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Comparison of Mucin Expression in Non-mucinous Breast Carcinoma and Benign Breast Tumor in Khartoum State

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Abstract

Globally, breast cancer continues to be the most prevalent cancer among women, and the second cause of cancer death among women worldwide. The lesions of the breast are characterized by varying amounts of mucin secretion, ranging from benign to malignant conditions. This is a retrospective comparative study, aimed to detect the presence of mucin and its types and intensity in non-mucinous breast carcinoma compared to benign neoplasms. One hundred blocks were previously diagnosed as non-mucinous breast carcinoma and another 100 benign neoplasms were taken from different hospitals in Khartoum state. The sections were stained using combined PAS-alcian blue at different pHs and examined using a light microscope.

Both types of neoplasms had mucin. Acidic mucin revealed a higher proportion, predominately sulphated mucin, which constituted 65% of benign and 93% of malignant, whereas carboxylated mucin was detected in 50% of benign and 72% of malignant. Meanwhile, neutral mucin was found in benign more often than malignant, constituting 30% and 23% of each type respectively. According to the intensity of mucin, all benign neoplasms showed low intensity of both neutral and acidic mucin, while malignant neoplasms exhibit all degrees of intensity (low, moderate and heavy) for acidic mucin and only low intensity for neutral mucin. The study concluded that malignant breast tumors are characterized by a raised amount of mucin compared to benign tumors, particularly acidic types with the highest sulphated mucin proportion; hence, the research suggests that greater emphasis should be placed on acidic mucins when evaluating the histopathological characteristics of neoplasms and as an ancillary tool.

Keywords: Breast cancer • Alcian blue • PAS • Acid mucin • Neutral mucin • Mucin intensity • Khartoum state

Introduction

Breast cancer is the most common type of cancer diagnosed and is the main cause of cancer death among women worldwide [1]. Breast cancer was the fifth leading cause of cancer deaths globally, with an estimated 2.3 million cases and 685,000 deaths in 2020, in women, it accounted for approximately 24.5% of all cancer cases and 15.5% of all cancer-related deaths, ranking first in morbidity and mortality in most parts of the world [2]. In 2020, Incidence rates range from <40 per 100,000 women in some Asian and African countries to over 80 per 100,000 in Australia/New Zealand, North America and parts of Europe. It is estimated that by 2040, due to population growth and aging alone, the burden of breast cancer will increase to more than 3 million new cases and 1 million deaths per year [3].

Generally, the Incidence rates of breast cancer are higher in Northern America, Australia/New Zealand, and Northern and Western Europe, and lower in most African and Asian countries [4]. In developed countries such as the United States, about 232,340 females will be diagnosed with breast cancer and 39,620 will die from it in 2013 [5]. In numerous Western nations, the mortality rates associated with breast cancer are steadily declining. This trend is largely credited to a combination of early detection through mammographic screening and improved treatment [6]. Conversely, there is a rising trend in mortality rates across numerous countries in South America, Africa, and Asia [6]. The International Agency for Cancer Research (IARC) reported that in 2012, the rate of breast cancer occurrence varied from 27 cases per 100,000 women in central Africa to 39 cases per 100,000 women in southern Africa [7]. The incidence of the breast cancer according to the patient statistics of the Radiation and Isotope Center of Khartoum (RICK), Breast cancer is the first most common cancer among Sudanese females (RICK, 2020).

Sex plays a significant role in the risk factors for breast cancer, which is 100 times more prevalent in women than in men, Established risk factors for breast cancer include early age at menarche, later age at menopause, advanced age at first birth, fewer number of children, less breastfeeding, menopausal hormone-replacement therapy, oral contraceptives, but also alcohol consumption, excess body weight, and physical inactivity [7], iodine deficiency in the diet also elevates the risk [8]. Viruses represent a significant risk factor closely associated with breast cancer. Additionally, both tobaccos smoking, whether through positive or negative exposure and high-dose of ionizing radiation elevate the risk. Furthermore, mutations in the BRCA-1, BRCA-2, and P53 genes have been significantly associated with breast carcinoma [9]. Women who inherit a deleterious germ line BRCA1 or BRCA2 mutation face high lifetime risks of developing breast cancer by age 80, which are estimated at 72% and 69%, respectively [10].

