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Immunohistochemical Survey of Invasive Ductal Carcinoma of the Breast, using ER, PR, HER2 and KI-67 biomarkers, in Uyo, Nigeria

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Abstract

Background: Breast's Invasive Ductal Carcinoma (IDC), which is the commonest type of malignancy in females worldwide, can be characterized using immunohistochemistry in view of personalized cancer therapy. In this study, we aimed to determine the pattern of immunohistochemical profiles of IDC using oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 receptor (HER2) and proliferative index (Ki-67) biomarkers in our tertiary healthcare facility in Uyo, Akwa Ibom State, Nigeria given the dearth of its data in our environment.
Materials and methods: We carried out a retrospective hospital-based immunohistochemical study of archival IDC tissue blocks over a four- and half-year period. Using systematic random sampling method, 64 formalin fixed paraffin embedded (FFPE) IDC tissue blocks were selected for this study. We carried out immunohistochemical evaluation using ER, PR, HER2 and Ki-67 biomarkers. Subsequently, we presented the results and classification schemes as text, tables, graphs, and photomicrographs.
Results: We found that the proportion of expressions were ER-negative (88.7%), PR-negative (87.3%), HER2-negative (68.3%) and Ki-67 (<20%) being 83.6% respectively. The immunohistochemical-based classification which was done using combined immunohistochemical profiles of ER/PR/HER2 and ER/PR/HER2/Ki-67 biomarkers respectively, revealed five immunohistochemical-based subtypes. These subtypes were ER-positive luminal A (ER+/±PR+/HER2-) [5.56%], ER-positive luminal B (ER+/±PR+/HER2+) [5.56%], HER2-overexpression (ER-/±PR+/HER2+) [16.66%], Triple negative (ER-/PR-/HER2-) [66.67%] and Unclassified subtypes (ER-/PR+/HER2-) [5.56%]. Furthermore, these five subtypes were further subcategorized into low (Ki-67 <20%) and high (Ki-67 ≥20%) proliferation subtypes accordingly.
Conclusion: The commonest pattern of immunohistochemical profile expression of IDC in Uyo was found to be the Triple negative subtype.

Keywords: Immunohistochemistry, Immunohistochemical profiles, immunohistochemical-based classification, Invasive Ductal Carcinoma (IDC) of the breast, immunohistochemical biomarkers, Triple negative breast cancer (TNBC) subtype.

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Introduction

Breast carcinoma is one of the commonest types of female malignant neoplasms globally and in Nigeria.[1-4] Importantly, the commonest

histopathologic type of malignant breast neoplasm (MBN) is the Invasive Ductal Carcinoma (IDC) of the breast; this applies globally, in Nigeria and in Uyo (this study's location).[5–11] Notably, MBN is the commonest cause of cancer death in Nigeria.[12–16] This high burden of MBN incidence, morbidity and mortality in Nigeria is traceable to low socio-economic status, poor health seeking behaviour and low level of health education.[17,18] In our environment, the main predisposing factors to developing MBN are age (i.e. women in premenopausal age group) and positive family history.[17,18] Tragically, these patients present late to the hospital with large aggressive tumours, and ultimately have poor clinical outcome.[17,18] The internationally accepted best practice for MBN management includes but not limited to the use of immunohistochemical profiling of MBN specimens (including IDCs) with consequent personalised targeted therapy.[19–25] The absence of this routine immunohistochemical evaluation of MBN/breast carcinoma specimens in our environment is tragic and is causatively linked to the pervading low socio-economic conditions. Unfortunately, the absence of routine immunohistochemical evaluation of IDCs, in our environment, has created the absence of local data on the immunohistochemical-based classification of IDC. Importantly, IDCs can be classified immunohistochemically into hormone receptor positive [expressing oestrogen receptor (ER) and/or progesterone receptor (PR)], human epidermal growth factor 2 receptor (HER2)-overexpression, and Triple Negative subtypes.[21,25–31] Furthermore, this immunohistochemical-based classification, derived from immunohistochemical profiling, provides the data on the biological heterogeneity of IDC in any given environment.[11,26,28,31–42] Hence, tragically, this absence means lack of data on the biological heterogeneity of IDC in our environment. Also, it is notable that routine use of immunohistochemical evaluation for IDC would have provided the basis for routine targeted therapy/personalized medical care of our IDC patients in view of their IDC biological heterogeneity. Importantly too, studies have demonstrated that routine targeted therapy can lead to reduction of morbidity and mortality in IDC patients, hence showing the need for such practice in our environment.[22–24,27,28,30,31,43]

