ISSN No. 2394-3971

Original Research Article

IMMUNOHISTOCHEMICAL PROFILE OF BREAST CARCINOMA IN UNIVERSITY OF CALABAR TEACHING HOSPITAL, CALABAR, SOUTH-SOUTH NIGERIA

***Kenneth A Omoruyi[1], Martins A Nnoli[1], Godwin A Ebughe[1], Godstime I Irabor[2], Zulu C Okoligwe[3], Samuel O Ejike[4]**

1. Department of Pathology, University of Calabar Teaching Hospital, Calabar , Cross River State, Nigeria.

2. Department of Pathology, Saba University School of Medicine, Saba, Dutch Caribbean Netherlands

3. Department of Histopathology, National Hospital, Abuja, Nigeria.

4. Department of Pathology, Federal Teaching Hospital, Abakiliki, Nigeria

Submitted on: February 2018
Accepted on: February 2018
For Correspondence
Email ID:
omoruyiken@yahoo.com

Abstract

Background: Breast cancer is the commonest cancer in the female. Immunohistochemistry of breast cancer hormone receptor (ER and PR) and Her2 are very useful tools for assessing the tumor cells behavior and making choice of chemotherapy.

Aims and Objective: The study aimed to describe the immunohistochemical profiles of breast carcinoma at University of Calabar Teaching Hospital (UCTH), Calabar.

Method: This is a descriptive study of immunohistochemistry of breast carcinoma in UCTH using archival paraffin-embedded blocks of breast cancer tissue. The records of cases diagnosed as breast cancer in a five year period (2010-2014) in the department of Histopathology of UCTH are stained immunohistochemically for Oestrogen receptor(ER), Progesterone receptor(PR) and Human epidermal growth factor receptor 2(Her2). The demographic variables and receptor status were collated and analyzed. The status is related to the histopathologic characteristics of tumor size, histologic grade, and histologic subtypes. The findings were presented in tables and charts and statistical significance of variables tested.

Result: One hundred and forty-seven (147) cases of breast cancer samples were analyzed. The mean age at diagnosis was 46.31+/-12.75 years old. The age ranged from 21-80 years old, the modal and median ages were 40 and 45 years respectively. Sixty-four percent (64%) were estrogen receptor positive. Progesterone receptor positive was 40.14% and Her2 positive was 21.09%.

Conclusion: The majority of the breast cancer is estrogen receptor positive but progesterone receptor and Her2 receptor negative.

Keywords: Immunohistochemistry, estrogen receptor(ER), progesterone receptor (PR), human epidermal receptor 2(HER2)

Introduction

Worldwide, breast cancer is the most common cancer for females and second most common cancer overall with more than 1,676,000 new cases diagnosed in 2012 (25% of female cases and 12% of the total).1 Breast cancer is a heterogeneous disease that shows a marked clinical and morphological diversity as well as variability in prognosis and response to therapy.

Globally, breast cancer biomarkers are increasingly becoming an important predictive and prognostic indicator that determines the mode of therapy and consequently prognosis and survival of breast cancer patient. The immunohistochemical profiles of breast cancer ensure that the receptor status of the tumor cells are elucidated and this gives a prediction of the biological behavior of cancer cell which aids are taking management decisions in breast cancer treatment. These hormone receptors (estrogen and progesterone), and other breast cancer tumor marker like HER2 which are determined by immunohistochemistry is also used as surrogates in deciding the molecular subtypes of breast carcinoma, which, however, is better determined by use the of Genome-wide microarray-based gene expression profiling studies. The prognostic and predictive indicator of clinical outcome of breast cancer is achieved by a clear understanding of the immunological profiles of the tumour cells. Women with ER+/PR+ tumour has a better survival statistics compared to tumours with ER+/PR-, ER-/PR+ or ER-/PR- which have higher risks of mortality and this is independent of other demographic and clinical tumor characteristics.2

The prevalence of hormone-receptor positive breast cancer varies from region to region as shown by these various researches: a combined study in Nigeria and Senegal using 6 regions showed ER+ tumours was 24%, PR+ 20% and HER2 17%,3 in Kumasi, Ghana ER+ (47.06%), PR+ (13.24%), HER2+ve (20.37%);4 in Eritrea in the horn of Africa, 40% was ER-,5 a comparative study of Norway and Ghana showed 85% and 76% ER+ breast cancer respectively;6 ER+ 33%, PR+ 18.3% in Tanzania;7 24% ER+, 34% ER-/PR+, 10% ER-v/PR+, 66% ER- and PR-, 26.5% overexpress HER2 in Kenya8 and 64% as ER/PR+ in a study in Saudi Arabia.9

