Original Article

The Immunohistochemical Profile of Breast Cancer in Indigenous Women of Southeast Nigeria

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Abstract

Objective: To evaluate the hormone receptor status of breast cancer in indigenous women of southeast Nigeria. **Materials and Methods:** We collected data on clinical parameters and histologic characteristics - from case notes and histology reports - of women diagnosed of breast cancer and paid for immunohistochemical analyses of their formalin-fixed, paraffin wax-embedded tissue cassettes for hormone receptor status. The relevant clinical data and histologic and immunohistochemical characteristics were analyzed using predictive analytical software, version 17 (IBM, SPSS Inc., Chicago, IL, USA). Comparisons of discrete data were done using Chi-square test, with levels of significance being set at $P \leq 0.05$. **Result:** Within the 6 years study period, 1,410 breast surgical cases were received; 28.2% (397/1,410) of which were diagnosed as breast cancers. Ultimately, 31% (123/397) of these women had IHC (ER, PR, and HER-2/neu) done on their specimens. The age range of the women was 24 to 85 years with a mean of 46.3 years. Approximately 61% (75/123) of the women were pre-menopausal. More than a third of the cancers were triple negative, 40.7% (50/123). **Conclusion:** We conclude that there is higher proportion of triple negative breast cancers compared to other parts of the world with therapeutic implications.

Keywords: Breast; Cancer; Immunohistochemistry; Indigenous; Southeast; Nigeria

Introduction

The 2012 GLOBOCAN report on breast cancer when compared to the 2008 GLOBOCAN report revealed an increasing incidence and mortality of breast cancer worldwide, with most of the deaths occurring in poor nations of sub-Saharan Africa. [1,2]

Studies have shown that breast cancer (BC) in women of African descent when compared to Caucasian women is characterized by earlier age at onset, a larger tumour size, and a higher grade at diagnosis with a corresponding poor prognosis. [3-5] One of the factors believed to contribute to these findings is 'intrinsic biologic factors'. [4,5]

On this intrinsic biologic factors, researchers have divided breast cancers into four subtypes depending on the presence or absence of oestrogen receptor (ER), progesterone receptor(PR), and human epidermal growth factor receptor 2(HER-2/neu). Luminal A cancers are (ER/PR +; HER-2/neu -), luminal B cancers are (ER/PR +; HER-2neu +), triple negative breast cancers [TNBC] (ER -,PR -, HER-2/neu -), and HER-2/neu + (ER -,PR -, HER-2/neu -). [6-8] Furthermore, using immunohistochemistry as surrogate for molecular markers, oncologists have categorized breast cancer into three main management groups: (i) those with hormone receptor-positive cancers who are managed with a number of oestrogen receptor (ER)-targeted therapy options ± chemotherapy; (ii) those with HER-2/neu + cancers, who will, in addition, receive HER-2/neu-directed therapy with trastuzumab or, in some situations, Lapatinib; and (iii) those with hormone receptor [ER and progesterone receptor (PR)]- negative and HER-2/neu negative breast cancers [that is, the so called Triple-Negative Group], for whom chemotherapy is the only modality of systemic therapy available. [9] This third group (triple-negative BCs) is found to preferentially affect the young and African-American women, of high histological grade, and of more aggressive clinical behaviour. [9]

Immunohistochemical profiling of breast cancers in Nigeria is done on a fee-for-service basis for purely economic reasons. Sequel to this, only few women with breast cancer get the desired best global standard clinical care of personalized treatment. Majority of the patients are treated empirically based on the predominant sub-type found from studies carried out in other parts of world. In Nigeria only few studies – carried out in other geographical regions - whose findings are albeit conflicting have been done. [10-13] As a result, we believe that there is a need to study the immunohistochemical characteristics of breast cancer in southeast Nigerian women.

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Material and Methods

This prospective multidisciplinary study conducted by some pathologists, radiologists, and surgeons at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, in southeast Nigeria from January 2010 to December 2015. NAUTH is a tertiary teaching hospital with four out stations. The predominant economic activity of the people of southeast Nigeria is commerce. This study was conducted at the Breast Clinic at NAUTH among women with palpable breast lump and non-palpable breast lump found at mammography by the radiologists in the study group. Breast surgeons in the study group documented the age, side of affected breast, size of tumour, last menstrual period, and type of biopsy (either needle core biopsy or open biopsy) amongst other clinical information for each patient in a case note opened at the Health Record Department of NAUTH. The surgical biopsies were promptly immersed in adequate volume (in the ratio of 1 ml of specimen to 10 ml of fixative) of 10% neutral buffered formalin in an appropriate fitting, correctly labelled container for fixation. Then each specimen with an accompanying completely filled consult form was sent to the Histopathology department of the hospital for subsequent tissue processing, embedding, routine staining, and histological evaluation by pathologists in the study group. The histologic diagnosis of cases diagnosed as carcinoma of breast read any of the following: low grade, intermediate grade, or high grade ductal carcinoma *in-situ*; low grade, intermediate, or high grade (based on the Nottingham modification of Scarff-Bloom-Richardson classification) invasive ductal carcinoma, non-specific or specific type (mentioned); or classical or pleomorphic invasive lobular carcinoma.

