Mucin 1(MUC1) Over Expression and Intracellular Localization Pattern in Invasive Ductal Breast Cancer using Immunohistochemistry

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Abstract

MUC1 glycoprotein is over expressed and its intracellular localization altered during breast carcinoma tumorigenesis. The present study aimed to screen mucin 1(MUC1) over expression and intracellular localization pattern in invasive ductal breast cancer. 40 formalin-fixed paraffin-embedded breast cancer blocks were collected from patients previously diagnosed with invasive ductal breast cancer. Patients' age range 23-55years with mean age 41 years .ER, PR and HER2 tumor markers were detected immunohistochemically, results revealed ER, PR and HER2 were positive in 12.5%, 10%, and 30 % respectively. From this investigation triple negative was 25 specimens (62.5%) and non triple negative was 15 specimens (37.5%) .MUC1 expression in triple negative were positive in 19/25 specimens (76%) and in non triple negative were positive in 10/15 specimens (67%).Intracellular localization pattern of MUC1expression was observed in five locations cytoplasm only, combinative cytoplasmic and membranous, combinative cytoplasmic and apical ,membranous and negative were detected in16, 9 ,2,2 and 11 specimens respectively .The study conclude no association between muc1 expression and types of invasive ductal carcinoma (triple negative and non triple negative). Cytoplasmic intracellular localization pattern of MUC1expression was most common type.

Key words: Breast cancer, Mucin1

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Introduction

Breast cancer is the most common malignancy in women. The American Cancer Society estimates that 231,840 new cases of invasive breast cancer (BC) will be diagnosed in the U.S. in 2015 and 40,290 women will die from this disease.[1] Breast cancer in African women is characterized by younger age at onset, advanced stage at diagnosis, and consequently poor prognosis [2] In Sudan lately, breast cancer incidence and mortality has been rising. Breast cancer in Sudan according to Radiation Isotope Center Khartoum (RICK) record during the period from 2010 to 2014 is 16.7%, 17.2%, 25% and 48% respectively of all cancer. Therefore, much effort is devoted to identifying factors of prognosis and therapeutic significance of breast cancer. MUC1 is a potential target for immunotherapy. Expression of MUC1 can play an important role in the development of resistance to chemotherapy. Also, MUC1 expression in cancer cells is associated with avoidance of immune defenses.[3]

Muc1 (episialin, epithelial membrane antigen, CA15-3 is a highly O-glycosylated mucin-like transmembrane glycoprotein encoded by a gene on chromosome 1q21[4].It is a sialylated transmembrane glycopratein with a large mucin-like domain consisting of 20-amino acid repeats which are rich in serines, threonines, and pralines[5]. MUC1 over expression strongly decreases cell-cell and cell-matrix interactions, and prevents epithelial cell aggregation by interfering with integrin-mediated adhesion and with E-cadherin- mediated cell adhesion[6-8] MUC1 is normally expressed on the apical surface of mammary epithelial cells However, in breast adenocarcinoma and a number of epithelial tumors, MUC1 is unregulated with aberrant expression over the entire cell surface [9-12.] This characteristic makes the MUC1 protein valuable as a marker in breast cancer diagnostics and prognosis.[10]Over expression of MUC1 enables cancer cells to avoid apoptosis, [13;14] and the MUC1 cytoplasmic tail binds to ER-a, b-catenin and p53, which

influence tumor growth.[15] Different studies suggest that the over expression of MUC1 is associated with an increased metastasis rate [16;17]. The presence of apical cellular localization is an indicator of intact MUC1 pathway that is associated with functional differentiation and good prognosis while the presence of other aberrant patterns of expression that are commonly seen in breast cancer is an indicator of defective MUC1 pathway that is associated with lack of functional differentiation and worse prognosis. [18]

Materials and Methods

This retrospective descriptive study was conducted in Radiation Isotope Center Khartoum (RICK) .40 formalin fixed paraffin embedded tissue blocks previously diagnosed as invasive ductal breast cancer were used. From each block 3 µm was cut using rotary microtome, samples were immunostained for detection of ER, PR, Her2 and MUC1. Sections were deparaffinized, then rehydrated through three different concentrations of alcohol then in distilled water. Antigen retrieval was carried out in PH 9.9 citrate buffer at 95^{o}C in water bath for 30 min. After that sections were cooled at room temperature. Then sections were washed in washing buffer for 5min. 0.3% H_2O_2 was used to block endogenous peroxidase for 10 min. Primary antibodies of ER, PR, MUC1 (Quartet Germany) and Her2 (Dako) were added for 30 min in sections respectively (each marker for facing section). Followed by primary antibody enhancer for 15min .Then secondary antibody label HRP was added for 15 min. DAB chromogen was added. Sections were counterstained with Mayer's acidic hematoxylin for 30 second. Then sections were dehydrated, cleared and mounted.

