Biologic Subtypes of Breast Cancer in Sokoto, Nigeria

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ABSTRACT

Most recently published works from across Nigeria and Africa point to a rising prevalence of breast cancer in these hitherto low incidence areas. The literature is also replete with studies on molecular markers of breast cancer amongst pre and post- menopausal women especially in the more advanced countries. Even though our centre has also witnessed a rising trend in the incidence of breast cancer, not much work has been done to elucidate the molecular biology of the disease. This study was therefore undertaken to determine the predominant receptor status of breast cancer among patients in Sokoto, North-western Nigeria. A one year retrospective study in which the case note of patients with histologically confirmed diagnosis of breast cancer between January and December, 2015 were reviewed. Parameters studied included demographic characteristics and receptor status of biopsy specimens. A total of forty six patients were seen in this study and all were females. The age range was 25-75 years. The mean age was 44.65 years. The median age was 45.00 years while modal age was 35.00 years. Seventy two percent (33) of the women with breast cancer were pre-menopausal while 28.26% (13) were post-menopausal. Immunohistochemical analysis shows that 47.8% (22) of cancers were ER positive, 41.3% (19) PR positive, 39.1% (18) ER/PR positive, and 43.5% (20) were HER 2/neu positive. Immunohistochemical classification based on ER, PR and HER2 gene expression showed that 24%(11) were Luminal A(ER+, PR+,HER2-), 22%(10) Luminal B(ER+,PR+,HER2+), 26%(12) HER2 type(ER-,PR-,HER2+) while 28%(13) were Basal-like(Triple negative) tumours. There was no statistically significant relationship between receptor status and age of patients. It was concluded that majority of the tumours in this study exhibited triple negative (basal-like) receptor characteristics in a predominantly premenopausal patient population.

Keywords: Breast cancer, receptor status, luminal, immunohistochemistry

INTRODUCTION:

Breast cancer is now the most common female malignancy in Nigeria, Africa and globally[1-5]. Reports from Nigeria have shown a consistent rise in the incidence of the disease[6,7]. Although there is a rising trend of breast cancer across Nigeria, overall incidence in black Africa and Asia is considered low in comparison to Western Europe and North America where incidence ranges between 50 to 100 per 100 000 women[8,9].

Breast cancer is classified into four groups based on immunohistochemical profile of ER/PR and HER2/neu expression, which correlates well with intrinsic gene expression microarray categorization as: ER/PR+, HER2-corresponding with Luminal A; ER/PR+, HER2+ corresponding with Luminal B; ER/PR-, HER2+ corresponding with HER2 over-expressed or enriched and ER/PR-, HER2 - corresponding with triple-negative/basal-like tumours[10-12].

In Nigeria as in most developing countries, late presentation of breast cancer with unfavorable prognosis is the norm[13-15]. More than two thirds of breast cancer patients in Nigeria present with advanced disease at the time of diagnosis[1-3,16]. The African patient is likely to present with a more aggressive and advanced tumor than her Western counterpart and is likely to die from the disease[15] Several works have shown that basal-like tumours occur significantly higher among pre-menopausal African-American and African women than Caucasian women[17-20]. Ikpatt *et al.* conducted a detailed pathological analysis to evaluate apoptotic activity in breast cancer from Nigerian (n = 300) and Finnish (n=285) women and concluded that tumours of Nigerian women had less tubular differentiation and higher mitotic to apoptotic index compared to Finnish women[21].

Patients with ER/ PR negative tumours are less likely to respond to endocrine therapy and more likely to have a poorer overall survival than women with ER/PR positive tumours[20,22]. Similarly, patients with HER2/neu positive tumours have been shown to have significant survival advantage with targeted therapy using the humanized monoclonal antibodies against HER2/neu[23]. Studies have also shown that advanced tumours are more likely to be hormone unresponsive compared to early tumours[24].