Therefore, in this study we aimed to retrospectively survey the immunohistochemical profiles of Invasive Ductal Carcinoma (IDC) of the breast, using oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 receptor (HER2) and proliferative index (Ki-67) biomarkers, in our tertiary healthcare facility in Uyo, Akwa Ibom State, Nigeria, over a four- and half-year study period. Furthermore, we aimed to carry out an immunohistochemical-based classification of IDC of the breast based on their expressed immunohistochemical profiles and subsequently determine the most common subtype of IDC of the breast in our environment. We will compare our findings with relevant literature and hence commence the filling of this IDC immunohistochemical knowledge gap in our environment.

Materials and methods

This study was a retrospective hospital-based cross-sectional immunohistochemical-based study. Our primary study material comprised of formalin fixed paraffin embedded (FFPE) tissue blocks of previously diagnosed cases of invasive ductal carcinoma (IDC) of the male and female breasts in the department of Histopathology, University of Uyo Teaching Hospital (UUTH), Uyo, Akwa Ibom State. Notably, UUTH, Uyo is a 500-bed government-owned tertiary healthcare facility serving the state and its environs. Our sample size was 64 FFPE tissue blocks. We selected these tissue blocks using systematic random sampling method; wherein every fourth sample in our sampling frame (which consisted of 236 cases of IDC within the study period of January 2010 to June 2014) were selected. We initially selected 59 cases using this method, subsequently, the last six cases in the sampling frame (to adjust for attrition during tissue blocks handling) were included, hence giving the final total of 64 FFPE tissue blocks. These samples included data from the department's Histopathology report files/register and their corresponding FFPE tissue blocks in department's archives. We validated these 64 selected blocks by cutting fresh histologic sections from them and staining them with hematoxylin and eosin (H&E) stain while adhering to the standard H&E staining protocol for light microscopic histopathological

evaluation. We subsequently took these selected/validated 64 blocks to the Institute for Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH), Ibadan, Oyo State, for immunohistochemical staining with ER, PR, HER2 and Ki-67 biomarkers while strictly adhering to standard immunohistochemical staining protocol.[22,24,44,45] Notably, the ER, PR and HER2 biomarkers/immunostains were sourced from Dako, an Agilent Technologies Company, Denmark. Also, the Ki-67 biomarker/immunostain was sourced from Thermo Scientific, a subsidiary of Thermo Fisher Scientific Inc, USA. The clone of the ER immunostain was ERα (estrogen receptor alpha) 1D5 monoclonal mouse antihuman antibody. The clone of the PR immunostain was PgR636 monoclonal mouse antihuman antibody. The HER2 immunostain clone was human epidermal growth factor receptor-2 (cerbB-2) oncoprotein monoclonal antihuman antibody. The clone of Ki-67 immunostain was 9C12B2 monoclonal antibody. The immunostaining for ER/PR/HER2/Ki-67 biomarkers on the 64 study cases were done in batches and serially. We carried out all the steps in this immunohistochemical staining procedure under the specified condition as stated in the manual accompanying the Dako and Thermo Scientific test kits respectively. We used the appropriate positive and negative controls for each staining batch. Thereafter, we evaluated these immunohistochemical slides using the ASCO/CAP standardized immunohistochemical scoring system (protocol and criteria) for ER, PR, HER-2 and Ki-67 biomarkers respectively.[22,24,44,45] Importantly, in accordance with these reviewed relevant literature, we considered ≥1% nuclear staining ER/PR biomarker expression as positive, 0 and 1+ membrane expression of HER2 biomarker as negative, 2+ membrane expression of HER2 as equivocal (an indication for Fluorescent in-situ Hybridization [FISH]) and 3+ membrane expression of HER2 biomarker as positive, and only nuclei expressing Ki-67 biomarker are eligible for mitotic index/proliferative fraction percentage estimation.[22,24,29,44,45]

We carried out this study according to the ethical guidelines of the World Medical Association Declaration of Helsinki 2000; thus, confidentiality was strictly ensured, and the research did not cause harm to any human subject.

We recorded all the data generated from this evaluation in a hard copy research notebook as well as in Microsoft excel spreadsheet. We, subsequently, analyzed these data using IBM SPSS 20.0 Statistical software. We, thereafter, presented our results, as text, tables, graphs, and photomicrographs.

