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Original Research Article

Molecular Biology of Breast Cancer in the Niger Delta Region of Nigeria: A Pilot Study

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Abstract: Breast cancer is the commonest cause of cancer related death among women globally. The incidence is lowest but increasing in Africa and accompanied by increased mortality. Different expression patterns of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor (HER) 2 have been identified. The aim is to determine the molecular subtypes of breast cancer and evaluate their association with tumour characteristics such as age at presentation, stage of disease and grade of tumour. A 2 year prospective study of all patients that presented at University of Port Harcourt Teaching Hospital. Data was collected and analysed using the Statistical Package for Social Sciences (SPSS) version 17.0. Eighty six patients were seen during the study period and they were all females. Their ages ranged from 26 to 83 and the mean was 46.1 ± 14.3 years. Infiltrating ductal carcinoma was the commonest histological type, and seen in 78 (90.7%) patients. Triple negative was the commonest receptor subtype and observed in 40 (46.5%) patients. Breast cancer receptor subtype was significantly associated with stage and grade of tumour but not with age at presentation. Breast cancer in Nigerian women occurs relatively in younger women and most are triple negative and aggressive. Given the young age of onset and aggressiveness of this disease, it will be imperative to identify women at risk and increase the awareness, target screening and develop prevention strategies.
Keywords: Breast cancer, molecular subtypes, Nigerian

INTRODUCTION

Breast cancer is the commonest cause of cancer related death among women globally [1]. The incidence is lowest but increasing in Africa and accompanied by increased mortality [1, 2]. It has been documented that Africans and African American women that with breast cancer are younger in age, present late to the hospital with higher grade tumours, more advance stage and negative hormonal receptor status and poorer prognosis when compared to Western European and Caucassian American women [3, 4]. Even though these disparities may be as a result of differences in socio economic status, access to screening and treatment decisions, the intrinsic biology of the cancer itself may play a role in the different outcomes [5, 6].

Knowing the receptor content is vital in the management of the breast carcinoma because different expression pattern of Oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor (HER) 2 have been identified [7, 8]. Over expression ER is more commonly observed in lower grade, smaller size tumours, more likely to be node negative and shows better survival outcome than ER negative cancers [7]. PR over expression is also associated with well differentiated tumours with good overall survival [9]. The ER over expression occurs in about 70-80% of invasive breast cancer at the time of diagnosis [10]. HER 2 over expression occurs in about 10-30% of invasive cancers [10] and is associated with high grade[11] and ER negative tumours [12] and poor survival [13]. Triple negative tumours are the most aggressive form and account for 10-17% of all breast cancers [14].

The aim of this study is to determine the molecular subtypes and evaluate their association with tumour characteristics such as stage of disease and grade of tumour and age at presentation.

PATIENTS AND METHODS

This is a 2 year prospective study (1st January, 2014 to 31st December, 2015) of all patients with carcinoma of the breast that presented to University of Port Harcourt Teaching Hospital (UPTH) through the surgical out patients' clinics and accident and emergency department. Data which included age, sex, menopausal status, parity, stage of disease, tumour grade, histological diagnosis and receptor status

[oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor (HER) 2] were analysed using the Statistical Package for Social Sciences (SPSS) version 17.0. Cases that are positive for either ER or PR or both are regarded as Hormone receptor (HR) positive. P value of < 0.05 or less was regarded as statistically significant. Differences in surgical and tumour characteristics between the various breast cancer subtypes were analysed using analysis of variance for continuous variables and chi square test for categorical variables. Excluded from the study were in situ carcinomas, sarcomas and secondary tumours. Informed consent and ethical approval were obtained from the recruited patients and the institution respectively.

TISSUE PROCESSING

Paraffin embedded block of tissues initially fixed with 10% neutral buffered formalin of trucut, incision, excision and mastectomy specimens were used. Controls and surgical sections were cut at 3 microns on the same slide. The sections were then allowed to drain for 20 minuites. Using hot plate at 60-70c, the slides were heated for 3 hours, thereafter, the sections were run through water and cleaned with xylene at double changes at 4 minuites intervals each. The sections were also passed through clean alcohol at double changes for 2 minuites each and then rinsed in water. The slides were then placed in a small container covered. Antigen retrieval was done using citrate buffer for 20-25 minuites at 100c in the pressure cooker. The slide rack and the retrieval solution were removed from the pressure pot and allowed to cool by passing through running gentle water. Next, the slides were sorted orderly in the humidity chamber according to the test to be done, 3% hydrogen peroxide was then applied for 15 minuites and then rinsed with PBS. A drop of inhibitor was then added for 15 minuites and then rinsed with PBS. A circle was inscribed around the tissue and the control using a hydrophobic pen. The primary antibody was added for 60 minuites and washed off with PBS. Also, add a drop of HRP on the slides for 20 minuites and properly washed with PBS. In addition, solutions 3 and 4 were mixed using equal measurement and applied for 3-5 minuites, then rinsed with PBS. A drop of copper which is an enhancer was added. Counterstaining was done using Harris haematoxylin for 2-3 minuites and rinsed. Slides were then passed through warm water for one minuite. Dehydration was done in absolute alcohol and slides were dried in the oven. Slides were reported using the College of American Pathologists recommended guidelines [15].

