

Immunohistochemical detection of p63 in breast cancer among Sudanese females

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Abstract

Introduction: p63 is a member of p53 family and known to be a marker of the myoepithelial cells. In breast cancer (BC) pathology, myoepithelial cells are indicative of the tumor invasiveness. The presence of myoepithelial cells is not always easily appreciated by routine Hematoxylin and Eosin stain. The use of p63 immunohistochemistry is a valuable tool to evaluate local invasiveness, precisely, and to differentiate between in situ and invasive carcinoma of the breast. In Sudan, detection P63 using IHC in BC, was not investigated before

Objective: The aim of this study is to detect p63 biomarker in breast cancer by using immunohistochemistry technique.

Materials and Methods: This is a descriptive retrospective cross-sectional study that was conducted between January 2022 to October 2022, at the Faculty of Medical Laboratory Science-Al-Neelain University and Radiation and isotope center of Khartoum (RICK).p 63 immunohistochemistry was performed in sixty Formalin-fixed paraffin-embedded samples of the patients with different lesions of the breast. Forty samples were confirmed histopathologically to have malignant breast lesions, twenty were assessed to be have benign lesions, the later were used as controls. Data was analyzed using was tested by chi-squared test. This study was reviewed and ethically approved by Al-Neelian University Ethical Review Board.

Results: Sixty breast biopsies were included in this study. Histopathologically, 40(67 %) samples were malignant and 20 (33%) samples were benign. Among the malignant tumours, 26/40 (65%) of the samples were invasive ductal carcinoma (IDC), 4/40 (10 %)were invasive lobular carcinoma(ILC) and 10/40 (25%) were carcinoma in situ(CIS).Regarding histological grades , the data of 10 samples was not available , 7/40 (23%) were grade I , 9/40 (30%) were grade II and 14/40 (47%) were grade III .The presence of p63 was detected in 21/40 (52.5%) , 20/20 (100%) of the malignant tumours and benign tumours , respectively (P~ value 0.000) . In Histological types of the malignant tumour, p63 was detected in 11/26 (42.3%) IDC, 10/10(100%) CIS, among ILC 4/0 no sample was positive for p63 (P-value ~0.000). In different histological grades, p63 was detected in 7/7 (100%) grade I, 4/9 (44%) grade II and grade 14/0 grade III, respectively (P- value~0.000.)

Conclusion: This study concluded that there is significant association in expression of p63 between benign and malignant tumours of the breast, with high frequency of the marker in benign tumours. It is also concluded that p63 is more frequent in grade I of the breast cancer among Sudanese patients.

Key words: Breast cancer, Myoepithelial, p63 expression, Sudanese.

Introduction

Breast Cancer (BC) is now the most commonly diagnosed cancer in the world. The most recent global cancer burden figures estimate that there were 2.26 million incident breast cancer cases in 2020 and the disease is the leading cause of cancer mortality in women worldwide.(Wilkinson et al., 2022).

(BC) incidence rates have been slowly increasing by about 0.5% per year since the mid-2000s, attributed at least in part to continued declines in the fertility rate and obesity (Siegel et al.,2022). Precise diagnosis is important to

predict the prognosis and select the treatment (Oluogunl *et al.*, 2019). In pathological diagnosis of BC, differentiation between *in-situ* and invasive carcinoma considered to be crucial. Myoepithelial cells of breast tissue are an identifier of the tumor micro invasiveness (Barbareschi et al., 2001). P 63 a member of P53 proteins, is known to be a myoepithelial cells marker (Barbareschi et al.,2001). The use of p 63 immunohistochemistry is a useful tool to differentiate between invasive and non-invasive lesion of the breast. It is well-documented that p 63 is expressed by normal and benign lesions of the breast and lost in

malignant (Wang et al.,2002).. In Sudan, detection P63 using IHC in BC, was not investigated before.

This study aimed to detect p 63 biomarker in different pathological lesions of the breast by using IHC and to assess it as a diagnostic marker of BC.

Materials and methods

This a descriptive cross-sectional study that was conducted from January 2022 to October 2022., at the Faculty of Medical Laboratory Science-Al-Neelain University and Radiation and isotope centre of Khartoum (RICK). The study was reviewed and ethically approved by Al-Neelian University Ethical Review Board.

Sixty Formalin-fixed paraffin-embedded samples of the patients with different types of breast lesions. Forty samples were confirmed histopathologically to have malignant breast lesions, samples were assessed to be have benign lesions, the later were used as comparative group.

From each sample, two sections of 3 µm thickness, were obtained. One section was stained by Haematoxylin and Eosin and the other one was used to perform IHC. For IHC , the sections were rehydrated. Heat induced epitope retrieval using Tris/EDTA pH 9.0 buffer was done followed by endogenous peroxidase inactivation using aqueous hydrogen peroxide. Incubation with primary and secondary antibody followed by Diaminobenzediene (DAB) chromogen and counterstaining was carried out.(Zytomed). Brown cytoplasmic deposit is considered to be positive. Prostate tissue that known to be positive for p63 was used as positive control. The presence of p63 in different histological types and grades of breast cancer was tested by chi-squared test. Software Statistical Package for the Social Sciences (SPSS) version 20 and p-value has been calculated. The p-value <0.05 has been considered statistically significant. The results then obtained have been compared statistically.

