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SURVIVORSHIP PATTERNS OF HISTOPATHOLOGICAL VARIANTS AND MOLECULAR SUBTYPES OF BREAST CANCER IN A TEACHING HOSPITAL IN NIGERIA
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ABSTRACT

***Objective*: To study the relationship of histopathological characteristics, molecular subtypes of breast cancer and survival in a low resource setting.**
***Design*: Tumours from prospectively ascertained patients newly diagnosed with breast cancer were analyzed. Formalin-fixed and paraffin-embedded sections were constructed into tissue micro-arrays and immunostained with five anti-bodies. Five molecular subtypes were determined.**
***Settings*: The study was conducted jointly in the Department of Pathology of University of Ilorin Teaching Hospital, Ilorin in Nigeria and at the University of Chicago in the United States.**
***Subjects:* The study included a total of 203 histologically confirmed breast cancer patients whose pathological specimens were processed in the Department of Pathology of University of Ilorin Teaching Hospital, Ilorin, Nigeria between January 2003 and December 2007.**
***Results*: Mean age at diagnosis was 49.2 (SD ±11.9) years. Median time from symptom onset to cancer diagnosis was six months. Median follow-up time was 8.3 months. Median tumour size at diagnosis was 6cm. The proportion of ER+, PR+, HER2+ tumours were 27%, 16% and 30%, respectively. The most common molecular subtype was basal-like (25.1%) followed by unclassified (24.0%), luminal A (20.5%), HER2+/ER- (19.3%) and luminal B (11.1%). Luminal A and B had best prognosis while basal-like and unclassified had worst prognosis. ER+ patients had longer duration of symptoms to diagnosis (median 8 months) than ER- patients (5 months) but ER+ patients had smaller tumours (median 5cm) than ER- patients (6cm, p=0.02). Recurrence-free survival was best for stage 1 and worst for stage 4 tumours. About 32.6% of patients had loco-regional and/or metastatic recurrence.**
***Conclusions*: In consecutive breast cancer cases in Nigeria, almost half of patients were triple negative. Luminal A and B subtypes had best prognosis while triple negative had worst prognosis. The delay in breast cancer diagnosis and higher proportion of late stage of breast cancer underscores need for prompt diagnosis and initiation of treatment, especially hormonal therapy for ER positive patients.**

INTRODUCTION

Breast cancer is a complex disease with heterogeneity between tumours and within tumours. Major differences exist in incidence and mortality rates across populations. Black women have a lower incidence rate of breast cancer but poorer survival than white women (1). Breast cancer in African Americans is more likely to be early-onset, high grade and estrogen receptor (ER) negative compared with breast cancer in white Americans (2, 3). A British study (4) also found that black patients presented at younger ages with higher frequency of grade 3 ER negative tumours and had poorer outcomes than white patients with breast cancer. In Africa, several studies (5) have investigated the distribution of ER status, and a meta-analysis showed that breast cancer patients living in Western (35%) and Eastern (41%) Africa had lower proportion of ER+ tumours than patients living in North-western (54%), North-eastern (63%), and Southern (60%) Africa. Tumour characteristics that differ between black and white patients may explain these differences in outcome. The striking difference in subtypes distribution across populations suggests heterogeneity in etiology. Luminal A and B subtypes are hormone receptor positive and have favorable clinical outcome (6). Human epidermal growth factor receptor 2 (HER2)-positive/ER-negative subtype is characterised by overexpression of HER2 and basal-like subtype is negative for ER, progesterone receptor (PR) and HER2; both subtypes had poorer outcomes before the advent of trastuzumab as molecularly targeted therapy for HER2-positive breast cancer. Immunohistochemical (IHC) markers have been used to define these subtypes with similar prognostic value (7, 8). Basal-like, or more generally triple-negative (ER negative/PR negative/HER2 negative) breast cancer, is repeatedly more prevalent in African Americans than in their white counterparts (8, 9). Tumour subtype has been associated with grade, and possibly tumour stage. These factor impact ultimately on prognosis depending on the molecular subtype.

It remains unclear why African women, especially sub-Saharan African women, are more likely to have aggressive subtype of breast cancer, including basal-like breast cancer. We hypothesized that delayed diagnosis might be one reason for population difference in breast cancer subtype distribution. In addition, the prognostic value of breast cancer subtypes in African breast cancer patients was also not well documented. This study aims to investigate the distribution of breast cancer subtypes, evaluate the contribution of clinical and pathological factors to patient survival, and explore the relationship between delay in cancer diagnosis and breast cancer subtypes.