PROGESTERONE RECEPTOR

Anti-Progesterone Receptor (PR) (1E2)
Rabbit Monoclonal Primary Antibody
Ref: 790-2223
Lot: f07130

HER-2

Ventana anti-Her2/neu
(4BS) Rabbit Monoclonal Primary Antibody
Ref: 790-4493
Lot: Eo7785

OESTROGEN RECEPTOR:

Anti- Oestrogen (ER) (SPI)
Rabbit Monoclonal
Primary Antibody
Ref: 790-4324
Lot: f06378

GTIN: 04015630972241

Manufacturer:
Ventana Medical Systems Inc.
Only immunohistochemistry was used to determine HER-2

RESULTS

There were 86 patients with breast cancer during the study period and they were all females. Their ages ranged from 26 to 83 years and the overall mean was 46.1 ± 14.3 years. Infiltrating ductal carcinoma was the commonest histological type and seen in 78 (90.7%) patients. The remaining 8 (9.3%) patients had infiltrating lobular carcinoma. Sixty (69.8%) were premenopausal while 26 (30.2%) post-menopausal. Eighty two (95.3%) patients were multiparous and they all breastfed for a minimum of 1 year while only 4 (4.7%) of them were nulliparous. Fifty (58.1%) patients presented to the hospital at advanced stages (stages 3 and 4) while 36 (41.9%) reported at stages 1 and 2. Fifty four (62.8%) tumours were histological grade ll, 26 (30.2%) grade lll and 6 (7%) grade l. See table 1. Fifty four (62.8%) had surgery. Other treatment modalities are also shown in table 1.

The triple negative was the commonest receptor subtype and observed in 40 (46.5%) patients. Twenty eight (32.6%) patients were classified as ER-positive, 12 (14%) as PR-positive and 28 (32.6%) as HER-2 positive. See table 2. The average age of presentation for ER/PR positive cases was 43.4 years, while that of HER-2 positive and triple negative breast were 47.5 and 46.1 respectively. The differences between the groups were not significant (P= 0.180).

Tumour stage was significantly associated with breast cancer receptor subtypes. A significant (P=0.001) proportion of ER/PR positive tumours were stages 1 and 2 whereas a significant (P=0.001) proportion of triple negative and HER-2 positive tumours were stages 3 and 4. Tumour grade was also significantly associated with breast cancer receptor subtypes. A significant (P= 0.000) proportion of HER-2 positive and triple negative tumours were grade lll compared to the ER/PR positive tumours. However, the

percentage of triple negative tumours that are grade lll are much less than that of grade ll (30% versus 65%). There were more ER/PR positive tumours that are histologically grade l compared to HER-2 positive and triple negative types but the difference was not statistically significant (P= 0.250). See table 3.

Table 1: Clinico Pathological Features of Breast Cancer

FEATURES	NO OF PATIENTS (%)
AGE:	
≤50	62 (72.1)
>50	24 (27.9)
MENOPAUSAL STATUS:	
PRE MENOPAUSAL	60 (69.8)
POST MENOPAUSAL	26 (30.2)
PARITY	
NULLIPAROUS	4 (4.7)
MULTIPAROUS	82 (95.3)
STAGE:	
1	4 (4.7)
2	32 (37.2)
3	26 (30.2)
4	24 (27.9)
GRADE: 1	6 (7)
2	54 (62.8)
3	26 (30.2)
BREASTFEEDING >1YEAR	
NO	0 (0%)
YES	86 (100)
TREATMENT	
SURGERY	54 (62.8)
Modified Radical mastectomy	36 (41.9)
Simple mastectomy	18 (20.9)
CHEMOTHERAPY	76 (88.4)
RADIOTHERAPY	37 (43.0)
HORMONE THERAPY	23 (26.7)
IMMUNOTHERAPY	4 (4.7)

