of stained cells

| P63 Score	%age of p63 stained cells
0	0%
1	<25%
2	25-50%
3	>50%

The data was analyzed by SPSS version 23. For numeric variables, mean and standard deviation were calculated. p63 was described as percentage and frequency and diagnostic accuracy was calculated.

RESULTS

Frequency	p63 Scores				Total
	0	1	2	3	
n	-	18	10	22	50
%age	-	36%	20%	44%	100%

The mean age of the patients was 29±11.33 years. 34 patients (68%) were within the age range of 15 – 30 years, 12 patients (24) were within 31 – 45 years and four patients (8%) were >45 years. (Graph-I)

Out of 50 cases, fibroadenoma (class-II) was seen in 22 cases (44%), atypical cells (class-III) were seen in 13 cases (26%), suspicious cells (class-IV) were seen in 8 cases (16%) and malignant cells (class-V) were seen in 7 cases (14%). (Table II)
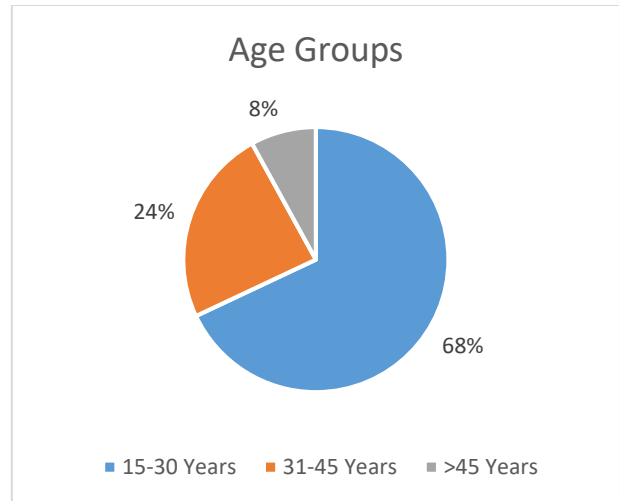
Graph-I: Number of patients and their age groups

Table 1I: Distribution of cases diagnosed after staining with H & E.

| Class	N	% age
I	0	0%
II	22	44%
III	13	26%
IV	8	16%
V	7	14%
Total	50	100%

Expression of p63 was seen and scoring was done. 18 cases (36%) expressed <25% (score 1), 10 cases (20%) expressed 25-50% (score 2), 22 cases (44%) expressed >50 (score 3) (Table III)

**Table 1III:** Distribution of cases according to p63 expression

Class	n	%age
I	0	0%
II	22	44%
III	10	20%
IV	8	16%
V	10	20%
Total	50	100%

Out of 50 cases, class-II smear was seen in 22 cases (44%), class-III smear was seen in 10 cases (20%), the class-IV smear was seen in 8 cases (16%) and class-V smear was seen in 10 cases (20%) (Table IV).
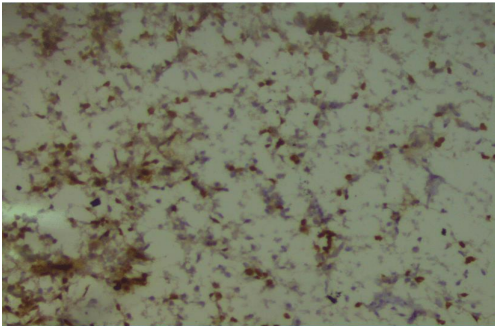
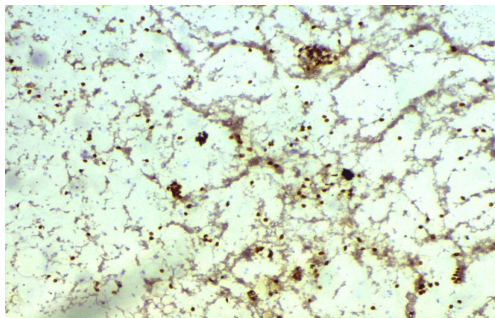**Table 1V:** Distribution of cases diagnosed after staining with p63
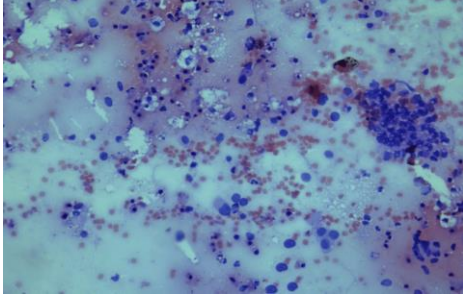
FNAC smear of breast class V stained with H&E (20x)
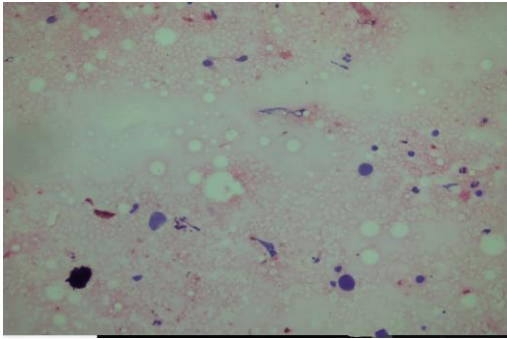
FNAC smear of breast class IV stained with H&E (20x)

FNAC smear of breast stained with P63 immunostain showing myoepithelial cells >25%. (20x)

FNAC smear of breast stained with P63 immunostain showing myoepithelial cells <25%. (20x)

DISCUSSION

Myoepithelial cells consist of the basal layer of ductal and lobular lining cells and this architecture is lost in malignancy so p63 can be used as a reliable marker as it stains the myoepithelial cells [5, 8]. In our study, 15 (30%) cases with malignancy were diagnosed by FNAC procedure with H&E staining. By using p63 staining, the observed malignancy positive cases were 18 (36%). 03 (6%) cases having suspicious morphology were diagnosed as malignancy positive by using P63 staining. These results were comparable with study of Wang et al. (2001) which suggested that myoepithelial cells were converted into naked nuclei and also consistent with the results of Barbareschi [8, 11].

p63 showed strong immunoreactivity and was localized to the nuclei of myoepithelial cells and three samples showed high background that impaired the cytologic staining. In a study by different authors, the main part of p63 immunoreactivity in different slides was p63 positive malignancy [8]. These studies showed (5–15%) p63 positive malignant cells of 4.6% and 11% of breast cancers respectively. In another study by Werling et al. (2003), p63 positive malignant cells (1–5%) are present in 12 specimens i.e. 23% and more positive cells (5%) in different specimens i.e. 13% [12]. In a study by Harton et al few of the suspicious cases were included in benign cases and few atypical cases were classified in malignancy on FNAC diagnosis [13].

CONCLUSION

It is concluded that p63 is a key diagnostic tool for breast lumps. P63 immunostaining has more diagnostic accuracy as compared to H & E staining in FNAC breast lumps. P63 immunostaining can be used in routine FNAC of breast lumps for rapid diagnosis.

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