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Triple Negative Evaluation of FFPE (Formalin Fixed Embedded Tissue) Breast Cancer Tissues in A Tertiary Health Institution of Bayelsa State, Nigeria

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Abstract

Breast cancer is a common disease worldwide, with over two million cases reported in 2018. Various therapy modalities use biological characteristics that influence treatment and outcomes to provide individualized care. Some biomarker advancements, such as hormone receptors, vascular endothelial growth factors, and HER2/neu receptors, have significantly contributed to the biological characterization of breast cancer. Triple-negative breast cancer is defined as malignancies that do not express hormone responsiveness or HER-2. Approximately 12% of women with breast cancer will have triple-negative illness, which is more common among non-Hispanic black women regardless of age, but is detected at a younger age than other subtypes. Women with triple-negative illness are more likely to be diagnosed at a later stage (stage III or IV). The pathogenesis of breast cancer is complex and poorly understood, however many risk factors are known. This cross-sectional study spanned from 2010 to 2022. In this study, the prevalence of triple-negative breast cancer in archived breast cancer cases at Federal Medical Centre Yenagoa, Bayelsa State, was analyzed using the Haematoxylin and Eosin Staining Technique on breast cancer samples collected over the study period. This study, done at Bayelsa State's Federal Medical Centre Yenagoa, analyzed 178 breast specimens. Ninety-six percent (96%) of the patients were IDC, and 5% were TNBC. The average age at presentation was 48.21 years, with 59.1% of patients being under 50 years. 40% of the patients exhibited HER-2/neu positive, and 65% had estrogen receptor (ER) positivity. The study was conducted in a single medical institution, and specific types of lesions were not included. Other potential limitations include comparing findings with various techniques and diagnostic protocols.

Keywords: Triple Negative, Breast cancer, Formalin Fixed Paraffin Embedded (FFPE), Estrogen receptors (ER), Progesterone receptors (PR), Human Epidermal Growth Factor receptor 2 (HER2).

INTRODUCTION

Breast cancer is a prevalent global disease, with over two million cases diagnosed in 2018 (Musa *et al.*, 2020). The biological factors that determine treatment and results are utilized by various therapy modalities to deliver individualized care (Waks & Winer 2019). Some developments in biomarkers, such as hormone receptors, vascular endothelial growth factors, and HER2/neu receptors, have made significant progress in the biological characterization of breast cancers. According to Beckwitt (2018), invasive breast cancer is the second most prevalent cause of cancer-related death for women in the United States, with over 250,000 new cases discovered each year. Biomarkers, such as gene tests, multi-analyte profiles, and BRCA1/2 mutation testing, are crucial for the diagnosis and treatment of breast cancer patients (Duffy *et al.*, 2015). To better care for women who are at risk, of having screenings, or receiving therapy for breast cancer, clinicians should be

knowledgeable about the disease. In the United States, the incidence of breast cancer is rising in literally all ethnic groups, with the incidence of estrogen-positive breast cancer rising in every ethnic group. Infiltrating ductal carcinomas (IDCs) constitute up to 80% of invasive breast cancers; invasive lobular carcinomas are the second most common kind (Igari *et al.*, 2022). Triple-negative breast cancer refers to malignancies that do not express hormone receptivity or HER-2. About 12% of women with breast cancer will have the triple-negative disease, more common among non-Hispanic black women, independent of age, but tend to be diagnosed at earlier ages than other subtypes. Women with triple-negative disease are also more likely to be diagnosed at a later stage (stage III or IV) Watkins (2019). The pathophysiology of breast cancer is multidimensional and still poorly understood, but certain risk factors are known. Advancing age and female sex are the most common risk factors, while genetic mutations, specifically BRCA 1 and 2, account for about 10% of breast cancers. Other known risk factors include a history of ductal carcinoma in situ, high body mass index (BMI), first birth at age greater than 30 years or nulliparity, early menarche (before age 13 years), family history of breast or ovarian cancer, late menopause, and postmenopausal hormone therapy use (Jackson *et al.*, 2020). The four breast cancer subtypes are associated with specific histologies and prognoses. Breast cancer is classified by its anatomical origin, hormone receptivity, and human epidermal growth factor receptor 2 (HER-2) expression (Watkins 2019). HER2 is an important biomarker and target of therapy for approximately 30% of breast cancer patients. Mammography and ultrasonography are used as initial imaging modalities (Weaver & Leung 2018). MRI may be used in specific circumstances, such as in patients with dense breasts, those with a history of breast cancer, those who are being evaluated for contralateral disease, and those at high risk for breast cancer (Onega *et al.*, 2022). In this study, the prevalence of triple-negative breast cancer in archived breast cancer cases in Federal Medical Centre Yenagoa, Bayelsa State was assessed using Hematoxylin and Eosin Staining Technique on breast cancer samples only with the period of study.