A similar variation in prevalence of biomarkers (ER,PR,HER2) is noticed in works done in Nigeria as buttressed by the following studies: multiple studies in Ibadan showed ER+ 65.1%, PR+ 54.7% and HER2+ve of 79.9%10, ER+ 65%, PR+ 60%, HER2 91%, 17% was triple negative11 and 22.8 - 25.0% as ER+12; ER+ 7%, PR+ 2.1%, HER2+ 3.8% and 87% triple-negative in Lagos13; 25% ER+, 27.8% PR+ and 25% HER2+ in Jos14; in Ilorin ER+ 27%, PR+ 16% and HER2+ 30%15; in two cohort studies in Nigeria and Senegal the hormone receptor frequencies were ER+(24% and 28%), PR+(20% and 17%), Her2(17% and 16%)3 respectively. A study in Benin show HER2 positivity of 10.8%.16 Titiloye et al in a Nigerian cohort study reported ER+ of 38%, PR+ 21%, HER2 over-expressing 4%, 53% was triple negative,17 in a comparative study between British and Nigerian women ER+ was 58.8% and 26.5%, PR+ 52.9% and 29.4%, and HER2 44.1% and 23.5% respectively,18

Materials and Method

Study design and material:

This was an archival cross-sectional study that determined the immunohistochemical profiles of breast carcinoma in UCTH, Calabar. The archival material was paraffin-embedded tissue blocks of breast carcinoma diagnosed in the department of histopathology, UCTH, Calabar. Basic information like age at diagnosis, year of diagnosis, sex and histopathological characteristics of the breast cancer were collated from the medical records.

Tissue ribbons made from paraffin-embedded blocks were mounted, deparaffinized and rehydrated. Antigen retrieval was done using heat in citrate buffer. Blocking of endogenous peroxidase activity is done after which the primary antibody is applied and incubated at room temperature for 1hour. The excess antibody is rinsed off with phosphate buffered saline and the section is then covered with the secondary antibody, incubated for 1hour and rinsed with a gentle stream of streptavidin-horseradish peroxidase reagent diluted with phosphate buffer saline. This is incubated for 30 minutes at room temperature and then the section is immersed in freshly prepared chromogen/substrate reagent solution diaminobenzidine/hydrogen peroxide for 2-7 minutes. The sections are mounted and interpreted using a control. The Allred grading score is used for ER and PR while HER2 is assessed by observing the completeness of the membrane staining.

Sample size:

The sample size of the study was comprised of all the histological specimen that was diagnosed with breast carcinoma seen in UCTH in the period 1st January 2010 to 31st December 2014.

Data analysis:

This was done using the current version of the US Centre for Disease Control (CDC) statistical software Epi-info 7 with descriptive and inferential statistics. The mean age, age range, and sex distribution were determined, the frequency (percentage) distribution of the biomarker(ER, PR and HER2) of breast carcinoma was determined and this immunological profile was correlated with histopathologic characteristics. Frequency tables, graphs, and charts were used to display the findings.

Criteria for selection:

Blocks of paraffin-embedded tissue specimen diagnosed with breast carcinoma during the study period of 1st January 2010 to 31st December 2014 were included in this study.

Exclusion criteria:

All the cases that the tissue blocks could not be gotten from the departmental store and all the cases that the immunohistochemistry result came out as null were excluded from the study.

Ethical consideration:

Ethical clearance for this study was obtained from the health research ethics committee of the University of Calabar Teaching Hospital, Calabar, Cross River state, Nigeria.

Conflict of interest: The author has no conflict of interest.

Results:

General findings

For five years study period of 1st January 2010 to 31st December 2014, nine thousand six hundred and forty-seven histology samples were received in the Department of Histopathology, University of Calabar Teaching Hospital. One thousand 0ne hundred and fifty-four of these samples were breast tissue and two hundred and sixty-nine representing 23.3% were diagnosed with breast cancer. A total of one hundred and

forty-seven (147) met the inclusion criteria and was included in the study analysis.

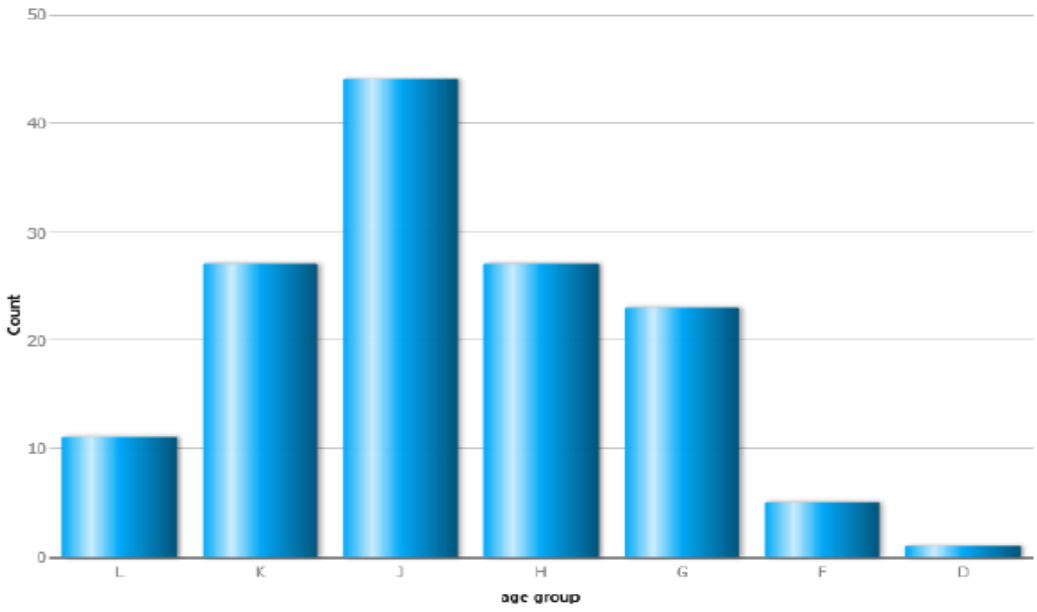
Socio-demographic characteristics of subjects

