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Prevalence of Molecular Subtypes of Breast Carcinoma in University of Calabar Teaching Hospital using Immunohistochemistry as surrogates for Intrinsic DNA gene characteristics.

Kenneth A.Omoruyi[1], Martins A. Nnoli[1], Godwin A. Ebughe[1], Godstime I. Irabor[2], Edoise M. Isiwele[3]

[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 Surgery, University of Calabar Teaching Hospital, Calabar , Cross River State, Nigeria.*

***Abstract*:**
Background:*Breast cancer is a heterogeneous disease that shows inter and intra-lesion variation. The classification into molecular subtypes has made chemotherapeutic management of breast cancer easier and patient-specific with excellent outcomes. Immunohistochemistry are used as surrogates for intrinsic DNA gene in most resource poor countries.*
Aimsand objectives: *This study aimed to describe prevalence of molecular subtypes of breast carcinoma using immunohistochemistry as surrogates for characteristics seen with intrinsic DNA gene.*
Methodology:*The immunohistochemistry for oestrogen receptor, (ER), progesterone receptor (PR) and Human epidermal receptor2 (HER2) of the breast carcinoma diagnosed in the university of Calabar Teaching Hospital in a five year period from 1st January 2010 to 31 December 2014 were collated. An algorithm is developed to determine the molecular subtypes. Luminal A (ER+/PR-, ER-/PR+. HER2-), Luminal B (ER+/PR-, ER-/PR+ and HER2+), Basal-like (ER-, PR-,HER2-) and Her 2 Type (HER2+, ER-, PR-)). The prevalence of each subtype is determined and each tumour characteristics in terms of age of subject, tumour size, histologic grade and histologic type is described. The findings are presented in charts and tables and statistical significance determined.*
Results: *Luminal A is (52.38%), Triple negative (26.53%), Luminal B (12.93%) and Her2 positive (8.16%). The Luminal molecular subtype accounted for 65.31%. All the males had Luminal A subtype.*
Conclusion: *The most common molecular subtype is Luminal A.*
Keywords: *Basal-like. Her 2 type, Immunohistochemistry, Luminal A&B, Molecular subtypes.*

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I Introduction

The implications of molecular classification in therapeutic era of breast cancer was generally accepted in the 2011[1] and 2013[2] St Gallen International Breast Cancer Conference. The molecular classification has proved to be more useful than histopathological classification as a predictive factor for different treatment because it provides an objective and reproducible assessment of prognostic features of breast carcinoma. A classification system based on gene expression analysis was proposed by Perou et al, [3] in this system of classification, breast cancer consist of four major molecular classes. These are luminal-like, basal-like, normal-like and HER2 positive subtypes. This classification was confirmed in a follow up experiments using larger number of cases.[4]

Immunohistochemistry is used as a surrogate for DNA micro-array classification to identify molecular subtypes of invasive breast cancer.[5-7]This method is a feasible alternative because many of the cases of invasive breast cancer occur in places where analysis of prognostic factors need to be economical, easy and reproducible.[8] Although immunohistochemical markers represent over simplification of the molecular complexity but it draws attention to fundamentally different nature of ER+ and ER- breast cancers.[9]Five surrogate immunohistochemical markers (oestrogen Receptor(ER), Progesterone Receptor(PR), Human Epidermal factor receptor 2 (HER2), Cytokeratin 5/6(CK5/6) and Epidermal Growth Factor Receptor(EGFR) are currently used for molecular classification. Using these biomarkers, the molecular sub-classes of breast cancer are: Luminal tumours that are hormone receptor positive; HER2 tumours with HER 2 over expression and Basal-like tumours with ck5/6 and/or EGFR.[10-12]Using these markers, at least five classes[13] of breast cancer subtypes are recognised: Luminal A, Luminal B, HER 2, Basal-like and Normal-like (unclassified).