MATERIALS AND METHODS

Study Area and Site

Bayelsa State was created out of the old River State on the 1st of October, 1996. There are eight (8) local government areas, and the capital is located in Yenagoa metropolis (Ambily *et al.*, 2022). The State population as estimated by the 2006 census is 1,704, 515 people out of which 874, 084 were males and 830, 432 females. The State is a coastal region with mangrove swamps, heavy rain forests, and a short period of dry season that lasts from November to March. The boundaries are the Atlantic Ocean on the West and South, neighboring States of Rivers on the East, and Delta on the North. It has four major cultural ethnic groups identified as Izon, Nembe, Ogbia, and Epie-Attisa with their respective languages (Amadi, 2020). The people are mostly Christians with few traditional worshippers. According to Samuel *et al.*, (2021), Bayelsa State shares hugely from poor health indicator ranking of the country's health system of 187th position out of 191 countries in the world published in the year 2000. The major occupations are farming, fishing, palm oil milling, lumbering, palm wine tapping, and local gin making. The State has a land mass of 12,110 square kilometers with more than three-quarters occupied by water. The land topography is two (2) meters below the sea level. It is located within Latitude 4015N and 5023S and longitude 5022 and 605 East of the equator. Bayelsa State is the second largest producer of crude oil in Nigeria and has the largest gas reserve and oil wells in Nigeria. Microtomy and Hematoxylin/Eosin staining technique and procedures were carried out in Niger Delta University Teaching Hospital, NDUTH, Okolobiri, Yenagoa, Bayelsa State, Nigeria.

Study population

A retrospective assessment of all previously reported formalin-fixed paraffin-embedded (FFPE) archived breast tissue blocks previously diagnosed with various pathologies from 2010 to 2022 in the institution's histopathology laboratory served as the study samples.

Research design

The research was a retrospective and a cross-sectional study. Therefore, the data on the relationship between tumor markers and primary breast cancer and other variables of interest, as they exist in our defined population, was collected at a single point in time within the periods of ten years review. This model was by Hennekens and Buring, (1987).

Sample Size Determination and Sample Selection

The formula of Naing *et al.*, (2006) was used to determine the sample size for the study. Therefore, applying the formula:

$N = Z^2pq/d2$

Where:

N = the calculated sample size (for a population greater than 10,000)
Z = the standard (alpha) normal deviate usually set 1.96 which correspond to 95% confidence level
p = the disease prevalence in the population study.
sq= 1.0-p
d = degree of accuracy (precision) desired usually set at 0.05
N= 187 based on a reported prevalence of 14.7%

Inclusion and Exclusion Criteria

All previously diagnosed breast cancer tissue blocks between 2010-2022 with adequate information such as the patient's age, clinical details, and available tissue mass or blocks were selected, while tissue blocks with another form of malignancy and dates above and below the time frame were excluded.

Sampling Methods

The convenience sampling method was used to select the formalin fixed paraffin embedded breast cancer tissue blocks (2010 – 2022) from the histopathology archives of the center. Convenience sampling is a non-probability sampling that involves the samples drawn from that part of the population that is close to hand (Scholtz, 2021).

LABORATORY PROCEDURE:

Preparation of Breast Cancer Tissues

The method of Bancroft and Gamble, (2008) was adopted in preparing the breast tissues. The tissue block selected was sectioned into four (4) microns using a rotary microtome. The serial sections were floated in a water bath at a temperature of 550C for 2 min. The floated sections were picked using labeled grease-free frosted end slides. Seventy-one slides were submitted for Ehrlich's hematoxylin and eosin staining.