Results

We found that the mean age of our study cases was 43.7 ± 11.8 years within an age range of 26 to 72 years (Table 1). We also found that the age group most affected by IDC was 31 – 45 years, constituting 50% of our cases (Table 1).

On immunohistochemical evaluation of our study cases, using ER, PR, HER2 and Ki-67 biomarkers, we found that for ER and PR similar proportions of the IDC tissue blocks were hormone receptor positive [11.3% for ER and 12.7% for PR

Table 1: Descriptive statistics of the age and sex distribution of the study cases

Variable	Male	Female	Minimum	Maximum	Mean	Std. Deviation
Age	0	64	26	72	43.7	11.805
Sex	0	64				
Total	0	64				

Age groups	Frequency	Percentage (%)
<31	8	12.5
31-45	32	50
46-60	16	25
>60	8	12.5
Total	64	100

Table 2: Frequency distribution of immunohistochemical hormone receptor (oestrogen receptor [ER] and progesterone receptor [PR]) status or expression of our study cases as well as the immunohistochemical human epidermal growth factor 2 receptor (HER2) overexpression of our study cases in terms of Scoring patterns.

Hormone Receptor (ER And PR) Status	Number	Percentage
ER Positive	7	11.3
ER Negative	55	88.7
Total	62	100
PR Positive	8	12.7
PR Negative	55	87.3
Total	63	100
HER2 Overexpression Status	**Number**	**Percentage**
Score 0	23	36.5
Score 1+	20	31.7
Score 2+	8	12.7
Score 3+	12	19
Total	63	100

Table 3: Frequency distribution of combined immunohistochemical (IHC) expression of ER, PR, and HER2 biomarkers in individual cases in terms of the immunohistochemical-based classification of invasive ductal carcinoma (IDC) of the breast (with the exclusion of all cases with HER2 score 2+ which are candidates for further characterization with Fluorescent in-situ Hybridization [FISH]).

Molecular Classification	Combined IHC Profiles	Number	Percentage
ER-Positive Luminal A Subgroup 1	ER+/PR+/HER2-	3	5.56
ER-Positive Luminal A Subgroup 2	ER+/PR-/HER2-	0	0
ER-Positive Luminal B Subgroup 1	ER+/PR+/HER2+	0	0
ER-Positive Luminal B Subgroup 2	ER+/PR-/HER2+	3	5.56
HER2 Overexpression Subgroup 1	ER-/PR-/HER2+	7	12.96
HER2 Overexpression Subgroup 2	ER-/PR+/HER2+	2	3.7
Triple Negative Subgroup	ER-/PR-/HER2-	36	66.67
Unclassified Subgroup	ER-/PR+/HER2-	3	5.56
Total		54	100

respectively], that 19% of them can be categorized (Score 3+) as positive for HER2 overexpression and, that they majorly showed Ki-67 expression of low proliferation rates (83.6%), using <20% or ≥20% scoring pattern (Table 2, Figure 2 and 3).

On combined ER/PR/HER2 immunohistochemical profiles of our study cases, we found Triple Negative Subgroup (ER-/PR-/HER2-) of IDC to be the most common subgroup (66.67%) of IDCs. This Triple Negative Subgroup was followed by the HER2 Overexpression Subgroup 1 (ER-/PR-/HER2+) accounting for 12.96% of our cases (Table 3). Furthermore, on combined ER/PR/HER2/Ki-67 immunohistochemical profiles of our study cases, we also found the Triple Negative Subtype Low Proliferation (ER-/PR-/HER2-/Ki67 <20%) to be commonest subtype of IDC (60.38%) (Table 4). This Triple Negative Subtype Low Proliferation is followed by HER-2 Over-Expression Subtype Low Proliferation (ER-/PR-/HER2+/Ki67 <20%) and

Table 4: Frequency distribution of combined immunohistochemical (IHC) expression of ER, PR, HER2 and Ki-67 biomarkers in terms of the immunohistochemical-based classification of invasive ductal carcinoma (IDC) of the breast (with the exclusion of all cases with HER2 score 2+ which are candidates for further characterization with Fluorescent in-situ Hybridization [FISH]).

