Original Article

Diagnostic Challenge of Carcinoma Breast on Core Biopsy - Role of p63 and Cytokeratin 8/18

Role of p63 and Cytokeratin 8/18

Rubina Gulzar, Ruqaiya Shahid, Shazia Mumtaz, Yusra Memon, Farheen Danish and Talat Mirza

ABSTRACT

Objective: The goal of this study was primarily to test the diagnostic utility of immunohistochemical stains p63 and cytokeratin 8/18 in the differentiation of malignant and non malignant lesions to prevent unnecessarily surgical intervention .

Study Design: Comparative / cross sectional study.

Place and Duration of Study: This study was conducted at the Department of Pathology, Dow University of Health Sciences, Karachi from 1 January 2015 to 31 January 2016.

Materials and Methods: Immunohistochemical stains p63 and cytokertin 8/18 were performed on 182 cases on needle core breast biopsies. Patients' name, age, histology numbers, diagnosis. type of tumor,grade of tumor, and expression of p63 and cytokeratin 8/18 were recorded with special emphasis on myoepithelial layer integrity and foci of invasion.

Results: Total number of cases were 182. Mean age was 42 years(27-70), Malignant lesions113(62.1%),Benign lesions 18(9.8%), Fibroepithelial lesion10(5.5%).Papillary lesions4(2.2%), Benign breast tissue37(20.3%). Most common malignant lesion was infiltrating ductal carcinoma 98(53.8%)followed by infiltrating lobular carcinoma 3(1.6%), mucinous carcinoma 2 (1.1%)& ductal carcinoma in situ were 10(5.5%). Benign lesions were peri ductal mastitis/chronic granulomatous mastitis 10(5.5%),sclerosing adenosis 3(1.6%)and ductal hyperplasia 5(2.7%).

Conclusion: The responsibility of the pathologist is to provide accurate diagnosis thus placing the patient in the appropriate therapeutic algorithm.

Key Words: Carcinoma breast, Core biopsy, p63 & cytokeratin 8/18 expresion.

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INTRODUCTION

Breast cancer is the most common cancer in females with a reported incidence of 1.67 million in 2012.[1]

Breast cancer is the most frequent cancer in women in Karachi, accounting for one-third of the cancers in the females and its incidence is second highest in Asia after Israel. According to a recent study in Pakistan (Ahmed S et al. 2013), breast cancer is one of the top malignancies (19.7%) in females.[2,3]

Previously Fine needle aspiration Cytology (FNAC) was the established cell collection technique for the diagnosis of breast cancer. However, for the last two decades it is largely replaced by core needle biopsy(CNB).[4]

Core biopsy provides an accurate pre-operative diagnosis and is a successful method of choice with 96% sensitivity and 99% specificity.[5]

CNB is certainly more reliable than cytology and is less invasive than surgical biopsy, and allows the best therapeutic treatment options. However in everyday diagnostics, pathologist encounters cases in which the distinction between benign and malignant cases is challenging. This is because the morphologic features become more challenging due to limited available material, in such type of cases definitely requiring ancillary studies, to reach an accurate diagnosis.[6]

The Breast ducts and acini contain two types of epithelial cells, inner luminal and outer basal/ myoepithelial cells. These cells can be distinguished by their immunophenotype. Cytokeratin (CK) 8/18 is expressed in the luminal layer, whereas CK5/14 and the transcription factor p63 characterizes the basal epithelial layer.[7]

The fundamental step for carcinogenesis is the loss of myoepithelial layer and loss of architecture which can easily be demonstrated by the use of immune-histochemistry.[6]

There is no study to date that compares the utility of p63 and cytokeratin 8/18 immunostains in the workup of clinically challenging core biopsy cases. Therefore, the goal of the present study is to assess the diagnostic

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utility of p63 and cytokeratin 8/18 in the dististiction of benign, insitu and invasive malignant cases.

MATERIALS AND METHODS

Case Selection: It is a comparative, cross sectional, prospective study performed at Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences, Karachi, from 1st Jan 2016 to 31 Jan 2017. This includes 182 cases of core breast biopsies in which there was high clinical suspicion of breast carcinoma but these were not conclusive on H &E and required further IHC stains. IHC CK 8/18 and p63 were applied. All other type of biopsies including mastectomse cases.ies, lumpectomies, excision and wedge biopsies were excluded.

Immuno-histochemistry: Four millimeter thick sections were deparaffinized in xylene and dehydrated. Antigen retrieval was done by boiling target DAKO Envision retrival solution (high PH 505) for 40mins at 96-99°C. Endogenous peroxidase activity was blocked by treatment with DAKO Envision flex peroxidase blocking reagent. The slides were incubated for 20-30mins at room temperature in humidity chamber with appropriate dilutions of primary antibodies along with their positive and negative controls. The slides were then incubated with secondary antibody (Envision horse reddish peroxidase) for coupling reaction for 20-30mins at room temperature. The substrate (Diamino benzidine +Chromogen) was used to produce crisp brown color at the site of target antigen. Hematoxylin (1-2 dips) was used as a counter stain. Controls of p63 and cytokeratin 8/18 positive stains were applied on the same slides.

