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THE CHANGING IMMUNOHISTOCHEMICAL PROFILE OF BREAST CARCINOMAS IN NNEWI, SOUTH-EAST NIGERIA: OUR EXPERIENCE

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ARTICLE INFO | **ABSTRACT** | **ORIGINAL RESEARCH ARTICLE**

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Introduction: GLOBOCAN statistics have shown a rising incidence of breast cancers (BCs) in Nigeria, with previous studies in our region showing predominance of triple-negative category. The molecular classification of BCs, as refined in 2015 by St. Galen's consensus, will help prognosticate and better personalize treatment even in resource-limited countries.

Objective: The aim of this study was to evaluate the immunohistochemical (IHC) status of BCs diagnosed in Nnewi, southeast Nigeria.

Materials and Methods: We analyzed all the morphologically diagnosed cases of BCs that were evaluated IHC for hormone receptors (HR) status, HER2, and Ki67 from two histopathology laboratories serving Nnewi and environs over 6 years using SPSS software, version 20.

Results: A total of 13275 surgical specimens were received, 2888 of which were solid malignancies and 2485 were breast tissue specimens (974 (39.20%) of which were BCs). BCs accounted for 33.73% of all solid cancers. Only 142 had IHC done on the tissue blocks. The age ranged from 21 to 80 years with modal age in the 5th decade and mean age of 49.90±12.1 years. The majority (59.2%) of the BCs were HR-positive and only 17.1% of BCs in women ≤40years were triple-negative (TN). HER2 BCs and triple-negative breast cancers (TNBCs) were commoner in women aged > 40 years.

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Conclusion: There is higher proportion of hormone receptor positive breast cancers in this study compared to previous study in our locality and other parts of the country. The higher proportion of triple negative breast cancers in other studies may be due to pre-analytic factors.

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INTRODUCTION

Breast cancers (BCs) are a heterogeneous group of diseases with varying biological profiles, morphology, clinical course, and therapeutic response.[1] The global cancer statistics show that BCs occupy the first position in incidence (11.7%) and fourth in mortality (6.9%) worldwide,[2] but ranks first both in incidence (22.7%) and mortality (18.1%) in Nigeria.[3] A look at the GLOBOCAN 2018 [4] and 2020 [3] statistics shows an increasing incidence of BCs in Nigeria.

The past few decades have witnessed a paradigm shift from the traditional to molecular classification of BCs to help define the basis for the heterogeneity of BCs. Gene expression profiling has been used to define, sub-classify, prognosticate and direct therapy in BCs.[5] Analogous to molecular profiling, immunohistochemical expression of hormone receptors (HR) including Estrogen and Progesterone receptors; HER2-neu and Ki-67 categorize BCs into molecular subgroups.[6] This 'St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer', released for clinical purposes to guide treatment decision-making for adjuvant therapies, defined IHC surrogates to distinguish the different molecular subgroups of BCs without a requirement for molecular diagnostics.[6] The different molecular subgroups include luminal A, Luminal B, HER2 over-expressed, and Triple-negative BCs. This classification was refined in 2015 to help prognosticate and better personalize treatment even in resource-limited countries.[7]

The Triple Negative BC category is noted to account for only about 10-15% of BCs overall but occurs with increased frequency in African-American women.[8] A review of the data from several studies appears to reveal a regional variation in the immunohistochemical profile of breast carcinomas, even within a country.[9-14] A previous study done in our center showed a preponderance of TNBC.[13]

Although the contribution of IHC in clinical practice is well established, it is acknowledged that the IHC method is significantly influenced by several factors, largely pre-analytic.[15] These factors reported to influence the outcome of IHC include cold ischemia time, fixation time, paraffin, storage time in paraffin, storage temperature, age of the cut sections, antigen retrieval technique, etc.[15-19] This study aimed to evaluate the IHC status of breast cancers diagnosed in Nnewi, southeast Nigeria, to define their molecular characteristics.

MATERIALS AND METHODS:

This is a descriptive study of formalin-fixed paraffin-embedded tissue blocks with a confirmed diagnosis of breast cancer in the histopathology department of the Nnamdi Azikiwe University Teaching Hospital and Pathocon Specialist Laboratory and Research Institute, both in Nnewi, Anambra over six years (from January 2015 to December 2020). Nnewi is the second-largest city in Anambra State, South-east Nigeria, with an estimated population of 391,227 according to the 2006 census.[20]

