Ki67 Expression, Relation to Breast Cancer Morphology, Molecular Sub-Types, And the Expression of Estrogen, Progesterone Receptors, And HER2 In Sudanese Women

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ABSTRACT

Background: Worldwide 19.3 million new cases of breast cancer (BC) will be diagnosed in 2020, and the disease will claim 10 million lives]. BC incidence rapidly increases globally and varies significantly by country. BC counts 11% of all new cancer cases and 23% of all female malignancies. This study was carried out to evaluate Ki67 expression, its relation to breast cancer morphology, molecular sub-types, and the expression of estrogen, progesterone receptors, and HER2 in Sudanese women.

Methods: Paraffin-embedded tissue samples from patients with BC diagnosed at the Department of Pathology, Gezira University, Wad Medani, between 2016-2022. One hundred twenty-five cases were retrieved, and 94 met the inclusion criteria. Histological sections 3-5 µm thick for each patient were stained with hematoxylin &eosin to confirm the morphology and to ensure the presence of normal tissue in the vicinity of the tumor; we used the standard methods for assessing estrogen (ER)and progesterone (PR) HER_2 and Ki-67.

Result: The study examined 94 invasive breast carcinomas in patients between 28 and 75, with a mean age of 47.6 years. Almost all studied cases were invasive ductal carcinoma (95.7). The mean tumor size was 43 mm, and the majority (57%) was grade 2. The rate of ER-positive tumors was 33%, and PR-positive tumors were found in 18%. Coexpression of ER and PR was found in only 10 cases (11 %). Ki67 was more than 20% in 51% of the studied group. Molecular type frequencies were Luminal-A(12%), luminal-B(16%), Her2/neu (25%), and triple-neagtive(41%). (table 1). There was a significant relationship between Ki 67 (>20%) and menopause, tumor grade Tumor type, and Molecular diagnosis(P-value 0.006, 0.0001,0.0375 and 0.0001), respectively.

Conclusion: This study detected that Ki 67 is highly prevalent in breast cancer in Sudanese women. Moreover, the hormone receptor status is similar to the incidence in central and western Africa and higher than in east Africa.

Keywords: Breast cancer, Ki-67, Moleculr type of breats cancer, Sudan

INTRODUCTION

Worldwide 19.3 million new cases of breast cancer (BC) will be diagnosed in 2020, and the disease will claim 10 million lives[1]. BC incidence rapidly increases globally and varies greatly by country[2]. BC counts 11% of all new cancer cases and 23% of all female malignancies[3]. Breast cancer, in general, is much more frequent in developed countries compared to Africa. However, its incidence is rising in most African countries, equalling or even topping the number of cervical carcinomas[4]. BC is exceptionally varied regarding signs and symptoms, histopathology, markers prognosis, and response to treatment[5]. Around 70% of Sudanese women‘s breast cancer cases occur in ages younger than 50 years[6]. The main problem with BC in Africa is that tumors are diagnosed with advanced disease due to substantial delay in presentation, and diagnosis, a complex issue mediated by patients and the health system in both Sub-Saharan and North Africa[3,7]. Recent findings indicate that more than three months between recognition and diagnosis is linked to advanced stages at presentation and a worse prognosis[8]. This certainly is related to infrastructural deficiencies and traditional attitudes resulting in late detection, with figures estimating two-thirds of patients in stages III and IV[9]. There is, however, still a debate about whether the poor prognosis of BC in African women is simply the consequence of late detection and limited resources for therapy or whether there is a genetic susceptibility for more aggressive phenotypes[10]. Despite significant progress in understanding factors contributing to racial differences, tumor genetics, and outcome, multiple disciplinary efforts are required to reduce or remove them[11]. HER_2Amplification varies from 20%-35% in breast cancer to around 80 ductal carcinomas in situ, which is essential for treatment and prognosis. The ductal carcinoma showed more propensity to show HER_2 positivity than lobular carcinoma, and high-grade carcinomas were HER_2 ppositive compaired tolow-grade tumors. The authors said any positive of HER_2 in grade 1ducatl or lobular caricom a required reevaluation of tumor grade and type. Effective targeted preventive programs are strongly encouraged to reduce the BC disease burden worldwide[2]. Ki67 is an independent prognostic marker to detect survival, prognosis, and response to therapy[12]. It is used to differentiate between LumenalA and Lumuenal B breast cancer[13] and is associated with minimal inter-laprotary variation[14]. Given that sub-Saharan African countries have more opportunities to implement modern health care, a deeper understanding of African women‘s breast cancer molecular characteristics relative to the more thoroughly researched western countries is essential to improve the prognosis and management of breast cancer. Therefore, we have studied Ki67 expression and its relation to morphology, the expression of estrogen and progesterone receptors, and HER2 in 94 cases of BC diagnosed in Wad Medani, Sudan.