Evaluation of immunohistochemistry: In the majority of benign breast glands, p63 antibodies demonstrated intense basal cell-specific nuclear immunostaining. In questionable foci, positive staining was taken as an evidence of benignity and deeper levels of H&E were examined. Negative staining of an entire suspicious focus was taken as presumptive evidence of malignant process; as long as the morphology was in agreement with the diagnosis. CK8/18 scored positive if any cytoplasmic and/or membranous tumor cell staining was observed

Statistical Analysis: Data was analyzed using SPSS version 20, and descriptive statistics were calculated as mean and median for age of patients and frequency and percentages for different types of breast lesions.

RESULTS

Total number of selected cases was 182. On all these cases IHC stains CK8/18 and p63 were applied for diagnosis. Mean age of the patients was 42 years (27-70).

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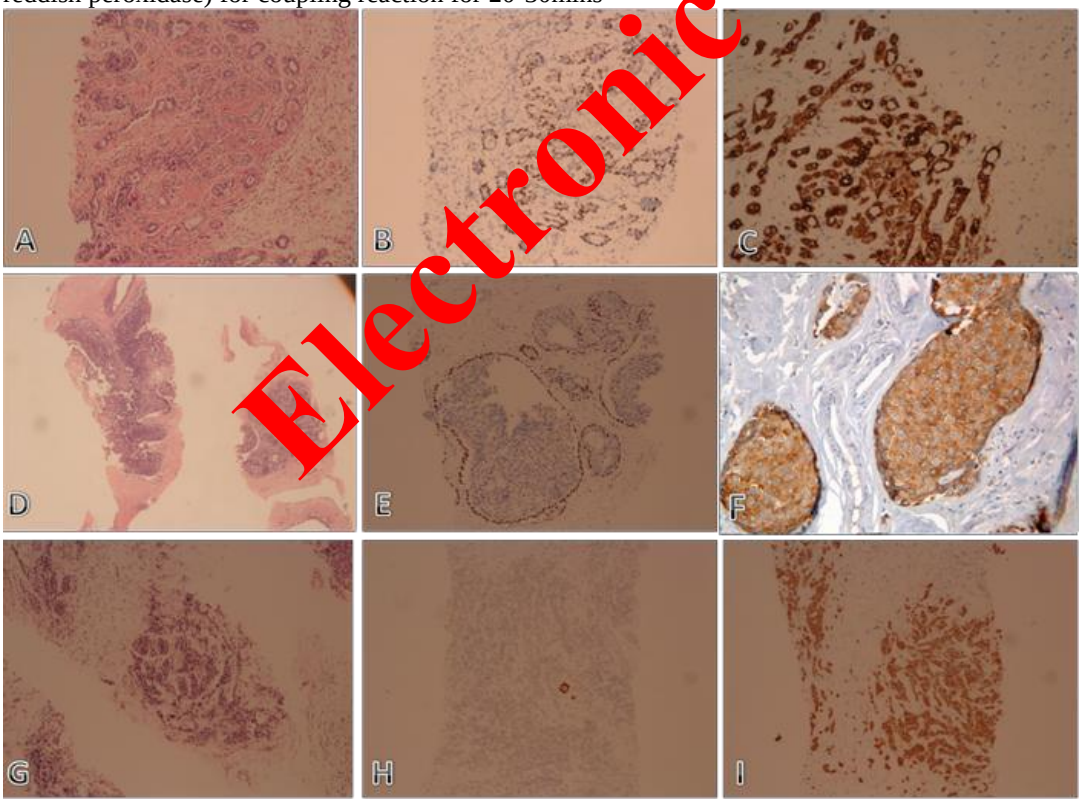

A: H&E of scelerosing adenosis. (original magnification,X100). B: Strong p63 immunoreactivity in the peripheral rim of myoepithelial cells.(original magnification,X200) C: Cytokeratin 8/18 in the epithelial cells. (original magnification,x200). D: H&E of Ductal Carcinoma Insitu solid type. (original magnification,X100). E: P63 stain myoepithelial cells. (original magnification,x200). F: Cytokeratin 8/18 stain epithelial cells. original magnification,x200). G: H&E of Invasive ductal carcinoma (IDC NOS). (original magnification,X100). H: Complete loss of p63 stain in myoepithelial cells. (original magnification,X200). I: Strong positive staining for CK8/18 in malignant sheets.(original magnification,X200)

Of these cases lesions were diagnosed as malignant 113(62.1%), 18(9.8%) were diagnosed as benign, 10(5.5%) were fibro epithelial lesions, 4(2.2%) were papillary lesions and 37(20.3%) were ultimately diagnosed as benign breast tissue.