Breast tissues with histological diagnosis of BC, with IHC request and whose samples have been promptly fixed in 10 % neutral buffered formalin not exceeding 72 hours were recruited into this study. The cases were grossed within 72 hours of surgery and the tissue blocks were stored at room

temperature. The cases with a request for immunohistochemistry were prospectively enrolled in the study. Data were extracted from patient records and requisition forms. The morphological classification was done according to the fifth edition of the World Health Organization breast tumor book 2019.[21] Histological grading was performed using the Nottingham classification system.[22] Immunohistochemical stainings was done for Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor2 (HER2) and Ki-67. The criteria set by ASCO (American Society of Clinical Oncology) were followed[23, 24]

- ER and PR-positive nuclei greater than 1% were considered hormone receptor-positive.
- HER2 was scored based on a 0 to 3 scale, a score of 3+ was considered positive and a score ≤2 was negative. Fluorescence in situ hybridization for HER2 amplification was not performed due to unavailability and cost.

Breast cancer subtypes were defined according to the IHC expression of ER, PR, HER2, and Ki67 count as follow:[6]

Luminal A= ER + /PR+, HER2−, Ki67 ≤ 20%,
Luminal B = ER + /PR+, HER2 +, any Ki67 or ER + /PR+, HER2−, Ki67 > 20%,
HER2 enriched= ER−, PR−, HER2+
Triple negative= ER−, PR−, HER2−.

Statistics

Statistical analysis and data processing was performed with the software SPSS version 20.

RESULTS

Breast specimens accounted for 2485 out of a total of 13275 surgical specimens received over the 6 year study period. A total of 2888 solid malignancies were diagnosed of which 974 were breast carcinomas accounting for 33.73% of all solid malignancies. Hence, breast carcinomas accounted for 39.20% of the breast specimens received in the pathology laboratories. Only 142 (14.58%) of the breast cancer tissue specimens had IHC done on the tissue blocks **Figures 1A-1D**

The yearly trend shows a gradual increase in IHC requisition between 2015 and 2020. However, there is a decline in the IHC requisition after 2018 when interpreted as the percentage of diagnosed cases. (See figure 2)

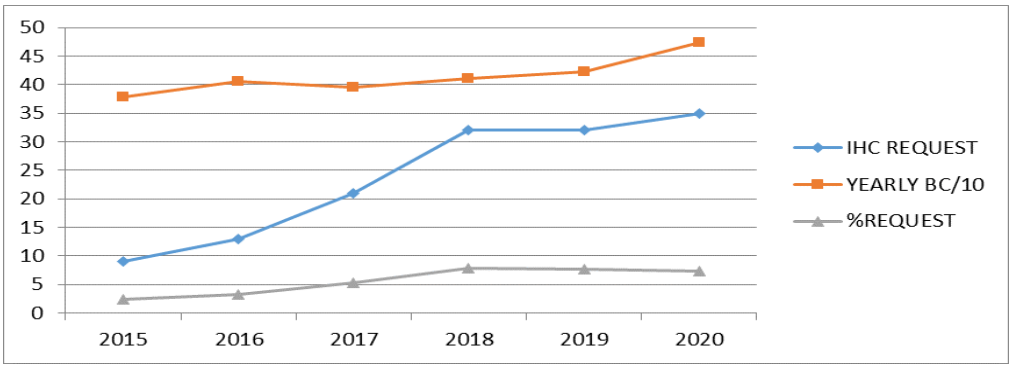

Figure 2: Yearly trend for Immunohistochemistry requisition
(IHC REQUEST= Yearly immunohistochemistry requisition; YEARLY BC/10= Breast carcinomas diagnosed per year/10; %REQUEST= Percentage of the diagnosed breast carcinomas with immunohistochemistry request.)

The age range of the clients diagnosed with breast carcinomas was 21 to 80 years with a modal age range in the 5th decade and mean age of 49.90±12.1 years. For women aged ≤40years, most (77.14%) BCs are HR-positive while only 14.29% are TN. (see table I).

Table I: The age range distribution of the different molecular categories

	LUMINAL A	**LUMINAL B**	**HER2 BC**	**TNBC**	**TOTAL**
Mean age	48.58±12.45 yrs	46.78±11.37 yrs	52.74±9.13 yrs	53.29±12.97 yrs	
Median age	45.0 yrs	46.5 yrs	54.0 yrs	52.0 yrs	
21-30	3	1	0	1	5
31-40	10	13	3	4	30
41-50	13	13	3	12	41
51-60	11	8	11	9	39
61-70	6	3	1	8	18
71-80	2	2	1	4	9
Total	**45**	**40**	**19**	**38**	**142**

HER2 BC= HER2 Breast cancers, TNBC= triple-negative breast cancers

Most cancers (59.86%) were estrogen receptor-positive, 44.37% were progesterone-receptors positive and 26.76% expressed HER2. 5 cases were equivocal for HER2 immunostaining. (See Table II)

