Pathophysiology 24 (2017) 191–196

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Breast cancer biomarkers at Niger delta university hospital: Comparisons with national and international trends and clinical significance☆

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ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 3 May 2017
Accepted 22 May 2017

Keywords:
Breast cancer
Premier study
IHC
Surrogate

ABSTRACT

Breast cancer biology is heterogeneous and patients with the same diagnostic profile have markedly different clinical outcomes. Gene expression profiling and its surrogate IHC markers classified breast cancer into four molecular subtypes with different prognosis, targeted therapies, and/or clinical outcomes. The present study was conducted to investigate breast cancer subtypes among women in NDUTH Bayelsa state and compared them with similar studies in other populations of international and national origin. Archived formalin fixed paraffin embedded tissue blocks were retrieved from January 2010-December 2014 provided 20 cases of microscopically confirmed invasive ductal breast carcinoma that were evaluable for histology and IHC (ER, PR, HER2, and Cytokeratin 5/6) immunostain. All the patients were females and most of them were under 50 years at presentation. The molecular subtypes were luminal A (54.1%), luminal B (25.0%), HER2 (12.5%), basal-like (4.2%), and unclassified (4.2%). Triple negative carcinoma (basal-like and unclassified combined) was 4.2%, ER+ (65%) and (PR + 70%).Breast carcinoma in Bayelsa state women presents at a younger age. We note that the prevalence of the molecular subtypes of breast carcinoma appears to have variation in geographical distribution. The reasons for these differences could be technical, quality of fixation and processing, varying staining techniques, and different criteria in scoring and reporting. This study indicates that the majority of breast cancer in NDUTH lies at the two ends of the molecular spectrum, that is, luminal A (54.1%), sensitive to hormonal therapy and with a good prognosis, and "triple negative" (4.2%) not hormonally sensitive and with a poor prognosis.

1. Introduction

Breast cancer affects more than one million patients annually in the world and is a leading cause of mortality worldwide [23] and is the commonest site-specific malignancy affecting women in Nigeria and worldwide with several reports indicating a rising incidence [23,26]. The increasing incidence and significance of breast cancer mortality highlights the need for development of new therapeutic and highly targeted treatment. Breast cancer receptor status most commonly defined by estrogen-receptor (ER), progesterone-receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses in the clinical setting has major implications for breast cancer prevention strategies and patient management [23]. Studies of these markers in African women with breast cancer in Sub-Saharan Africa (SSA) have had extremely variable findings; reported percentages of estrogen receptor negative (ERN) tumors range from 30% to 40% [1,5,6] to >70% [31] in comparison to corresponding percentages in the United States: 35% in breast cancer

☆ This retrospective study investigated the subtypes of breast cancer in female cancer patients that attended Niger Delta University Teaching Hospital (NDUTH), Bayelsa state, Nigeria, from January 2010 to December 2014.Formalin fixed paraffin embedded tissue blocks archived of these subjects retrieved and twenty cases of confirmed ductal breast carcinoma were evaluated by histological and IHC (ER, PR, HER2, and Cytokeratin 5/6) techniques. Results show that the molecular subtypes were luminal A (54.1%), luminal B (25.0%), HER2 (12.5%), basal-like (4.2%), and unclassified (4.2%). Triple negative carcinoma was 4.2%, ER+ (65%), and PR+ (70%). The prevalence of the molecular subtypes of breast carcinoma varies geographically. Findings suggest that breast carcinoma in Bayelsa state women presents at a younger age comparatively and predominate of the luminal A subtypes, which is sensitive to hormonal therapy, with a good prognosis, and the triple negative class, which is hormonal insensitive, with a poor prognosis. Biomarkers evaluations in the pathology laboratory are critical for the effective management of breast cancers.

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http://dx.doi.org/10.1016/j.pathophys.2017.05.002
0928-4680/

patients aged 40years and decline to 15% −20% by age 70years and is slightly higher in black than in white American women [3].

Breast cancer is regarded as a biologically heterogeneous disease and patients with the same diagnostic and clinical prognostic profiles can have markedly different clinical outcomes. This difference is possibly caused by the limitation of the current taxonomy of breast cancers, which classify molecularly distinct diseases into clinical classes based mainly on morphology. A partial explanation for this disparity in behavior is found in studies using modern techniques including molecular profiling to examine the biology underpinnings of breast cancer [15,40]. Molecular profiling has provided biological evidence for heterogeneity of breast cancer through the identification of intrinsic subtypes. Analysis of gene expressions suggest that breast cancers can be divided into molecular subtypes which have distinct clinical features, with markedly differing prognosis and clinical outcomes [23,30].These subtypes consist of two estrogen receptor (ER) positive types (Luminal A and Luminal B), and three ER-negative types human epidermal growth factor receptor-2[HER2] expressing, basal like and normal breast-like [26,29]. Luminal A tumors, characterized by positive ER, and negative Her-2 show the most favorable clinical features among the five subtypes [9,23]. Basal like tumors typically show low expression of ER and HER-2, and exhibit high expression of genes characteristic of the basal epithelial cell layer, including expression of Cytokeratin 5/6 (CK5/6). This subtype is more prevalent in patients with BRCA1 mutation [29] while the HER-2+ tumors fall into at least 2 distinct groups: HER-2+/ER- and HER2+/ER+ [29,30].