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METHODS

This was a one year retrospective study at the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Northwest Nigeria between January and December 2015 in which the case note of patients with confirmed diagnosis of breast cancer were retrieved and reviewed. Parameters studied included demographic and receptor characteristics.

Inclusion/Exclusion criteria: All histologically confirmed malignant breast lesions from both sexes with receptor status were included**.** Histologically confirmed malignant breast lesions without receptor status were excluded.

Receptor characteristics: Our center started routine immunohistochemistry to determine receptor status of breast cancer in 2015. All consecutive breast specimens received in 10% buffered formalin were processed with auto processors. Paraffin-embedded sections at 3-4 ìm were routinely stained with haematoxylin and eosin (H & E) stains to determine their suitability for immunohistochemistry. Immunohistochemical assays were performed on representative paraffin embedded blocks by deparaffinizing and rehydrating the section with xylene. This was followed by heat-induced epitope retrieval where slides were heated in a buffer for 40 minutes to unmask antibodies and antigen. The slides were subsequently incubated in 0.03% hydrogen peroxide for 5minutes to block endogenous peroxidase activities, followed by incubation for 20 minutes in a protein-blocking solution (Protein Block Serum-Free Solution) to reduce nonspecific background. Slides were then incubated for 10 minutes with 3, 31 di-aminobenzidinechromogen (for enzymatic immunodetection) and counter stained with haematoxylin and covered. Results of the immunostaining were scored semi-quantitatively and presented as receptor status. Comparison of patient's age with receptor status was carried out for any association using the Pearson's Chi square test. A *P-value* of less than 0.05 was considered significant.

Data Analysis: Results were analyzed using the Windows statistical package for Social Sciences (SPSS) version 20.

RESULTS

A total of forty six cases were seen and analysed in this study and all were females. The age range was 25-75 years. The mean age (±Standard Deviation) was 44.65 (±12.55) years. The median age was 45.00 years while modal age was 35.00 years (Table 1). Seventy two percent (33) of the women with breast cancer were pre-menopausal while 28.26% (13) were postmenopausal. Immunohistochemical analysis shows that 47.8% (22) of cancers were ER positive, 41.3%(19) PR positive, 39.1%(18) ER/PR positive, and 43.5%(20) HER 2/neu positive (Table 2). Immunohistochemical classification based on ER, PR and HER2 gene expression showed that 24%(11) were Luminal A (ER+, PR+,HER2-), 22%(10) Luminal B (ER+,PR+,HER2+), 26%(12) HER2 type (ER-,PR-,HER2+) and 28%(13) were Basal-like (Triple negative) tumours. Table 3 showed that there was no statistically significant relationship between receptor status and the age of patients.

Table 1:Age distribution of patients with breast cancer

Age group (Years)	Frequency	Percentage
20 - 29	2	4.35
30 - 39	16	34.78
40 - 49	15	32.61
50 - 59	4	8.70
60 -69	6	13.04
≥70	3	6.52
Total	46	100

Table 2: Receptor status of breast cancer in Sokoto in 2015

Status	ER	PR	ER+PR	HER2/ NEU
N	46	46	46	46
Positive	22 (47.8)	19 (41.3)	18 (39.1)	20 (43.5)
Negative	24(52.2)	27(58.7)	28 (60.9)	26 (56.5)

Key: ER: Oestrogen Receptor, PR: Progesterone Receptor, HER2/NEU: Human Epidermal growth factor Receptor 2.