Results

Sixty breast biopsies were included in this study. Forty (67 %) samples were malignant and twenty (33%) samples were benign. The age of the patients with malignant tumours ranged from 21-60 years, while the age of the patients with begin lesions was 21-40years, 40(67 %) samples were malignant and 20 (33%) samples were benign. Among the malignant tumours, 26/40 (65%) of them were invasive ductal carcinoma (IDC) , 4/40 (10 %) were invasive lobular carcinoma(ILC) and 10/40 (25%) were carcinoma in situ(CIS).Regarding histological grades, 7/40 (23%) were grade I , 9/40 (30%) were grade II and 14/40 (47%) were grade III .The presence of p63 was detected in 21/40 (52.5%) , 20/20 (100%) of the malignant tumours and benign tumours , respectively (P~ value 0.000) .Among different histological types of the malignant tumour p63 was detected. 11/26 (42.3%) IDC, 10/10(100%) CIS , among ILC 4/0 no sample was positive for p63 (P-value ~0.000). Regarding, histological grades, p63 was detected in 7/7 (100%) grade I, 4/9 (44%) grade II and grade 14/0 grade III, respectively (P- value~0.000) (table.1)

Discussion

p63 is a homolog of p53, and has been shown to be expressed exclusively in myoepithelial cells in normal breast and can be very useful in differential diagnosis. p63 IHC is useful in helping to distinguish invasive carcinoma from benign proliferations with a similar morphological appearance. (Zaha et al.,2014). The current study aimed to detect p63 in different lesions of the breast and to assess it diagnostic role to differentiate between these lesions.

Table.1 Characteristic of the studied patients and immunohistochemical detection of p63 in breast biopsies

Tumour types				
Benign		Malignant	Total	
20(33%)		40(67%)	60(100%)	
Age				
Age group		Benign	Malignant	Total
21-40		19	14	33
41-60		1	21	22
more than 60		0	5	5
Total		20	40	60
Histological types				
IDC		ILC	ISC	Total
26(65%)		4(10%)	10(25%)	40(100%)
Histological grades				
I		II	III	Total
7(23%)		9(30%)	14(47%)	30 (100%)
Detection of p63 in benign and malignant tumours				
	Positive	Negative	Total	P-value
Benign	20(100%)	0	20(100%)	0.000**
Malignant	21(25.5%)	19(47.5%)	40(100%)	
Detection of p63 in different histological types of the malignant tumours				
P63	Positive	Negative	Total	P- value
IDC	11	15	26	0.000**
ILC	0	4	4	
ISC	10	0	10	
Total	21	19	40	
Detection of p 63 in different histological grades of breast cancer among studied patients				
P 63	Positive	Negative	Total	P-value
Grade I	7	0	7	0.000**
Grade II	4	5	9	
Grade III	0	14	14	
Total	11	19	30	

In this study, sixty samples were included. Forty (67 %) samples were malignant and twenty (33%) samples were benign. The results revealed that BC affects older patients. This result is agreed with reports worldwide in (Albrektsen et al.,2010) ductal tumors, and malignant sarcomas, mainly phyllodes tumors, seemed to occur at higher frequency in women diagnosed <2 years after first childbirth. The proportions of medullary tumors and Paget disease were particularly high among women diagnosed 2-5 years after last birth. The high proportion of poorly differentiated tumors in women with a recent childbirth was partly explained by young age.

Histopathologically, IDC is predominant that accounts for 65 % of the studied samples followed by ILC and ISC which account for 10% and 25%, respectively. The histopathological pattern of the studied sample is similar to those reported by many investigators. (Saini et al.,2021).

In this study the majority of the cases were grade II and III (77%). Advanced presentation of the disease in Sudan could be due to the lack of awareness and early detection methods in addition to the shortage and coast of the heath care,the majority of the cases gradeI grade II similar to (Oluogun et al.,2019).

p63 IHC is known to be good diagnostic tool of BC with 100% specificity to differentiate benign and malignant lesions. In this study, high frequency of p63 was detected in being lesions in comparison to malignant and the result is statically significant (P-value 0.001).p63 as myoeptelial marker that reflects the invasiveness of the cancer, is expressed by benign tumour and lost in malignancy (Barbareschi et al., 2001).