We sent paraffin wax-embedded tissue cassettes of breast cancer patients who paid the fee for oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2/neu) over expression to an outside laboratory facility for immunohistochemistry. At the facility the tests were done manually using Leica Novocastra antibodies (NCL for ER & PR; and CBII for HER-2/neu) protocol with in-house control tissue blocks, Leica polymer detection, pressure cooker as the antigen retrieval instrument with retrieval solution Ph 6.0 for 20 minutes; phosphate buffered saline as the wash buffer. The tests were performed in humidity chamber. Based on the immunohistochemical diagnosis of the cases we sub-classified the breast cancers into the following: Luminal A cancers (ER/PR +; HER-2/neu -), luminal B cancers (ER/PR +; HER-2neu +), triple negative breast cancers [TNBC] (ER -,PR -, HER-2/neu -), and HER-2/neu + (ER -,PR -, HER-2/neu -).

The relevant clinical data and histologic and immunohistochemical characteristics were analyzed using predictive analytical software, version 17 (IBM, SPSS Inc., Chicago, IL, USA). Comparisons of discrete data were done using Chi-square test, with levels of significance being set at $P \leq 0.05$.

Ethical approval was obtained from the ethical committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi.

Results

A total of 1,410 breast specimens were received from the Breast Clinic for histological assessment within the study period. This constituted 24.3% of 5,801 surgical specimens received in the Histopathology Department of the hospital within the period. Breast cancer cases accounted for 397 (28.2% of all breast cases). Only 123 (31%) of these (all females) had immunohistochemistry – ER, PR, and HER-2/neu – done on their buffered 10% formalin paraffin-embedded tissue blocks. The average age at diagnosis was 46.3 years (standard deviation, ± 12.3; range, 24-85years). The majority of the cancers (61.0%) occur before 50 years of age with the peak frequency (31.7%) occurring in the 40-49 years age range [Table 1]

Table 1: Frequency distribution of breast cancer patients by age groups.

Age Groups	Frequency (%)
20-29	7 (5.7)
30-39	29 (23.6)
40-49	39 (31.7)
50-59	33 (26.8)
60-69	7 (5.7)
70-79	6 (4.9)
80-89	2 (1.6)
Total	123 (100.0)

Table 2 illustrates clinicopathological characteristics of the patients as regards side of lesion, type of biopsy, tumour size, histologic subtype, and tumour grade. The left breast was more affected at 59.3% (73/123). As regards tumour size at presentation, none of the patients had a tumour size less than 2cm; the majority, 53.7% (66/123) had a tumour size greater than 5cm. The most common type of tumour biopsy is incisional or excisional biopsy, 63.4%; 78/123. Invasive ductal carcinoma, not otherwise specified (NOS), was the commonest histological diagnosis, 92.7% (114/123). Grade 2 (moderately differentiated) was the most common tumour grade, 51.2% (63/123). Altogether, grade 2 and 3 cases accounted for approximately up to 80% (96/123) of breast cancers.

Table 2: Distribution of breast cancer by clinico-pathological features.

Features	Frequency	Percent
No. of Cases: 123		
Anatomic Side of Tumour		
Right Side	49	39.8
Left Side	73	59.3
Both Sides	1	0.8
Tumour size		
<2 cm	-	-
2-5 cm	57	46.3
>5 cm	66	53.7
Type of Biopsy		
Needle Core	8	6.5
Incision or Excision	78	63.4
Mastectomy	37	30.1
Histological subtype		
Invasive Ductal Carcinoma, Not Otherwise Specified	114	92.7
Invasive Ductal Carcinoma; Metaplastic Variant	3	2.4
Invasive Lobular Carcinoma	3	2.4
Mucinous (Colloid) Carcinoma	1	0.8
Invasive Papillary Carcinoma	1	0.8
Ductal Carcinoma *In-Situ*	1	0.8
Tumour grade		
Grade 1	27	22
Grade 2	63	51.2
Grade 3	33	26.8

When compared to the other two receptors, most cancers, 42.3% (52/123) were oestrogen receptor-positive. Among the oestrogen-positive cases, the majority stained 3+, 42.3% (22/52). Progesterone-receptors were present in 37.4% (46/123) of the cancers. Among the progesterone positive cases, the majority stained 3+, 39.1% (18/46). Most of the cancers in this study, 88.6% (109/123) were HER-2/neu-negative while 11.4% (14/123) stained positive. Among the HER-2/neu cancers, nearly 10% (10/123) of the reviewed cases stained 2+ (equivocal of HER-2/neu) as seen in Table 3.