Although the immunohistochemical surrogates have been adopted for gene expression studies, it is important to recognise that the molecular classes defined by immunohistochemistry correspond only partially to molecular classes defined by gene expression profiles.[9] Regional and ethnic variations exists in molecular class prevalence pattern.[14] The Luminal type for example in a study in Nigeria is 80.2%;[15] in Saudi Arabia 19.9%;[14] in western region 70.28-78.6%[10-11,16-17]and North Korea 44.5%.[18]

Luminal A

This is the most common subtype and represents 50-60% of all breast cancer. The cells resemble luminal cells lining the mammary duct. These tumours frequently have low histological grades. Low degree of nuclear pleomorphism, low mitotic activity and includes special histological types (these are tubular, invasive cribriform, mucinous and lobular). It is characterised by higher levels of ER, lower level of proliferation genes, expression of luminal epithelial cytokeratins(CK) 8 and 18, other luminal associated markers including ER1, genes associated with ER function such as LIV1(zinc transporter ZIP6 or SLC39A6; solute carrier family 39 zinc transporter, membrane 6), hepatocyte nuclear factor 3 alpha(FOXA1), X-box binding protein1(XBP1), GATA binding protein3(GATA3), B cell lymphoma 2 (BCL2), erbB3 and erbB4.[16]Immunohistochemical biomarkers that characterize Luminal A are ER+ve, and/or PR+ve, HER2 –ve, CK5/6 –ve and EGFR –ve and low Ki67 (proliferating cell nuclear antigen).[19] Less than 15% have p53 gene mutation. Tumour are generally grades 1 and 2 with good prognosis and fairly low recurrence rates.[17-18,20-21] Variations in the frequency occur with region: a study in Saudi Arabia had 3.9%,[22] amongst Chinese women was 60.8%[10] and 68.8%[23] in two separate works in Indian women 37.4%,[24] it was 27% and 33%[25] respectively in Nigeria and Senegal, in Eritrea 55%;[26] in Ugandan women 38%.[27] Another Nigerian study was77.6%[14]showing that variation also occurs even within the same country; variation with age is also seen in Luminal A subtype: Study in young women less than 40 years showed 33% of this type[28] but 25%[29] in women less than 35 years though 55.4% of total studied population was Luminal A subtype. It also varies with race/ethnicity and menopausal status: A study in Carolina in African American showed 36% in premenopausal women and 59% in post-menopausal women and 54% for non-African American.[19]

Luminal B

Luminal B tumours comprise 15-20% of breast cancer and have a more aggressive phenotype, higher histological grade, proliferative index and a worse prognosis.[30] The cells resemble cells of the lining of the mammary duct. It has a higher recurrence rate and lower survival rates after relapse when compared to Luminal A. The immunohistichemical biomarkers that characterize Luminal B are ER+, and/or PR+, HER2+ or HER2– with ki67 of greater than 14%. The genes expressed by Luminal B include avian myeloblastosis viral oncogene homolog (v-MYB), gamma glutamyl hydrolase(GGH), lysosome-associated transmembrane protein 4-beta(LAPTMB4), nuclease sensitive element binding protein1(NSEP1) and cyclin E1(CCNE1). HER2 associated genes ERBB2 AND GRB17 are seen in 30-50% of cases.[31] It also express growth receptor signalling genes.[32] The women are younger than Luminal A.[21] It also have a poorer prognosis than luminal A.[17-18,20-21,33]Other characteristics are poorer tumour grade, larger tumour size, lymph node positivity and p53 gene mutation. There is also variation with region, race/ethnicity, age and menopausal status as shown by these literatures: in Indian women it was 11.1%,[24] amongst Chinese women was 7.8%[10] and 4.3%[23] in two separate works, in Saudi Arabia 16.0%[22], 5% each in Uganda;[27] and Eritrea.[26] It was 2% and 3% respectively[25]in Nigeria and Senegal and 2.6% in another Nigerian study was luminal B;[14] it was 35%[28] in a study in US of women less than 40 years, 11.8% of the total but 14.3% was in women less than 35 years[29]when stratified by age.