MATERIALS AND METHODS

Materials for the study were made up of tissue blocks from patients with breast cancer that were processed in the Department of Pathology of University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria between January 2003 and December 2007. All samples were formalin-fixed and paraffin-embedded (FFPE) according to routine surgical practice. They were exported to the Center for Clinical Cancer Genetics and Global Health, University of Chicago. The study was approved by the Institutional Review Boards at both institutions.

Pathologic features, including histologic diagnosis, histologic grade, tumour size, and axillary lymph node metastasis, were extracted from histology reports and evaluated by the pathologist. Whole sections of archival slides stained with hematoxylin and eosin were evaluated for volume-corrected mitotic index expressed as mitoses per square millimeter, mean nuclear area, and fraction of fields with tubular differentiation. The histology grading of invasive breast cancer was performed using the modified Scarff-Bloom-Richardson system (10).

Tissue microarray (TMA) was carried out at the Human Tissue Resource Core within the Department of Pathology at the University of Chicago Medical Center. Tissue microarrays were constructed from formalin-fixed and paraffin embedded (FFPE) tumour samples and adjacent normal histological epithelium, which serve as an internal positive control. Cores were precisely arrayed into a new recipient paraffin block using the automated tissue microarrayer ATA-27 (Beecher Instrument, Silver Spring, MD) with the method described by Kononen *et al* (11) (Figure 1). Paraffin specimens were cut into 4-μm sections and mounted on positively charged slides. The slides were deparaffinised and rehydrated in xylene followed by graded alcohols, then washed in Tris-buffered saline.

Immunohistochemical assays were performed using a DAKO immunostainer (DAKO, Carpinteria, CA) with anti-bodies and antigen unmasking. Slides were incubated in 0.03% hydrogen peroxide for five minutes to block endogenous peroxidase activity, followed by incubation for twenty minutes in a protein-blocking solution (Protein Block Serum-free solution, DAKO) to reduce nonspecific background. Envision reagents (DAKO) were used as a detection system. Slides were then treated for five minutes with 3-3'-diaminobenzidine chromogen, counterstained with haematoxylin, and coverslipped. Appropriate negative controls for the immunostaining were prepared by omitting the primary antibody step. The results of immunostainings were scored semiquantitatively using Reiner's four-point scale based on intensity and percentage of IHC reaction

(12) (Figure 2). Epidermal growth factor receptor (EGFR) and HER2 staining were evaluated according to manufacturer's instructions (DAKO).

Consistent with previous publication (8), breast cancer subtypes were defined as luminal A (ER positive and/or PR positive, HER2 negative), luminal B (ER positive and/or PR positive, HER2 positive), basal-like (ER negative, PR negative, HER2 negative, CK5/6 positive, and/or EGFR positive), HER2 positive/ER negative (HER2 positive, ER negative, PR negative), and unclassified (negative for all five markers). A tumour was considered ER and/or PR positive if 10% or more of the cells examined had estrogen and/or progesterone receptors.

Questionnaires were administered to collect data from follow-up patients. Patients not attending clinics were traced through their addresses and phone numbers while contacts were made with public and private hospitals from which specimens were sent to our laboratory. Information on diagnosis and treatment were obtained from patients' case files.

Descriptive analysis of data includes proportion, mean, standard deviation, median and interquartile range. We examined the association of ER status, subtypes and duration from first symptom and breast cancer diagnosis using Wilcoxon rank-sum test or Kruskal-Wallis rank test. Fisher's exact test was used to examine the relationship between breast cancer subtypes and other histological factors. Recurrence-free survival was estimated using the Kaplan-Meier method and the association between clinicopathological factors and recurrence-free survival using log-rank tests.

RESULTS

A total of 203 histologically confirmed breast cancer patients whose specimens were processed in the Department of Pathology of University of Ilorin Teaching Hospital, Ilorin, Nigeria between January 2003 and December 2007 were included in this study. The most common histologic type, as shown in Table 1, was infiltrating ductal carcinoma, which accounted for 81.3% of cases. The good prognostic histologic types including colloid, intraductal and medullary carcinomas were relatively few with 8, 8 and 3 cases respectively. For histologic grades of tumours, 10.3% were grade I (low grade), 40.2% were grade II (intermediate grade) and 49.5% were grade III (high grade). Mean age at diagnosis was 49.2 (SD ±11.9, range 21-92) years and the commonest age group affected was 40-49 years (Table 1).