Most common malignant lesion was Infiltrating Ductal Carcinoma 98(53.8%) followed by Infiltrating lobular Carcinoma 3(1.6%), Mucinous Carcinoma 2 (1.1%) & Ductal Carcinoma in Situ were 10(5.5%).

Benign lesions were peri-ductal mastitis/chronic granulomatous mastitis 10(5.5%), sclerosing adenosis 3(1.6%) and ductal hyperplasia 5(2.7%).

DISCUSSION

Breast lesions have diverse morphological appearances. The differential diagnosis of breast carcinoma in needle biopsy includes an array of possibilities, from normal structures such as small benign ducts, acini or sclerosing adenosis to atypical ductal hyperplasia and Ductal Carcinoma in situ.

Reis-Filho JS et al(2002) for the first time, evaluated the diagnostic utility of p63 staining in differentiating in situ and invasive malignancies on fine needle aspiration.[7]

In malignant tumors the immunohistochemical stain p63 showed complete loss of myoepithelial layer and Cytokeratin 8/18 highlighted foci of invasion. Our study highlights the usefulness of combination of these two stains in differentiating benign from malignant and in situ from invasive carcinomas.

In year 2007, Shamloula MM and collogues observed similar finding in the excision biopsy specimens with high sensitivity of p63 and Cytokeratin 8/18.[8]

According to authors' knowledge no previous study has evaluated the utility of p63 and Cytokeratin 8/18immunohistochemical staining in benign and malignant breast lesions on core needle biopsy.

Total ten cases of carcinoma in situ were identified that included solid, comdo and cribriform type all shows intact myoepithelial highlighted on immune-histochemical stain p63 and show no foci of invasion on cytokeratin 8/18 many articles support our findings.[8,9] However as our specifically emphasis on core biopsy which mostly have small amount of biopsy material and p63 have patchy nuclear positivity to exclude the invasion between these patchy areas cytokeratin 8 /18 is extremely helpful.(Figure D,E &F) .Because it highlighted small minor foci of invasion and two of the cases which were reported as carcinoma in situ with minor foci of invasion subsequently on excisional biopsies showed invasive ductal carcinoma with in situ component.

Fibroepithelial breast lesions include fibroadenoma and Phyllodes tumors, which are further characterized into benign, borderline and malignant. Role of core needle biopsy role in correctly differentiating these fibro epithelial lesions is limited and is solely based on assessment of stromal cellularity of these lesions. Recent studies have shown that the proliferative indices ki-67 and p 53 may have a role for more accurate preoperative diagnosis.[10,11] Role of p63 and Cytokeratin 8/18 is not established to distinguish fibro adenoma and Phyllodes tumors.

IHC marker p63 was helpful in differentiating the spectrum of papillary lesions in our study. Four cases of papillary lesions were identified and p63 highlighted fibro vascular cores in three cases; whereas, one case showed patchy loss in cores. In all cases excision of the lesion was recommended. Liberman et al reviewed in his research 35 patients who were diagnosed as papilloma on core needle biopsy, but turned out to be malignant on excision so instead statement on papiloma with atypical hyperplasia we simple recommended excision biopsy.[12]

We found that 9.9% of benign breast lesions comprised of chronic granulomatis mastitis, sclerosing adenosis & usual ductal hyperplasia. All of these are mimickers of breast carcinoma clinically and radiologically. Mammographic presentation of the chronic granulomatis mastitis and adenosis were suspicious of carcinoma but low power appearance of intact lobular architecture, lack of cytological atypia and intact myoepithelial layer and support from the IHC combination helped in establishing the correct diagnosis.

Previous studies have reported that sclerosing adenosis was associated with invasive carcinoma on excision biopsy in 28-30% of cases.[13,14] Reaching the correct diagnosis on core needle biopsy is important for appropriate management of the patient.[14]

CONCLUSION

We recommend an immunohistochemical panel approach based on the differential diagnostic scenario as the best practice for distinguishing breast cancer and its mimickers on needle core biopsies to avoid un necessary mastectomies and surgeries. The availability of myoepithelial markers, p63 and cytokeratin 8/18 provide significant role in such type of cases. We also recommend that intermediate level sections be prospectively obtained on charged (gelatinized) slides for potential immunohistochemistry and additional morphologic evaluation.

Author's Contribution:

Concept & Design of Study: Rubina Gulzar
Drafting: Farheen Danish, Talat Mirza
Data Analysis:
Revisiting Critically: Ruqaiya Shahid, Shazia Mumtaz, Yusra Memon
Final Approval of version: Rubina Gulzar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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