RESEARCH ARTICLE



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Mucin Level as a Potential Biomarker for Breast Cancer **Diagnosis**

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Background: Breast cancer is the second leading cause of cancer death and a health problem worldwide. Secreted mucins are upregulated in ductal adenocarcinoma of the breast, however, the use of mucin as breast cancer biomarker has not been established before. This study aimed to determine the use of mucin level as a potential biomarker for breast cancer diagnosis.

Materials and methods: This was a retrospective, cross-section study involving 40 women subjects with breast cancer. Mucin level was examined with a combination of Alcian blue/periodic acid Schiff (AB/PAS) technique applied to each specimen. The results obtained were statistically analyzed using SPSS.

Results: Results of neutral mucin detection showed that among breast cancer subjects, 16 cases (40%) were neutral mucin score (+), 23 cases (57.5%) were neutral mucin score (++), and 1 case (2.5%) was neutral mucin score (+++). Meanwhile, 10 cases (25%) were acid mucin negative, 17 cases (42.5%) were acid mucin score (+), 11 cases (27.5%) were acid mucin score (++), and 2 cases (5%) were acid mucin score (+++). The most frequent type of mucin was the combination of acid and neutral mucin (30 cases; 75%) and neutral mucin were 10 cases (25%).

Conclusion: Detection of mucin level can be used as an alternative technique for the diagnosis of breast cancer complementary to other types of special stains.

Keywords: AB, PAS, breast cancer, histological grade, mucin level

Introduction

Breast cancer is one of the most common oncologic diseases worldwide, and has become the most frequent cause of death among women.^{1,2} Although breast cancer incidence in Sub-Saharan African countries is lower than in developed countries, African women are more likely to be diagnosed

at later stages of the disease, thus increase the risk of death from breast cancer. This is due to the lack of awareness by women, accessibility to screening methods, and availability of African-based research findings that would influence governmental decision making.³

Large numbers of cancer types were identified using functional assays and biomarkers.4 Mucins are

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high molecular weight glycoproteins that are involved in regulating diverse cellular activities both in normal and pathological conditions. Mucin activity and localization are mediated by several molecular mechanisms, including discrete interactions with other proteins.⁵

Both membrane-bound and secreted mucins are upregulated in ductal adenocarcinoma of the breast. In breast cancer tissues, MUC1, MUC3, and MUC4 among the membrane bound mucins; and MUC2, MUC5AC, MUC5B, and MUC6 among the secreted mucins, show significant up-regulations compared to the normal breast epithelium. In addition to up-regulation of gene transcription, the copy number of MUC1 was also shown to be significantly increased in breast cancer cells, however, not in the non-neoplastic breast ductal cells.⁶

Most breast carcinomas express MUC1, MUC3 and MUC4. Among the various mucins expressed in breast cancer, MUC1 and MUC3 are potential prognostic indicators. MUC1 is also related to patient outcome. Patients with overexpression of mucins show shorter survival life. Overexpression of MUC1 indicates a poor prognosis in patients with breast cancer and is associated with MUC1 promoter methylation status. 8

Mucin plays an important role in the process of tumor progression, invasion and metastases, as well as protection against the host immune response. However, the study regarding the use of mucin as breast cancer biomarker has not been established before. It is necessary to assess the mucin level to be used as an alternative technique for the diagnosis of breast cancer complementary to other types of special stains. This study aimed to determine the mucin level as a potential biomarker for breast cancer diagnosis.

Materials and methods

Study Design and Samples Collection

This was a retrospective, cross-sectional study, conducted in Soba University Hospital, Khartoum, Sudan. Forty women with breast cancer attending the hospital were recruited as the subjects. Specimen included pre-existed formalin fixed paraffin embedded tissue. Combination of Alcian blue/periodic acid Schiff (AB/PAS) technique was performed to detect the mucin level. Ethical approval was obtained from the National University Ethical Research Committee (No. NU-RES/09-026-07, date 1/9/2021) in accordance with the Declaration of Helsinki Principles. The agreement was taken from Soba University Hospital before the sample and data collection.

Staining Procedure

Combination AB/PAS technique was performed. Samples were dewaxed in xylene and rehydrated through graded ethanol to distilled water. Slides were stained in the AB solution for 30 minutes, followed by rinsing in running tap water for 5 minutes and then briefly in distilled water. Slides were oxidized with periodic acid for 5 minutes. The slides were rinsed in running tap water for 5 minutes. Sections were covered with Schiff reagent for 15 minutes. The slides were rinsed in running tap water for 10 minutes, and counterstained with hematoxylin. The slides were then incubated in bluing solution, and followed by final rinsing in tap water for 5 minutes. Slides were dehydrated in graded ethanol, cleared with xylene, and mounted the slides with a miscible medium.

Evaluation Method

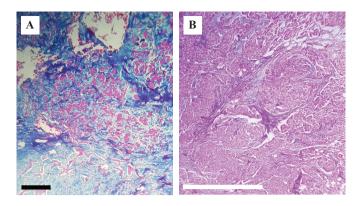
The method used for evaluation of mucin level in breast cancer patients followed the previously described methods. 10,111 Meanwhile, the evaluation categories were following the earlier study performed. Both secreted and membrane-bound mucins have been demonstrated to be expressed during the progression from normal ducts to invasive adenocarcinoma. The (+) indicating that mucin was detected and (-) indicating that mucin was undetected.