Table 2: Expression of ER, PR And HER 2 Markers In Breast Cancer

MARKER CASES (86)	POLSITIVE FREQUENCY (%)	NEGATIVE FREQUENCY (%)
ER	28 (32.6)	58 (67.4)
PR	12 (14)	74 (86)
HER 2	28 (32.6)	58 (67.4)
TRIPLE NEGATIVE	40 (46.5)	46 (53.5)

Table 3: Baseline Characteristics of Tumore Type

	HORMONE RECEPTOR POSITIVE (ER+ve/PR+ve)	HER 2+ve	TRIPLE NEGATIVE	P VALVE
AGE (**Mean**)	43.4 ± 10.4	47.5 ± 14.6	46.1 ± 14.7	0.180
STAGE				
Early (1 and 2)	18 (64.3%)	10 (35.7%)	14 (35%)	0.001
Advance (3 and4)	10 (35.7%)	18 (64.3%)	26 (65%)	0.001
GRADE				
I	4 (14.3%)	2 (7.1%)	2 (5%)	0.250
II	22 (78.6%)	14 (50%)	26 (65%)	0.000
III	2 (7.1%)	12 (42.9%)	12 (30%)	0.000

DISCUSSION

In agreement with findings from other African studies where the mean age is about 48 years and > 65% are premenopausal, most of the patients in our study were young (mean age of 46.09 ± 14.3 years) and premenopausal at presentation [16,17]. Some workers have documented that the biology of breast cancer in white women is different from that in African and African women where the disease is commoner and most of the patients are found to be young. The experience is the same in Nigeria as the life expectancy is < 50 years.

Following immunohistochemistry, most of the tumours (46.5%) were found to be triple negative and this is much higher than the prevalence of triple negative breast cancer observed among Caucasian women (< 20%) and African American women [18, 19]. Triple negative breast cancers are heterogeneous with poor response to hormonal therapy and chemotherapy and poor prognosis. In this study, the triple negative subtype is found to be more associated with advance stage (3 and 4) tumours and significantly (P = 0.000) associated with grades ll and lll tumours. These pathological features are associated with poorer prognosis and worse overall disease free survival. Triple negative breast cancers are poorly differentiated histologically and are characterized by an aggressive clinical history. The high rate of relapse associated with triple negative cancers has been attribute to the fact that there are no specific treatment guidelines for them and they are being managed with the standard treatment for all breast cancers [20]. Therefore, more research is required in the specific management of the various subtypes particularly the triple negative as this will be of great benefit to breast cancer patients of African origin.

From our study, only about 32.6% of cases account for ER positive, 14% PR positive and 32.6% HER-2 positive breast cancers. Some other studies of breast cancer markers in Sub Saharan Africa have had extremely variable findings with reported percentage of ER negative tumours ranging from 40% [14, 21] to 70% [22]. Comparatively, corresponding percentages in the black American population were 35% in breast cancer patients aged 40 and 15 to 20% by age 70 [23]. ER/PR positive breast cancer was significantly associated with stages 1 and 2 tumours (P = 0.001) and grade 2 tumours (P = 0.000). As previously reported, ER positive cancers are associated with a lower grade, smaller size, more likely to be node negative and better prognosis than ER negative cancers [7]. As was reported, poorly differentiated tumours have decreased ER expression [24, 25]. This indicates that less differentiated tumours have decreased dependence on oestrogen and consequently, reduced sensitivity to hormone therapy [24, 25]. This explains the better survival outcomes associated with ER positive breast cancer as they also derive benefit from endocrine therapy.

In this present study, HER-2 positivity were more associated with grade ll (P = 0.000) and advanced stage (3 and 4) tumours (P = 0.001). In a similar study, the frequency of HER-2 overexpression decreased significantly in low grade (grade l) tumours and also in patients with high grade (grade lll) tumours [26]. In a study of 3655 breast cancer cases, Lal *et al* [27] reported that HER 2 amplification and overexpression are limited to invasive breast carcinoma of intermediate to high grade. Under normal physiological conditions, HER 2 is inactive; however, once activated, it may enhance tumour growth, invasion and metastasis [28] which may explain HER 2 association with intermediate to high grade tumours and advanced stages.

CONCLUSION

Breast cancer in Nigerian women occurs relatively in younger women and most are triple negative and aggressive. Given the young age of onset and aggressiveness of this disease, it will be imperative to identify women at risk and increase the awareness, target screening and develop prevention strategies.

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