Median time from symptoms onset to cancer diagnosis was 6 months (interquartile range 3-12 months). Clinical stage 4 tumours were 31.6% and stage 3 was19.8%. Median tumour size was 6cm. Of the 203 patients, the proportions of ER+, PR+ and HER2+tumours were 27%, 16%and 30% respectively. With respect to age, ER+ proportion was 22% in patients less than 50 years and 32% in patients above 50 years. Table 2 shows the molecular subtypes of breast carcinoma based on the histologic types. There were 171 cases with complete immunohistochemical results that were included in the classification. Commonest breast cancer subtype was basal-like (25.1%), followed by unclassified (24.0%), luminal A (20.5%), HER2+/ ER-(19.3%) and luminal B (11.1%). It is pertinent to note that many of the slow growing breast carcinomas with good prognosis were ER+. Such was the finding with mucinous/colloid carcinoma wherein out of the 8 cases recorded, 6 were ER+ while only 2 were ER-.

Patients with ER+ tumours had longer duration of symptoms (median 8 months) than patients with ER- tumours (5 months) but ER+ patients had smaller tumours (median 5cm) than ER- patients (6cm, P=0.02). In patients with shorter duration of symptoms (<1year) ER- tumours were larger than ER+ tumours while there was no difference in tumour size between ER- and ER+ tumours in patients with longer duration of symptoms (Figure 3).

Spirited effort was made to follow up patients from those attending clinics to those traced to their homes through their addresses or phone numbers as well as patients being managed in public or private hospitals but whose specimens were sent to our laboratory for histopathological diagnosis. Of the 101 patients whom we successfully followed up, 8 died within 1year and 6 months of follow up. Thirty three patients (32.6%) had loco-regional and/ or metastatic recurrence. Patients that could afford it had further surgery, chemotherapy and radiotherapy when applicable but only 3 patients are still being followed up 10-12 years after the diagnosis of breast cancer. More patients died or absconded from clinic at the onset of follow-up. As expected, recurrence-free survival was the best for stage I and the worst for stage IV tumours (Figure 4). Luminal A and B subtypes had best prognosis while basal-like and unclassified subtypes had worst prognosis but the difference was not statistically significant (Figure 5).

Table 1
Demographic and pathological characteristics of breast cancer

Histological variants	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	Age Unspecified	Total	%
Infiltrating ductal carcinoma, no special type (NST)			9	34	54	36	24	5		1	2	165	81.3
Infiltrating lobular carcinoma					5	1	2	3		1	4	16	7.9
Colloid/Mucinous carcinoma				3	1	3	1					8	3.9
Intraductal carcinoma			1	2	2	3						8	3.9
Medullary carcinoma					2		1					3	1.5
Adenoid cystic carcinoma											1	1	0.5
Paget's disease								1				1	0.5
Infiltrating ductal carcinoma (mixed type)						1						1	0.5
TOTAL			10	39	64	44	28	9		2	7	203	
%			4.9	19.2	31.5	21.7	13.8	4.4		1.0	3.5		100.0

Table 2
Molecular subtype based on histologic variants

	Basal- like	Unclassified	Luminal A	Her2+/ER-	Luminal B	TOTAL
Infiltrating ductal carcinoma	41	35	25	26	17	144
Infiltrating Lobular carcinoma	1	1	2	2	1	7
Intraductal carcinoma		1	1	4	1	7
Colloid/Mucinous carcinoma		2	6			8
Medullary carcinoma	1		1			2
Paget's disease of the nipple				1		1
Adenoid cystic carcinoma		1				1
Infiltrating ductal carcinoma (Mixed type)			1			1
TOTAL	43	41	35	33	19	171
%	25.1	24.0	20.5	19	11.1	100.0

Figure 1
Tissue microarray construction

Figure 2
IHC staining with 5 bio markers

Figure 3
Box plot of tumour size by estrogen receptor status and duration of symptom to diagnosis