Molecular Classification	Combined IHC Profiles	Number	Percentage
ER-Positive Luminal A Subtype Low Proliferation	ER+/PR+/HER2-/Ki67 <20%	2	3.77
ER-Positive Luminal A Subtype Low Proliferation	ER+/PR-/HER2-/Ki67 <20%	0	0
ER-Positive Luminal B Subtype Low Proliferation	ER+/PR+/HER2+/Ki67 <20%	0	0
ER-Positive Luminal B Subtype Low Proliferation	ER+/PR-/HER2+/Ki67 <20%	2	3.77
HER-2 Over-Expression Subtype Low Proliferation	ER-/PR-/HER2+/Ki67 <20%	7	13.21
HER-2 Over-Expression Subtype Low Proliferation	ER-/PR+/HER2+/Ki67 <20%	1	1.89
Triple Negative Subtype Low Proliferation	ER-/PR-/HER2-/Ki67 <20%	32	60.38
Unclassified Subtype Low Proliferation	ER-/PR+/HER2-/Ki67 <20%	0	0
ER-Positive Luminal A Subtype High Proliferation	ER+/PR+/HER2-/Ki67≥20%	1	1.89
ER-Positive Luminal A Subtype High Proliferation	ER+/PR-/HER2-/Ki67≥20%	0	0
ER-Positive Luminal B Subtype High Proliferation	ER+/PR+/HER2+/Ki67≥20%	0	0
ER-Positive Luminal B Subtype High Proliferation	ER+/PR-/HER2+/Ki67≥20%	1	1.89
HER-2 Over-Expression Subtype High Proliferation	ER-/PR-/HER2+/Ki67≥20%	0	0
HER-2 Over-Expression Subtype High Proliferation	ER-/PR+/HER2+/Ki67≥20%	1	1.89
Triple Negative Subtype High Proliferation	ER-/PR-/HER2-/Ki67≥20%	4	7.55
Unclassified Subtype High Proliferation	ER-/PR+/HER2-/Ki67≥20%	2	3.77
Total		53	100

Table 5: Frequency distribution of the commonest combined immunohistochemical expression of ER/PR/HER2 and ER/PR/HER2/Ki-67 biomarkers respectively in individual cases in terms of the immunohistochemical-based classification of invasive ductal carcinoma (IDC) of the breast in our Tertiary Healthcare Facility, Uyo, South-South, Nigeria.

Molecular Classification	Combined IHC Profiles	Number	Percentage
Triple Negative Subgroup	ER-/PR-/HER2-	36 out of 54	66.67
Triple Negative Subtype Low Proliferation	ER-/PR-/HER2-/Ki67 <20%	32 out of 53	60.38
Triple Negative Subtype High Proliferation	ER-/PR-/HER2-/Ki67≥20%	4 out of 53	7.55

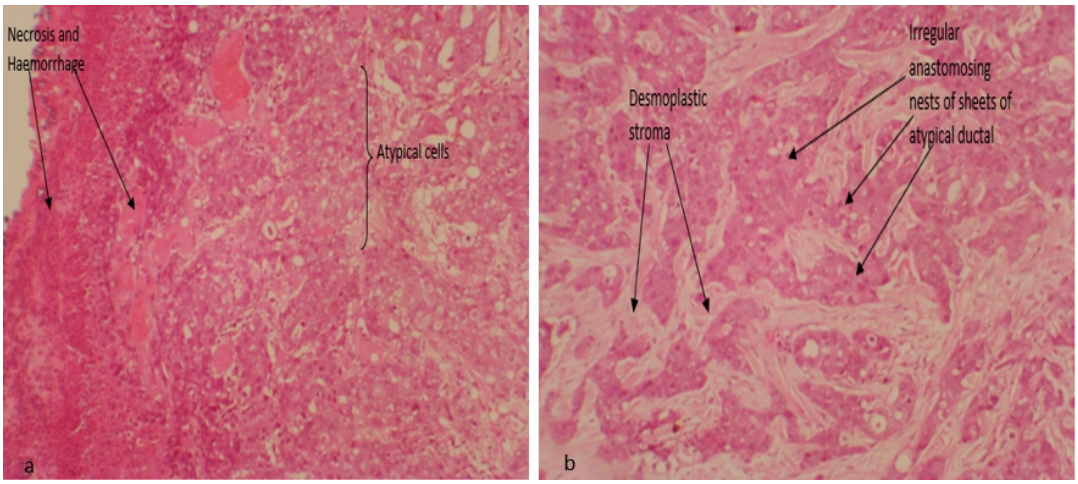

Figure 1: (a) Histopathological section of invasive ductal carcinoma of the breast tissue showing sheets of atypical cells admixed with areas of necrosis and hemorrhage (on the left-hand side of the photomicrograph) [H&E stain; Mag. X100]. (b) Histopathological section of invasive ductal carcinoma of the breast tissue showing irregular anastomosing nests of sheets of atypical ductal cells within a desmoplastic stroma [H&E stain; Mag. X100].