Basal-like/Triple negative

The basal-like subtype represents from 8-37% of all breast cancer depending on the proportion of poorly differentiated grade 3 cases included in the population studied.[34][34] The cells are similar to the basal cells and normal myoepithelial cells[35] surrounding the mammary ducts. The tumour are associated with high histological and nuclear grade, poor tubules formation and the presence of central necrotic and fibrotic zones, pushing borders, conspicuous lymphocytic infiltrate and medullary features with exceptionally high mitotic and proliferative indices. Most of the tumours are infiltrating ductal tumours with solid growth pattern, aggressive clinical behaviour and high rate of brain and lung metastasis.[36]Immunohistochemical biomarkers that characterize basal-like are ER-, PR- and HER 2- but CK5/6+ve and/or EGFR+ve, CK 14, 17 and Laminin positivity. They also over express P-cadherin, fascin, caveolins 1and 2 and apha-beta crystallin. Most have p53 mutation, evidence of genomic instability and inactivation of the retinoblastoma(Rb) pathway; it occur in the young and common in African American,[18,20] it accounted for 21.2% of breast cancer in all ages but 57.1% in women less than 35 years.[29] There is variation with race and menopausal status as showed in a study in Carolina, it accounted for 39% of breast cancer in premenopausal African Americans and 14% in postmenopausal African American and 16% in non-African Americans.[19]Variations also occur with age; in

young women less than 35 years was 57.1%and 21.2%[29]when it was not stratified by age. Most breast cancer with BRCA1 inheritance breast are triple negative.[37-39]The tumour are often aggressive with poor prognosis.[18-19,23,29,40] The percentages are varied in most studies; in two separate works in Saudi Arabia 10.0%[22] and 24%,[35] in China 18.9%[10]and 22.5%[23] in two different studies, in Indian women 7.5%,[24] comparative study in Ghana and Norway 22% and 7% respectively,[3] 42.7% in Kumasi, Ghana,[41] in Senegal 23%,[25] 25% in Eritrea,[26] in Uganda 34%.[27] Studies in various part of Nigeria have shown different percentage of breast cancer being basal-like: 15.8%[14] in Ibadan, 65%[42] in Abia, 87%[43] in Lagos, 25% in Ilorin.[44]

Her 2 type

HER2 positive cancer accounts for 15-20% of breast cancer subtype. HER2 positivity confers more aggressive biological and clinical behaviour. It is characterised by high expression of the HER2 gene and other genes associated with the HER2 pathway and/or HER2 amplicon located in the 17q12 chromosome. The tumours are highly proliferative, 75% have a high histological and nuclear grade and more than 40% have p53 mutations.[45] These tumour are HER2 positive, ER, PR, EGFR and ck5/6 negative.[11,46] The carcinomas associated with Paget's diseases are usually poorly differentiated, ER negative, and overexpress HER2/neu.[47]The tumour have poor prognosis and higher sensitivity to neo-adjuvant therapy.[22]Various studies have shown variation in the percentage of this tumour sub-type with age, race/ethnicity and menopausal status: 11%[28] in a study of young women; in Carolina, USA[19]this subtype did not vary with race and menopausal status but it was 11.6% of total and 3.6% in women less than 35 years,[29] in china 12.5%[10] and 4.6%[23] in two separate works in Indian women 29%,[24] 26.5% in Kijabe, Kenya,[48] 5% in Eritrea,[26] in Uganda 22%,[27] in two studies in Saudi Arabia it was 17.3%[22]and 23%[35] respectively; in Nigeria and Senegal 15% and 14% respectively,[25] 40%[14] in another Nigerian study.

