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The Breast Cancer Steroid/HER-2 Receptor Profile Immunohistochemistry in Imo State: A Private Center Experience

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ABSTRACT

Background: Breast cancer, the most frequent cancer in women, has been reported to be quite widespread in Africa, with aggressive forms of the disease being the most common. Nigeria's infrastructure is inadequate for the estimation. Indigenous African women have a low rate of hormone receptor positivity, and triple negative breast cancer is the most common phenotype. The expression of hormone receptors and human epidermal growth factors 2 is critical for determining the prognosis, management approaches, and decision-making in breast cancer. In Imo state, there is a scarcity of information on hormone receptors (ER and PR) and HER 2 expression. The goal of this study is to look into the molecular subgroups of breast cancer based on surrogate immunohistochemical markers.
Methods: Clinicopathologic information on female breast cancer patients was obtained from our computer database. From 2017 to 2019, the immunohistochemical biomarker results on oestrogen, progesterone, and HER2 were evaluated in a group of female patients diagnosed with breast cancer at Rose Medical Services.
Results: There were 131 cases involved in this study. The mean age of the patients was 47.07±0.05 of all breast cancers. The oestrogen receptors, progesterone receptors, and HER2 positive rate were 65.6%, 46.6%, and 13.7% of breast cancers respectively. The proportion of luminal A subtype, Luminal B subtype, HER2 over expression and TNBC were 64.1%, 13%, 3.1%, and 19.8% respectively.
Conclusion: Despite the small sample size of our investigation, we were able to conclude that hormone receptor positive subtypes may prevail in Imo state breast cancer patients, followed by TNBC, and then HER2 positive subtypes. Our findings demonstrate the enormous range of breast cancer receptor markers and subtypes found across the United States and beyond. A larger prospective breast cancer investigation is recommended to confirm the current findings.

Keywords: *Hormone receptors, Oestrogen, Progesterone, Human epidermal growth factor 2, Triple negative breast cancer*

INTRODUCTION

Human breast cancer remains a global public health challenge, and currently the predominant malignant disease of the women folks.[1-3] It accounts for one fourth of the entire cancers and second cause of malignancy induced death, both in developed and developing countries.[4-6] According to the WHO, 2.3 million women were diagnosed with breast cancer in 2020, with 685 000 deaths worldwide. As of the end of 2020, there were 7.8 million women

alive who had been diagnosed with breast cancer in the previous five years, making it the most common cancer in the world. [7]

The cancer burden in Africa is expected to rise in the coming decades, owing primarily to rising life expectancy, changes in lifestyle associated with economic development, and longer survival of HIV/AIDS patients receiving antiretroviral therapy, as HIV/AIDS patients have a significantly higher risk of developing cancer than the general population. [8]

Regrettably, death rate due to breast cancer is found most common among African Americans, far much more than those of Asian extract. Breast cancer rates in Sub-Saharan African women are lower than in Western women, according to studies. [3]

However, as documented by Awadelkarim et al., the majority deaths due to the disease takes place among the women from this region and are more at risk of dying younger due to inadequate medical care. .[9]

In comparison to non-Hispanic whites, reports show that African countries, including Nigeria, are replete with early onset breast cancer cases, large tumour size with high grade at diagnosis; often associated with late presentation; and subsequent poor prognosis.[10,11]

According to Weigelt et al., tumours of the breast are diverse in their natural history and responses to therapy due to variation in transcriptional programs. Thus making proper diagnosis and sub typing necessary, before commencement of treatment.[12]

Gene analysis in pathology now plays a central role in diagnostic workout [13], and breast cancer is not left out. Gene expression profiling technology has been used in the separation of breast cancer into molecularly discrete subtypes, which has been surrogated by immunohistochemical technique[14,15], and the subtypes include: luminal A (oestrogen receptor(ER) positive and/or progesterone receptor(PR) positive, human epidermal growth factor receptor 2(HER2) negative, luminal B(ER positive and /or PR positive, HER2 positive), HER2 over expression(ER negative, PR negative, HER2 positive and triple negative breast cancer (TNBC) which are mostly basal – like.[16.1] Tumour size, nodal involvement, histologic type and grade, and immunohistochemistry expression status of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor 2 (HER2) are the major established prognostic and predictive factors for breast cancer but steroid/ HER2 receptors determination play important role in therapeutic decision.[17-19, 1]