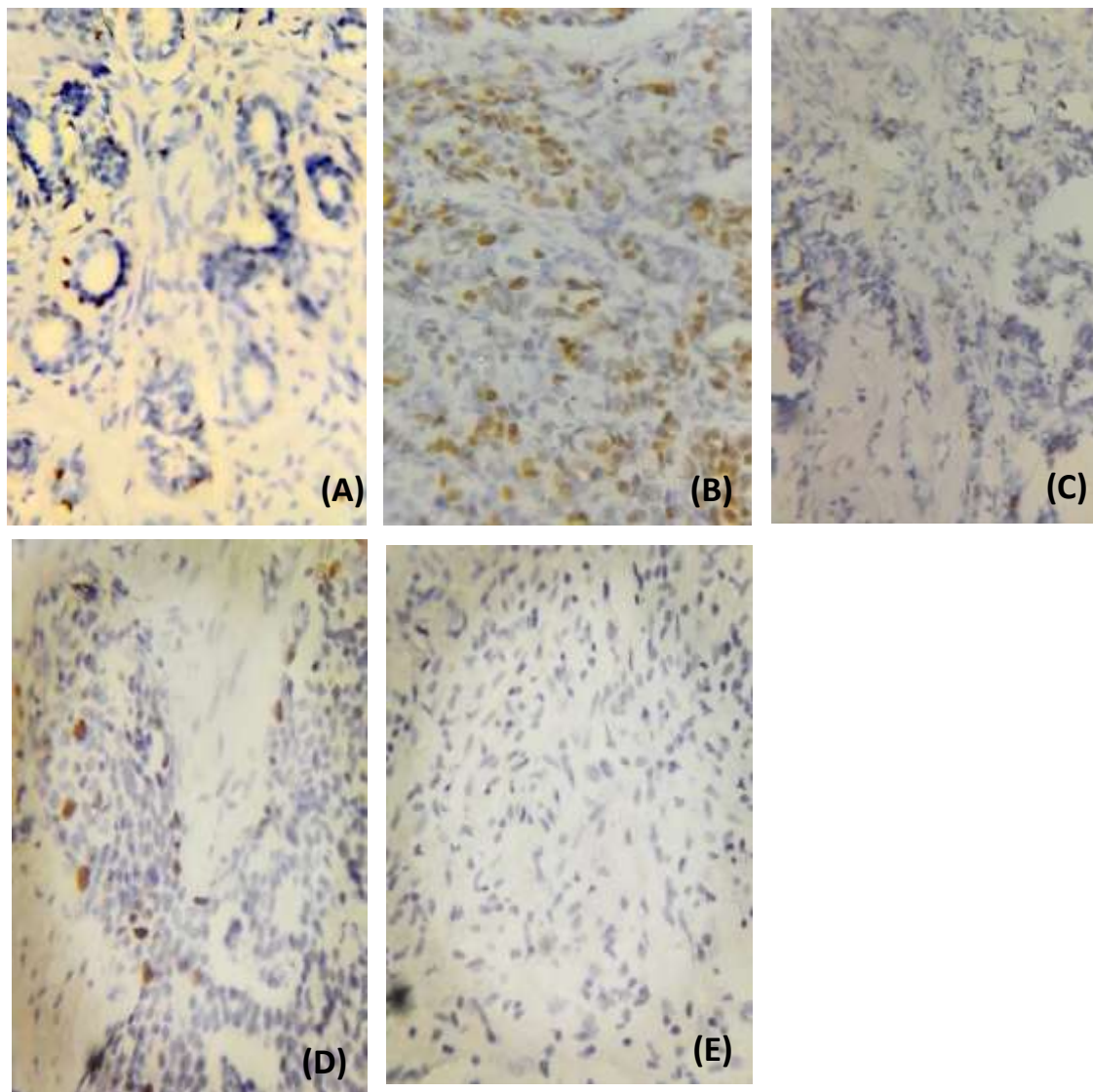


Fig.1. p63 expression in breast Tumour. **A.** p63 immunoreactivity in of myoepithelial cells in begin tumourx400. **B.** p63 positive neoplastic epithelial cells in invasive ductal carcinoma in situ. **C.** p63 positive neoplastic epithelial cells in invasive ductal carcinoma grade I. **D** p63 positive neoplastic epithelial cells in invasive ductal carcinoma grade II. **E.** completely divided of p63 expression in invasive ductal carcinoma grade III (X10).

This result was reported by Wang X et al., 2002 who investigated benign and malignant lesions of breast for p63 expression and found that p63 was exclusively expressed in the myoepithelial cells of normal breast, partially expressed in ductal hyperplasia, rarely expressed in carcinoma in situ and not at all expressed in invasive carcinomas. (Wang et al.,2002).

A comparative study conducted by Yanping and Qiurong 2006 revealed that all benign lesions were positive for p63. In malignant lesions, 26 cases including 19 cases of DCIS and 7 cases of intraductal papillary carcinoma were positive for p63 and remaining 19 IDC cases were negative for p63.

Verma et al., 2018 conducted study on total of 151 cases and found that the expression of p63 in malignant cases showed 100% negativity and in benign cases 88.6% showed positivity and 11.4% were negative. Further in benign category, study done by Ranjan et al., 2021 on 98 cases, revealed 100% positivity for p63 in benign cases while malignant cases show only 17.6% positivity for p63.

In comparison to IDC and ILC, p 63 was detected all CIS studied samples (P-value ~0.001). Also , p63 is detected in all samples with grade I and all samples of grade III showed positive p63 expression .These results confirmed the importance of p 63 IHC in identifying the invasiveness of the tumour regardless to its histological type.

Conclusion

This study concluded that IDC is the commonest histological type. By using IHC , 63 marker is more frequent in benign tumours with significant difference that could serve as diagnostic marker to identify the invasiveness of BC.

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