Table 1: The age distribution of subjects

Age(years)	Frequency	Percentage
20-29	12	8.16
30-39	30	20.41
40-49	47	31.97
50-59	29	19.73
60-69	23	15.65
70-79	5	3.40
80-89	1	0.68
Total	147	100%

The mean of patient age at diagnosis is 46.31 years (SD+/-12.75) old. The age range is from 21-80 years old. The modal and median ages are 40 years and 45 years respectively. The patients' age is stratified into three major groups. These are an age less than 40 years old that has 42 cases (28.57%); age 40 to 55 years old that has 73 cases (49.66%) and age more than 55 years old that has 32 cases (21.77%). The number that is premenopausal(less than 55 years) is 115 cases (78.23%). The modal age group is 40-49 years and has 47 cases (31.97%).

Figure 1: Bar chart showing age distribution of breast cancer in subjects.



Key: L=20-29yrs; K=30-39yrs; J=40-49yrs, H=50-59yrs; G=60-69yrs, F=70-79yrs, D=80-89yrs

Table 2: The proportion of estrogen receptor status of breast cancer in UCTH

Receptor status	Frequency	Percentage
Positive	94	63.95
Negative	53	36.05
Total	147	100

Table 2 shows that 94 cases (63.95%) are estrogen receptor positive and 53 cases (36.05%) estrogen receptor negative.

Figure 2: Pie chart showing the proportion of estrogen receptor status.

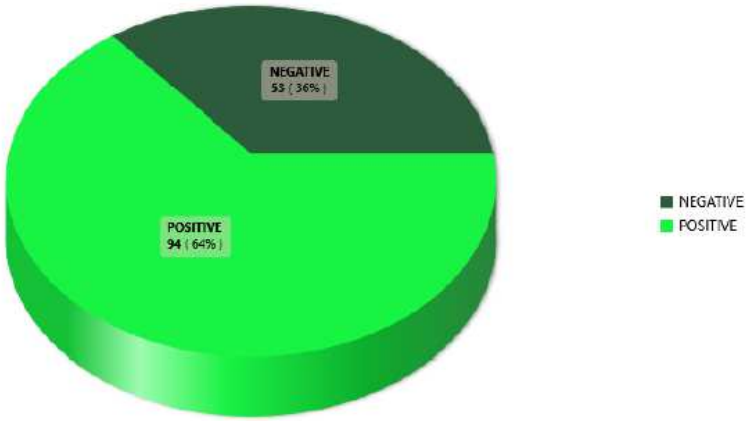


Table 3: The proportion of progesterone receptor status of breast cancer in UCTH

Receptor status	Frequency	Percentage
Positive	59	40.14
Negative	88	59.86
Total	147	100

Table 3 shows that 59 cases (40.14%) are progesterone receptor positive and 88 cases (59.86%) are progesterone receptor negative.

Figure 3: Pie chart showing proportion of progesterone receptor status

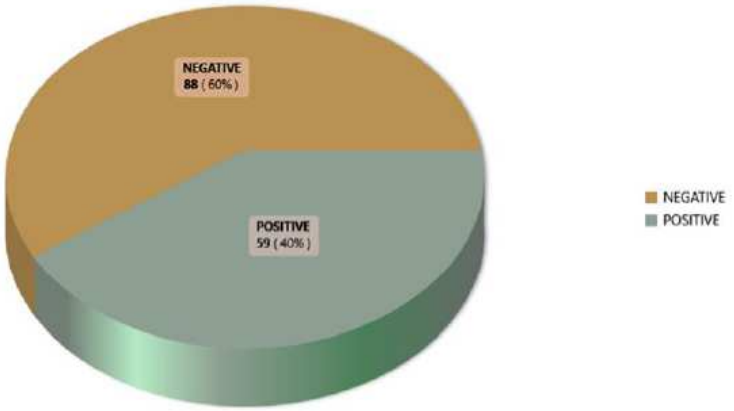


Table 4: The proportion of Human epidermal growth factor receptor2 status of breast cancer in UCTH

Receptor status	Frequency	Percentage
Positive	31	21.09
Negative	116	78.91
Total	147	100

Table 4 shows that 31 cases (21.09%) are Her2 receptor positive and 116 cases (78.91%) are Her2 receptor negative.