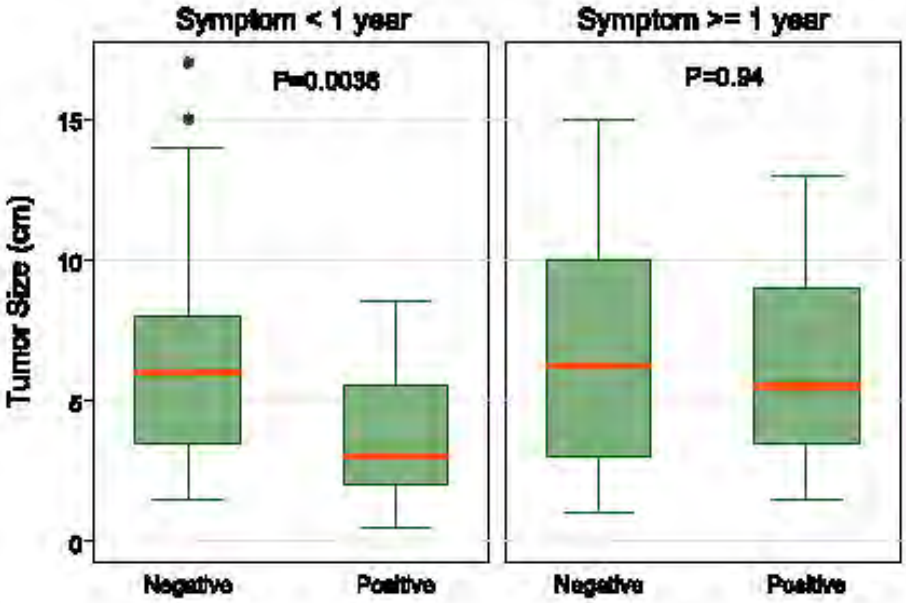


Figure 4
Recurrence-free survival by pathological stage

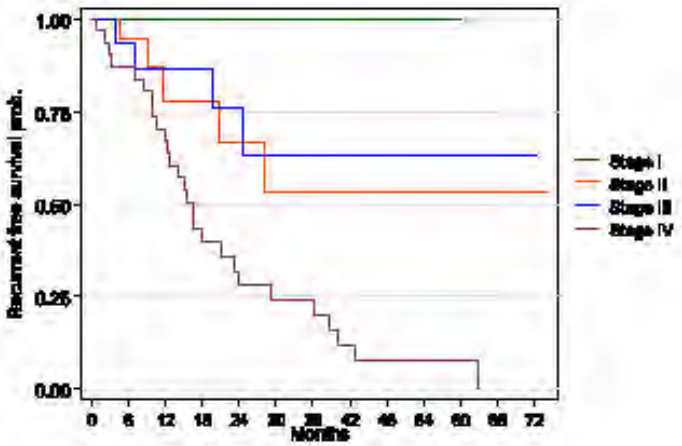
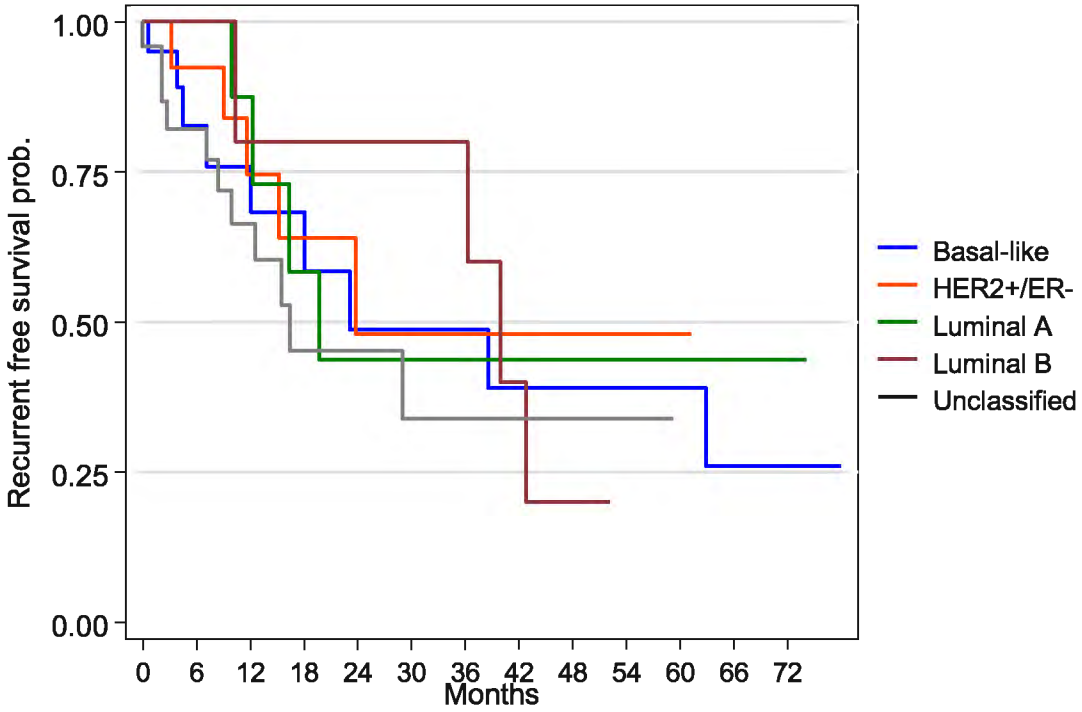