Normal breast-like or unclassified type

This accounts for about 5-10% of all breast carcinomas. They are poorly characterised and have been grouped into the classification of intrinsic subtypes with fibroadenoma and normal breast samples. They express genes characteristics of adipose tissue presenting an intermediate prognosis between Luminal and basal-like cancers and usually do not respond to neo-adjuvant chemotherapy. These tumours are penta-negative that is negative for ER, PR, HER 2, ck5/6 and EGFR. There is over expression of PIK3R1 and AAKR1C1, with other genomic alterations. It has a good prognosis but exhibit low pathologic complete remission rate of 6.0%. Study in Nigeria and Senegal shower a frequency of 28% and 27% respectively,[25] in Eritrea it accounted for 10%,[26] in Saudi Arabia 42.8%.[22] Differences in prevalence patterns occur in the study of western and other regions, mainly in Luminal and unclassified subtypes.

II Materials and method

Study design and material

This was an archival cross sectional study that used the immunohistochemical results of breast carcinoma diagnosed in the department of Histopathology ofUniversity of Calabar Teaching Hospital (UCTH) as surrogates to classify breast carcinoma into molecular subtypes. The archival material was paraffin embedded tissue blocks of breast carcinoma diagnosed in a five year period starting 1st January 2010 to 31 December 2014. Basic information like age at diagnosis, year of diagnosis, sex and histopathologic characteristics were collated from the medical records. The frequency (percentage) of the molecular subtypes (luminal a, luminal b, basal-like and her2 overexpressing) in University of Calabar Teaching Hospital, Calabar, was determined. This was done using immunohistochemical (hormonal markers) as surrogates as follows: Luminal A(ER+/PR-, ER-/PR+. HER2-); Luminal B(ER+/PR-, ER-/PR+ and HER2+); HER2 type(HER2+, ER-, PR-) and Basal-like(ER-, PR-,HER2-).

Sample size:

The sample size of the study was comprised of all the histological specimen that was diagnosed of 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) of the molecular subtypes (luminal a, luminal b, basal-like and her2 overexpressing) in University of Calabar Teaching Hospital, Calabar determined using immunohistochemical (hormonal markers) results for PER, PR and Her2 as surrogates 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 that immunohistochemistry could be done on 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.

III Results

For five years study period of 1st January 2010 to 31st December 2014, nine thousand six hundred and forty seven histology samples was 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 as 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

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%

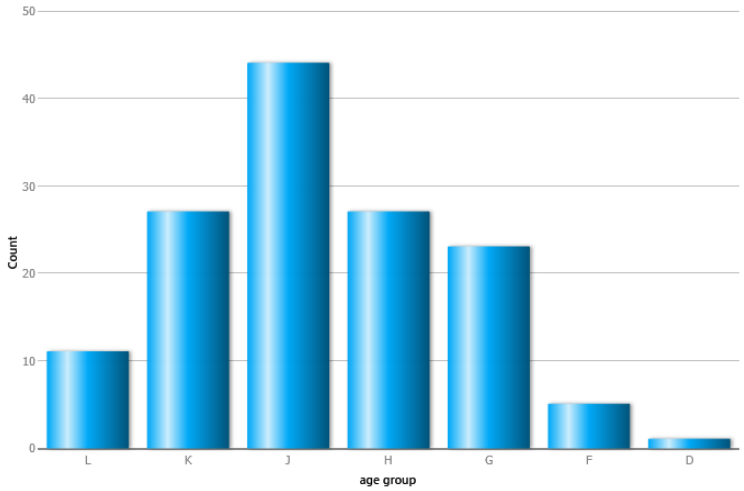
The mean of patient age at diagnosis is **46.31 years (SD+/-12.75)** old. The age range is from 21-80 years old. The modal and median ages are 40 years and 45 years respectively. The patients' age is stratified into three major groups. These are age less than 40 years old that has 42 cases (28.57%); 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%).

Table 2: The relationship between sex and molecular subtype of breast carcinoma.