Haematoxylin and Eosin Staining (Breast Tissues)

Ehrlich's hematoxylin and Eosin method was used for histological studies by Bancroft and Gamble, (2008). The slides were stained and read to confirm the previous diagnosis. Primary histopathological evaluation was made on 5 µm thick sections stained with usual Hematoxylin– Eosin. The classification was made according to WHO's criteria, and the majority (n = 19) of the cases were invasive ductal carcinoma, two were invasive papillary carcinomas and one was mucinous carcinoma. The Scarff–Bloom–Richardson system, which is the modified Elston–Ellis's system was used in grading the invasive ductal carcinoma.

Principle

The principle of reaction is based on the chemical theory of dye; where the acidic component of the tissue is stained by the basic dye (hematoxylin) blue and the basic component (cytoplasm) is stained by the acidic dye (Eosin) pink.

Procedure

The breast tissues were sectioned using rotary microtomes into serial sections and stained. The slide sections were dewaxed and hydrated in water, drained, and transferred to hematoxylin solution (primary stain) for 20 min until the nuclei stained blue. Rinse in water and immediately subject to two seconds differentiation using 1% acid alcohol, rinse to stop the reaction, and blue in Scot's tap water to restore the nuclear stain. Dehydrate in three changes of alcohol (70%, 95%, Absolute) and stain in 1% Eosin (cytoplasmic stain) dehydrate in two changes of alcohol (95% and Absolute) and clear in xylene and mount in DPX.

Statistical Analysis

The raw data was coded in a spread sheet for each analysis on statistical software package Graph Pad prism 5 (Graphed Software Inc., 2014). Data was presented in percentage.

RESULT

Table 4.1: Descriptive Statistic of Breast Disease among Subject Studied

Variables	No. Observed	Percentage
Tumor type		
Malignant	71	37.9
Benign	116	62.1
Gender		
Male case	04	2.2
Female cases	183	97.8
Histologic type		
Ductal carcinoma	68	95.8
Lobular carcinoma	03	4.2
Anatomical position		
Right breast	61	33.5
Left breast	63	34.5
Bilateral	05	26.7
No documentation	58	31.0

Table 4.1 described the selected variable among breast cancer patient studied. Malignant breast cancer accounted for 37.9% of breast disorders studied within the given period, while 62.1 % were benign lesions. Majority of the malignant lesions were of ductal carcinoma (95.8%) and 4.2% are lobular carcinoma. Data revealed that right breast lesions accounted for 33.5%, left breast (34,5%) while bilateral was 26.7%. poor documentation of clinical information was 31.0%

Table 4.2: Distribution of Breast Disease Based on Age (Years)

Age	Malignant		Benign		
≤15-29	7(9.8)	49(77.7)	5(25.0)	1(50.0)	7(21.8)
30-39	18(25.3)	8(12.6)	8(40.0)	1(50.0)	6(18.7)
40-49	14(19.7)	3(4.7)	4(20.0)	(0.0)	8(25.0)
50-59	20(28.2)	1(1.5)	2(10.0)	(0.0)	5(15.6)
60-69	5(7.0)	(0.0)	(0.0)	(0.0)	4(12.5)
70-79	4(5.6)	1(1.5)	(0.0)	(0.0)	1(3.1)
≥80	1(1.4)	(0.0)	(0.0)	(0.0)	(0.0)
No Age	2(2.8)	1(1.5)	1(5.0)	(0.0)	(0.0)
Total	71(37.9)	63(54.3)	20(17.2)	2(1.7)	31(27.5)

Fibroadenoma Fibrocystic Phylloid Other lesions

The distribution of breast lesions based on age.as presented in table 4.2, shows antiparallel trend between the malignant and benign cases. There was an increase in the percentage of malignant breast lesions until 5th decade and started dropping from 60th decade to the 8th decade. The opposite was observed in benign lesions as the increases were observed within the age of 15-29th year of age. Fibroadenoma was the most common form of benign disorder recorded in the present study followed by fibrocystic changes with 54.3% and 17.2% respectively.

