Hormonal and HER2 Receptor Immunohistochemistry of Breast Cancer in North-Eastern Nigeria: a preliminary report

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Abstract:
Background: *Morphologically identical breast carcinoma can display divergent clinical outcomes and response to therapies, withdifferences in incidence, clinical picture and mortality rates across various patient populations. This could be attributed to differences in the molecular characteristics of the carcinoma. Recently, gene expression profiling and its surrogate Immunohistochemistry (IHC) markers classified breast cancer into 4 molecular subtypes, and these receptor-defined subtypes are a major determinant of treatment options and disease outcomes. However, there remains an uncertainty regarding the frequency of the various subtypes in Africa. To date, there has been no comprehensive study of the molecular subtypes of breast cancer in our environment.*
Objective: *To conduct a preliminary study on Estrogen (ER), Progesterone (PR) and Her2 receptor status of breast cancers at the University of Maiduguri Teaching Hospital, North-Eastern Nigeria.*
Materials and Methods: *A prospective study spanning three years and four months was done on breast cancers seen at a surgical unit at the University of Maiduguri Teaching Hospital, from November 2012 to March 2016.Standard histological and immunohistochemicalanalysis were done for ER, PR and Her2 status.*
Results*: A total of 50 cases of breast cancer were seen in the unit over a period of three yearsand four months with an age range of 20 – 75 years, and a mean age of 46.1 ±13.4 SD (years). Of these, 38 had available Immunohistochemistry results for receptorstatus. Proportion of ER+, PR+ and Her2+ tumors were 36.8%, 34.2% and 21.1% respectively. Phenotypic classification based on ER, PR and Her2 immunohistochemistry showed that more than half were Triple Negative (52.6%, 20/38), while 10 cases (26.3%) were Luminal Type A, 5 cases (13.2%) were Luminal Type B, and 3 cases (7.9 %) showed overexpression of Her2.*
Conclusion*: We observed that breast cancer in our cohort occurs at a young age group, mostly pre-menopausal, with more than half of the patients having thetriple negative phenotype. While a significant proportion are ER positive, majority are hormone receptor negative, favoring chemotherapy over hormonal treatment. There is need for effective screening programs, and immunohistochemistry of breast cancers should be standard and routine.*
Keywords:*Breast cancer, immunohistochemistry, Hormonal receptor, Nigeria*

I. Introduction

Breast cancer is the leading cause of cancer-related death in women worldwide [1]. The incidence and clinical outcomes of breast cancer differ across various populations, age and racial groups, with higher incidence rates reported in developed countries, amongst white caucasians and older women [2]. In many developing countries, however, its incidence has recently been found to be on the increase [3, 4], and in Nigeria, breast cancer has overtaken cervical cancer as the leading cause of cancer mortality in women [4,5]. In our center, there has been a steady increase in the prevalence of breast cancer overthe last decade, from 12.5%in 2003 [6], 13.9% in 2005 [7] and 17.1% in 2009 [8].

Breast cancer in the African woman has a high mortality, characterized by late presentation with advanced stage at diagnosis, younger age, higher grade and more negative hormonal status with poorer prognosis when compared toCaucasian American and European women [2]. In the USA, African-American women diagnosed with breast cancer have a 5-year relative survival of only 77.7%, compared with 89.7% for the Caucasian women [9]. Data indicate that African-American women have a relatively higher frequency of breast cancer that is high-grade and are hormone receptor negative, and in general, these cancers are more biologically aggressive than those in Caucasians [10]. While these low survival rates in low-income countries may be attributed to socio-economic status, ineffective early detection programs, poor access to timely health

care services and the lack of adequate diagnostic and treatment facilities, the inherent biology of the disease itself may play a role in the adverse outcome.

Today, breast cancer is considereda heterogeneousdisease exhibiting a spectrum of clinical and morphological diversities, posing a major challenge in its treatment and outcome. Traditional histological classification of breast cancer has been useful in categorizing the various types, with recognized factors which predict its behavior and management, including tumor size, lymph node involvement and distant metastasis. But this disease is known to have diversity between tumors of even the same histological type, and even within the same tumor. Individual breast tumors may exhibit strikingly distinct clinical characteristics in different patient and ethnic populations, with divergent clinical courses despite having similar histopathologic appearances.

The use of immunohistochemistry to further characterize breast cancer globally has introduced a new dimension to current knowledge of the disease. In most tertiary institutions in Nigeria, however, molecular profile of breast cancer by immunohistochemistry was introduced only in the last decade or two. To date, there has been no comprehensive study on the hormone and Her2 status of breast cancer in our environment.The aim of this study was to make a preliminary assessment of the ER, PR and Her2 receptor status of breast carcinoma at the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria, and to relate these findings with the patients' age, menopausal status and tumor grade.