SEX			Molecular subtype			
	Frequency	Percentage	Luminal A	Luminal B	Basal-like	Her2 positive
F	144	97.96	74	19	39	12
M	3	2.04	3	0	0	0
Total	147	100	77	19	39	12

Table 2 shows that 144 cases (97.96%) is female and 3 cases (2.04%) is male. Based on immunohistochemistry result, Luminal A is 77 cases (52.38%); Luminal B is 19 cases (12.93%); Basal-like (Triple negative) is 39 cases (26.53%) and Her 2 positive is 12 cases 8.16%). All the males have the Luminal A molecular subtype. The Luminal types (A and B) molecular subtype of breast cancer is 96 cases (65.31%).

Figure 1: Bar chart showing age distribution of subjects with breast carcinoma.



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

Table 3: The age range, mean age and standard deviation for molecular subtypes of breast carcinoma.

Molecular subtype	frequency	Percent	Mean age	Std dev.	Age range	Modal age
Luminal A	77	52.38	51.58	12.54	21-80	50
Luminal B	19	12.93	50.84	8.22	40-64	40
Basal-like	39	26.53	33.71	5.97	22-47	35
HER2 positive	12	8.16	46.17	6.63	33-59	42
Total	147	100				

The Luminal A molecular subtype mean age is 51.58 years with standard deviation of 12.54, age range is from 21-80 years and a modal age of 50 years. Luminal B subtype mean age is 50.84 years with standard deviation of 8.22, age range is from 40-64 years and modal age of 40 years. The Basal-like molecular subtype mean age is 33.71 years with standard deviation of 5.97, age range is from 22-47 years and modal age of 35 years. Her2-positive subtype mean age is 46.17 years with standard deviation of 6.63, age range is from 33-59 years and a modal age of 42 years.

Figure 2: Pie chart showing Molecular subtype of breast carcinoma in UCTH, Calabar.

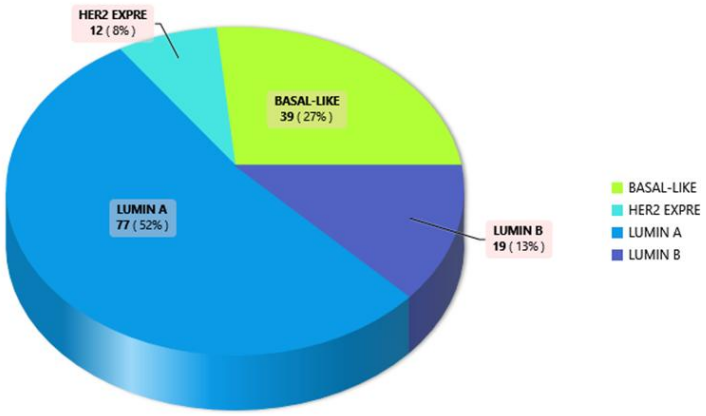


Table 4: The relationship betweenMolecular subtype of breast carcinoma and age group distribution.

Molecular subtype	Frequency	Age group distribution					
		20-39 years		40-55 years		56-89 years	
		Frequency	Percentage	Frequency	percentage	Frequency	Percentage
Luminal A	77	9	21.43	42	57.53	26	81.25
Luminal B	19	0	0	14	19.18	5	15.62
Basal-like	39	32	76.19	7	9.59	0	0
Her2 positive	12	1	2.38	10	13.70	1	3.13
Total	147	42	100	73	100	32	100

Table 4 shows that 42 case (28.57%) are in the age group less than 40 years. Majority of this number, 39 cases (76.19%) are Basal-like molecular subtype while 9 cases (21.43%) are Luminal A and 1 case (2.38%) is Her2 molecular subtype. The age group 40-55 years is 73cases (49.66%). Many of this number, 42 cases (57.53%) are Luminal A, 14 cases (19.18%) are Luminal B, 7 cases (9.59%) are Basal-like and 10 cases (13.70%) are Her2-positive. The age group more than 55 years is 32 cases (21.78%), majority of this number, 26 cases (81.25%) are Luminal A, 5 cases (15.62%) are Luminal B and 1 case (3.13%) is Her2-positive.