Table 4.3: Tumour Markers over Expression among Subjects Studied

Tumour markers	No examined	Over expression	Prevalence (%)
Estrogens(ER)	71	Positive score 46 +2= 12 +3= 18 Negative score 25	65.0 25.0 40.0 35.0
Progesterone(PR)	71	Positive score 50 + 2=10 + 3=25 Negative score 15	70.0 20.0 50.0 30.0
EGF2	71	Positive score 28 +2= 4 +3=7 Negative score 17	40.0 15.0 25.0 60.0
Cytokeratin 5/6	71	11	15.0

Table 4.3 shows percentage breast hormone receptors and oncogenes expressions with estrogen receptor positivity is to negativity as (65:35%) Progesterone (70:30%), epidermal growth factor-2 (15:0%) with 25% equivocal and Ck5/6(15.0%).

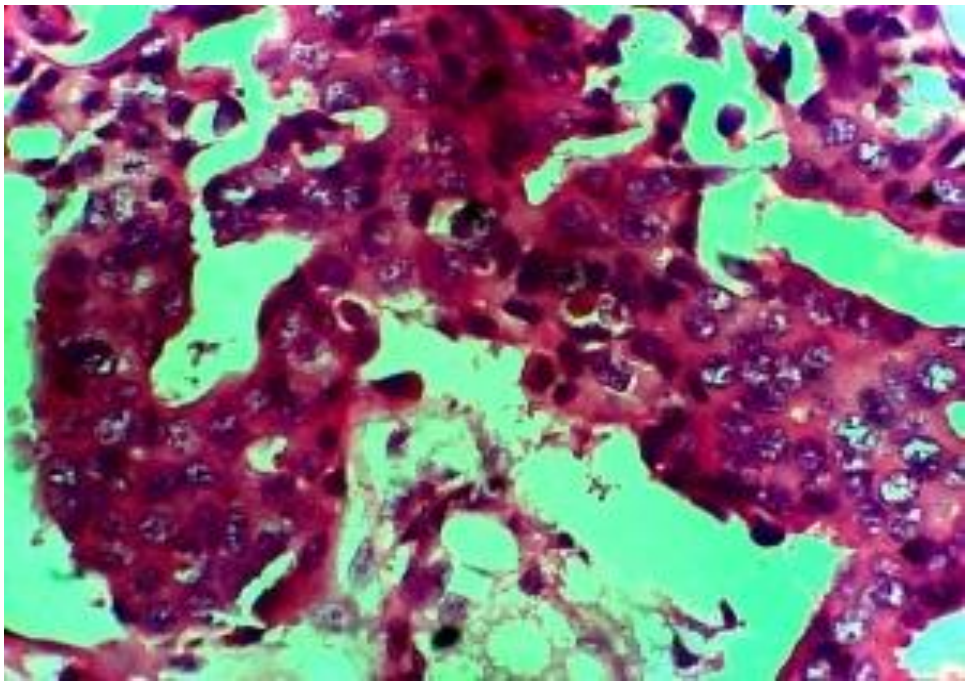

Plate 4.1: H&E

Triple Negative (H/E, ER-, PR-, HER2-) X 400magnification

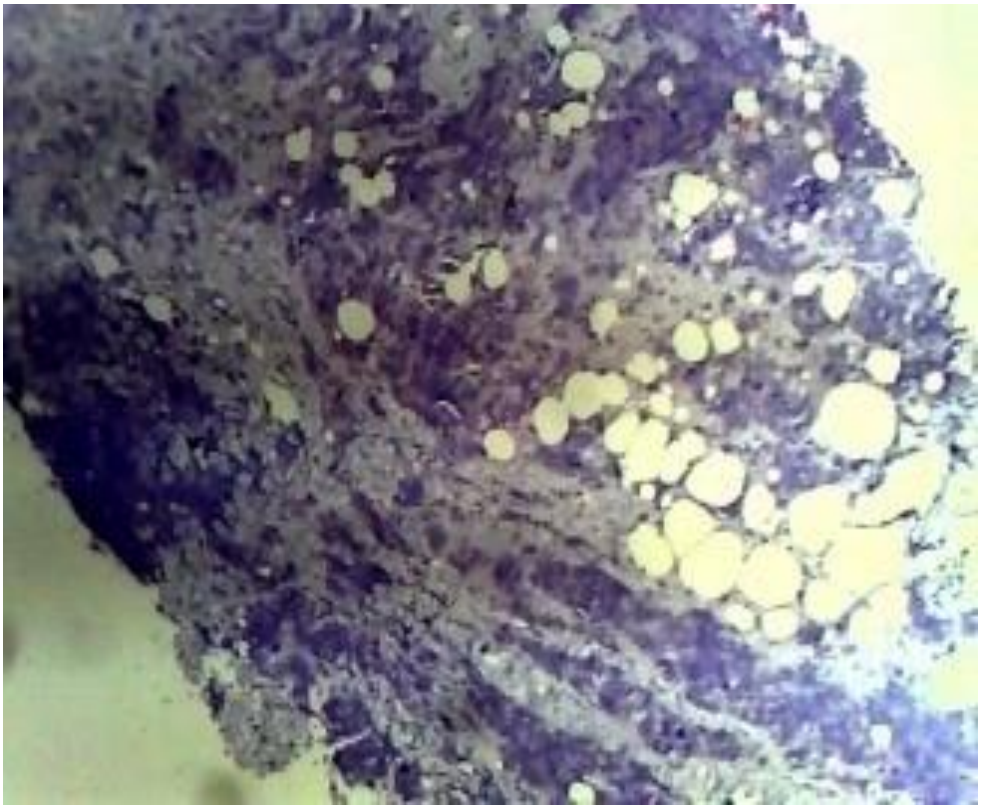

Plate 4.2: ER-

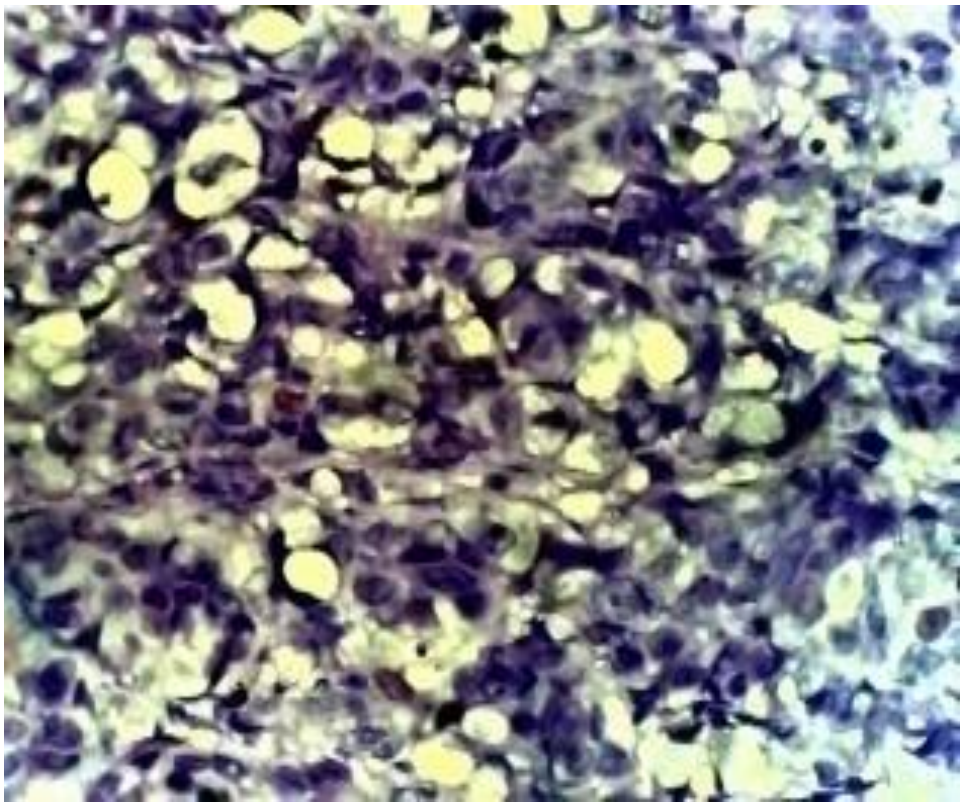

Plate 4.3: PR-

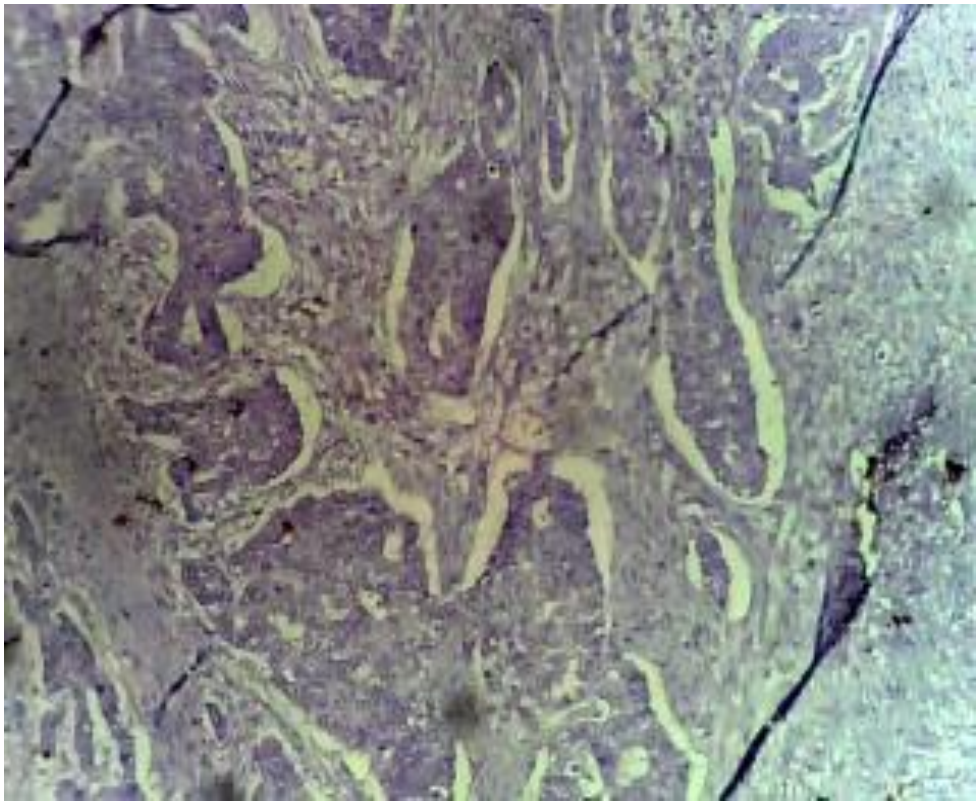

Plate 4.4: Her2-

DISCUSSION

Breast cancer is a heterogeneous disease with a growing number of recognized biological subtypes. Prognostic indicators based on currently available clinical and histopathological variables such as tumor size, tumor grade, lymph node status, and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations (Yersal & Barutca 2014). However, these indicators are still inadequate within a given patient population with a specific predicted