Figure 4: Pie chart showing proportion of Her2 receptor status

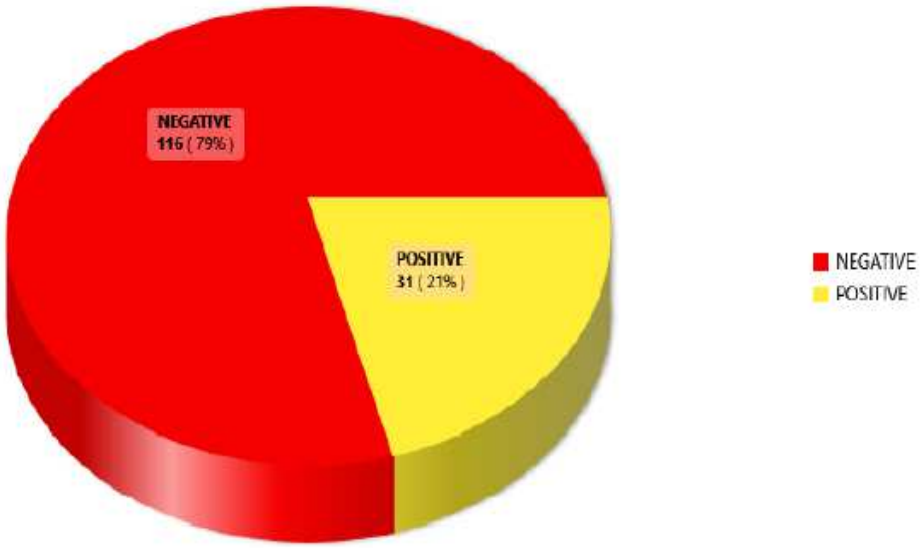


Table 5: Relationship between histologic type and receptor status.

Histologic type	Hormonal receptor status							
			oestrogen receptor		Progesterone receptor		Her2 receptor	
	Frequency	Percent	Positive	Negative	positive	negative	Positive	Negative
Ductal	146	99.32	93	53	58	88	31	115
Lobular	1	0.68	1	0	1	0	0	1
Total	147	100	94	53	59	88	31	116

Table 5 shows a majority of breast carcinoma are estrogen receptor-positive but progesterone and Her2 receptor-negative. There is no association between histological type of breast cancer and hormone receptor status.

Table 6: Relationship between histologic subtype and receptor status.

Histologic type	Hormonal receptor status							
			oestrogen receptor		Progesterone receptor		Her2 receptor	
	Frequency	percent	Positive	negative	Positive	Negative	positive	Negative
Invasive ductal NOS	136	92.52	88	48	58	88	31	115
DCIS	6	4.08	5	1	1	0	0	1
Special type	5	3.40	1	4	1	4	1	4
Total	147	100	94	53	59	88	31	116

Table 6 shows that the majority of the ductal type are generally estrogen receptor positive and progesterone receptor and Her2 receptor negative. The lobular type is estrogen and progesterone receptors-positive. The special type is mainly estrogen receptor, progesterone receptor, and Her2 receptor negative. The ductal carcinoma in-situ is majorly estrogen receptor positive.

Table 7: Relationship between age group distribution and receptors status.

Age group (years)			Hormone receptor status					
			Oestrogen Receptor		Progesterone Receptor		HER2 receptor	
	Frequency	Percent	Positive	Negative	Positive	Negative	positive	negative
20-39	42	28.57	8	34	4	38	1	41
40-55	73	49.66	55	18	35	38	24	49
56-89	32	21.77	31	1	20	12	6	26
Total	147	100	94	53	59	88	31	116

The table 7 shows that the young age (less than 40 years) is 42 cases (28.57%) and the cancer are majorly receptor-negative. The age group 40-55 years is 73 cases (49.66%) and the cancers are majorly receptor-positive. The age group 56-89 years (more than 55years) is 32 cases (21.77%) and the cancers are estrogen receptor and progesterone receptor positive but Her2 receptor negative.

There is an association between age group less than 40 years and estrogen receptor(ER) ($p<0.001$), progesterone (PR) ($p<0.001$) and Her2 receptor ($p<0.001$) receptor. There is also an association between age group 40-55years and ER ($p=0.006$) and Her2 ($p=0.001$). There is an association between age greater than 55 years and ER (<0.001) and PR ($=0.004$). Statistical analysis showed highly significant differences between breast cancer hormone receptor status and age group.

Table 8: Relationship between tumor size and receptor status

Tumour size			Hormone receptor status					
			Oestrogen Receptor		Progesterone Receptor		HER2 receptor	
	frequency	percent	Positive	Negative	Positive	negative	positive	Negative
1.5-1.9	6	4.08	4	2	4	2	1	5
2-5	111	75.51	74	37	43	68	23	88
5.1-12.5	30	20.41	16	14	12	18	7	23
Total	147	100	94	53	59	88	31	116

The range of tumor size is from 1.6cm to 12cm and a mean of 4.04cm with a standard deviation of 1.98. The modal size of a tumour is 3.2cm and the median size is 3.4cm. Six cases (4.08%) is tumour size of 1.5-1.9cm (less than 2cm); 2-5cm is 111 cases (75.51%) and 5.1-12.5cm (greater than 5cm) is 30 cases (20.41%). This is shown in table 8.