II. Patients and Methods

A prospective study of fifty patients in the unit with histological diagnosis of breast carcinoma at the University of Maiduguri Teaching Hospital was done over three years and four months, from November 2012 to March 2016. The tertiary hospital caters to a varied population from across various states within the North-Eastern region of Nigeria, as well as from neighboring countries like Cameroon, Chad and Niger. No sampling was done. Inclusion criteria were all cases of breast carcinoma with tissue biopsy and conclusive histological diagnosis. Those who had result of the receptor status by immunohistochemistry available made up the cohort. The patients' demographics including age and sex, and relevant clinicopathological parameters such as menopausal status andtumor grade were captured on a proforma.

Cancer tissue from either core tissue biopsy of the breast tumor histologically confirmed to be breast carcinoma, or from mastectomy specimen were routinely fixed in buffered Formalin for 24 hours. Histologic diagnoses were made from Hematoxylin and Eosin stained sections, and Formalin-Fixed Paraffin Embedded (FFPE) wax tissue blocks were prepared within 72 hours of obtaining the specimen. Sections from each of the representative block were subjected to a standard Immunohistochemistry protocol for Estrogen Receptor, Progesterone receptor and HER2 receptorusing the DAKO immunostainer (DAKO, Carpinteria, CA). Analysis for positivity and intensity of staining were done. Those for Her2 were considered positive with a staining of +3, or negative (0, +1 and +2). The ones with borderline results (+2) were considered Her2 negative as there is no facility to subject it to Fluorescent In-Situ Hybridization (FISH) technique for further confirmation.Breast cancer sub-types were defined as Luminal A (ER+ and/or PR+, Her2 -), Luminal B (ER+ and/or PR+, Her2+), Triple Negative (ER -, PR -, Her2-), Her2+ subtype (Her2+, ER-, PR-).The data was entered onto a spreadsheet and statistical analysis was done using SPSS version 20.The level of statistical significance was put at $p \leq$ to 0.001.

III. Results

Over the course of the study period, a total of fifty (50) patients with a histological diagnosis of breast carcinoma were managed by the unit. Only one (2%) male patient was seen; the rest were females (49, 98%), of whom a greater proportion were pre-menopausal (31, 62%), and six (12%) were nulliparous. The age ranged from 20- 75 years, with a mean age of 46.1 ±13.4 SD (years). The highest incidence occurred in the age group 50 - 59 years (14, 28%), followed closely by those in the 30 - 39 years age group (13, 26%).Nearly half of the patients were below the age of 40 years (17, 44.7%).The distribution of the cases in the various age groups are shown in Fig. 1.

The commonest histological type was Invasive Ductal Carcinoma (Not Otherwise Specified, NOS), accounting for 82%. Six (12%) were Invasive Lobular Carcinoma, while there were one (2.0%) each of Mucinous carcinoma, Invasive Papillary carcinoma and Invasive Ductal Carcinoma, squamoid differentiation (TABLE 1). Nineteen of the cancers were graded, using the modified Scarf-Bloom-Richardson (SBR) grading system, of which 7 (36.8%) were SBR Grade 2, while majority (63.2%) were poorly differentiated (SBR Grade 3).

All these patients had their tumor receptor status analyzed by Immunohistochemistry, of which thirty-eight patients (76%) had their results available for analysis, and are included in this study. Fourteen (36.8%) of the patients had tumor positive for Estrogen receptor (ER positive), while 24 (63.2%) were negative. Thirteen (34.2%) were Progesterone Receptor positive (PR positive), while 25 (65.8%) were negative. Eight (21.1 %)

had over-expression of Her 2 receptors, and 30 (78.9%) were negative. TABLE2 shows the distribution of the ER, PR and Her2 receptors.

Phenotypic classification based on ER, PR and Her2 immunohistochemistry showed that more than half (20, 52.6%) of the patients were Triple Negative (ER-, PR-, Her2-). The next commonest molecular subtype was the Luminal A (ER+, PR+/-, Her2-), with 10(26.3%), followed by Luminal B (ER+, PR+/-, Her2+) with 5 cases (13.2%), and 3 (7.9%) Her2 positive (ER-, PR-, Her2+). The only male patient had the Luminal A sub-type. Figure 2 depicts the distribution of the molecular subtypes.

Out of the 14 cases positive for ER, 12 were also positive for PR. Twelve out of the 13 patients who were PR+ were also positive for ER. Pearson Chi square test shows a strong association between ER and PR positivity, X^2 (1, N = 38) = 0.829, p < 0.001. However, there was no association between Her2 and ER, X^2 (1, N = 38) = 0.141, p = 0.399, nor between Her2 and PR, X^2 (1, N = 38) = 0.172, p = 0.302.