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MATERIALS AND METHODS

Ethical approval for this study was issued by the university of Gezira faculty of medicine ethical committee (2021/18). Paraffin-embedded tissue samples from patients with BC diagnosed at the Department of Pathology, Gezira University, Wad Medani, between 2016-2022.. One hundred twenty-five cases were retrieved, and 94 met the inclusion criteria. Histological sections 3-5 µm thick for each patient were stained with hematoxylin &eosin to confirm the morphology and to ensure the presence of normal tissue in the vicinity of the tumor. All histological slides were re-evaluated, classified according to the actual WHO classification, and graded according to Elston and Ellis by the author and proofed by prof Mohammadani. Immunohistochemistry was performed using an automated machine LEICA Model No: BOND- MAX serial number M496234. Estrogen receptors monoclonal Rabbit antihuman Estrogen receptors α-clone EP1- ready to use (Aglint technologies. Inc,5301 stevens creek Blvd. Santa Clara, CA95051 United States. Manufactured in the United States). Progesterone receptors clone (1E2) Rabbit monoclonal antibodies (Roche, Germany). For Ki-67, ER, and BR, the protocol used was as follows; Peroxide Block (5 minutes), followed by three washes (2 minutes each), primary antibody (15 minutes), followed by three washes, post-primary antibodies(8 minutes), followed by three washes, Polymer (8 minutes) followed by three washes, Mixed DAB Refine(10 minutes) followed by three washes with deionized Water, and hematoxylin (5 minutes) 3 washes. To minimize daily variation, slides were immunostained together in a single run. Normal tissue at the tumor margin served as an internal control for ER and PR, and positive control for the staining process was generally carried on each slide. Inclusion criteria all invasive carcinoma with no previous treatment with either chemo or radiotherapy. Exclusion criteria: Poorly fixed specimens and biopsy not sufficient for immunohistochemistry stain. Evaluation of immunohistochemical staining. Negativity for estrogen and progesterone receptor was scored only in cases with positive internal control; the Allred score was used for quantification. The DAKO score was used for Her2/neu; the tumor score correspondent to the completeness of cell membrane staining and the intensity of stain as follows (0: no stain,1: incomplete membrane stain with weak intensity,2: complete membrane staining with moderate intensity, and 3: intense and complete membrane immunoreactivity). The 3+ positives were considered for statistical analysis. For ER PR and Ki-67, the percentage of neoplastic cells with an evident immunoreactivity was determined among 2000 cells in a minimum of 10 high-power fields using an X40 lens (X400)[15]. The number of Ki-67 positive cells was scored in percentage; Ki67 less than 20% is regarded as low, while a value more than 20% is considered high and used for statistical coreelations[16,17].

Molecular Subtypes : The St. Gallen international expert consensus in 2013 devived Molecular type into Luminal-Aluminal-B, Her2/neu, and triple-negative depending on ER, PR, HER_2, and ki67 status[18].

Statistical Analysis: Statistical correlation between immunohistochemical expression data analyzed by chi-square. Below 0.05 result of p~ vale was typically deemed to be significant. The statistical package spss v 23.0 was applied to all calculations.

RESULTS

The study examined 94 invasive breast carcinomas in patients between 28 and 75, with a mean age of 47.6 years. A minority of case showed positive family history for BC(17%), and the majority were sporadic. Almost all studied cases were invasive ductal carcinoma (95.7). The mean tumor size was 43 mm, and the majority (57%) was grade 2. The rate of ER-positive tumors was 33% (figure 1A), and PR-positive tumors were found in 18% (figure 1B). Coexpression of ER and PR was found in only 10 cases (11 %). Twelve tumors only expressed ER (13%), and two were PR-positive. Ki67 was more than 20% in 51% of the studied group (figure 1F). Molecular type frequencies were Luminal-A (12%), luminal-B (16%), Her2/neu (25%) (figure 1D) and triple-neagtive(41%) (table 1). There was a significant relationship between Ki 67 (>20%) and menopause, tumor grade Tumor type, and Molecular diagnosis(P-value 0.006, 0.0001,0.0375 and 0.0001), respectively, but no correlation between high ki67 score and Age at diagnosis, family history, age at menarche and Tumor size table 2.