Majority of the cancers, 43.9% (54/123) were luminal type A, closely followed by basal like/triple negative type, 40.7% (50/123). The remaining two molecular types, Luminal type B cancers and HER-2/neu overexpressing cancers, were 4.9% (6/123) and 10.6% (13/123) respectively [Table 4] There was statistical correlation ($P \leq 0001$).

Table 5 illustrates the correlations of various clinicopathological features with the molecular type (based on the cancers' immunohistochemical evaluation for steroid hormone receptors and HER2-/neu). There is no significant correlation between tumour molecular type and mean age at presentation (P: 0.190), tumour histologic subtype (P=0.397), tumour histological grade (P=0.993), or tumour size (P=0.605).

Discussion

Immunohistochemistry of breast cancers is not routinely determined in the developing countries such as Nigeria despite the invaluable prognostic and therapeutic information it provides. This is attributed to the limited resources and the relatively high cost of IHC testing. [14,15] In this study, the cost of IHC was the reason why only 31% of these women seen at our centre had IHC done on their tissue blocks. This low percent is comparable to the 18.7% reported by Nwafor et al. [13] in Nigeria.

In this study, most of the cancers did not express ER (57.7%) and PR (62.6%). This is similar to non-expression of ER (75.0%) and PR (62.2%), ER (61.6%) and PR (70.0%), and ER (53%) reported by Gukas et al. [10] in Jos, north central Nigeria, Titiloye et al. [12] in Ile-Ife, southwest Nigeria, and Galukande et al. [16] in Kampala, Uganda respectively, but different from the findings of Adebamowo et al. [11] in Ibadan, southwest Nigeria, Nwafor et al. in Lagos, southwest Nigeria, and Awadelkarim et al. [5] in Khartoum, Sudan where most of the cancers were ER+ (65.1%) and PR+ (54.7%), and ER+ (54.2%) and PR+ (50%), and ER+ (64.0%) and PR+ (67.0%) respectively. The differences in the findings from these studies are not likely due to biologic differences but to differences in specimen collection, processing, and variable IHC techniques and interpretation. All these studies however show non-expression of the HER-2/neu by most of the cancers. In this study, 77.2% of the reviewed cases were negative for HER-2/neu including the nearly 10% that stained 2+. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend fluorescence *in situ* hybridization (FISH) confirmation for all cases judged to be 2+ by immunohistochemistry. [17] This was not done for lack of the facility for FISH in Nigeria and for

Table 3: Immuno-histochemical Staining Reactions For ER, PR, and HER-2/neu.

Marker	% (Frequency) Positive	Negative
Oestrogen Receptor	**42.3% (52/123)**	**57.7% (71/123)**
Strength of Positivity:		
1+	28.8% (15/52)	
2+	28.8% (15/52)	
3+	42.3% (22/52)	
Progesterone Receptor	37.4% (46/123)	62.6%(77/123)
Strength of Positivity:		
1+	23.9% (11/46)	
2+	37.0% (17/46)	
3+	39.1% (18/46)	
HER-2/neu	11.4% (14/123)	88.6% (109/123)
Categories of Negativity:		**Comment:**
0 staining	87.2% (95/109)	Unequivocally negative
1+ staining	3.7% (4/109)	Unequivocally negative
2+ staining	9.2% (10/109)	Equivocally negative

HER-2/neu = Human epidermal growth factor receptor 2.

Table 4: Recognized molecular types of breast cancer and their relative frequencies based on their steroid and HER-2/neu Receptors IHC Status.

Major Group	Components	Frequency (%)	Molecular Type
No. of Cases: 123			
ER/PR+, HER-2/neu -	ER+/PR+, HER-2/neu-; ER+/PR-, HER-2/neu-; ER-/PR+, HER-2/neu-	54 (43.9)	Luminal A
ER/PR+, HER-2/neu +	ER+/PR+, HER-2/neu+; ER+/PR-, HER-2/neu+; ER-/PR+, HER-2/neu+	6 (4.9)	Luminal B
ER/PR-, HER-2/neu +	ER-/PR-, HER-2/neu+	13 (10.6)	HER -2/neu over expression
ER/PR-, HER-2/neu -	ER-/PR-, HER-2/neu-	50 (40.7)	Basal like/Triple Negative

ER = Oestrogen Receptor; PR = Progesterone Receptor; HER -2/Neu = Human Epidermal Growth Factor Receptor 2; IHC = Immunohistochemistry.