Data were collected from patients file, and data were analyzed using statistical package for social science (SPSS), mean, frequency and chi square were calculated. Ethical clearance was taken from the ethical committee of the Faculty of Medical Laboratory Science of ALNeelain University.

Results

40 specimens were diagnosed as invasive ductal carcinoma. Patients' age range between 23-55 years with mean age 41years ,22 patients were less or equal 40years (young patients) and 18 patients were more than 40years (old patients) as showed in table1. ER, PR and HER2 were

positive in 12.5%, 10%, 30 % and negative 87.5%, 90%, 70% respectively as listed in table 2. From this investigation triple negative was 25 specimens (62.5%) and non triple negative was 15 specimens (37.5%). In table 3 MUC1 expression in triple negative were positive in 19/25 specimens (76%) and in non triple negative were positive in 10/15 specimens (67%). Intracellular localization pattern of MUC1expression was observed in five locations cytoplasm only, combinative cytoplasmic and membranous, combinative cytoplasmic and apical, membranous and negative were detected in16, 9 ,2 ,2and 11 specimens respectively as described in table 4 and figure 1.

Table (1) Relation between age group and type of invasive ductal BC

cases	Age group		p.value
	less or equal 40	more than 40	
triple negative	14	11	
non triple negative	8	7	0.9
Total	22	18	

Table (2) Immunohistochemical results of ER, PR and HER2

Results	ER		PR		HER2	
	Frequency	%	Frequency	%	Frequency	%
Positive	5	12.5	4	10	12	30
Negative	35	87.5	36	90	28	70
Total	40	100	40	100	40	100

Table (3) Relation between MUC1 expression and types of invasive ductal carcinoma

		MUC1result		p.value
		MUC1 positive	MUC1 negative	
cases	triple negative	19	6	0.5
	non triple negative	10	5	
	Total	29	11	

Table (4) Intracellular localization pattern of Muc1expression

Intracellular localization pattern of Muc1	**Frequency**	**(%)**
cytoplasm only	16	40
combinative cytoplasmic and membranous	9	22.5
combinative cytoplasmic and apical	2	5
membranous	2	5
Negative	11	27.5