Though many studies on breast cancer have been carried out in Bayelsa state using routine heamatoxylin and eosin (H and E)-based on morphological diagnosis with occasional special histochemical staining method, and other ancillary methods, investigations such as immunohistochemistry were hardly ever used to classify breast cancer in Niger Delta University Teaching Hospital Okolobiri (NDUTH) Bayelsa state Nigeria, as it is done in other centers in Nigeria. The present study was conducted to investigate breast cancer subtypes in Bayelsa state women and compared them with similar studies in other populations of international and national origin. Information derived would provide a baseline for research and help in formulating management policies for breast cancer management in the hospital.

2. Material and methods

2.1. Study area

This is a retrospective single-centre hospital based study carried out in Niger Delta University Teaching Hospital (NDUTH) Okolobiri, Bayelsa State, South–South Nigeria. The hospital serves as a referral centre in Bayelsa State and equally serves as a training institution for Medical and Allied medical sciences students of the state own university and other colleges of health. Bayelsa State is located within Lat. 4°15N and Lat. 5°23 south and Long. 5°21 and 6°51 East of the equator, bounded by the Atlantic ocean by the south of Nigeria. Bayelsa has the highest collection of the Ijaws tribe in Nigeria and is a second largest producer of crude oil in Nigeria with the largest gas reserve and oil well. Her major occupation is fishing and civil service. The result obtained was analyzed using simple descriptive statistical methods, Graph-Pad Prism 5 (GraphPad Software, San Diego, CA).

2.2. Sample collection

A total of ninety formalin-fixed and paraffin embedded tissue blocks (20 malignant cases and 70 benign cases) were collected from the department of histopathology laboratory Niger Delta University Teaching Hospital Okolobiri from January 2010 to December 2014. The twenty (20) malignant cases were further subjected to immunohistochemical testing.

2.3. Methodology

The histological subtypes of invasive breast carcinoma and Nottingham histological grade were defined using the routine (Heamatoxylin and Eosin) morphological evaluation. Immunostaining was done for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER-2/neu), and Cytokeratin 5/6 (CK5/6) using the manufacturer's antibodies and protocol. ER and PR were considered positive if 1% or more of the nuclei of invasive carcinoma cells were stained.HER-2/neu stains were scored as 0, 1+, 2+, and 3+ using the College of American Pathologists guidelines [35]. Specifically, 3+ represented uniform intense membrane staining of more than 30% of the tumour cells, and considered positive for HER-2/neu over expression. CK5/6 was scored positive if any (weak or strong) cytoplasmic and/or membranous invasive carcinoma cell staining was observed. Color development and background staining was visualized using the 3, 3-diaminobenzadine chromogen and heamatoxylin as counter stain, respectively. Appropriate negative controls for immunostaining were prepared by eliminating the primary antibody step.

Combination of IHC markers used to define breast cancer molecular subtypes in this study were as follows; luminal A (ER positive and/or PR positive, Her2 negative), luminal B (ER positive and/or PR positive, Her-2 positive), Her-2 type (ER negative, PR negative, Her-2 positive), basal-like (ER negative, PR negative, Her-2negative, and CK5/6 positive), and normal like (ER, PR, Her2, and CK5/6 negative). By this definition, the "triple negative" (ER, PR, and Her-2 negative) tumor incorporate both the basal-like and the unclassified categories.

The immunohistochemical method is the Avidin Biotin Complex (ABC) method also referred to the Avidin biotin imuunoperoxidase method. The antibodies used for this work were manufactured by Novocastra products owned by LEICA. The antibody dilution factor was 1:100 dilutions for all the antibody markers. Cells with specific brown colors in the cytoplasm, cell 4.membrane or nuclei depending on the antigenic sites are considered to be positive. The heamatoxylin stained cells without any form of brown colors are scored negative. Non specific binding/brown artifacts on cells and connective tissue were disregarded.

2.4. Results

A total of 90 cases of Primary breast tumour comprising of 70 benign cancer cases and 20 malignant cancer cases covering a 4 year period were included in the study. Patients Mean age was 49.4 and 48 years for benign and malignant cases respectively. The result was presented in photomicrographs and tables.

Table 1 shows histopathological subtypes with 23.3% of patients presented with fibroadenoma, 11.1% had fibrocystic change disease, 18.8% of the patients had infiltrating ductal carcinoma while only 2.8% patients with papillloma and inflammatory disease was 5.6% while poor documentation accounted 26.6% (evidence of the cases registered but no result and/or tissue blocks).

Table 2 shows the prevalence of breast tumor by gender. The ratio of female to male was 94.4/4.4 female to male respectively. The prevalence of breast pathology was highest within the ages of 21–30 years (28.8%) followed by 31–40 years (27.7%) and least from 51 and above.

Table 3 shows immunohistochemical breast cancer subtypes as follows: luminal A subtype (54.1%), Luminal B subtype (25.0%), Her2+ (12.5%), basal-like (4.2%) and normal breast like 4.2%.