risk of recurrence, there are always patients whose actual clinical outcome doesn't match that predicted by the indicator. Thus, attempts have been made to use molecular profiling to create more accurate prognostic indicators to address these issues (Freitas *et al*., 2021). Hence the importance of this study in Federal Medical Centre Yenagoa, Bayelsa State.

Breast cancer is the most frequently diagnosed cancer in females in developed countries, affecting 1 in 8 women in the United States (Smolarz *et al*., 2020). Developing countries are not lagging. We received about 178 breast specimens during this period and after excluding biopsies, recurrences, sarcomas, and secondary and benign lesions. Males accounted for 2.2% of total cases and 97.8% were female in agreement with (Hellgren, 2023). Reports on outcomes in male breast cancer compared to female breast cancer are conflicting. Most former studies report worse outcomes for male breast cancer compared to female breast cancer, but some indicate similar or even better survival for male breast cancer patients (Wu *et al*., 2016). The mean age at presentation was 48.21 years and 59.1% of cases were ≤50. Younger age at presentation as compared to the Western population (Sofi *et al*., 2012) was seen in our series which was in concordance with studies done in India and other countries. The left breast was involved more commonly than the right in concordance with Naeem *et al*., (2012). Invasive ductal carcinoma (IDC) was the most common histologic type comprising 95.8% of all invasive breast cancers, invasive lobular carcinoma (ILC) is less common and accounts for 4.2% of all invasive breast cancers (Engels *et al*., 2014). Fibroadenoma was the most common benign lesion in the present study in agreement with (Gupta *et al*., 2019). Triple-negative breast cancer (TNBC) accounts for 5% of all invasive breast cancers studied. Although the specific pathogenesis of TNBC has not been found, studies suggest risk factors may lead to its occurrence among the black and white population (Watkins 2019). This prevalence is low compared to (Stark *et al*., 2010) in a hospital-based study that reported a higher prevalence of TNBC in Ghanaian women (79%) compared with and high compared with African-American (32%) and white American women (10%)). Different prevalence has been worldwide, 10-13% in white patients (Amadou *et al*., 2014), 23-30% in African-American patients (Jiao *et al*., 2014), 82% in Ghana (Sakyi *et al*., 2022), 39% in Saudi Arabia (Moghimi *et al*., 2018), 19.3% in Chinese Mainland (Ma *et al*., 2012), and 15.9% in Taiwan, 10-19.2% in Hispanic which is much similar to the Japanese series (8-14%) (Kwong *et al*., 2010) and 31.7% in Abuja-Nigeria (Madukwe and Oboma, 2016).