Table II: Immunohistochemical Staining Reactions for ER, PR, and HER-2

	ER		PR		HER2		
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Equivocal**
21-30	4	1	3	2	1	4	0
31-40	23	7	16	14	6	22	2
41-50	26	15	21	20	8	30	3
51-60	19	20	13	26	18	21	0
61-70	9	9	7	11	3	15	0
71-80	4	5	3	6	2	7	0
Total	**85**	**57**	**63**	**79**	**38**	**99**	**5**

ER = Estrogen Receptor; PR =Progesterone Receptor; HER2 =Human Epidermal Receptor Type 2.

The majority (59.86%) of the breast carcinomas were in the luminal while TNBCs accounts for 26.76% of breast carcinomas (*Figure 3*).

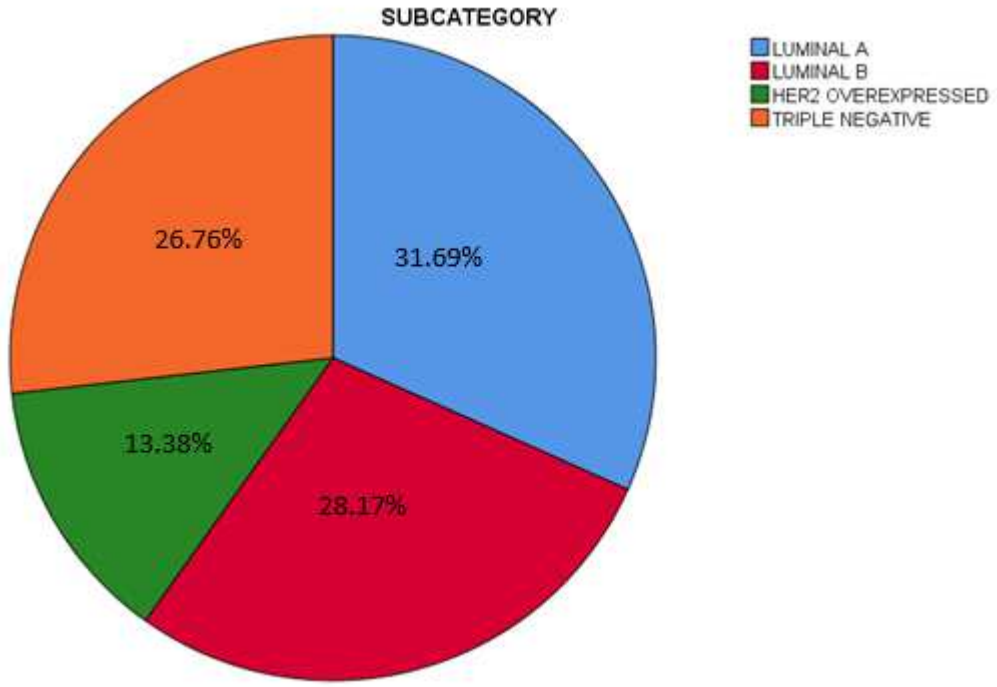

Figure 3: showing the proportion of the molecular classes of breast carcinomas

TNBC was noted to be the predominant (44.44%) subcategory after the sixth decade (figure 4).