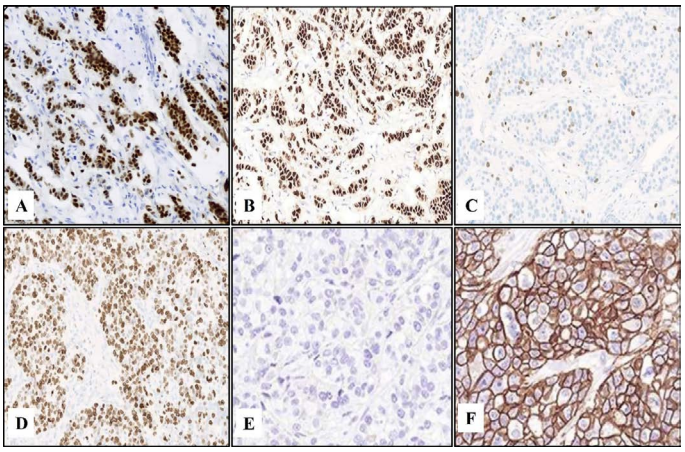

Figure 1: Showed an immunohistochemical stain of studied cases A) strong positivity of ER. B) PR nuclear positivity C) Ki-67 positivity less than 20%. D) Ki-67 positivity of more than 20%. E) HER2 negative. F) HER2 complete membrane strong positivity with DAKO score 3

Table 1: Basic clinicopathological tumor criteria

Variable		Frequency	Percent
Age at diagnosis	< 50	52	55.3%
	>50	42	44.7%
Family history	Negative	78	83.0%
	Positive	16	17.0%
Age at menarche	<15 years	34	362%
	>15 years	28	29.8%
	Uncertain	32	34%
Diagnosis	DEC	90	95.7%
	lobular	1	1.1%
	mucinous	2	2.1%
	papillary	1	1.1%
Grade	1	18	19.2%
	2	52	55.3%
	3	24	25.5%
ER	Negative	64	68.1%
	positive	30	31.9%
PR	Negative	78	83.0%
	positive	16	17.0%
HER2	Negative	67	71.3%
	positive	27	28.7%
Ki67	<20%	46	48.9%
	>20%	48	51.1%
Triple-negative	Yes	41	43.6%
	No	43	56.4.%
Molecular diagnosis	luminal-A	12	12.8%
	luminal-B	16	17.0%
	Her_2/neu+	25	43.6%
	triple-negative	41	26.6%

Table 2: Correlation between KI67 and clinicopathological tumor criteria

Variable		Ki67		P- Value
		Less than 20%	More than 20%	
Age at diagnosis	>50 years	24(52.2%)	28(58.3%)	0.347
	<50 years	10	10	
Age at menarche	Uncertain			0.549
	< 15	14(53.5%)	20(55.6%)	
	> 15	12(46.2%)	16 (44.4%)	
Menopause	Yes	26(56.5%)	14(29.2%)	0.006
	No	28(58.3%)	34(70.8%)	
Tumor size	≥ 2	6 (13.0%)	6 (12.5%)	0.601
	2.1 - 5.0	34(74.0%)	32(66.7%)	
	>5.0	6 (13.0%)	10(20.8%)	
Family history	Yes	8 (17.4%)	8 (16.7%)	0.571
	No	38 (82.6%)	40 (83.3%)	
Tumor grade	1	14 (30.4%)	4 (8.3%)	0.0001
	2	28 (60.9%)	24 (50.0%)	
	3	4 (8.7%)	20 (41.7%)	
Tumor type	IDC	42(91.3%)	48(100%)	0.037
	Others	4 (8.7%)	0 (00.0%)	
Molecular diagnosis	luminal-A	0 (00.0%)	12(25.0%)	0.0001
	luminal-B	16(34.8%)	0 (00.0%)	
	Her2/neu+	12 (26.1%)	13(27.1%)	
	triple-negative	18(39.1%)	23 (47.9%)	