Human epithelial tumors express a variety of mucin glycoproteins on their cell surfaces. Mucins are a group of high molecular weight glycoproteins characterized by numerous O-glycosylated tandem repeat domains, which differ in their quantity, length, and degree of O-glycosylation. A number of human secretory mucin genes, referred to as MUC genes, have been identified. Through cDNA cloning, at least 19 distinct human mucin genes have been recognized, including MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, and MUC20 [11], among which MUC1 gene encodes a membrane form of mucin-like O-glycoprotein or epsilon, that is highly expressed in breast carcinoma [12]. The unique properties of the MUC1 protein render it an important marker for diagnosing and prognosis in breast cancer. Mucin is categorized into acid and neutral types, with additional subdivisions for the acid category [13], acidic mucins exhibit selective staining with alcian blue, which is dependent on the pH level, whereas neutral mucins can be identified using PAS, colloidal iron mucicarmine, or metachromatic dyes [14].

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Materials and Methods

Study design

This is a retrospective descriptive comparative study aimed to detect mucin expression and its type in non-mucinous breast carcinoma in comparison with benign breast neoplasm's breast.

Sample collection

One hundred blocks (Formalin fixed paraffin wax embedded tissues, which obtained previously from women with breast neoplasm) of non-mucinous breast carcinoma with different pathological types as study group and 100 of benign neoplasms as comparative group, were selected randomly from different hospitals in Khartoum state (Laboratory Administration histopathology department, Radioisotope Centre Khartoum, Suba University Hospital and Omdurman Hospital).

Three sections of 5um thick were cut from each formalin fixed paraffin wax embedded tissue of non-mucinous breast carcinoma and non-malignant neoplasms, using rotary microtome and mounted on labeled glass slides after floating on water bath at 40ºC, then sections were placed in an oven 75ºC for 15 minutes to fix the tissues on the slides and melt the wax to facilitate the later deparaffinization before the subsequent staining technique, control sections were prepared as the same.

Sample staining

Alcian blue was prepared at three different pHs; pH 1 for sulphated acidic mucin, pH 2.5 for all acidic mucin and pH 3.2 for carboxylated mucin as described in Bancroft JD and Gamble M [13]. Then the Sections were stained using combined alcian blue PAS as described in Bancroft and Gamble [13] as follows:

Sections were deparaffinized and rehydrated, treated with diastase solution for one hour at 37ºC and washed in distilled water then stained with alcian blue solution for five minutes, washed in water and rinsed in distilled water, then treated with 1% aqueous periodic acid for five minutes, rinsed in distilled water, further treated with Schiff's reagent for 15 minutes, washed in running tap water for ten minutes, then nuclei was stained with Mayer's Haematoxylin for 2 minutes, washed in water, dehydrated, cleared, and mounted in D.P.X.

Result assessment

The quantity of mucin evaluated as: Intensive (85-100%) = (+++), Moderate (50-85%) = (++), Low (Less than 50%) = (+) and absent (0).

Data analysis: Data analysis was carried out by Computerized by using statistical package of social since, software version 21.0, Chi squire test.

Results

This study aimed to identify the type of mucin histochemically in non-mucinous breast cancer compared to benign neoplasms. The non-mucinous breast carcinoma was classified as follows; non-invasive ductal carcinoma 18 (18%), non-invasive lobular carcinoma 15 (15%), invasive ductal carcinoma 55 (55%) invasive lobular carcinoma 4 (4%), tubular carcinoma 4 (4%), medullary carcinoma 4 (4%), while benign neoplasms had two categories; 65 (65%) which were diagnosed as fibro adenoma and the remaining 35 were non-fibro adenoma benign tumors. The ages of the study population including study and comparative groups (200) ranging from 16 to 80 years old with a mean of age 48years the majority of the them were among the age group 20-60 years old which constituted 159 (79.5%).

