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IS THE BIOLOGY OF BREAST CANCER IN AFRICA CHANGING?
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ABSTRACT

Background: **Incidence of breast cancer (BC) is increasing in Africa, with higher case-mortality compared to non-African settings. Prior studies have shown that BC in Africa has a much higher proportion of estrogen-receptor (ER) negative and triple negative (TN) cancers, subsets with poorer prognosis regardless of the setting. However, there is growing evidence that these differences may partly be attributed to prior study designs and resources.**
Objectives : **To determine the status of hormone receptor ER, PR and growth factor Her2 status on breast cancer.**
Design: **A prospective study.**
Setting: **Histopathology and immunohistochemistry laboratary at Moi Teaching and Referral Hospital**
Subjects: **Tissue specimens from 100 breast cancer patients.**
Results: **Patients mean age at the time of diagnosis was 45 years, 98% of cases were in women, 90% were infiltrating ductal carcinoma, and the majority were poorly differentiated. Sixty-two percent were ER positive 44% were PR positive and 22% were Her-2 /neu . Twenty-four percent of cases were TN.**
Conclusion: **With improved access to in-country reliable IHC, our study supports the growing data that African breast cancer is not radically biologically different from breast cancers outside Africa.**

INTRODUCTION

Breast cancer (BC) is evolving into the primary cause of cancer death amongst women in Africa. GLOBOCAN data shows BC incidence is rising between their 2008 and 2012 analyses by as much as 50% (1,2). BC incidence to mortality ratio is higher amongst women in Africa, ranging from approximately 40% in North and South Africa to 50% in East and Middle Africa, compared to North America, where it is 16% (2). Several potential causes have been proposed for this observation, including poor access to healthcare, late presentation of disease, and biologically more aggressive disease (3). The first two potential causes are amenable to corrective actions by governmental and non-governmental healthcare providers - however, if Africa has a higher proportion of biologically more aggressive disease, strategies to reduce BC mortality will need to account for this from the start.

The hypothesis that African BC is biologically more aggressive has evolved over the last two decades, driven largely by pathological studies of estrogen receptor (ER), progesterone receptor (PR) and Her2 expression. For instance, ER-negative status has been reported to range from 67% to 76% in retrospective pathology studies from Nigeria, Tanzania, and Ghana, as compared to around 20% amongst cancers from white Americans (4–6). Similarly, rates of triple negative breast cancer (TNBC), in which ER, PR, and Her2 are all negative, have been reported as significantly higher amongst African populations. Data from Nigeria, Senegal and Ghana report incidence of TNBC ranging from more than 50% to more than 80%, as compared to less than 20% in white Americans(6,7).

Prior studies in Kenyans with BC have reported similar incidence of poor prognosis expression profiles. Studies from Kijabe and Nairobi have found ER-negative and TNBC in 66-73% and 28% of cases, respectively (8,9). As Bird *et al* highlighted, this has enormous implications to the management of BC in a

resource limited setting such as Kenya. The guidelines by Breast Health Global International recommend in the absence of immunohistochemical staining (IHC) for hormone and Her2 status, BC patients should be empirically started on tamoxifen (10). However, if ER- and triple-negative tumours are as common as reported, then the majority of the expenditure on tamoxifen would be wasted.

Given the therapeutic implications of receptor status in breast cancer, and the fact that to-date, reports from Africa in general, and Kenya, in particular have been small case-series, analysis of additional breast cancers in Kenya are warranted. To this end, our group performed a prospective analysis of receptor status expression of breast cancers diagnosed in Western Kenya between 2009 and 2013.

MATERIALS AND METHODS

Moi Teaching and Referral Hospital (MTRH), the second largest governmental referral and tertiary care facility in Kenya, has a nascent cancer care programme. In addition to the development of chemotherapy and palliative services, this program has invested development of diagnostic services, including an immunohistochemistry (IHC) laboratory opened in 2009. The service is the second IHC provider in Kenya, and is the only UKNEQAS-validated laboratory in the country (http://www.ukneqas.org.uk/). ER, PR, and Her2 staining are performed weekly as a routine component of breast cancer diagnosis.

From September 2009 to May 2013, 100 consecutive cases with well-fixed paraffin embedded tissue blocks of breast carcinoma were prospectively identified. All specimens had been fixed with 10% buffered formalin for 24 hours. Following standard procedures for dehydration, clearing and paraffin embedding, the paraffin block was sectioned and routine haematoxylin and eosin (HE) staining was performed for diagnosis.

Subsequently, four 3-5 micrometer thickness sections were cut from each block and mounted on positive charged slides for IHC and left to dry overnight, deparaffinized, and rehydrated. Staining was performed using the following antibodies: ER (Clone EP1, DAKO, Denmark); PR (clone PgR 636 DAKO Denmark); and Her2 (c-erb-2 oncoprotein, Dako Denmark) using the Dako Envision Flex Kit (DAKO, Denmark) as per manufacturer's instructions. All antibodies were at a dilution of 1:300 for 20 minutes. Amplified signal was demonstrated by diaminobenzidine and counterstained with haematoxylin. An in-house composite block for positive staining, mid staining and negative staining was constructed and was used in each batch of IHC staining, to assure quality control.