The mean age of patients with the Triple Negative sub-type was 45.4 ± 13.6SD (years), with age range from 27 – 75 years. Fifty percent of these patients were below the age of 40 years, with the highest occurrence in the age group of 30 – 39 years (7, 35%), followed by 50 – 59 years (6, 30%). The mean age of the Luminal A sub-type was 45.4 ± 14.0 SD (years), with age range from 29 – 71 years. One third (30%) are below the age of 40 years. The mean age of Luminal B sub-type was 43.6 ± 16.1 SD (years), with age range 29 – 68 years. Those with Her2+ subtype have a mean age of 51.7 ± 10.4SD (years), with age range from 40 – 60 years, and none of them were below the age of 40 years.
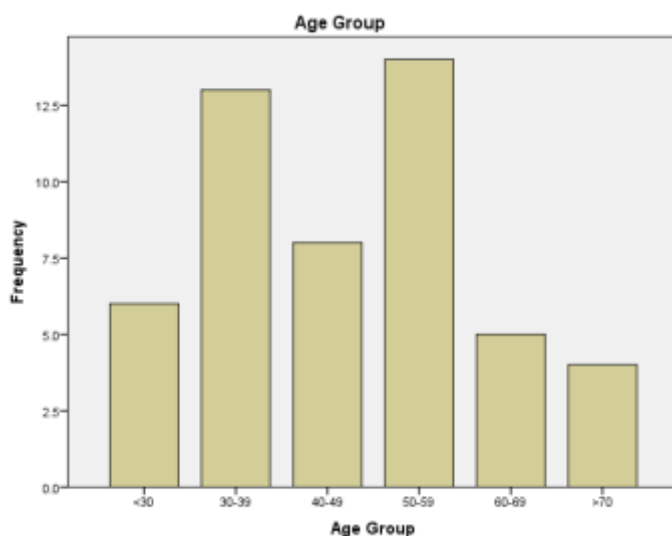
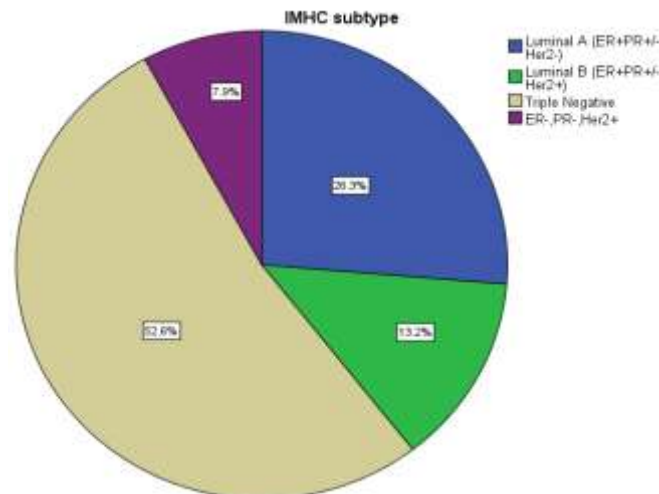**Figure 1:** Age distribution of Breast cancer cases (n=50).

Figure 2: Distribution of the various molecular sub-types of breast cancer (n=38).

Table 1: Histological types of Breast Cancer

Histological type	Frequency (n = 50)	Percentage
Invasive Ductal Carcinoma (NOS)	41	82%
Invasive Ductal Carcinoma (squamoid differentiation)	1	2%
Invasive Lobular Carcinoma	6	12%
Invasive Papillary Carcinoma	1	2%
Mucinous carcinoma	1	2%

Table 2: Expression of ER, PR, and HER2 markers in breast carcinoma

Marker Cases (38)	Positive		Negative	
	Frequency	Percentage	Frequency	Percentage
ER	14	36.8%	24	63.2%
PR	13	34.2%	25	65.8%
HER2/neu	8	21.2%	30	78.9%

IV. Discussion

Our study showed a mean age of breast cancer to be 46.1 years, with a bi-modal peak of age distribution, the highest being within the age group 50 – 59 years, followed by the younger age group 30 – 39 years. Nearly half (44.7%) of the patients were below the age of forty years, with 62% being pre-menopausal. Only one male was seen (2%). The relatively young age of patients in our cohort has been observed in other studies [2, 11, 12, 13]. This highlights the need for effective screening programs at a younger age group in our environment than what is obtained in the west. The frequency of screening should be increased in women above the age of forty years.