There is association between age and molecular subtype: age and Luminal A ($p=<0.001$ for <40 years and > 55years); age and Luminal B: <40 years ($p=0.0018$) and 40-55years ($p=0.028$); age and Basal-like: $p<0.001$ for< 40 years, 40-55 years and >55 years and age and Her2-positive 40-55 years $p=0.017$.

Table 5: The relationship between Molecular subtype of breast carcinoma and Tumour size group

Molecular subtype	Tumour size group distribution					
	1.5-1.9cm		2-5cm		5.1-12.9cm	
	frequency	Percentage	frequency	Percentage	frequency	Percentage
Luminal A	4	66.66	59	53.15	14	46.67
Luminal B	0	0	15	13.51	4	13.33
Basal-like	1	16.67	29	26.13	9	30.00
Her2 positive	1	16.67	8	7.21	3	10.00
Total	6	100	111	100	30	100

Tumour size group 1.5-1.9cm (less than 2cm) is 6 cases, of these number, Luminal A is 4 cases (66.66%), no Luminal B and 1 case (16.67%) each for Basal-like and He2-positive molecular subtype. Tumour size group 2-5cm is 111 cases comprising of Luminal A which is 59 cases (53.15%), 15 cases (13.51%) are Luminal B, 29 cases (26.13%) are Basal-like and 8 cases (7.21%) are Her2-positive. Tumour size group greater than 5cm is 30 cases consisting of 14 cases (46.67%) that are Luminal A, 4 cases (13.33%) are Luminal B, 9 cases (30%) are Basal-like and 3 cases (10%) are Her2-positive.

Table 6: The Tumour size range, mean, mode and standard deviation for molecular subtypes of breast carcinoma.

Molecular subtype	Frequency	Percent	Tumour size Mean	Std dev.	Tumour size range	Modal tumour size
Luminal A	77	52.38	3.85	1.82	1.6-11	3.2
Luminal B	19	12.93	4.13	1.59	2.2-9	3.0
Basal-like	39	26.53	4.45	2.53	1.6-12	2.5
HER2 positive	12	8.16	3.81	1.44	1.8-6.2	3.2
Total	147	100				

The mean tumour size is highest for the Basal-like molecular subtype and is 4.45cm and standard deviation of 2.53, tumour size range is from 1.6-12cm and the modal tumour size is 2.5cm. The mean tumour size of Luminal A molecular subtype is 3.85cm and standard deviation of 1.82, tumour size range is from 1.6-11cm and the modal tumour size is 3.2cm. The mean tumour size of Luminal B is 4.13cm and standard deviation of 1.59, tumour size range is from 2.2-9cm and the modal tumour size is 3.0cm. The Her2-positive has the lowest mean tumour size and is 3.81cm with standard deviation of 1.44, tumour size range is from 1.8-6.2cm and the modal tumour size of 3.2cm.

Table 7: The relationship between Molecular subtype of breast carcinoma and Histologic grade.

Molecular subtype	Histologic grade					
	Grade 1		Grade 2		Grade 3	
	frequency	percentage	frequency	percentage	frequency	Percentage
Luminal A	6	54.55	38	55.88	27	44.26
Luminal B	1	9.09	11	16.18	7	11.48
Basal-like	3	27.27	13	19.12	22	36.06
Her2 positive	1	9.09	6	8.82	5	8.20
Total	11	100	68	100	61	100

Table 7 shows that grade 1 tumour is 11 cases. Luminal A has the highest grade 1 (well differentiated) tumour and are 6 cases (54.55%), Luminal B is 1 case (9.09%), Basal-like is 3 cases (27.27%) and Her2 positive is 1 case (9.09%). The grade 2 tumour is 68 cases. Luminal A also has the highest number of cases in grade 2 (moderately differentiated) tumour and are 38 cases (55.88%), Luminal B is 11 cases (16.18%), Basal-like is 13 cases (19.12%) and Her2 is 6 cases(8.82%). The grade 3 tumour is 61 cases. Luminal A has the highest grade 3 (poorly differentiated) tumour and these are 27 cases (44.26%), Luminal B is 7 cases (11.48%), Basal-like is 22 cases (36.07%) and Her2 positive is 5 cases (8.20%).