DISCUSSION

Cancer of the breast is a growing health burden throughout the world[19]. The majority of medical understanding about breast cancer comes from studies conducted in North America and Europe;however, recent studies have revealed key distinctions between the morphology and minfistation of cancer in African women[20]. Our results showed high negativity of ER, 77%, in line with studies from; Ghana, 75%[21] and Nigeria, 75%[22], 65%, and 61% in Kenya and Mali, respectively[23]. Meta-analysis and systemic review revealed factors like young age, tissue collection method, retrospective studies, early diagnosis, and being from south Africa contributed to hormone receptor-negative breast cancer[23]. In this study, HER_2 positivity was 27%, agreeing with other studies, which showed HER_2 positivity (DAKO score 3+) is more variable, ranging from 18% to 82%;[24-26]. Because most studies do not report positive internal and process controls, whether these figures represent biology or are just fixation artifacts is questionable[27]. Even though black women experience breast cancer at a lower rate than white women in America, new statistics revealed that the mortality in black women is still higher than in other ethnic groups. The death rate is tenfold higher in women under 50, and a 5- survival rate for all molecular subtypes is lower for stage 1. The gap between black and other groups is absolute for HER_2 negative and ER&PR positive receptors at 96%[28]. In a 2017 study by Asmarom et al. comparing Sudanese and german women, BC behaviors and markers concluded that the Sudanese women had aggressive tumors with negative receptors even in grade I and II cancer[20]. To reduce breast cancer mortality by alleviating racial differences through the facilitated entrance to high-tech screening programs and management through collaboration between the health system, community shareholders, and upholding organizations. There is a substantial possibility of improved breast cancer prognosis by advocating early diagnosis and upgrading the treatment centers[22]. In the current study, Molecular type frequencies were Luminal-A (12%), luminal-B (16%), Her2/neu (25%), and triple-neagtive (41%). The percent of triple-negative is in agreement with west African countries: Nigeria (46.7%), Senegal (46.7%), Togo (37.6%) and Ganah (44%), North African countries Eygpt (44.79%) and Sudan (34.5%), east Africa Kenya (36.7%) and Rwanda (37.7). Higher present were reported in west African countries: Nigeria(63%), Senegal (46.7%), and Ganah (82.20%), and one study from north Africa, Tunisia (66.25%)[29,30]. In contrast, a lower frequency of triple-negative was reported mainly in central, east, and southern African countries: Congo (4.20%)[31], Kenya (20%), Ethiopia (23%), Botswana (21.1%), and South Africa (21.7%)[29,32], this variation in triple-negative frequency across Africa cannot be explained by demographic variation only, a vailed protocol for assessing hormone status including pre and post-analytical quality control is highly recommended. In this study, almost 50% of the studied group showed high Ki67 expression (>20%). This agrees with studies from the democratic republic of Congo 48.42%[31] and south africa, which revealed high Ki67 expression in advanced tumors (48%)[33] and Nigeria (55%)[34] Ki67 in Morocco (72.3%)[35]. Ki76 became more available and cost-effective, and its assessment has been recommended for routine use to predict the prognosis of triple-negative breast cancer. Ki67 is indicative of proliferation, and its percentage may affect the treatment of receptor-negative tumors with high Ki67; the responses to chemotherapy are better since it affects dividing cells more than non-dividing ones[36]. Ki67 anticipated poor prognosis as an independent factor and can be used as a predictive factor for stage I triple-negative tumors[16]. Ki67 can differentiate between luminal A and B breast cancer and high Ki67 predictor survival. It can be used as a guide in determining treatment choice, especially when its expression is more than 20%. Ki67 is correlated with grade II&III, stage III&IV BC, and receptor-negative tumors[37]. The strength of this study is that 1) all specimen are treated preanalytical regarding fixation concentration of formalin, slicing, and duration of fixation; all immune histochemistry were done in a single run and for the first time in our setting in a fully automated machine which granite the quality of stain. The limitation of this study is that we did not examine cytokeratins 5 and 6 for the detection of putative stem cells and basal keratins and p63 to detect myoepithelial cells and FISH to assess equivocal score (Dako 2).

CONCLUSION

This study detected that Ki 67 is highly prevalent in breast cancer in Sudanese women. Ki 67 expression contributed to tumor biology and survival rate in black women; therefore, assessing Ki 67 in every breast cancer in Sudanese women might improve treatment strategy and change the outcome of breast cancer. Moreover, the hormone receptor status in Sudanese women is similar to the incidence in central and western Africa and higher than in the east and south Africa.

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