Table 9: Relationship between histologic grade and receptor status.

Histologic grade			Hormone receptor status					
			Oestrogen Receptor		Progesterone Receptor		HER2 receptor	
	frequency	percent	Positive	Negative	positive	Negative	positive	negative
1	11	7.86	7	4	7	4	2	9
2	68	48.57	48	20	45	23	17	51
3	61	43.57	33	28	20	41	12	49
Total	140	100	88	52	72	68	31	116

Table 9 shows that grade 1 (well differentiated) is 11 cases (7.86%); grade 2 (moderately differentiated) is 68 cases (48.57%) and grade 3 (poorly differentiated) is 61 cases (43.57%). Majority of grades 1 and 2 are estrogen and progesterone receptors positive while grade 3 are generally progesterone and Her2 receptor negative.

There is an association between grade 2 tumors and ER (p=0.018) and PR (p=0.0004). There is also an association between grade 3 tumors and PR (p=0.009). The analysis also showed a significant difference between estrogen receptor and grade 2 tumors and between progesterone receptor and grades 2 and 3 tumors.

Discussion:

Breast cancer is the commonest cancer in a female with an increasing incidence rate worldwide1,19 and thus has become a cancer of serious public health concern. The St. Gallen 13th and 14th International Breast Conference took into cognizance this high incidence and thus came out with guidelines for the diagnosis and treatment options for early breast cancer and lymph node-positive breast cancer respectively.20,21 These management guidelines took into cognizance the biology of tumor cells as detected by DNA Micro-arrays that assess the intrinsic gene expression profile of the breast carcinoma. The conference also recommended tailored therapy towards precision treatment via individualized treatment decision. Breast cancer is a heterogeneous disease that has marked intra and inter tumor variations. It has diverse morphologic and biologic features, variable clinical outcome and response to therapeutic options. This heterogeneity has been a major drawback in coming out with a clear-cut therapeutic regimen for each molecular subtype of

breast cancer. Despite this heterogeneity, the management, prognostication and prediction of response to therapy of breast cancer is based chiefly on the tumour cell biology which involves the assessment of hormonal receptor, epidermal growth factor receptor, Her 2 receptor, cytokeratin5/6 and proliferative marker ki-67 of the breast carcinoma. The receptors are routinely tested for using immunohistochemistry as a surrogate for the intrinsic biologic characteristics. The hormone receptors are estrogen receptor(ER), progesterone receptor (PR) and human epidermal growth factor receptor (Her2). These biomarkers predict the molecular subtypes and possible choice and outcome of therapy.

The age at diagnosis of breast cancer is a very important prognostic factor.22 The age range of breast cancer in the study is 21-80 years. This is in agreement with many other studies that the age also falls between the third and ninth decade of life.23,18,24,25 However, this is at variance with some reports that the lower limit is the second decade of life26,27,28 and those that the lower limit is the fourth decade.29 The age at diagnosis reported in many studies in Africa and Asia countries are about a decade lower than values in Europe and America.30,31-3,18

The mean age of this study is 46+/-12 years. This is in tandem with the majority of works in other parts of the world,3,4,9,27 and also in Nigeria12,23,34-8,39,40,17-8 in which the mean age is in the fifth decade of life. The median age of this study is 45 years and peak age group is 40-49 years which is similar to other studies.4,41,17,24,25,28,42 The mean age and peak age group both fall in the fifth decade of life. The ages are the reproductive stage of the woman's life. During this period the breast is exposed to the effects of the estrogen and progesterone hormones that promote its growth and development, usually any actively proliferating group of cells can be a fertile ground for cancer development. This may partly explain why breast cancer is commoner in this age group in Africans and Asians but may not explain why the age of Americans and Europeans are a decade higher. The life expectancy is lower in African and poor Asian countries (42-48 years), it means that their population in most cases will have a large number of the inhabitants being young, this may also explain why younger women will present with breast cancer as opposed to US America and European countries where the life expectancy is higher and the proportion of older women will be higher. This may partly explain the difference in peak age group between this study, other African and Asian studies and the western world. The strong association of age with breast cancer was also statistically confirmed in this study, similar to other reports associating age at presentation to an increased risk factor for breast cancer.22 This association remained even after correcting with other factors like tumor size and histologic grade that also affects breast cancer. Immunohistochemical analysis of the hormonal receptor status showed that 63.95% are estrogen receptor positive while 36.05% was estrogen receptor negative. Progesterone receptor positive breast cancer was 40.14% and 59.86% was progesterone receptor negative. Her2 receptor positive was 21.09% while 78.91% was Her2 negative. This high proportion of ER (60%) positive and low Her2 (22%) positive were similarly reported in Carolina Breast cancer study43 but PR (56%) positive was equally high which is different from this study. These values are at variance with various studies that have been done on hormone receptor (ER and PR) and Human epidermal growth receptor (Her2)3-9 around the world. Similarly, a wide variation in the percentage of each biomarker was reported in Nigerian studies. 3, 10, 11, 13, 14, 15, 16,

17, 18,24 The reasons for this variation may not be unconnected with the pre-analytical errors like delay in fixation, using the wrong fixative and prolonged fixation of more than 72 hours of samples that will require immunohistochemistry. Also, the reviewed results are from studies that are either retrospective or prospective research works. The pre-analytical error that may have resulted in variation in results obtained will more likely be associated with retrospective results, in which the sample may be fixed in 10% neutral buffered formalin for more than 72 hours thereby reducing the chances of unmasking and identifying the hormone marker or Human epidermal receptor 2(Her2) during antigen retrieval process of analytic stage. Some of the studies also had their results for Her2 confirmed with Fluorescence In-Situ Hybridization (FISH) and quantitative real-time polymerase chain reaction (qRT-PCR) which was not the case in this study.