IV Discussion

The use of immunohistochemistry of hormonal receptor and Her2 receptor status as surrogate for the molecular classification of breast cancer has been the practice in many resources-strapped countries. Although this does not completely capture all the biological characteristics of a molecular subtype as described by intrinsic gene expression profile using DNA micro-array but approximates to it and is thus used for the classification. The receptors used are a sis panel biomarker, these are hormone receptors like oestrogen receptor(ER) and progesterone receptor (PR), Human epidermal growth receptor 2(Her2), Epidermal growth factor receptor (EGFR), Cytokeratin 5&6 and proliferative marker ki-67. This research work attempted this classification using only the hormone receptors and Her2 in the following way: Luminal A (ER+/ PR+ or ER+/PR- or ER-/PR+ and Her2 -); Luminal B (ER+/PR+ or ER+/PR- or ER-/PR+ and Her2+), Basal-like or Triple negative (ER-, PR- and Her2-) and Her2-over-expressing (ER-, PR-, and Her2+). This has the drawback of not being able to separate the unclassified or normal breast-like subtypes and proper characterization of the Luminal types with ki-67.

The study found Luminal A to be 52.38%, Luminal B was 12.93%, Basal-like/Triple Negative was 26.53% and Her2 over-expressing was 8.12%. The Luminal subtype accounted for 65.31% of total breast carcinoma. This is at variance with reported studies from many centres, for instance it has a range of 70.28-78.6% in various reports from around the globe;[10-11,16-17] 44.5% in North Korea;[18] and 30%[49]in West African study. Similarly, studies done in many Nigerian centres also reported varying values of Luminal subtype of breast cancer.[50-52] The percentage of the other molecular subtypes in this study were equally at variance with both international values[14,28-32,35,41,43,53-54] and local reports.[15,25,50,55-56]Irianiwati also reported Luminal A as the highest molecular subtype in Indonesia women, this was followed by Triple negative subtype but Luminal B was the lowest[57] as opposed to this study that had Her2 over-expressing as the lowest but the other reports have different molecular subtypes as their highest and lowest molecular subtypes. For instance reports from Ilorin,[50]Lagos[56] and Abia[55] had basal-like/triple negative as their highest molecular subtypes while it was Luminal A in Ibadan.[15]

The study showed that the mean age for each of the molecular subtype was smallest for triple negative breast cancer 37.71+/-5.97 years, this is at variance with Carolina breast cancer study that had the unclassified subtype as the smallest mean age and the mean age of basal-like/triple negative was 46+/-10 years and was the second lowest.[10] Many reports are in agreement with this study in Triple negative having the lowest mean age and has also be reported as the commonest molecular subtype of cancer in the young especially in women of African descent.[10,32,53,55] The reason adduced for this is the high prevalence of inherited breast cancer genes like the BRCA gene which predispose them to have breast cancer at a younger age. Other abnormal gene like PTEN, ATM, may also be contributory. The mean age for the other molecular subtypes are 51.58+/-12.54 years for Luminal A, 50.84+/-8.22 years for luminal B and 46.17+/-6.63 years for Her2 over-expressing subtypes.

The Luminal A subtype had the highest mean age while Luminal B and Her2 over-expressing was in between. This compares well with report from Carolina breast cancer study in which Luminal A also had the highest mean of 52+/-12 years, followed by Luminal B which was 50+/-12 years.[10] This also conforms with previous reports that Luminal B patients are generally younger than Luminal A patients.[27] The modal age for triple negative was 35 years and was also the lowest for all the molecular subtypes while Luminal A was the highest (50 years), Luminal B and Her2 over-expressing were 42 years.