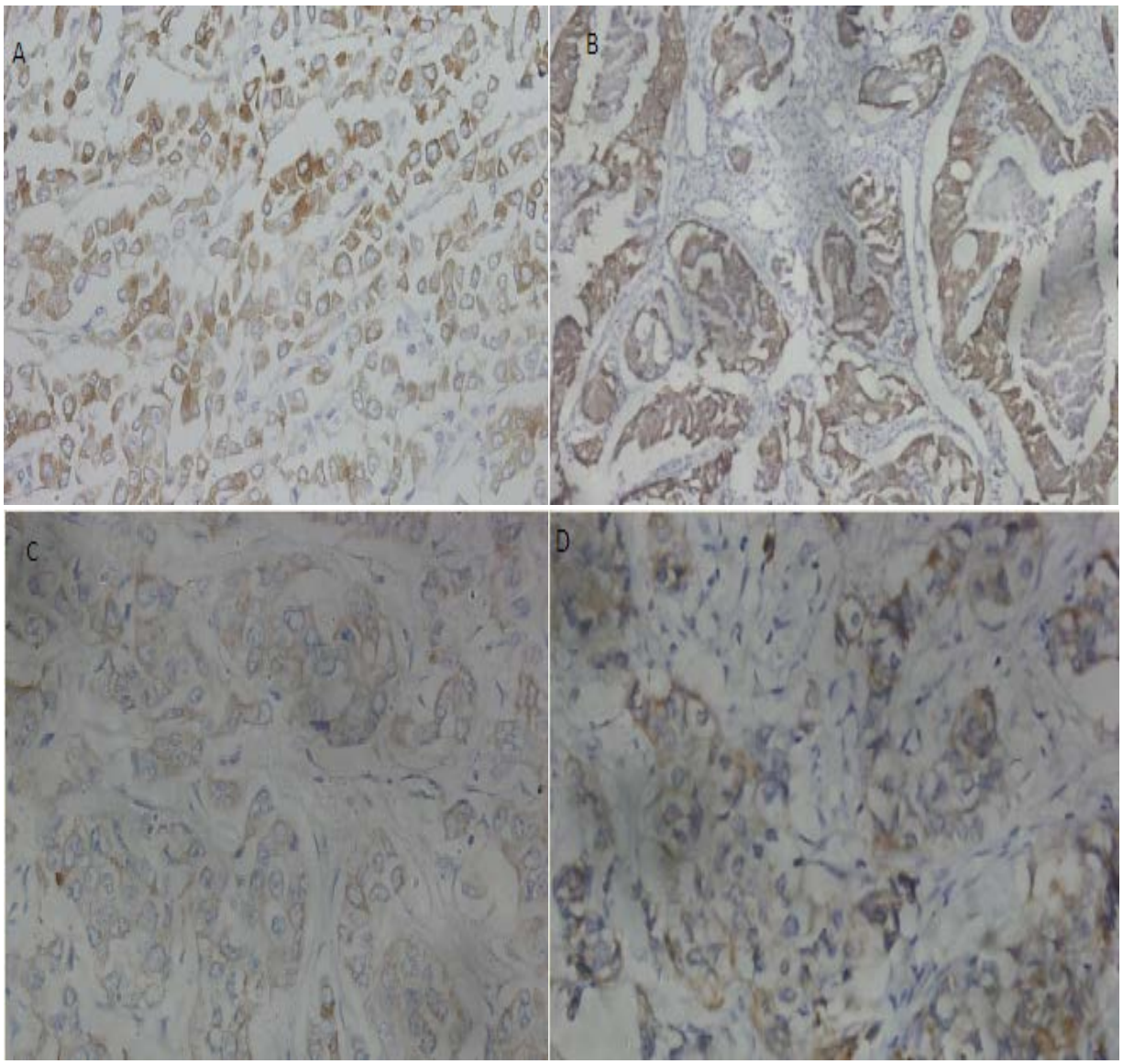

Fig. 1. Intracellular localization pattern of MUC1expression (A) cytoplasmic, (B) Combinative cytoplasmic and apical, (c) combinative cytoplasmic and membranous, and (D) membranous IHC staining.

Discussion

In this study Patients' age range between 23-55 years with mean age 41years. The majority of patients were under 40 years. Grade of invasive ductal carcinoma was found as follow 35/40 specimens were grade3 and 5/40 specimens were grade 2. Some studies clarify that breast cancer

in African women is characterized by younger age at onset, advanced stage at diagnosis, and consequently poor prognosis. Of all breast cancer subtypes, The basal-like and triple-negative phenotypes are found in greater proportion in black and Hispanic with a characteristic sharp decrease in survival during the 3–5 years after diagnosis, and with a much lesser likelihood of distant relapse at 10 years than is seen in patients with ER-positive tumors .[19] In this study 25 specimens (62.5%) were triple negative BC and 15 specimens (37.5%) were non triple negative. MUC1 was expressed in 19/25 triple negative and in 10/15 in non triple negative. Most 18/40 intracellular localization pattern of MUC1expression in our study was cytoplasm only of whom12 specimens were triple negative and 6 specimens were non triple negative. This results is different from Sung-Im Do et al results, who found MUC1 expression was associated with estrogen receptor (ER) expression and negative MUC1 expression was associated with triple negativity [20].Also Misato Iizuka, et al demonstrated that cytoplasmic localization of MUC1 protein varies between breast cancer subtypes, he found it is negative in TN tumors [21]. But Alan Siroy, et al found that 94% of early-stage high grade TNBC with a basal-like phenotype expresses MUC1[22] . Apical membrane MUC1 expression was associated with smaller tumors, lower tumor grades, PR positivity and increased overall survival [23] ,and aberrantly localized MUC1 in BC cytoplasm or the non-apical membrane was associated with a worse prognosis [24].

Conclusion

The study concludes that, no association between MUC1 expression and status of breast invasive ductal carcinoma. Cytoplasmic intracellular localization pattern of MUC1expression was most common type.