Pathologists from Brown University (Providence, RI, USA) and Indiana University (Indianapolis, IN, USA) provided guidance and support to MTRH pathologists in the interpretation of the IHC results. Tissue sections stained locally and the UKNEQAS scheme slides were assessed by UKNEQAS. ER and PR status was scored according to the Allred score, and Her2 scoring was done according to criteria set by DAKO (12). All statistics were performed in MS-Excel (Microsoft, Redmond, WA, USA) and are descriptive.

RESULTS

The clinical and morphologic information pertaining to the 100 consecutive cases is presented in Table 1. Briefly, 98% of patients were women, and the mean age at the time of biopsy was 45 years. Infiltrating ductal cell carcinoma accounted for 90% of the cases. Ninety four percent of the cases were histologically graded, with 90% found to be grade 3, or poorly differentiated.

All cases were successfully stained for ER, PR and Her2 status with consistent positive and negative controls. Aggregate results, regardless of morphology, are presented in Table 2. Thirty eight percent were ER negative, 66% were PR negative, and 22% were Her2 positive. Only 24% were negative for all three stains. There was no apparent relationship between ER, PR and Her2 expression and patient age or grade of the tumour.

Table 1
Clinical, Morphological, and IHC Results from 100 Consecutive Breast Cancers in Moi Teaching and Referral Hospital

Age at time of biopsy		48.7 (15-84) years
Sex (Male:Female)		2:98
Histologic diagnosis	Infiltrating ductal carcinoma	91%
	Infiltrating lobular carcinoma	1%
	Lobular Carcinoma	4%
	Metastatic ductal cell carcinoma	2%
	Mucinous Carcinoma	2%
Morphologic Grade*	Grade 1	1%
	Grade 2	3%
	Grade 3	90%

6% of cases were not graded

Table 2
Receptor Status of Tumours

Receptor	
ER +	62%
PR +	44%
Her-2 +	22%
ER +, PR +	37%
ER -, PR +	7%
ER+, PR -	25%
ER -, PR -, Her2 +	7%
ER +, PR +, Her-2 +	6%
ER -, PR -, Her-2 -	24%

ER, estrogen marker; PR, progesterone marker; Her-2, Human epidermal growth factor receptor 2; +, positive; -, negative.

DISCUSSION

The rising incidence of breast cancer in developing countries has made it one of the top causes of cancer death in women in Africa (13). Unlike developed nations where BC is commonly diagnosed in early stages, in Africa advanced stage presentation with concomitant high mortality is common (10). While implementation of cancer care programs across Africa promise to make screening and treatment more accessible, thus may lead to earlier presentation of disease, altering the incidence to mortality ratio will also be dependent upon understanding the biology of BC in African women. Even in highly resourced medical environments, ER negative and in particular TNBC carry a poor prognosis – if these are more common in SSA than in North American and European populations, regional strategies that are biologically appropriate must be developed.

The hypothesis that BC in Africa is biologically more aggressive arose from studies performed on stored pathologic specimens from pathology archives across Africa. Studies from specimens originating in Tanzania, Nigeria, Ghana and Kenya indicated that ER negative and TN BC is between two- and four-times more common than those reported in non-African populations (4,5,8,14,15). In guidance documents proposing cost effective strategies for management of BC, empiric tamoxifen has been proposed in the absence of routine hormonal receptor status determination (10).

However, as other authors have pointed out, if ER negative disease were more common in Africa than in non-African populations, not only would tamoxifen be ineffectual, but empiric use of even a cheap drug would waste the limited healthcare resources available for cancer care in many African countries (8). Further, if TNBC represents the majority of breast cancer in Africa, a successful strategy to contain mortality would depend not on simply implementation of successful strategies from resourced settings, but global improvement in successful treatment of TNBC. However, it remains unclear if the prevalence of receptor-negative and triple-negative disease is as common as earlier reports found.

We report that 32 and 24% of the specimens tested in our externally certified laboratory were ER negative and triple negative, respectively. This is significantly lower than prior reports from Africa (60-70% ER-negative), but in line with more recent studies. Studies from South Africa, Namibia, and Ethiopia report ER-negative tumours ranging from 20-35%, and triple negative tumours ranging from 20-21%. Sub-group analysis comparing black versus white women found only minor differences between these populations (6% more ER-negative and 3% TNBC in blacks) (16–18). Geographically closer to our population, Sayed *et al* reported that amongst 301 breast cancers at a Nairobi hospital found 27% were ER-negative and 20% were triple negative (19).

Our results fit with this recent trend in studies of receptor status amongst African BC – the distribution of poor prognosis disease is only slightly higher in Africans than in prior reports from the US and Europe. While it is possible that the biology of disease has changed in the last 20 years, it is far more probable methodological issues hampered the reliability of earlier studies. As more cancer infrastructure has been developed in Africa, studies are less reliant on retrospective pathologic specimens, under variable storage conditions, and with poor control of the initial collection and fixation. Indeed, a recent review and meta-analysis found consistent bias towards negative staining in studies utilising archived specimens – with 10% (4-17%) lower ER-positive tumours in those

studies (20).

Overall, our study of the expression of ER, PR and Her2 in 100 consecutive BCs in Western Kenya is additional evidence that African breast cancer is not radically biologically different compared to American and European populations.

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