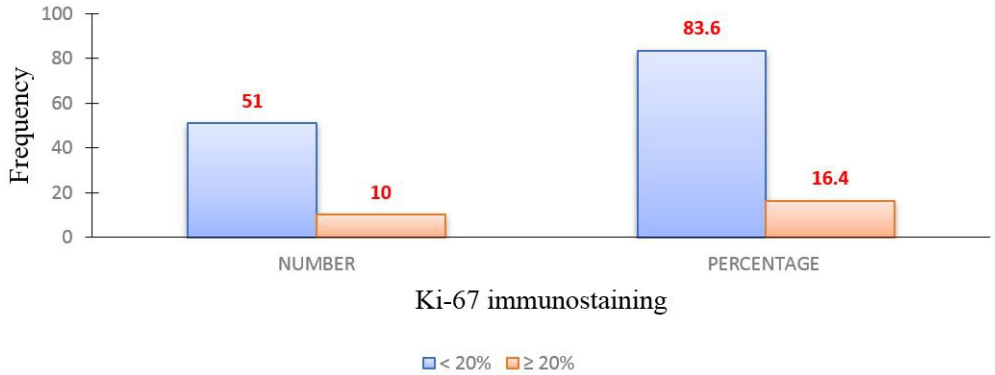

Figure 2: The distribution of immunohistochemical expression of Ki-67 in our study cases using Scoring Pattern of <20% or ≥20%.

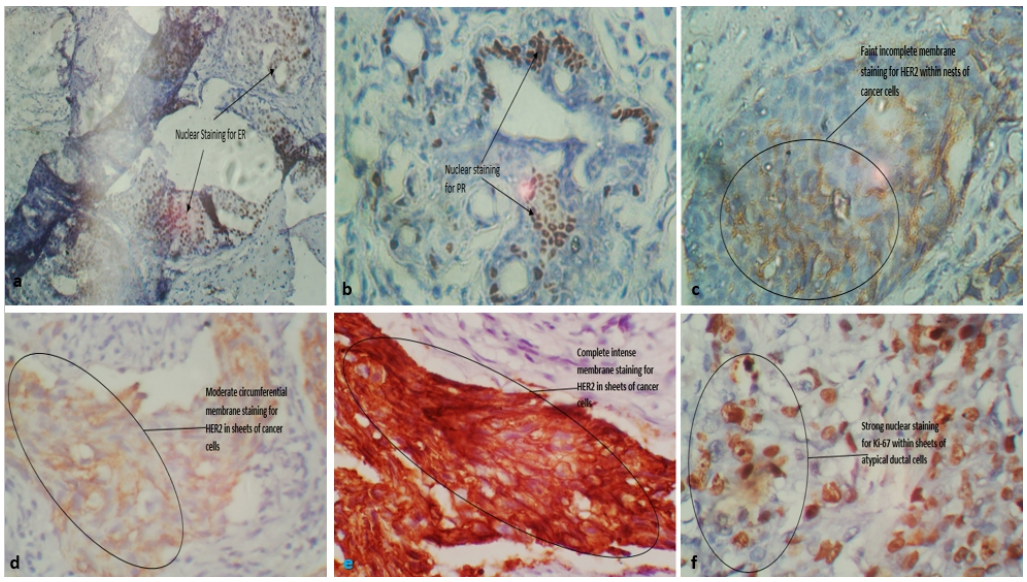

Figure 3: (a) Immunohistochemical section of female IDC tissue showing strong nuclear staining for ER within nests of cancer cells [mag. X40]. (b) Immunohistochemical section of female IDC tissue showing nuclear staining for PR within sheets of atypical ductal cells [mag. X40]. (c) Immunohistochemical section of female IDC tissue showing faint incomplete membrane staining (score 1+) for HER2 within sheets of cells [mag. X400]. (d) Immunohistochemical section of female IDC tissue showing moderate circumferential membrane staining (score 2+) for HER2 within sheets of atypical ductal cells [mag. X400]; this is a candidate for further characterization with FISH. (e) Immunohistochemical section of female IDC tissue showing complete intense membrane staining (score 3+) for HER2 within sheets of atypical ductal cells [mag. X400]. (f) Immunohistochemical section of female IDC tissue showing nuclear staining for Ki-67 within sheets of atypical ductal cells, of ≥20% nuclear staining [mag. X400].