Hormone receptor markers (ER&PR), together with HER2, are often utilized in the sub typing of breast cancer, while cytokeratin can function in the confirmation of the diagnosis.[20]

According to Sengal et al., hormone receptor positivity is low among women of African descent, with dominant hormone receptor negatives known to be associated with aggressive types of breast cancer. [21].

Thus, in order to gain insight into breast cancer receptors and molecular subtypes patterns among IMO state women in Nigeria, we analysed breast cancer reports of patients who use our facility, since there are scarce publications regarding these receptors around here. In our centre, our consultant pathologists collaborate to diagnose breast cancer, which is then followed by immunohistochemical evaluation, and here we present the pattern of these receptors/subtypes.

MATERIALS AND METHODS

2.1 ACCESSION AND PREPARATION OF SLIDES

From 2017 to 2019, we analysed breast cancer cases in our private centre, Rose Medical Services, in Imo State, Nigeria. It involves 131 female breast cancer patients who were treated with radical mastectomy, lumpectomy, or TRUE cut biopsies, in the majority of instances. Patients' samples arrived in buffered formalin, along with request forms that included the patient's name, age, clinical information, nature of specimen, and treating doctor's name. The breast samples that were obtained were

formalin fixed, dehydrated in graded alcohols, cleaned in xylene, and embedded in paraffin wax. After that, the blocks were processed and sectioned using a microtome. Graded alcohols and water treatment were used to hydrate dewaxed portions. Fixation pigments were removed when necessary, followed by a 5-minute staining with Harris haematoxylin. The slides were briefly cleaned under running water before being differentiated in 1% acid alcohol and then returned to water. Scott's tap water was used to "blue" the samples once more, and then the samples were returned to tap water until the portions were "blue" once more (10-15 minutes).

The sections were then stained with 1% Eosin for 1 minute. They were cleaned again in tap water for 1-5 minutes. Finally, the slides were dehydrated and subjected to changes in xylene before being mounted with dibutyl phthalate xylene (DPX) and coverslips. Nuclei turned blue/black, cytoplasm turned pink, muscle tissue turned deep pink, and fibrin turned deep pink as a result.

2.2 Histopathological diagnosis and classification

Histomorphological diagnosis of the cases was carried out through the collaborative effort of two consultant pathologist in our center, before sending the positive representative sample blocks to Biomolecular Labs, Enugu for immunohistochemical characterization, and based on hormone receptors/HER2 profile status we classified the breast cancer cases into subtypes as follows: Luminal A (ER+ and/ PR+ and HER2-);Luminal B(ER+ and /PR+ /HER2+); HER2 over expression(ER- and PR-/HER2+); and Triple negative /basal like (ER- and PR-/HER2-). The data from our work were subjected to descriptive statistical analysis.

2.3 Source of information

Prior to the commencement of this study, patients' informed consent was sort for and obtained. The relevant clinicopathological information of the patients including age, primary diagnosis, and hormones/HER2 receptors' marker profiles were retrieved from the centre's database and in review of this study, literature search was done through wide google search for contemporary information on the topic.

2.4 Inclusion and exclusion criteria

Our study comprised patients who had a complete clinical and histological diagnosis of breast cancer and whose samples had been fixed in 10% neutral buffered formalin for no more than 48 hours. Patients who did not meet the above criteria, on the other hand, were excluded from the study.

2.5 Ethical Approval

All authors hereby declare that this study was started after receiving ethical approval from our institution's ethical board, and that it was carried out in compliance with the Helsinki Declaration of 1964's ethical norms.