Table 5: Distribution of breast cancer by clinico-pathological features.

Features	Molecular Type				P
	Luminal A (n= 54)	Luminal B (n= 6)	HER-2 OE (n= 13)	Basal-like/Triple Negative (n= 50)	
Mean ages	44.78±10.99	42.17±8.59	45.77±11.70	49.44±13.59	0.190(>0.05)
Site of Tumour					
Right	17	2	6	24	0.539 (>0.05)
Left	37	4	7	25	
Both	-	-	-	1	
Tumour size					
<2 cm	-	-	-	-	0.200(>0.05)
2-5 cm	27	4	8	18	
>5 cm	27	2	5	32	
Type of Biopsy					
Needle Core	4	-	1	3	0.683(>0.05)
Incision or Excision	35	5	9	29	
Mastectomy	15	1	3	18	
Histological subtype					
IDC, NS	52	4	11	47	
IDC, MV	-	1	-	2	
ILC	1	-	1	1	0.397(>0.05)
MC	-	-	-	-	
IPC	-	-	1	-	
Ductal Carcinoma *In-Situ*	-	1	-	-	
Tumour grade					
Grade 1	10	1	4	12	0.480(>0.05)
Grade 2	33	2	6	22	
Grade 3	11	3	3	16	

HER-2 OE = Human Epidermal Receptor Type 2 Over-Expression; IDC, NOS = Invasive Ductal Carcinoma, No Special Type; IDC, MV = Invasive Ductal Carcinoma, Metaplastic Variant; ILC = Invasive Lobular Carcinoma; MC = Mucinous Carcinoma; IPC = Invasive Papillary Carcinoma.

the exorbitant cost of sending and conducting FISH HER-2/neu testing abroad. Erroneous or inconclusive HER-2/neu test results may lead to inappropriate patient management and will only enhance the difficulties of managing the introduction of new treatments and technologies in a resource-limited healthcare setting (like Nigeria). [18]

Luminal A breast cancer subtype was the commonest, 43.9% and the majority of them, 70.4% were seen in patients below 50 years, though there was no significant association between molecular subtype and age group in decade. The 43.9% found in this study is similar to the rates of 41.4% [12] and 39.6% [13] reported in studies done in southwest Nigeria but lower than rates of 77.6%, 53.7%, and 55.4% reported by Adebamowo [11] in southwest Nigerians, Onitilo [19] in South African Blacks, and Ihemelandu [20] in America from African American women, respectively. The reasons while there is a difference in the rate of luminal A breast cancer sub-type between this study with most of the studies done in the other regions in Nigeria, [10,12,13] and the rate in Blacks in South Africa and America could be partly explained by reproductive factors, since parity and early age at first birth are associated with reduced risk of ER+ cancers only. [21]

Human epidermal growth factor type 2 over-expressed breast cancer subtype was seen in 10.6% of cases, with a slightly lower mean age of 45.8±11.7 years compared to the mean age of 46.3±12.3 years for all breast cancers. This study rate of 10.6% is within the range of 4.0% to 12.5% reported in previous studies in Nigeria. [11-13] HER-2/neu over-expressed breast cancer subtype is reported to represent prognostically the worst subtype of breast cancers. [21]

The triple negative/basal-like subtype of breast cancers was the second most common type, 40.7% and there is no difference in rate for patients below or equal to and above 50 years of age. Basal-like breast cancer subtype constitutes approximately 50% (48.5%) of grade 3 cancers in the reviewed cases, though there is no statistical correlation between basal-like subtype and their age distribution or tumour grade. The 40.7% rate found in this study is almost triple the rate, 15.8% reported by Adebamowo et al. [11] in southwest Nigeria, and about double the rates, 29.2%, 20.4%, and 21.2% reported also in southwest Nigeria, Blacks in South Africa and in America, respectively. [19,20] However, this study's rate is lower than the rate, 53.4% reported by Titiloye et al. [12] in southwest Nigeria. This study rate of 40.7% is within the range of 15.8%-to-53.4% reported in studies in southwest Nigeria. The wide range observed in the studies reported from the same ethnic and geographical region in Nigeria is highly unlikely to be due to biological or socio-cultural differences but more probably due to other factors like quality of fixative, time interval between collection of specimen and fixation, length of fixation before processing, potency of IHC reagents, variable IHC techniques, erroneous or inconclusive HER-2/neu test results; and skewed sample size – 152, 58, and 48 samples reviewed by Adebamowo et al., [11] Titiloye et al., [12] and Nwafor et al. [13] respectively.

Conclusion

We conclude that there are a higher proportion of triple negative

breast cancers in women of southeast Nigeria than the proportion in women from the other parts of the world with therapeutic implications.

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Conflict of Interest

All authors disclose that there was no conflict of interest.

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