The majority of the invasive ductal carcinoma in this study had high ER-positivity but a different finding was reported by Javaria et al that documented 91.3% were ER/PR negative44 but invasive lobular carcinoma had high ER/PR positivity similar to what this study found. The difference in hormone receptor and Her2 status may be due to the different methods used in handling the tissue at the stage of pre-analytical, analytical and post-analytical of immunohistochemistry and the inconsistencies observed in these stages of immunohistochemistry between and within the same laboratories can explain the variations documented by the various studies in identifying ER/PR and Her2 status. Pre-analytic errors may result from delayed placement of sample in fixative, use of wrong fixative and prolonged fixation time more than 72 hours. Analytic errors may include antibody selection, antibody validation, and antigen retrieval method used and post-analytic errors may be due to wrong positive and negative controls, interpretation and reporting of the result.

The age-specific comparison showed that majority of a patient less than 55 years had estrogen receptor-positive tumors. This is different from a study done in Kuwait45 in which majority of a patient less than 55 years had a low proportion of estrogen-positive tumors. The study report is also at variance with work in Pakistan that documented a high percentage of ER/PR negative tumor in women greater than 45 years.44

The study showed that most of the breast cancer found in the very young patients (less than 40 years) were progesterone receptor-negative (90.48%) and Her2 receptor-negative (97.62%). This is at variance with a study done in Riyadh that had a low percentage of progesterone receptor-negative (39.4%) and Her2 (44%)receptor-negative in women less than 45 years.44 The percentage of progesterone receptor-negative (50%) was just half in the young, although less than 30 years was used,45 but that of Her2 receptor-negative was (25%). The breast cancers of the age group 40-55 years equally have a low proportion of progesterone receptor-positive and Her2 receptor-positive in this research work. This is at variance with a report in Kuwait that had a very high proportion of Her2 (78.8%)45 receptor-positive for breast cancer in patient 30-55 years.

The majority of the tumors in this study that had tumor size of 2-5cm were progesterone receptor-negative (61.26%) and Her2 receptor-negative (79.28%). This, however, is at variance with a report from Kuwait in which Her2 receptor-positivity (84.3%) and progesterone receptor (67.4%) are very high45 and the estrogen receptor-negativity was lower (19.1%). Tumour size greater than 5cm also have a very high percentage of progesterone receptor negativity (60%)

and Her2 receptor negativity (76.67%) in this study and this is in tandem with the Kuwait study for progesterone receptor (76.9%) but at variance with the Her2 receptor (7.7%) negative and 92.3% positive.45 These variations observed with receptor status and tumour sizes may also be as a result of pre-analytic fixation, analytic antigen retrieval or post analytic interpretation and reporting of results of immunohistochemistry for hormone receptors and for the Her2 receptor,
and further confirmation of Her2 with Fluorescence in-situ Hybridization (FISH) that was not done for this study would have affected the proportions of positive and negative results.

The study showed that majority of the cancers were grades 2 (48.57%) and 3 (43.57%) tumors, grade 2 was the highest and grade 1 (7.86%) was the lowest in the proportion of the breast cancers. Comparative analysis of receptor status and tumor grade revealed that most grade 1 tumors were estrogen receptor-positive (63.64%) but progesterone receptor-negative (63.64%) and Her 2 receptor negative (81.82%). The same high estrogen receptor positivity and low progesterone and Her2 receptor-negativity were observed in grades 2 and 3. Javaria et al also reported a high percentage of a low-grade tumour in ER/PR positive tumour and high-grade tumour in ER/PR negative tumours44 but at variance with Saleh and Abdeen report in Kuwait that had high progesterone receptor positive for grade 1, high Her 2 receptor positivity for grades 2 and 3, high oestrogen receptor negative for grades 2 and 3 and a marginal high progesterone receptor positive for grade 3.45Irianiwati et al also reported grade 2 as the commonest tumour in its work on breast cancer in Indonesian women similar to although the value was less than this study finding- 40%.29

Saleh and Abdeen also reported a right side preponderance of 53.61%, the left side is 42.17% and bilateral is 4.22%.45 Comparative analysis with receptor status showed a high estrogen receptor positivity, low progesterone receptor and Her2 receptor positivity for both the right and left-sided tumors in this study. This is different from the high positivity for estrogen and Her2 receptors and low progesterone receptor on the right side and high progesterone, estrogen and Her2 receptors on the left side45 reported in Kuwait.