The majority of study group (100) is in age group 41-60 which accounted 47 (47%), most of them were ductal carcinoma (Table 1). However, the most frequent histological grade of invasive ductal carcinoma as shown in Table 2 is grade II, which represents (52.8%), followed by grade I (32.7%) and lastly grade III (14.0%).

All types of mucins were detected in both neoplasms, with the highest expression of sulphated mucin (93%) in malignant and (65%) in benign, followed by the carboxylated type which accounted for 72% in malignancy and 50% in the benign, while neutral mucin was detected in control more than in cases, accounted 30% and 23% respectively. The highest expression of mucin types was seen in invasive ductal carcinoma as shown in Table 3.

Table 4 and Figure 1 shows the intensity of neutral mucin among the study group, majority of non-invasive ductal carcinoma, non-invasive lobular carcinoma and invasive ductal carcinoma showed no reactivity with an account of 16/18(88.9%), 14/15(93.3%), 43/55(78.2%), respectively, on another hand tubular carcinoma and medullary exhibit low reactivity (3/4, 75%) rather than absent (1/4, 25%), with a total of 23% with low reactivity which slightly lower than comparative group (fibro adenoma and other non-malignant conditions), that represent 30%.

The intensity of acid mucins among study group compared to comparative

Table 1. Description of the histological classification according to the age group among study group (cases).

Age Group	Histological Diagnosis						Total
	N.I.D.C	N.I.L.C	I.D.C	I.L.C	Tubular Carcinoma	Medullary Carcinoma	
<20	2	0	0	0	0	0	2
20-40	6	6	23	0	0	0	35
41-60	7	8	25	2	3	2	47
61-80	3	1	7	2	1	2	16
Total	18	15	55	4	4	4	100

N.I.D.C: Noninvasive Ductal Carcinoma
N.I.L.C: Noninvasive
I.D.C: Invasive Ductal Carcinoma
I.L.C: Invasive Lobular Carcinoma

Table 2. Frequency of histological grades in invasive ductal carcinoma.

Histological Grade	Frequency	Percent
Grade 1	18	32.7
Grade 2	29	52.7
Grade 3	8	14.5
Total	55	100

Table 3. Descriptions of mucin types in relation to histological diagnosis among study group compared to comparative group.

Histological Diagnosis	Neutral Mucin	Acid Mucin	
		Carboxylated	Sulphated
Noninvasive ductal carcinoma	2(2%)	13(13%)	16(16%)
Noninvasive lobular carcinoma	1(1%)	7(7%)	11(11%)
Invasive ductal carcinoma	12(12%)	43(43%)	55(55%)
Invasive lobular carcinoma	2(2%)	3(3%)	4(4%)
Tubular carcinoma	3(3%)	3(3%)	4(4%)
Medullary carcinoma	3(3%)	3(3%)	4(4%)
Total	23(23%)	72(72%)	93(93%)
Fibroadenoma	19(19%)	32(32%)	43(43%)
Others	11(11%)	18(18%)	22(22%)
Total	30(30%)	50(50%)	65(65%)

Table 4. The intensity of neutral mucin in relation to histological diagnosis among cases and comparative group using periodic acid schiff's reagent.