RESULTS

The study involved 131 breast cancer positive female patients with age range 23-72 years and mean age 47.07± 0.05 years. Invasive ductal carcinoma was the most common, 86.7%. The breast receptor markers profile in our study, demonstrated the presence of 46.6%(61) progesterone receptors; 65.6%(86) oestrogen receptors; 13.7% (18) HER-2 and 19.8%(26) TNBC. (see Table 1).

Table 2: Shows the predominance of hormone receptors positive malignancy of the breast 77.1%(101) over hormone receptors negatives. The hormone receptors positive of the type ER+/PR+ has the incidence of 73%(96); ER-/PR+ has 4%; and then nil for ER+/PR-.

Table 3: shows the percentage distribution of several molecular subtypes. The most common subtype was Lumina A, which accounted for 64.1 percent (84 cases), followed by TNBC, which accounted for 19.8 percent (26 instances) of the malignant breast samples. Only 13% (17 cases) involve luminal B, while Her2 overexpression cases account for 3.1 percent (4 cases).

Overall, the luminal A molecular subtype was the most prevalent. The distribution of Luminal A peaked in the fourth and fifth decades of life, then dropped to one in the seventh. TNBC was most common in the third decade of life, followed by the fourth decade. In our analysis, Luminal B made up 13% of all molecular subtypes of breast cancer, with the highest frequency occurring between the ages of 41 and 50.Her2 overexpression was the least prevalent subtype of breast cancer, accounting for only 3% of cases [4].

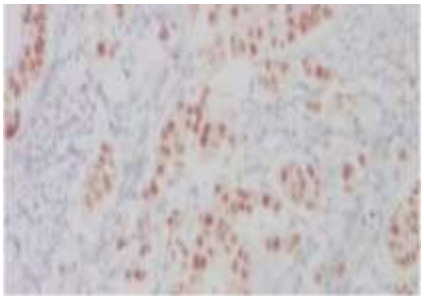

Figure 1.A. Immunohistochemical demonstration of Oestrogen nuclear receptors in a breast cancer section(x40).

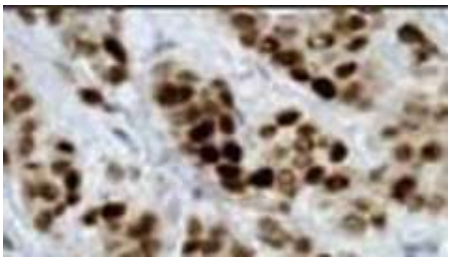

FIGURE 2. Immunohistochemical demonstration of progesterone receptors in breast carcinoma(x40)

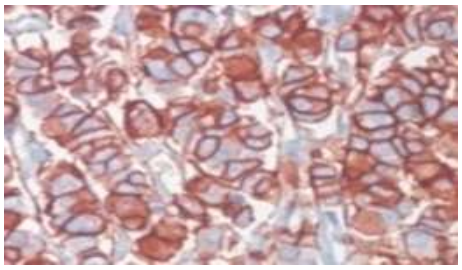

FIGURE 3 Immunohistochemical demonstration of strong circumferential membrane staining of HER2 over –expression of breast cancer (40)

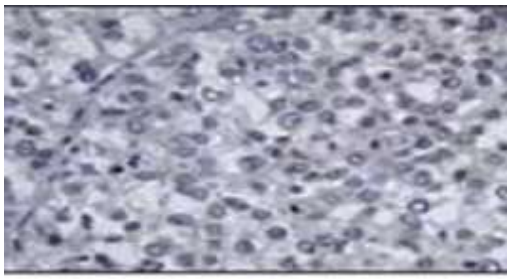

FIGURE 4 Section of the breast cancer showing lack of any of the aforementioned receptor markers(x40).