Conclusion:

Breast carcinoma with positive estrogen and progesterone receptors are frequently seen in post-menopausal women. Breast carcinoma of the invasive ductal histologic types is more likely to be estrogen receptor and progesterone receptor positive. Hormonal receptor (estrogen and progesterone) positivity is equally common in carcinoma diagnosed in patients with grade 2 and grade 3 tumors.

References:

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC cancer Base No.11[internet] Lyon, France: International Agency for Research on Cancer, 2013.
2. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumour characteristics and prognosis: a prospective cohort of breast cancer patients. Breast cancer res. 2007, 9(1):R6.
3. Huo D, Ikpatt F, Khramtsov A, Dangou JM, Nanda R, Dignam J, et al. Population differences in breast cancer: Survey in indigenous African women reveals over representation of triple-negative breast cancer. J Clin Oncol 2009; 27:4515-21.

4. Ohehe-Yeboah M, Adjei E. Breast cancer in Kumasi, Ghana. Ghana Med J 2012; 46(1):8-13.
5. Tesfamariam A and Roy I. Molecular Biology of breast cancer in the Horn of Africa: Case series-A pilot study of breast cancer from Eritrea. ISRN Pathology 2013; Article ID787495:01-07.
6. Adjei EK, Owusu-Afriye O, Awuah B, Stalsberg H. Hormone receptor and Her 2 expression in breast cancer in sub-sahara Africa. A comparative study of biopsies from Ghana and Norway. Breast J 2014; 20(3):308-11.
7. Mbonde MP, Amir H, Akslen LA, Kitinya JN. Expression of estrogen and progesterone receptors, ki67, p53 and BCL-2 proteins, CathepsinD, Urokinase plasminogen activator and Urokinase plasminogen activator receptor in carcinomas of the female breast in an Africa population. East Afri Med J 2001; 78(7):360-65.
8. Bird PA, Hill AG, Houssami N. Poor hormonal receptor expression in east Africa breast cancer: Evidence of a biologically different disease? Ann Surg Oncol 2008; 15(7):1983-88.
9. Satti MB. Oestrogen receptor/progesterone receptor and human epidermal growth factor receptor 2 status in breast cancer: A 9 years study at Princess Noorah Oncology Centre, Saudi Arabia. Histopathology 2011; 59:537-42.
10. Adebamowo CA, Famooto A, Ogundiran TO, Aniagwu T, Nkwodimmah C, Akang EE. Immunohistochemical and molecular subtypes of breast cancer in Nigeria. Breast Cancer Res. And Treat 2008; 110(1):183-88.
11. Adebamowo CA, Ogundiran T, Akang E. Epidemiology of Triple negative breast cancer in Nigeria women. J ClinOncol 2006; 24:10504.
12. Ogundiran TO, Ezeome ER. Epidemiology, clinical presentation, and management of advance Breast Cancer in Nigeria. In the proceeding of Society of Oncology and Cancer Research of Nigeria (SOCRON) 2008 conference, pp 1-42.
13. Makanjuola SBL, Ayodele SD, Obafunwa JO Oludara MA, Popoola AO. Breast cancer receptor status assessment and clinicopathological association in Nigerian women: A retrospective analysis. J Cancer Res Ther 2014; 2:122-27.
14. Gukas ID, Jennings BA, Mandong BM, Igun GO, Girling AC, Manasseh AN, et al. Clinicopathological features and molecular markers of breast cancer in Jos, Nigeria. West Afri J Med 2005; 24(3):209-13.
15. Adeniji KA, Huo D, Khramtsov A, Zhang C Olopade OI. Molecular profiles of breast cancer in Ilorin, Nigeria. 2010 ASCO Annual meeting. J ClinOncol 2010; 28(15s):abstr1602.
16. Ugiagbe EE, Olu-Eddo AN, Obaseki DE. Immunohistochemical detection of HER2/neu over-expression in breast carcinoma in Nigeria: a 5-year retrospective study. Niger J Clin Pract 2011; 14(3):332-37.
17. Titiloye NA, Omoniyi-Esan GO, Adisa AO, Komolafe AO, Afolabi OT, Adelusola KA. Breast cancer in Nigeria Cohort: Histopathology, Immunohistochemical profile, and Survival. Postgraduate Medical Journal of Ghana 2013; 2 (2):83-87.
18. Gukas ID, Girling AC, Mandong BM, Prime W, Jennings BA, Leinster SJ. A comparison of Clinicopathological Features and Molecular Markers in British and Nigerian Women with Breast