Triple Negative Subtype High Proliferation (ER-/PR-/HER2-/Ki67 ≥20%) accounting for 15.1% and 7.55% of our study cases respectively (Table 4). Notably, we found the Triple Negative Subtype of IDC to be the commonest subtype of IDC in our tertiary healthcare facility using two types of immunohistochemical-based classification of IDC (Table 5)

Discussions

Importantly, we found that 50% of the selected cases were within the 31 – 45 years' age group, with a mean of 43.7 years, and this constituted the peak age group of the sample population. Smaller proportions of patients were found in the extremes of the age grouping which is consistent with the findings of other studies, wherein IDC was found

rarely in women less than 30 years of age but more commonly with increasing age.[46] This peak age group corresponds to women of premenopausal age in their active reproductive years. Possibly, this group of patients have been exposed to the implicated hormonal risk factor such as early age at menarche. The works of Boyle, and Ariyaratne and Dilesha also corroborate this assumption.[46,47] This peak age group and mean age were also similar to the findings of other African studies as regards age of IDC patients. Nggada, Yawe, Abdulazeez and Khalil found a peak age range of 40 – 49 years within an age range of 17 – 85 years, and Forae, Nwachokor and Igbe found peak age of 40 – 49 years and a mean of 46 years.[8,9] Notably, all our selected cases were found to be females, this could indicate that male MBN is rare in Uyo, Akwa Ibom State, and this is in contrast to findings in studies from northern parts of Nigeria; wherein approximately 9% of cases of MBN were found amongst males.[9,10,48] Furthermore, Kene, et al found the peak age group to be 30 – 49 years with a mean of 44.5 years, and Dauda, Misauno and Ojo found the peak age group amongst the female patients to be 21 – 40 years with a mean age of 43.9 years.[10,49]

Our results show that most (88.7%) of the study cases evaluated for expression of ER biomarker were negative. This finding by implication denotes that most of the IDC in Uyo, Akwa Ibom State follows the ER-independent pathway in its development or pathogenesis which studies have established to result from direct genotoxic effects of oestradiol.[36,50] This lack of ER expression also possibly denoted some other pathways influencing the development of IDC in our environment. The categories of such pathways include that of microRNAs, hedgehog signalling, tumour suppressor genes like p53, BRCA1, BRCA2 and NEK2/PBK/MELK implicated in triple negative breast cancer subtype.[51–54]

Additionally, most (87.3%) of the tumours we evaluated immunohistochemically for the expression of PR biomarker were negative. The implications of this, is very similar to those earlier mentioned concerning ER biomarker expression, as they are both hormonal receptors responsible for the normal physiology of the breast. Hence, both receptors can be affected by the same pathogenetic pathway in the development of MBN (particularly IDC). However, a study has shown that the level of PR expression is directly proportional to the treatment outcome in terms of response to hormonal therapy in IDC.[25] Tragically, majority of our study cases were hormone receptor negative, giving them bad prognostic and predictive indices; since, the implicated molecular agent is yet unknown and hence it's impossible to constitute a molecular therapeutic target for them. These findings in our study of proportions of ER-positivity (11.3%)/PR-positivity (12.7%) however contrasted with the findings in some other studies with sample sizes ranging from 99 to 648 cases; wherein 70%/32.1% and 65.1%/54.7% proportions for ER-positivity/PR-positivity were respectively found.[38,40]

Importantly, on evaluating our study cases with HER2 biomarker, according to the scoring scale of 0, 1+, 2+ and 3+, we found that majority (36.5%) of them had score 0. Furthermore, on grouping these scoring scales into positive (Scores 3+) and negative (Scores 0 and 1+) status, we found that most (68.2%) of our study cases were HER2-negative. This finding implies that a reasonable proportion of IDCs in Uyo, follows a pathway other than amplification/over-expression of human epidermal growth factor 2 receptor gene/protein in its pathogenesis. These IDCs (breast cancers) will rather follow the pathogenetic pathways earlier mentioned for ER-negative expression in Uyo. This finding is however consistent with the findings in other studies; with 4% to 32.1% HER2-positivity.[38] This finding also brings to fore the difficulty in treating IDC patients who are HER2-negative; hence, it connotes a poor prognostic and predictive index since these patients cannot benefit from trastuzumab (Herceptin) therapy.