TABLE: 1. Age and Receptor Marker's Distribution

	CANCER PATIENT	PR POSITIVE	ER POSITIVE	HER-2 POSITIVE	NEGATIVE
21 - 30	7	6	6	2	0
31 – 40	27	12	16	3	8
41 – 50	44	20	31	9	6
51 – 60	34	19	27	2	4
61 – 70	12	3	4	1	4
>70	7	1	2	1	4
TOTAL	131	61	86	18	26

TABLE 2. HORMONE RECEPTORS

	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-
NUMBER OF PPATIENTS	96	-	5	30
PERCENTAGE %	73	0	4	23

Table 3.AGE AND DISTRIBUTION OF BREAST CANCER SUBTYPES

Breast cancer subtype	Number of patients	21-30	31-40	41-50	51-60	61-70	>70
HER-2	4(3%)	0	2	2	0	0	0
LUMINAL A	84(64%)	5	17	28	27	6	1
LUMINAL B	17(13%)	2	1	8	2	2	2
BASAL-LIKE	26(20%)	0	8	6	4	4	4
TOTAL	131	7	28	44	33	12	7

DISCUSSION

The immunohistochemical classification of breast cancers based on hormone and HER2 receptor profiles is critical because it provides prognostic and therapeutic information for the patient's benefit. In this study, one hundred and thirty-one female breast cancer positive patients within the age range of 23-72 years, and mean age of 47.2± 0.05 years, were evaluated for

relevant receptors profile. The age in this report does not vary so much from 46.8± 11.5 years document by Adebamowo and Ajai, in the western part of Nigeria.[21] Also, in the same range are the mean ages for breast cancer patients studied in Morocco(46 years), which is another African country like Nigeria; India(44.6)- an Asian country[23,24]. According to Abdulrahman and Rahman[25], the average age of occurrence of breast cancer in Africa is 48 years, with premenopausal women accounting for the majority of cases, whereas the majority of women in Europe present after menopause.

This is intandem with our result, as over 60% of our breast cancer patients are below or equal to the age of 50 years. In the United Kingdom, the median age at presentation for Black women is 46 years, comparable to African women, and 67 years for white British women.[25] In this study, the peak age of incidence is 41-50 years.(See Table 1).Although Tanimowo et al[26] reported 30-39, the two studies only show younger ages for the development of breast cancer in Africans. Late motherhood, fewer children, late menopause, hormone replacement therapy, obesity, and adult weight gain have been associated with risk factors for an increase in HR-positive tumours in postmenopausal women.[27] The factors responsible for the early development of breast cancer in African women are unknown, but it is possible that it is linked to the relevant genes involved in this disease, such as BRCA1 and 2, as well as their variants [11]. Our study revealed that invasive ductal carcinoma is the commonest histological form of breast cancer (86. 7%), which is consistent with the previous studies[21,26].

The hormone receptor profile in our study shows that the 77% of the breast cancer cases are positive for hormone receptor markers. This is in conformity with the 65.1% reported by Adebamowo et al[28] indicating the preponderance of hormone receptors positivity in the locality. The incidence of ER and PR positivity in the malignancy of the female breasts are respectively 65.6% and 46.6%; while 13.7% manifested over expression of HER2 and 19.8% negative for the three receptor markers(triple negative breast cancer). The pattern of the incidence (ER>PR>HER2) is consistent with the recent study by Adeniji et al., [29], in which ER+ constitute 73%;PR 27% and HER2 18.3%.A Brazilian study revealed that 73% of the breast cancer is hormone receptor positive. [30] This report aligns with ours, which is 77%.(see Table 2).In terms of hormone receptor positivity, our findings differ from those of Tanimowo et al[26], since it contains ER (18%), PR (14.85), and HER2 (32 percent). This is proof of a country's variance in hormone receptor distribution.

McCormick et al., reported that The presence of ER in breast cancer tissue is a good indicator of a better prognosis, as increased expression is linked to a lower risk of cancer-specific mortality in both women of colour and white women, as well as a longer time of recurrence and a good response to therapy.[31].In breast cancer cells, IGF-I (insulin-like growth factor-1) inhibits PR expression via the phosphatidyl inositol 3-kinase/AKT/mammalian target of the rapamycin pathway, resulting in low PR expression and an indicator of activated growth factor signaling. According to Lamb et al. [32], ER and PR are members of one steroid receptor superfamily, sharing similar structures that include hormone binding, nuclear translocation, DNA binding and transactivation domains. After high affinity hormone binding, these receptors become active and operate as transcription factors by binding to the promoters of target genes at precise binding sites. They can also be activated in the absence of ligand because they can be phosphorylated by kinases like mitogen-activated protein kinase (MAPK) and protein kinase B (AKT), which are commonly over activated in cancerous processes. Activated receptors can then function as co activators, amplifying the effects of other transcription factors. Low or absent PR was linked to a poor prognosis