Variations in the incidence and prevalence of TNBC in women of African ancestry include differences in methods of case ascertainment, population age structure, genetic and lifestyle risk factor distribution, access to mammography screening, over-estimation of ER and PR negativity, poor or unreliable laboratory standards for tissue handling, type of fixation used, and the initiation and duration of fixation can affect the optimum performance of immunohistochemical testing. A population-based case study of black women from the USA and Caribbean showed that, although having more children was associated with an increased risk of TNBC, the association with breastfeeding was not significant (Ambrosone *et al*., 2014). Another large population-based, case-control study of 873 African-American and 1072 white women with breast cancer living in California reported a significant trend between longer durations of breastfeeding and reduced risk of development of TNBC (ptrend=0·02), and oral contraceptive use was associated with a 2·9 times increased risk of women aged 45–64 years developing TNBC. (Ambrosone *et al*., 2014) A study carried out in the USA shows no relation between breastfeeding and TNBC. The Her-2/neu positivity was found to be 40% in this study; reports from other parts of Nigeria have shown values of 30.8%, 22.0%, 11.4%, 20.8% from Lagos (Sanni *et al*., 2019), Maiduguri (Imam *et al*., 2017), Nnewi (Nwammuo *et al.*, 2017) and Benin (Udoh *et al*., 2023) respectively. Previous studies have shown that using various molecular and IHC procedures, the frequency of amplification or over-expression of Her-2/neu is 10 to 52% with an average of 15 to 25% (16). (Grunda *et al*., 2012) found no statistically significant difference in Her-2/neu expression in breast carcinoma occurring in African Americans and Caucasians, having recorded figures of 38.7% and 40%positivity for Her-2/neu, respectively (16). These differences in prevalence might be due to pre-analytical variables, automated equipment, and tissue fixation. It is noteworthy that Seshie and colleagues reported 25.5% Her2/neu positivity in a retrospective analysis of the breast cancer subtype done in Korle Bu Teaching Hospital, Ghana, West Africa (Seshie *et al.*, 2015). Studies by Yau and co-workers reported Her-2/neu expression of 21.0% in breast cancer cases seen in Hong Kong, while Mahyar and colleagues (Mahyar, 2014) observed 38% among Iranian women with early-stage breast cancer. A comparative multicenter study that ensures a uniform protocol and minimal analytical variations would be necessary to explain the variations observed. The role of estrogen receptors (ERs) and progesterone receptors (PRs) in breast carcinoma treatment is well established. In a systematic study of breast cancer receptor status measured at diagnosis in a South African public hospital, it was observed that the majority (63%) of tumors were ER-positive in black breast cancer patients. Based on >1,000 receptor-characterized tumors in South African public hospitals, ER positivity was 65% overall and 63% in black women very similar to our present study with an ER-positive prevalence of 65%. (Alagizy *et al*., 2022) equally recorded 77.8% estrogen-positive cases and 62.1% progesterone-positive all ductal carcinoma studied in the pathology department of King Abdulaziz University Saudi Arabia.

CONCLUSION

We noted 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, such as the quality of tissue fixation and processing, varying staining techniques, and different criteria in scoring and reporting. It is also possible that the molecular phenotypes of African breast cancer is biologically diverse, and the result of regional population genetic differences, as well as environmental factors.

Conflict of Interest: No conflict of interest

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