Notably, the immunohistochemical evaluation of the proliferative index of IDC in Uyo, using Ki-67 biomarker revealed that our study cases can be broadly categorized into the low proliferative index IDCs and high proliferative index IDCs using the St. Gallen recommended scoring scale of <20% or ≥20%. The low proliferation index IDCs (<20%) were found to constitute the majority in this scoring scale. This is consistent with the findings of some studies wherein low proliferation index IDCs constituted the majority.[28,37] Interestingly, this finding suggests that our study cases may be following the pathway of evasion of cell death or

apoptosis rather than rapid proliferation in its tumorigenesis. This is consistent with the findings in the landmark seminal works of Hanahan and Weinberg, who reviewed the hallmarks of cancer, amongst which is evasion of cell death or apoptosis.[55,56]

The immunohistochemical-based classification of invasive ductal carcinoma (IDC) of our study cases using combined ER/PR/HER2 biomarker expression profiles subclassified them into luminal A, luminal B, HER2-overexpression, triple negative and unclassified subgroups/subtypes of IDC. The relevant literature reviewed for this study generally showed these first four subtypes of IDC, however a study simultaneously carried out in Nigeria and Senegal showed that 28% of their study cases were of the unclassified subtype.[57] Furthermore, this study also reported that the unclassified subtype expressed vascular endothelial growth factor, B-cell lymphoma extra-large protein, and Cyclin E immunohistochemically, giving them a bad prognostic index. Notably, though, most of the studies reviewed, which conducted immunohistochemical-based classification of MBN, agreed with luminal A, luminal B, HER2-overexpression and triple negative breast cancer subtype categorization exclusively.[28,35,37–42]

The commonest immunohistochemical profile subtype of IDC in our study was the triple negative subtype, constituting 66.67% of the cases. This finding is in agreement with several studies done on IDC (breast cancer) patients of African descent or those of African Americans, wherein it was consistently found that triple negative subtype was the most common subtype of IDC/breast cancer amongst Africans as well as African Americans.[11,31,33,34,41,47,52,57–59] However, few African studies found contrary results wherein the ER-positive luminal A subtype was the commonest.[35,42] Thus, our findings is consistent with that of majority of relevant studies as regards commonality of triple negative breast cancer subtype amongst patients/individuals of African descent. This finding implies that most IDCs/breast cancers in Uyo will follow the ER-independent pathway as well as the NEK2/PBK/MELK pathways in its pathogenesis.[52] This finding is also consistent with this study's peak age group of women in premenopausal age and of African descent, as found in studies elsewhere.[31,33,47,57,59]

Notably, the triple negative subtype was followed by HER2-overexpression subtype, constituting 16.66% (on combination of its subgroups one and two). However, it is of note that none of the relevant literature reviewed followed this sequence of proportionality like ours in their immunohistochemical-based classification subtypes. This sequence of proportionality varied from study to study between third and fourth positions for HER-2-overexpression lesions. This implies that aside from the common pathogenetic pathway for IDC development in triple negative breast cancer subtype already mentioned, that the next pathogenetic pathway followed by IDC/breast cancer in Uyo could be that of HER2/neu. Notably, some of the relevant literature reviewed for this study showed that HER2/neu gene amplification and protein overexpression were implicated in the genomic instability underlying the development of HER2-overexpression subtype of IDC.[5,6] Consequently, HER2/neu serves as a molecular therapeutic target, hence patients with this subtype can be treated with trastuzumab (Herceptin) and the other newer agents, hence making it a good prognostic and predictive index, even though in the absence of the drug it becomes a negative prognostic and predictive factor.[22,24,25,43]

Importantly, ER-positive luminal B (5.56%) and ER-positive luminal A (5.56%), both constitute the hormone receptor subtypes in our study. These hormone receptor subtypes had the same proportion with the Unclassified subtype. This finding contrasted with what was found in other parts of the world and other African studies that had higher proportions of hormone receptor subtypes. However, in other parts of the world apart from Africa, the hormone receptor subtypes are usually ranked first.[38–40] Interestingly too, in some other African studies, by Huo et al and Galukande et al, the hormone receptor subtype usually ranked second or third in proportion.[41,57] However, in few African based studies, by Sayed et al, and Nwafor and Keshinro, the hormone receptor subtype, ranked first.[35,42] The import of this level of hormone receptor subtypes is that a low proportion of IDC in Uyo, go through the ER-dependent rather than the ER-independent pathways in its tumourigenesis. This finding also implies that even though hormone

receptor status is a good prognostic and predictive factor, only a small proportion of IDC patients in Uyo will benefit from targeted hormonal therapy with tamoxifen and the other newer agents.