Cancer. Clin Med Oncol. 2008; 2:347-51.
19. Stewart BW, Kleihues P. World Cancer Report 2003. Published by International Agency for Research on Cancer (IARC) press, Lyon 2003. ISBN9283204115.
20. Untch M, Gerber B, Harbeck N, Jackisch C, Marschner N, Mobus V, et al. 13th St Gallen International Breast Cancer Conference 2013: Primary Therapy of Early breast cancer. Evidence, controversies, consensus- the opinion of a German Team of Experts (Zurich 2013). Breast care 2013; 8(3):221-29.
21. Jackisch C, Harbeck N, Huober J, Von Minckwitz G, Gerber B, Kreipe HH, et al. 14th St Gallen International Breast Cancer Conference 2013: Primary Therapy of Early Breast Cancer: Evidence, controversies, consensus-Opinion Expressed by German Team of Experts. Breast Care 2015; 10(3):211-19.
22. Rauh C, Gass P, Heusinger K, Haeberle L, Jud SM, Hein A, et al. Association of Molecular subtypes with breast cancer risk factors: a case-only analysis. Eur J Cancer Prev, 2015; 24(6):484-90.
23. Ebughe GA, Ekanem IA, Omoronyin OE, Nnoli MA, Nwagbara VJ, Udosen JE, et al. Age-specific incidence of breast cancer in Calabar, Nigeria. International Journal of Tropical Disease and Health. 2016, 16(4):1-12.
24. Nwafor CC, Keshinro SO. Pattern of hormones receptor and Human epidermal growth factor 2 status in sub-sahara breast cancer cases: Private Practice experience. Nigerian Journal of Clinical Practice 2015; 18(4):553-58.
25. Forae GD, Nwachokor FN, Igbe AP. Histopathologic profile of breast cancer in an African population. Ann Med Health Sci Res 2014; 4(3):369-73.
26. Ihekwaba FN. Breast Cancer in Nigerian Women. Br J surg 1992; 79(8):771-75.
27. Galukande M, Wabinga H, Mirembe F, Karamagi C, Asea A. Molecular breast cancer subtypes prevalence in an indigenous subsahara African population. The Pan African Medical Journal 2014; 17:249.
28. Anyanwu SNC. Temporal Trends in breast cancer presentation in the third world. J Exp Clin Cancer Res. 2008; 27(1)17.
29. Irianiwati W, Dwianingsih EK, Triningsih E, Utoro T, Soeripto. Clinicopathologic features of Indonesian breast cancer with different Molecular subtypes. Asian Pac J Cancer Prev. 2014; 15(15): 6109-13.
30. Abdulrahman GO Jr, Rahman GA. Epidemiology of breast cancer in Europe and Africa. J Cancer Epidemiol 2012; Article ID 915610:01-05.
31. Agarwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of Breast Cancer in Asian Women. World J Surg 2007; 31 (5):1031-40.
32. Fregene A, Newman LA. Breast cancer in Sub-Saharan Africa: how does it relate to breast cancer in African–American women. Cancer 2005; 103(8):1540-50.
33. Anim JT. Breast Cancer in Sub-Saharan African Women. Afr J Med Sci 1993; 22(1):5-10.
34. Adebamowo CA, Ajayi OO. Breast Cancer in Nigeria. West Afr J Med 2000; 19(3):179-91.
35. Adebamowo CA, Adekunle OO. Case controlled study of the epidemiological risk factor for breast cancer in Nigeria. Br J Surg 1999; 86(5):665-68.
36. Anyanwu SN. Breast cancer in eastern Nigeria: a ten year review. West Afr J Med 2000; 19(2):120-25.
37. Ikpatt OF, Kuopio T, Ndoma-Egba R, Collan Y. Breast Cancer in Nigeria and

Finland: epidemiological, clinical and histological comparison. Anticancer Res 2002; 22(5):3005-12.
38. Ebughe GA, Ugare GU, Nnoli MA, Bassey IA, Nwagbara VJ, Udosen JE, et al. Histological Type and Tumour Grade in Nigerian breast cancer: relationship to menarche, family history of breast cancer, parity, age at first birth and age at menopause. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 2013; 7(5):58-63.
39. Adisa CA, Eleweke N, Alfred AA, Campbell MJ, Sharma R, Nseyo O, et al. Biology of breast cancer in Nigeria women: a pilot study. Ann Afr Med 2012; 11(3):169-75.
40. Dauda AM, Misauno MA, Ojo EO. Histopathological Types of Breast Cancer in Gombe, North Eastern Nigeria: a seven year review. Afr J Reprod. Health. 2011; 15(1):107-09.
41. Nggada HA, Yawe KD, AbdulazeezJ, Khalil MA. Breast cancer burden in Maiduguri, North Eastern Nigeria. Breast J 2008; 14(3):284-86.
42. Udo-Affah GU, Eru EM, Kalu C, Uruakpa KC, Inyang JI. The prevalence of breast cancer in University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar (GHC) from 2006 to 2010. IOSR Journal of Nursing and Health Science (IOSR-JNHS) 2014; 3(5):08-10.
43. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes and survival in the Carolina Breast Cancer study. JAMA. 2006; 295(21):2492-502.
44. Javaria I, Mohamma A, Alam AS, Ghadah MA, Bandar NA. Hormone receptor status of breast cancer in different age groups, lymph node status, histologic type and tumour grade, an experience at King Fahad Medical City, Riyadh. Pak J Surg 2014; 30(4):296-300.
45. Saleh F and Abdeen S. Pathobiological features of breast tumours in the state of Kuwait: a comprehensive analysis. Journal of carcinogenesis 2007; 6:12:01-12.