Histological Diagnosis	PAS		Total
	Low	Absent	
Noninvasive ductal carcinoma	2(11.1%)	16(88.9%)	18(100%)
Noninvasive lobular carcinoma	1(6.7%)	14(93.3%)	15(100%)
Invasive ductal carcinoma	12(21.8%)	43(78.2%)	55(100%)
Invasive lobular carcinoma	2(50%)	2(50%)	4(100%)
Tubular carcinoma	3(75%)	1(25%)	4(100%)
Medullary carcinoma	3(75%)	1(25%)	4(100%)
Total	23	77	100
Fibro adenoma	19(29.2%)	46(70.8%)	65(100%)
Others	11(31.4%)	24(68.6%)	35(100%)
Total	30	70	100(100%)

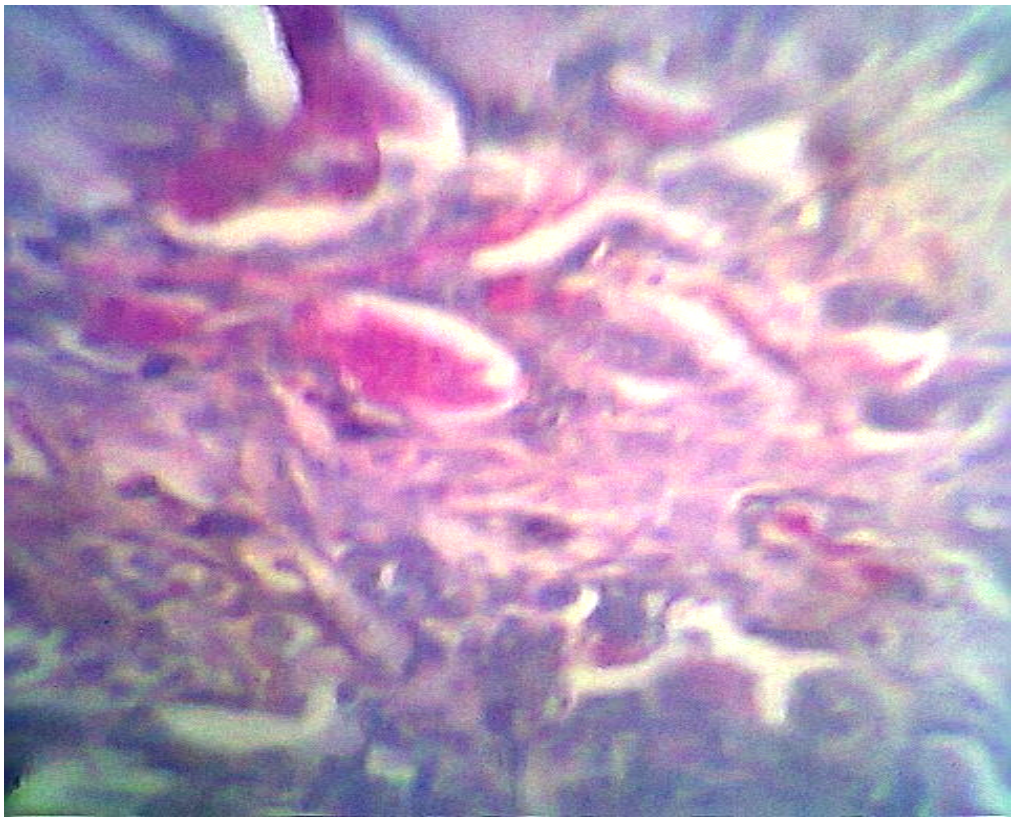

Figure 1. Breast section diagnosed as medullary carcinoma showing low intensity of neutral mucin using PAS and alcian blue (X40).

Table 5. Description of acid mucin intensity among histological diagnosis of the cases and comparative group using alcian blue at different pH.