and response to hormone therapy in primary breast cancer, implying an aggressive tumour phenotype and resistance to hormonal therapy. [33] It is important to emphasize that ER determination is important not only because it serves as an independent prognostic marker, but also because it is a predictor of therapy response. Endocrine therapy can be used to treat patients with breast cancers that have an ER expression level of more than or equal to 1%. [32] Clerk et al.[34] observed in 1984 that ER has been documented as an important predictor of prolonged disease-free interval as well overall survival for patients with primary breast cancer, and in cases of advanced disease, it also corresponds with responsiveness to endocrine therapy. As for PR, it has been demonstrated to be a more powerful prognostic indicator and predictor than oestrogen receptor. Overexpression of the HER2 protein and gene in breast cancer has been linked to aggressive tumour characteristics like early tumour relapse, axillary node positivity, high tumour grade, and large tumour size. [35]

The commonest molecular subtype in our study was luminal A (64.1%), followed by TNBC (19.9%), HER2 rich (3.1%), and luminal B (13%).(See Table 3).The pattern of tumour incidence in our study (Luminal A> TNBC>Luminal B>HER2 over expression) is consistent with Nwafor and Keshinro's [36], work in southern Nigeria. Although our figures are slightly higher, both studies show that Luminal A is more prevalent in breast cancer subtypes in our area, which is a good sign. Luminal's tumours (Luminal A and B) have a better prognosis than other breast carcinomas, and they respond to endocrine medications that block the ER (tamoxifen or fulvestrant) or reduce the endogenous generation of natural ligands.[32] The least subtype of breast cancer in our study is the HER2 over expression type, recording 3.1%.This is a far cry from what was reported by Tanimowo et al. [26], but consistent with the work of Nwafor and Keshinro, who presented Her2 over expression as the least common breast cancer phenotype[36].This finding suggests that these receptors can vary in prevalence, even within the same area. HER-2 is a universal partner for heterodimerization with other members of the HER receptor family (together with HER-1 (EGF R), HER-3, and HER-4) and a member of a family of transmembrane tyrosine kinase receptors (along with HER-1 (EGF R), HER-3, and HER-4). The activation of many signalling pathways by amplification of the gene c-erbB-2, result in over expression of the HER-2 protein, increases tumour growth, invasion, and survival. [37]

Minoza et al. [38] and Usman et al. [39] both reported TNBC subtype predominance in the northern area of Nigeria. This is not in line with the result of our study which has TNBC, only second to the highest type of breast cancer subtypes. This is another evidence of variability in prevalence of breast cancer subtypes, even within a country. TNBC cancers have the poorest prognosis, and there is no standard targeted therapy for them yet. Patients with TNBC positive tumours in Nigeria have an 8.3 percent BRCA1 mutation, whereas patients positive for steroid and HER2 receptors only have a 2.5 percent mutation, according to Zheng and colleagues. Furthermore, patients with TNBC cancers are marginally, but not significantly, more common among older patients at the time of diagnosis than patients with non-TNBC tumours, and TNBC is unrelated to tumour stage, according to Zheng et al.[11]. Breast cancer subtypes with HER2 overexpression have worse clinical, pathologic, and molecular progression, while the TNBC breast cancer subtype has the worst overall survival. [40].

In conclusion, we note that, despite the fact that our study's sample size is not very great, it was able to indicate that hormone receptor positive subtypes may predominate in Imo state breast cancer patients, followed by TNBC, and finally HER2 positive subtypes. Our research illustrates the wide variation in the distribution of breast cancer receptor indicators and subtypes across our

country and beyond. We advocate for the establishment of a robust cancer registry in Nigeria and Africa as a whole in order to provide more reliable and complete tumor epidemiology data, to verify

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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