Table 3: Relationship between receptor status and age at diagnosis

Variable	ER Status		χ^2	*p* value
	Negative	Positive		
Age Group(years)				
< 50	17 (50.0)	17 (50.0)		
≥50	10 (83.3)	2 (16.7)	2.139	0.144
	PR Status			
	Negative	Positive		
< 50	20 (58.8)	14 (41.2)		
≥ 50	12 (100.0)	0 (0.0)	3.617	0.057
	HER2/NEU Status			
	Negative	Positive		
< 50	19 (55.9)	15 (44.1)		
≥ 50	12 (83.3)	2 (16.7)	1.638	0.201

DISCUSSION

The study shows that breast cancer in our women is predominantly pre-menopausal (71.74%) in contrast to Caucasian women where the disease is mainly post-menopausal[25]. Most published reports from Nigeria and America revealed that breast cancer in African women occur a decade earlier than the Western average[25-27]. African-American women are also known to present at significantly younger age than their Caucasian counterparts[28,29]. Similarly, black British women presented significantly younger (median age of 46 years), than white patients (median age of 67 years)[30].The factors responsible for this are not fully understood although it may be due to mutations in the breast cancer genes (BRCA 1 and 2) their variants[29].

Studies have shown that pre-menopausal African-American women have higher prevalence of triple negative (basal-like) breast tumours, than their white counterparts [31] and this trend has also been seen among indigenous African women[13,20,26].

Our study showed a preponderance of triple negative (basal like) tumours at 28% (13). Reports from Jos, (North Central Nigeria); Ile-Ife, (South-Western Nigeria); Aba, (South-Eastern Nigeria) and other parts of Africa also showed similar pattern[13,32-36].

In Ile-Ife, Omoniyi-Esan *et al.* in a 5-year prospective study of 136 cases of breast cancer specimens showed that 45 cases (33.1%) of tumours were Basal-like (Triple negative), 30 cases (22.1%) were Her2 over expressing, 21 cases (15.4%) were Luminal B type and 20 cases (14.7%) were luminal A type[32]. The peak age group was 40-50 years in agreement with our study[32].

Adisa *et al.* also carried out a detailed analyses of tumour receptors and infiltrating macrophages (TAM) on 17 breast cancer specimens from Aba, South-East Nigeria and concluded that majority of the tumours were high grade (100% were grade III), triple-negative (65%), and occurred more commonly in young women (mean age 47 years)[33].

Similarly, Gukas *et al.* from Jos, North Central Nigeria in a review of 36 consecutive patients with breast cancer reported that there was a predominance of high grade, invasive ductal carcinomas which were likely to be ER/PR negative but *P*53 positive. They concluded that these features suggested a biologically aggressive form of breast cancer in Nigerian women with possibility of poor response to both hormonal therapy and chemotherapy[13]. Even though majority of our patients were premenopausal with predominant triple negative

receptors a comparison of age at diagnosis with receptor expression did not show any significant statistical relationship perhaps because a good proportion of the tumours were also ER and PR positive. In contrast to our study however, Nwafor and Keshinro in a retrospective analysis of 48 breast cancer specimens from Lagos, South-West Nigeria reported a higher proportion of luminal A (ER+/PR+,HER?), 19 (39.6%), tumours compared to other subtypes with majority of the patients aged 50 years and above[37]. Triple negative/basal-like cancers were the second most common type of cancer in their series accounting for 29.2% (64.3% of which were seen in age groups 30-49 years and 78.6% with tumour grades 2 and 3)[37]. There was however no statistical correlation between the triple negative groups and their tumour grades or age distribution[37]. Also, McCormack *et al.* in a case series report on breast cancer receptor status and stage at diagnosis concluded that although a greater proportion of black than non-black South African women had ER-negative or triple negativebreast cancer, majority in all racial groups were still predominantly ER-positive[38].

Previous reports have shown that women with luminal A tumors have better recurrence-free and overall survival than women with other molecular subtypes[38,39]. Similarly, Luminal tumors are known to have better survival outcome compared to HER2+ or triple negative tumors[39,40].

CONCLUSION

It was concluded that majority of the tumours in this study exhibited triple negative (basal-like) receptor characteristics in a predominantly premenopausal patient population. These features showed a biologically aggressive form of breast cancer in our women with possibility of poor response to both hormonal therapy and chemotherapy.

Limitations of the study: The manual system of storage of information by the medical records department makes retrieval of case files a challenging task. A two-year study period would have meant a larger sample size and longer period of evaluation.

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