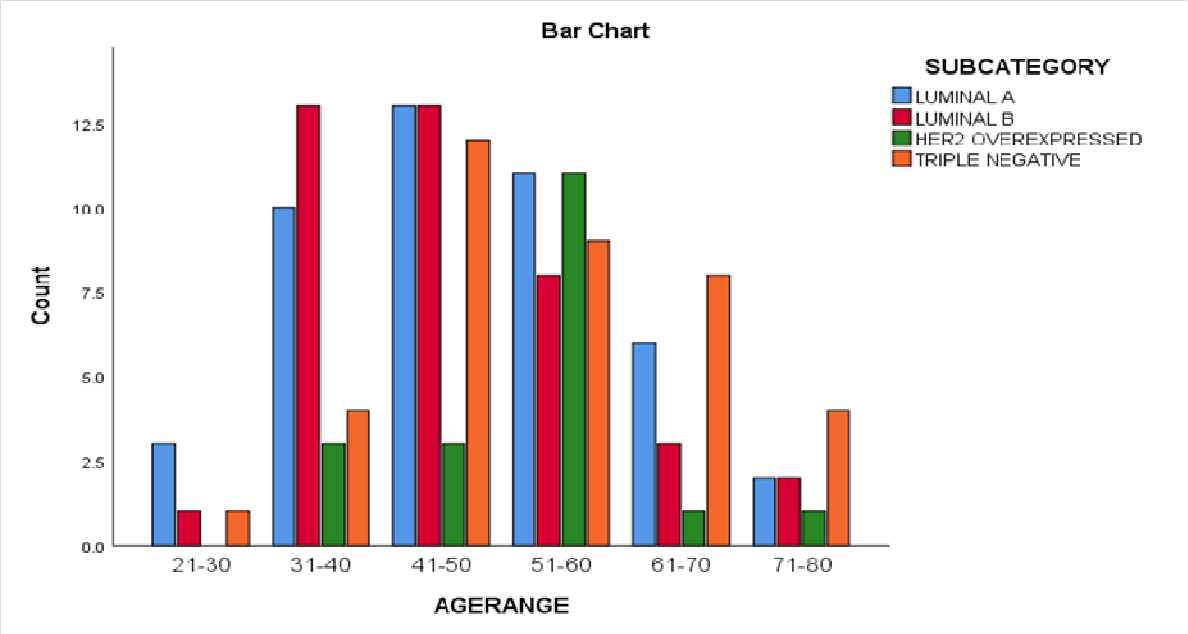

Figure 3: Bar chart illustrating molecular subcategories per age range

The clinicopathological characteristics of the patients as regards side of lesion, tumor size, and morphologic variant is as illustrated in Table III.

Table III: Distribution of breast cancer by clinicopathological features.

		Frequency	Percent
Anatomical side			
	Right	71	49.7
	Left	68	47.6
	Bilateral	3	2.1
Tumor Size			
	< Or =2CM	5	3.5
	>2-5cm	83	58.0
	>5cm	54	37.8
Morphologic Type			
	IDC NST	125	87.4
	ILC	5	3.5
	Mixed NST And Colloid Carcinoma	1	0.7
	Metaplastic Carcinoma	1	0.7
	Mucinous Carcinoma	1	0.7
	Neuroendocrine	2	1.4
	Secretory	1	0.7
	Invasive Papillary	3	2.1
	Invasive Micropapillary	3	2.1

IDC NST= Invasive Ductal Carcinoma, No Special Type; ILC = Invasive Lobular Carcinoma.

Table IV shows that most of the BCs were more than 2.0 cm in size at the time of diagnosis, and none of the TNBC was ≤2.0cm.

Table IV: Tumour size characteristics of the molecular subtypes at diagnosis

TUMOR SIZE	LUMINAL A	LUMINAL B	HER2 BC	TNBC	Total
≤2.0CM	4	0	1	0	5
>2.0-5.0CM	26	23	12	22	83
>5.0CM	15	17	6	16	54
Total	45	40	19	38	142

DISCUSSION

With advances in molecular research into diseases, an attempt at defining the heterogeneous nature of BCs has moved from cellular to molecular level. This molecular classification has not only aided diagnosis but also the provision of prognostic and therapeutic information for the individual patient, enhancing individualized customized therapy.[5,25] The role of the pathologist is therefore critical in patient management, providing not only a diagnosis but also information that predicts prognosis and aids therapy.[5] IHC is an invaluable tool for the pathologist to perform this role; however, it is not yet a routine in developing countries. This

could be attributable to the cost of, and not readily available IHC services in developing countries, including Nigeria where payment for most health services is by out-of-pocket payment. Although BCs accounted for 33.73% of all solid cancers in this study, only 14.58% of the 974 diagnosed breast cancer tissue specimens had IHC done on the tissue blocks. The previous study done in this center by Ukah *et al.*[13] and that by Nwafor *et al* in Calabar Nigeria,[26] reported similar low requisition.

In this study, most of the BCs were HR-positive with about 27% of the cases being in the triple-negative category. This is in contrast to the previous study in our center with only 42% HR positivity,[13] and other parts of the country and Ghana with a reported predominance of TNBCs,[11, 12, 27] Other studies in Nigeria,[26] and India [28] show predominance of HR-positive BCs. Although the study among Togolese women reported more TNBC category, there was HR positivity in 54% of the BCs.[12] Adebamowo *et al.*, similarly reported 80.2% HR positivity and only 15% TNBC in their studies, concluding that BCs in Africa follows the same pattern as that found elsewhere.[14] Studies have shown that there are limitations with IHC,[29] including technical issues, despite its contribution in pathology diagnostics. In the recent past, inadequate fixation including over-fixation, delayed fixation, and fixatives of doubtful qualities were common experiences in Nigeria. Evidence has shown that preanalytic factors including fixation, cold ischaemic time, etc can affect antigen retrieval and reactivity, hence leading to false-negative results.[30-32] Technical pitfalls in IHC may therefore account for some of the disparity, as this may be a limitation to the IHC procedures