Diagnosis	Alcian Blue at Different pH											
	PH 2.5 All Mucin				PH 1 Sulphated Mucin				pH3.2 Carboxylated Mucin			
	Heavy	Moder-ate	Low	Absent	Heavy	Moder-ate	Low	Absent	Heavy	Moder-ate	Low	Absent
N.I.D.C	5(5%)	6(6%)	5(5%)	2(2%)	6(6%)	6(6%)	4(4%)	2(2%)	2(2%)	2(2%)	9(9%)	5(5%)
N.I.L.C	0(0%)	0(0%)	12(12%)	3(3%)	0(0%)	0(0%)	11(11%)	4(4%)	0(0%)	0(0%)	7(7%)	8(8%)
I.D.C	23(23%)	10(10%)	22(22%)	0(0%)	0(0%)	19(19%)	36(36%)	0(0%)	9(9%)	9(9%)	25(25%)	12(12%)
I.L.C	2(2%)	0%	0(0%)	2(2%)	0(0%)	2(24)	0(0%)	0(0%)	1(1%)	1(1%)	1(1%)	1(1%)
Tubular carcinoa	4(4%)	0(0%)	0(0%)	0(0%)	4(4%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	3(3%)	1(1%)
Medullary carcinoa	4(4%)	0(0%)	0(0%)	0(0%)	0(0%)	4(4%)	0(0%)	0(0%)	0(0%)	0(0%)	3(3%)	1(1%)
Total	38(38%)	16(16%)	39(39%)	7(7%)	10(10%)	31(31%)	51(51%)	6(6%)	12(12%)	12(12%)	48(48%)	28(28%)
	100(100%)				100(100%)				100(100%)			
Fibroadenoma	0(0%)	0(0%)	60(60%)	0(40%)	0(0%)	0(0%)	43(43%)	22(22%)	0(0%)	0(0%)	32(32%)	33(33%)
Others	0(0%)	0(0%)	5(5%)	0(95%)	0(0%)	0(0%)	22(22%)	13(13%)	0(0%)	0(0%)	18(18%)	17(17%)
Total	0(0%)	0(0%)	65 (65%)	-35%	0(0%)	0(0%)	65(65%)	35(35%)	0(0%)	0(0%)	50 (50%)	50(50%)
	100(100%)				100(100%)				100(100%)			

N.I.D.C: Noninvasive Ductal Carcinoma
N.I.L.C: Noninvasive
I.D.C: Invasive Ductal Carcinoma
I.L.C: Invasive Lobular Carcinoma

Figure 2. Intensive reaction of acid mucin (Alcian blue pH 2.5) in section diagnosed as invasive ductal carcinoma (X40).

group, using alcian blue stain at different pH as shown in Table 5 and Figures 2-4.

First at pH 2.5: "The intensity of both carboxylated and sulphated", the majority of non-invasive ductal carcinoma and non-invasive lobular carcinoma showed moderate and low intensity respectively. However, in invasive ductal carcinoma, the majority showed intense reactivity, as well as all sections; represent tubular carcinoma, medullary carcinoma and fifty percent of invasive lobular carcinoma. While the comparative group exhibited low intensity.

Second at pH 1: "The intensity of sulphated acid mucins", the majority in non-invasive ductal carcinoma sections, showed moderate reactivity while low reactivity represented by majority of non-invasive lobular carcinoma. Whereas, all invasive ductal carcinoma showed reactivity and the majority was intense reactivity, also intense reactivity seen in all tubular carcinoma and medullary carcinoma, whilst all in invasive lobular carcinoma showed moderate reactivity. Concerning the comparative group the majority of them revealed low intensity.

Third at pH 3.2: "The intensity of carboxylated mucin", Invasive ductal carcinoma was the most common type (43%), classified as (9%) heavy