Importantly furthermore, using a combined ER/PR/HER2/Ki-67 immunohistochemical biomarker expression profiles, our IDC study cases in Uyo were further classified into two proliferation subtypes, namely: the low (Ki-67 <20%) and the high (Ki-67 ≥20%) proliferation subtypes. Our study cases were thus categorized into low and high proliferation subtypes of ER-positive luminal A, ER-positive luminal B, HER2-overexpression, Triple negative, and Unclassified subtypes accordingly. It is of note that the commonest subtype in this classification scheme was Triple negative low proliferation subtype constituting 60.38% of the cases, followed by HER2-overexpression low proliferation subtype constituting 13.21% of the cases. Notably, amongst the high proliferation subtypes, the Triple negative high proliferation subtype (7.55%) was also the commonest, followed by the Unclassified high proliferation subtypes, which constituted 3.77%.

To our knowledge, our study is among the few studies that conducted immunohistochemical-based classification of IDC/breast cancer with incorporation of proliferative index biomarker (Ki-67) in an African population. All MBN/IDC studies incorporating Ki-67 evaluation were all connected with the 2009, 2013 and 2015 St Gallen International Breast Cancer Consensus Conferences.[22,24,27,28] The St Gallen Consensus have variously noted the importance of Ki-67 as an immunohistochemical biomarker for the estimation of tumour proliferative fraction in MBN. The St Gallen Consensus (especially the 2013 edition) modified the criteria for definition of luminal A and luminal B subtypes by using variability in Ki-67 proliferation index. The follow up studies showed that most of the tumours were of low proliferation type. Hence, depending on the tumour type as well as the clinical setting, Ki-67 can provide both prognostic and predictive indices. However, the only drawback with implementation of routine use of Ki-67 is that it lacks standardization to ascertain its clinical validity, analytical validity, and clinical utility. Importantly, the use of 20% landmark in our immunohistochemical-based classification of IDC was an adaption from the works done by the St Gallen Consensus, though they only used Ki-67 for the luminal IDC subtypes unlike in this study where we used it across board. The St Gallen Consensus has noted that a Ki-67 low proliferation index is a good prognostic parameter. Thus, in our study's context, with the commonest subtype being Triple negative low proliferation subtype, it implies that even though a triple negative status conveys a poor prognostic picture, the low proliferation status will probably checkmate the intrinsic aggressive status of the triple negative status. This finding, however, implies that the challenges faced with treatment of triple negative breast cancer subtype will be present in our environment, given its lacks of established biological therapeutic target(s) for targeted therapy (personalized medicine).[24] Patients with TNBC will most likely benefit from the newer agents for treatment of TNBC subtype such as poly (ADP-ribose) polymerase-1 (PARP) inhibitors, bicalutamide, alvespimycin, SRC inhibitor dasatinib, phosphoinositol-3 kinase (PI3K)/mTOR inhibitor NVP-BEZ235, CTLA4 (Ipilimumab) or PD-1 (nirolumab).[24,30,31]

Finally, the limitations of our study are intrinsic to its nature/design as a retrospective study as well as being set in a resource-poor environment. The FFPE tissue blocks included in this study were fixed, in the past, using 10% buffered formalin as against the ideal 10% neutral buffered formalin (NBF). The duration of fixation as well as the cold ischaemic time data for our study cases were not accessible. We could not carry out FISH studies on IDC having score 2+ on HER2 evaluation. Furthermore, our study did not ascertain the percentage of our study case, who were already on one form of breast cancer chemotherapy or the other prior to tissue biopsy for histopathological and immunohistochemical evaluations.

The next step for us is to carryout prospective (preferably multicentred-based) IDC immunohistochemical and molecular biology studies, in the background of controlled pre-analytical variables and reasonable research funding. These more advanced techniques/modalities will enable us to extensively explore and characterize the IDC in our environment, hence enabling us to carry out excellent molecular classification of African IDCs

of the breast as well as unravelling consequent novel therapeutic targets for effective personalised cancer care

Conclusion

In conclusion, the commonest patterns of immunohistochemical profile expression of IDC in our Tertiary Healthcare Facility in Uyo were found to be the Triple negative subgroup (66.67%), Triple Negative Subtype Low Proliferation (60.38%) and Triple Negative Subtype High Proliferation (7.55%) respectively, using different immunohistochemical-based classification schemes. These tragic findings call for further molecular characterization of the TNBC subtype, using advanced molecular diagnostic techniques, in search an effective novel personalized therapeutic target since it is presently still elusive.

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Conflict of Interest

None to declare.

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