Studies of breast cancer sub-markers in Sub-Saharan Africa have had variable findings. Our study showed that the triple negative (TN) subtype accounted for the highest proportion of all cases, at more than half of the patients (52.6%). This conforms with reports from other studies in Nigeria, ranging from 33.1% to 87%. [2, 11, 12, 14, 15]. In 2006, however, Adebamowo et al [16] reported a lower incidence of TN cancers at only 17%, and a high proportion of ER positive tumors at 65%, comparable to what is obtained in the Caucasian population.

A significant proportion (36.8%) of the breast cancers in our study were Estrogen receptor positive (ER+). The average age at presentation of ER+ disease was 45.2 years, which concurs with a meta-analysis of breast cancer subtypes of indigenous populations in Africa in 2014, where ER+ disease was found to increase with increasing average age at diagnosis, with an average age of 31 – 46 years in sub-Saharan Africa [17]. Women of African descent, particularly of West African origin are known to have a higher prevalence of ER-negative tumors compared with white Europeans [9]. Metabolomic studies on Triple negative tumors amongst African-Americans in the USA in 2014 demonstrated that these tumors have some characteristic metabolic signatures that distinguish them from ER+ tumors, suggesting that these metabolic characteristics might offer new targets for treatment [10]. Interestingly, this high proportion of ER+tumors as seen amongst Caucasian women are also reported in East African-born black women [12, 18]. A study in Ethiopia [19] showed a high proportion of ER+ patients at 65%. Although majority of reports from Nigeria show a preponderance for triple negative cancers, it is worthy of note that a significant proportion of our patients (36.8%) are ER positive. Thus, in a resource-poor setting such as ours, the use of anti-estrogens may be justified even when receptor status of the cancer is not known. Expectedly, the expression of PR was significantly associated with ER expression, but neither of these receptors had any association with the Her2 receptor. This lack of correlation between the hormone receptors and Her2 receptor was also noted by Omoniyi-Esan et al [12].

There was little or no age difference in the mean ages of the three commonest phenotypes, with Triple Negative and Luminal A having an average age of 45.4 years, and Luminal B at 43.6 years. Although the Her2+ sub-type made up only 7.9% of our patients, it is interesting that it was seen in older patients with an average age of 51.7 years.In contrast, Makanjuola et al had noted that Her2+ occurred at a younger age group in their study, as it was for the Triple negative sub-type [15]. Eng [17]observed no clear age trends for Her2 disease in North-African studies, but in Nigeria, there appears to be a relatively lower incidence of Her2+ disease, ranging from 3.9% - 5.2%[14, 15, 20]. However, Adeniji et al [13]in Ilorin in 2010 reported that 19% of their study population had the Her2+ subtype, andrecently, Titiloye et al [11] reported of 19.6% having the Her2+ subtype.

Majority (63.2%) of the breast cancers in our study were poorly-differentiated (SBR Grade 3), and none was well-differentiated (Grade 1). This is comparable to other studies in Nigeria which also show a high preponderance of grade 3 cancers [4, 14]. In a study of African-Americans in the USA, the triple negative and Her2 subtypes were found to be associated with a higher prevalence of an advanced histological grade (Grade 3) [9].

The prevalence of breast cancer in our environment was difficult to establish at the time of this study as the general patient turnover during this period was adversely affected by the insurgency that involved the North-Eastern region of the country. As such, fewer cases were seen as majority of the population sought treatment elsewhere due to the instability of the region at this time. However, we still believe that this preliminary study serves as a representative of the overall population of breast cancer patients in our environment.

Immunohistochemistry for receptor status is still yet to be routine for breast cancers in Nigeria. With improvement in facility and resources, a clearer picture of the overall make-up of breast cancer amongst the Nigerian population will come into view. As knowledge of receptor subtypes has a significant impact on the clinical outcome and treatment options, there is need to emphasize on prioritizing this test, and ensuring high quality control in laboratory procedures for fixation and immunohistochemistry.

V. Conclusion

This study replicates previous findings that breast cancer in the Nigerian cohort occurs at a younger age than what is found in the west, involving mostly pre-menopausal women at their prime, with preponderance of the poorly-differentiated type. The need for effective enlightenment and screening programs at an earlier age cannot be over-emphasized. The Triple-Negative sub-type is over-represented in our environment (52.6%), with its attendant poor clinical outcome. This requires re-direction of the medical treatment modality from the traditional hormonal manipulation to the use of appropriate cancer chemotherapy in this cohort of patients. As this preliminary study heralds a more comprehensive overview of breast cancer in our environment, the glare of the burden that this disease imposes on our society at this time cannot be ignored.

Acknowledgements

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