In this study, most BCs occur in the fifth decade of life, with a mean age of 49.90±12.1 years. This is similar to the mean age of 48.8±0.53 years, 48±12.3 years, and 47.76±11.08 years reported in Sudan and Eritrea,[33] Nigeria[11] and India[34] respectively but less than the mean age of 63 years reported in Western countries like USA [35] and higher than 41 ± 13.5 years reported in Bangladesh, [36] and 45± 14years reported in Uganda.[37] The study was done among the Sudanese and Eritrean women also show a modal age of occurrence in the fifth decade.[33]

Epidemiological data show that TNBC mostly occurs in premenopausal young women under 40 years old.[38, 39] This is contrary to the findings of our study, with most (77.14%) BCs in women aged ≤40years being HR-positive while only 14.29% are TNBCs. TNBC was the predominant subcategory in women beyond the sixth decade of life.

The median ages for the BCs categories were below 50years for the luminal, but 54years and 52years for HER2 and TNBC respectively. HER2 BC and TNBC appear to be commoner in women >50 years of age. This is congruent with Taminowo *et al.*, who reported that TNBCs were less frequent in patients aged 40 years or younger (39.5 %).[39] An Indian study reported a higher rate of HER2 expression and TNBC in women younger than 50 years.[40] Adebamowo *et al.* reported no statistical association between age and molecular subtype of BC.[14]

Most of the BCs in this study are morphologic invasive ductal carcinoma, no special type. There was no significant difference in the side involved, however, only three cases of bilateral occurrence were noted. This is consistent with that reported in other studies both within and outside Nigeria.[11,39, 41]

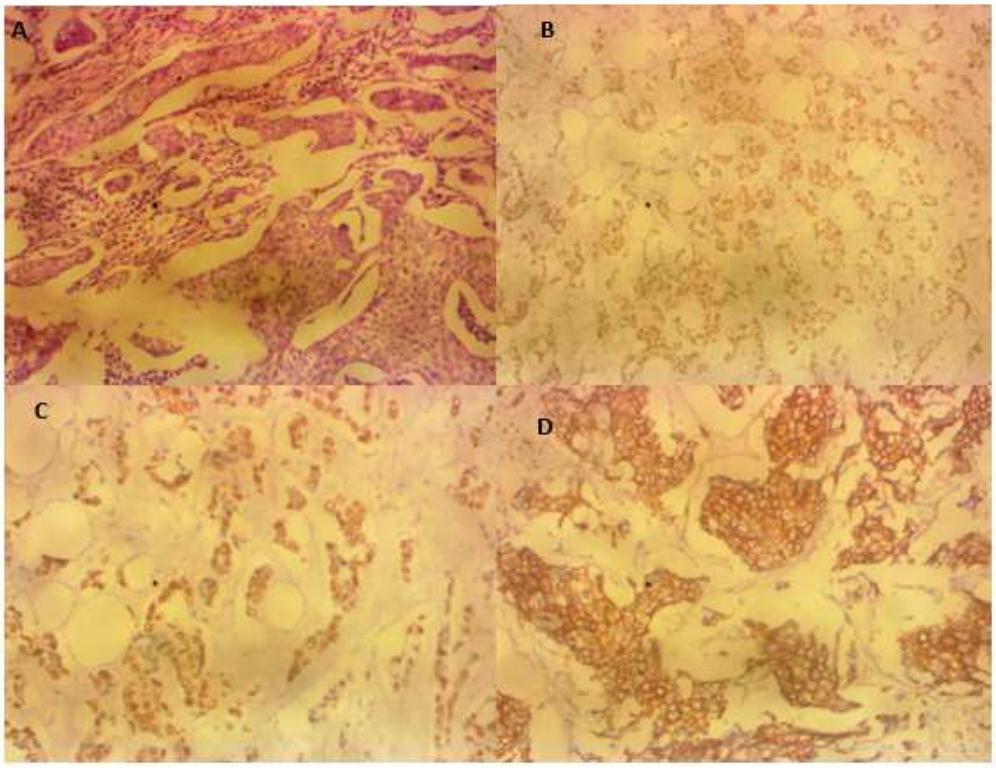

Figure 1: Photomicrograph of breast carcinoma: A=H&E; B= ER-positive; C=PR positive and D= HER2 positive (all images at x200 original magnification)

CONCLUSION:

Most BCs in our environment are HR-positive. There is a need to educate the clinicians on the need for prompt and proper tissue fixation, to reduce preanalytic errors and therefore false-negative immunohistochemical results.

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