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References

(1) American Cancer Society. What are the key statistics about breast cancer?2015.

(2) Elgaili et al. Breast burdecancer n in central Sudan. International Journal of Women's Health 2010.

(3) Suzuki Y SMHSeal. MUC1 carrying core 2 O-glycans functions as a molecular shield against NK cell attack, promoting bladder tumor metastasis. Int J Oncol 2012;40(6):1831-8.

(4) Gendler SJ CECADTJGBD. The locus of the polymorphic epithelial mucin (PEM) tumour antigen on chromosome 1q21 shows a high frequency of alteration in primary human breast tumours. Int J Cancer 1990;45(431):435.

(5) Gendler SJCALJT-PTDNPJBLPENLaDW. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 1990;265:15286-93.

(6) Wesseling J, Van der Valk SW, Vos HL, Sonnenberg A, Hilkens J. Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components. J Cell Biol 1995;129:255-65.

(7) Hilkens J, Ligtenberg MJ, Vos HL, Litvinov SV. Cell membrane-associated mucins and their adhesion-modulating property. TIBS 1992;17:359-63.

(8) Ligtenberg MJL, Buijs F, Vos HL, Hilkens J. Suppression of cellular aggregation by high levels of episialin. Cancer Res 1992, 52, 1992;52:2318-24.

(9) Gendler SJ SA. Epithelial mucin genes. Annu Rev Physiol 1995;57:607-34.

(10) Zaretsky JZ WMTIHMWDK. Expression of genes coding for pS2, c-erbB2, estrogen receptor and the H23 breast tumor-associated antigen. A comparative analysis in breast cancer. FEBS Lett 1990;265(1-2):46-50.

(11) Hilkens J VHWJBMSJvdVSCPC. Is episialin/MUC1 involved in breast cancer progression?. Cancer Lett 1995; 90(1):27-33 1995;90(1):27-33.

(12) Wreschner DH HMTISNHJZJKPWMLRDAea. Human epithelial tumor antigen cDNA sequences. Differential splicing may generate multiple protein forms. Eur J Biochem 1990;189(3):463-73.

(13) Ren J ADKDea. MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. Cancer Cell 2004 2004;5:163-75.

(14) Yin L KSKD. MUC1 oncoprotein blocks hypoxia-inducible factor 1a activation in a survival response to hypoxia.. J Biol Chem 2007;282:257-66.

(15) Mukhopadhyay P CSPMLIJMBSK. Mucins in the pathogenesis of breast cancer-implications in diagnosis, prognosis and therapy. Biochim Biophys Acta 2011;1815: 224-40.

(16) Wesseling J vdVSVHSAHJ. Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components. J Cell Biol 1995;(129):255-65.

(17) Kohlgraf KG GAHMMJBMKSea. Contribution of the MUC1 tandem repeat and cytoplasmic tail to invasive and metastatic properties of a pancreatic cancer cell line.. Cancer Res 2003;63:5011-20.

(18) Rahn JJ DLPMea .The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature. Cancer 2001;91:1973-82.

(19) J.Lachapelle and W.D.Foulkes. Triple-negative and basal-like breast cancer: implications for oncologists. Current Oncology 2011;18(4.(

(20) Sung-Im Do eal. Associations between the Expression of Mucins (MUC1, MUC2, MUC5AC, and MUC6) and Clinicopathologic Parameters of Human Breast Ductal Carcinomas. J Breast Cancer 2013;16.8-152:(2)

(21) Misato Iizuka YNFF ea. Altered intracellular region of MUC1 and disrupted correlation of polarity-related molecules in breast cancer subtypes. Cancer Sci 2015.

(22) Alan Siroy, Fadi W., and et al. MUC1 is Expressed at High Frequency in Early-Stage Basal-Like Triple Negative Breast Cancer. Hum Pathol 2013 Oct 4;44(10): 2159-66.

(23) van der Vegt B dRMPJPCHJdBGHW. The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma. J Histopathology 2007;51(3):322-35.

(24) Rahn JJ DLPMHJ. The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature. Cancer 2001;91:1973-82.

Abbreviations

Muc1 mucin1

BC breast cancer

ER estrogen receptor

PR progesterone receptor

HER2 human epidermal growth factor receptor 2

TNBC triple-negative breast cancer

IHC immunohistochemistry

HRP horseradish peroxidase

DAB 3,3'-Diaminobenzidine