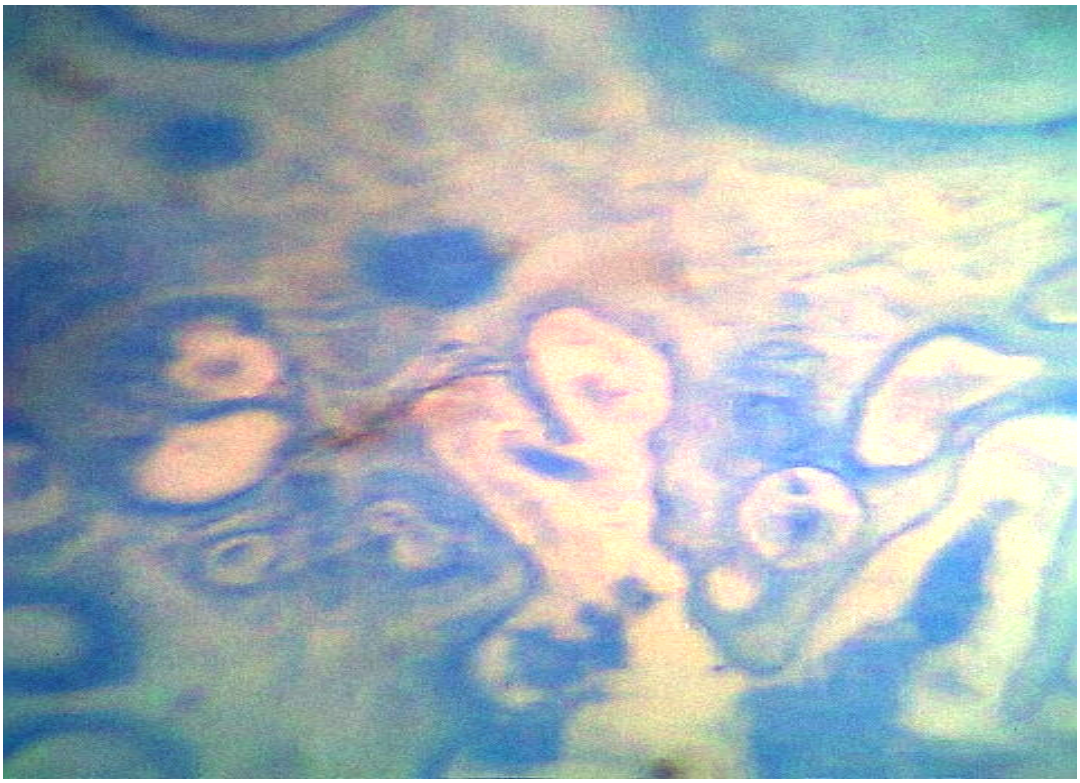

Figure 3. Moderate reaction for sulphated acid mucin using alcian blue pH 1.0 in breast tissue diagnosed as noninvasive ductal carcinoma, by light microscope, X40.