The study showed that irrespective of molecular subtype, breast cancer is commonest in premenopausal and peri-menopausal women, only a small fraction occurred in postmenopausal women and was mainly Luminal A subtype. The age range for the triple negative breast cancer was 22-47 years showing that all cases of triple negative breast cancer fell below the age 50 years. This is also similar to many reports that documented that the triple negative breast cancer is commoner in the young especially of African origin.[10,55] The age range for the other molecular subtypes are 21-80 years for Luminal A, 40-64 years for Luminal B and 33-59 years for Her2 over-expressing subtype. The study also showed that triple negative breast cancer is the commonest molecular subtype in the young as majority of patient with Triple negative 82.05% were actually less than 40 years while 17.95% were 40-55 years of age. A result that is slightly similar to this was reported in Carolina in which 64% of the basal-like/triple negative were pre-menopausal.[10] The majority of patients with Luminal A (54.55%), Luminal B (73.68%) and Her2 over-expressing (83.33%) are in the age group 40-55 years. Luminal A

also had a substantial proportion (33.77%) of age group greater than 55 years (postmenopausal) but the postmenopausal patient were 26.32% of Luminal B and 8.33% of Her2 over-expressing subtype. These are at variance with Carolina study that reported fairly high proportion of the all the molecular subtypes in postmenopausal women (Luminal A is 54%, Luminal B is 49%, Her2 over expressing was 45% and triple negative was 36%).[10]

The study showed that most of the cancer irrespective of molecular subtype had a tumour size that was 2-5cm; 76.62% of Luminal A, 78.95% of Luminal B, 74.36% of Triple negative and 66.67% of Her2 over-expressing subtypes had this tumour size. The proportion of tumour greater than 5cm were comparatively lower: 18.18% of Luminal A, 21.05% of Luminal B, 23.08% of Triple negative and 25% of Her2 over-expressing subtypes. The Triple- negative subtype has the highest mean for tumour size of 4.45cm +/-2.53 and it also had the tumour with the largest size of 12cm, these are in keeping with reports that the triple negative breast cancer have tumour with large size.[56,58] The mean tumour size for the other subtypes are 3.85+/-1.82cm for Luminal A, 4.13+/-1.59cm for Luminal B and 3.81+/-1.44cm for Her2 over-expressing. Luminal A has the highest percentage of tumour size less than 2cm of 66.67% and a similar finding was reported in Indonesian women.[57] Unlike in Indonesian women were Her2 over-expressing has the highest percentage of very large tumours, Luminal A has the highest in this study.

The majority of breast cancer in this study irrespective of molecular subtype had a very high percentage of grades 2 and 3 tumours. These two grades accounted for 92.14% of the total leaving grade 1 with just 7.86%. Grade 2 was slightly higher 48.57% than grade 3 (43.57%). This is in tandem with the study that reported a high percentage of high grade tumour in sub-Sahara African countries,[59-60] west African,[41,49]Eastern Africa[30,48] countries and the Carolina breast cancer study.[10] This is also the case in many studies done in Nigeria.[25,42,55,61-62] However, the proportion of grades 2 and 3 in each study is not exactly the same; 71-77% in Ibadan[42] were grades 2 and 3, 100% was grade 3 in Abia,[55] 70.6% was grade 3 in Jos,[61] 83% in Nigeria comparative study with Senegal.[25] A high proportion was also reported in African Americans.[10]

The study showed that all the male had Luminal A molecular subtype of breast cancer and had a mean age of 59+/-6.08 years as against 46.04+/-12.04 years for the female.

V. Conclusion

The Luminal A (52.38%) molecular subtypes was the commonest followed by Triple negative (26.53%), Luminal B (12.93%) and Her2 over-expressing (8.16%) in that order. The Luminal molecular subtype accounted for 65.31%. All the male breast carcinoma were Luminal A subtype. Breast carcinoma with large tumour size are of the basal-like subtype and are commonly seen in the young patients.

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