Figure 5
Recurrence-free survival by molecular subtype



DISCUSSION

In this study, majority of the patients at presentation were young and pre-menopausal or peri-menopausal with a mean age of 49.2years. This agrees with findings from previous studies within Nigeria (13, 14, 15) and other parts of Africa (16) which reported mean age of 48 years and approximately two-thirds of the patients were pre-menopausal. The proportion of ER+ tumours was 27%. This is slightly higher than the 24 - 25% values obtained in earlier studies (17, 18, 19) but consistent with the general finding that only about a quarter of breast cancers in Nigeria are ER+. Although molecular studies on breast cancer from women of African ancestry are sparse, most of the available reports reveal the preponderance of ER-, and indeed triple negative tumours (17, 18, 19). Conversely, more studies are available in African-American women, and these studies reveal that breast cancer in Africa-Americans is more likely to be early-onset, higher grade, and ER- compared to breast cancer in white American women (2, 3). Similar study among British women (5) also showed that black patients had a preponderance of ER- tumours compared to the white patients. The studies from the western world showing higher incidence of hormone receptor negative breast cancers among the African diaspora compared to the white women might as well be a pointer to why there is even higher incidence of hormone receptor negative breast cancer among indigenous African women compared to those in diaspora. Forty nine percent of women in the present study were triple negative and close to a third (31.6%) of the women presented in pathologic stage four when the tumours were of large sizes (average tumour size was 6cm), already ulcerated and fungating. Furthermore only 10.3% of the tumours were low grade while 49.5% were high grade and this lends credence to the fact that breast cancer in African women are often of high grade, ER- and patients present late. Very significant was the finding that average time from symptom onset to cancer diagnosis was six months. Indeed, some women presented in the hospital after three years or more of detecting lumps in their breast by which time such tumours might have metastasised. These factors account for the poor outcome of the indigenous African women with breast cancer. The striking difference in subtype's distribution across populations suggests heterogeneity in etiology. Tumour subtype is strongly associated with grade and these are good predictors of clinical outcomes independent of other prognostic factors (19, 20). The current study clearly shows that many of the good prognostic histologic variants of breast cancers such as colloid carcinoma are ER+.

Studies (1) among women born and raised in the United States revealed that black women have a lower incidence rate of breast cancer but poorer survival than white women. Socio-economic factors that lead to late stage at diagnosis and limited access to quality

health care contribute substantially to the disparity (2, 3). This is equally the predominant picture that is encountered among indigenous African women. Triple negative breast cancer is common among African women. In many parts of the continent, however, hormone receptor and HER2 status are hardly ever determined before commencement of treatment due to lack of resources. Capacity building/ manpower development and establishment of regional centers for immuno-histochemical studies in the continent have become imperative.

This study has some limitations. Being a retrospective study, data were extracted from patients' case files and pathology registers. Some vital data were not documented and that formed the basis of exclusion of some cases. Despite effort made at follow up and contact tracing, we could only get about half of the patients that were included in the survivorship study. In order to take care of these short comings associated with retrospective studies, our research team has resolved to embark on prospective study on breast cancer.

In conclusion, socio-economic factors that lead to late stage at diagnosis of breast cancer and limited access to quality health care contribute substantially to the disparity across populations. Breast cancer in black women is aggressive, triple negative and women present late with resultant poor prognosis. Since close to half of African women with breast cancer are triple negative, administration of anti-oestrogen without molecular studies should be discouraged. Regional centers should be established for proper histopathological and immuno-histochemical diagnosis to ensure appropriate and personalised therapy.

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