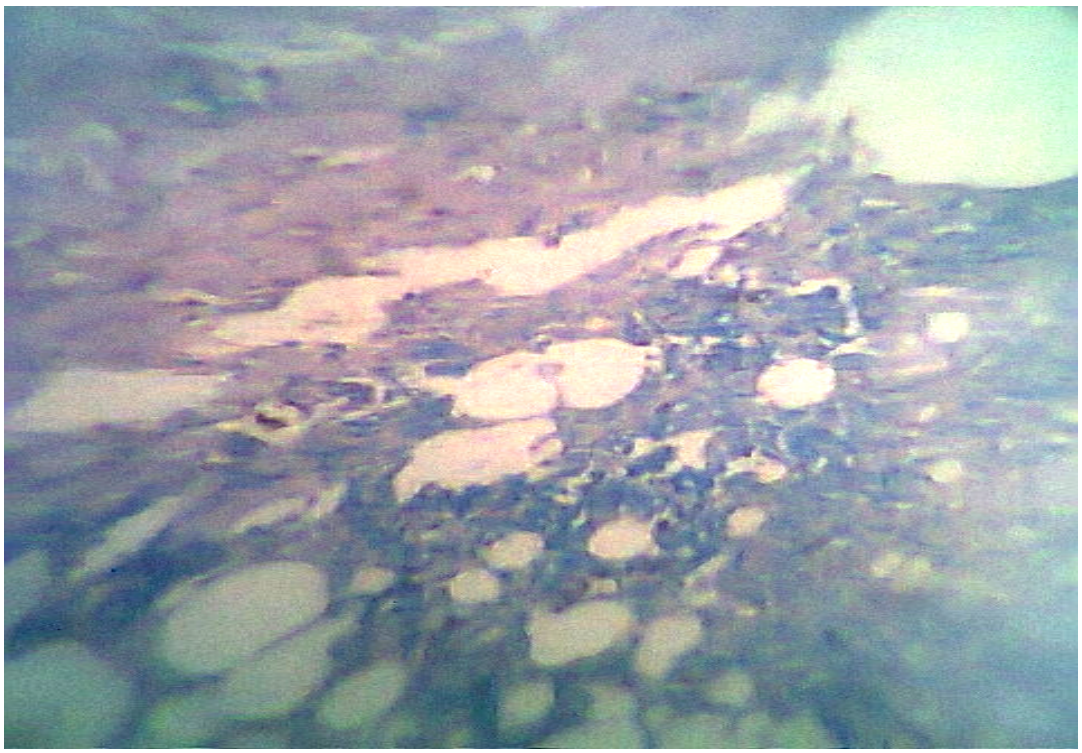

Figure 4. Low reaction for carboxylated acid mucin using alcian blue pH 3.2 in breast tissue diagnosed as invasive lobular carcinoma, by light microscope, X40.

intensity, (9%) moderate intensity and (25%) low intensity, in contrast most of NIDC as well as tubular and medullary carcinoma exhibited low intensity, with only low intensity being observed in the comparative group.

Discussion

The present study reveals that the highest incidence of breast malignant neoplasms was at the age group above 40 years old with a high incidence of invasive ductal carcinoma which accounted for 73%, this finding is not far away from the results of Elgaili EM, et al. [15] who studied the types, stages and ages of breast cancer at diagnosed women living in central Sudan encompassing al-Gaezira, Blue Nile, White Nile and Senar states. Their cases comprised 1255 women diagnosed with breast cancer, they found that (26%) of women were >50 years old and (74%) were <50 years old or premenopausal, they reported

that invasive ductal carcinoma was the most common (79%) breast carcinoma and getting breast cancer increases with age, similar results detected by several studies Majeed W, et al. and Abdihalim TS and Idris AAA [16,17].

This study showed that both types of breast neoplasms express mucin with an increased ratio in malignant (93%) than in benign (65%) these results agree with several previous results [18,19]. However, the highest expression of mucin types was seen in invasive ductal carcinoma and N.I.D.C., this result agreement with Oral O, et al. and Thai JN, et al. [20,21].

Our finding revealed that the rate of acid mucin was higher compared to neutral mucin expressed in breast carcinoma, particularly in invasive ductal carcinoma grade II, similar result was reported by many researchers [17,22]. The highest expression of acid mucin compared to neutral mucin was also noted by Tan PH, et al. [23]. Nevertheless, many findings show evidence that the most common sub-type of acid mucin encountered was sulphated mucin [18,24], this supports our finding as sulphated mucin existed in 93% and 65% of malignant and benign tumor respectively. Worth noting that sulphated mucin was detected in all IDC, ILC, tubular carcinoma and medullary carcinoma and the majority of NIDC and NILC exhibited 88.9% (16/18) and 73.3% (11/15) respectively. Therefore, the demonstration of sulphated mucin may aid in the identification of breast tumors.

Conclusion

Breast neoplasms are characterized by mucin expression, with an increased ratio in malignant types as opposed to benign ones, mostly of sulphated acidic mucin. Consequently, the study proposes that more consideration of acidic mucin be taken when assessing the histopathological characteristics of neoplasms, particularly sulphated mucin as an additional adjuvant tool in the diagnosis of malignant breast neoplasm.

Acknowledgement

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Conflict of Interest

There is no conflict of interest.

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