Data Analysis

Data analysis was performed by SPSS software version 23.0 (IBM Corporation, Armonk, NY, USA), by using Fisher's exact test. The *p*<0.05 was considered as statistically significant result.

Results

In this study, the average age of the breast cancer subjects was 51±13 years old and the median was 50 years old (30-80 years old). Most of the subjects were in the 40-60 years old group. The amount of mucin was scored as follows: strongly positive intense mucin (+++), moderate positive intense mucin (+++), and weakly positive intense mucin (+) according to the intensity of the stain (Figure 1). For the acid mucin detection, 10 cases (25%) were acid mucin negative, 17 cases (42.5%) were acid mucin score (++), 11 cases (25.5%) were acid mucin score (+++). The most frequent type of mucin was the combination of acid and neutral mucin, which was found in 30 cases (75%), and neutral mucin which was found in 10 cases (25%) (Table 1). Sixteen cases



(40%) were neutral mucin score (+), 23 cases (57.5%) were positive for neutral mucin score (++), 1 case (2.5%) was neutral mucin score (+++) (Table 2). This results showed the intensity and level of neutral mucin distribution in different blocks, revealed that the most common findings score (++) for neutral mucin. The grade of breast cancer in different samples was diverse. The majority of histological grade of breast cancer in this study was grade II (52.5%) (Table 3). There was no correlation between grade of breast cancer and mucin types (p=0.161), grade of breast cancer and level of acid mucin (p=0.195), as well as grade of breast cancer and level of neutral mucin (p=0.954) (Table 4).

Discussion

Subjects in this study were classified to different age categories, and the most frequent age group was 40-60 years old. This result is in accordance with previous reports mentioned that there are numerous risk factors, such as sex, aging, estrogen, family history, gene mutations and unhealthy lifestyle, which can increase the possibility of developing breast cancer.^{2,12}

The most frequent type of breast cancer was invasive ductal carcinoma grade II (52.5%). Acid mucin was common in malignant lesions of breast compared to benign lesions. Acid mucin tended to increase with age. These results are in agreement with many previous findings. ^{13,14} Acid mucin staining results are also in agreement with many previous reports. ¹⁵⁻¹⁸

Mucin is a membrane-bound O-glycoprotein that is expressed at the basal level in most epithelial cells. ¹⁹ Mucin has long been viewed as a tumor-associated molecule because of its frequent overexpression and aberrant glycosylation in most carcinomas. Mucin is overexpressed in >90% of breast carcinomas and other types of cancer, including ovarian, lung, colon, and pancreatic carcinomas. ²⁰

PAS. A: Acid mucin score (+++) and neutral mucin (++); B: Acid mucin score (+++) and neutral mucin (+). Black bar: 200 μm; White bar: 500 μm.

Mucin becomes re-expressed during the transition of primary breast tumor to metastatic lesion. Moreover, mucin re-expression enhances malignancy by promoting the survival and proliferation of non-adherent actively metastasizing cells. These observations pave the way for the assessment of mucin expression in primary breast tumor samples as a marker for metastatic disease.^{21,22}

Table 1. Distribution of subjects based on acid mucin levels.

Acid Mucin	n	%	
Negative	10	10 25.0	
(+)	17	42.5	
(++)	11	27.5	
(+++)	2	5.0	
Total	40	100.0	

Table 2. Distribution of subjects based on neutral mucin levels.

Neutral Mucin	n	%	
(+)	16	40.0	
(++)	23	57.5	
(+++)	1	2.5	
Total	40	100.0	

Table 3. Distribution of subjects based on breast cancer grades.

Cancer Grade	n	%
I	9	22.5
II	21	52.5
III	10	25.0
Total	40	100.0

	Variables	Cancer Grade (n (%))			1 .
Variables		I	II	III	<i>p</i> -value
Type of mucin	Acid and neutral mucin	9 (30.0)	14 (46.7)	7 (23.3)	0.161
	Neutral mucin	0 (0)	7 (70.0)	3 (30.0)	0.161
Acid mucin	Negative	0 (0)	7 (70.0)	3 (30.0)	
	(+)	4 (23.5)	9 (52.9)	4 (23.5)	0.195
	(++)	3 (27.3)	5 (45.5)	3 (27.3)	
	(+++)	2 (100.0)	0 (0)	0 (0)	
Neutral mucin	(+)	3 (18.8)	9 (56.3)	4 (25.0)	
	(++)	6 (26.1)	11 (47.8)	6 (26.1)	0.954
	(+++)	0 (0)	1 (100.0)	0 (0)	

Table 4. Correlation between type & level of mucin with histological grades.

Conclusion

Detection of mucin level can be used as an alternative technique for the diagnosis of breast cancer complementary to other types of special stains.

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^{*}p<0.05; Fisher's exact test.