Original Research Article

IMMUNOHISTOCHEMICAL PATTERN OF BREAST CANCER IN MAIDUGURI, BORNO STATE

Key Words: IMMUNOHISTOCHEMICAL, PATTERN, BREAST, CANCER MAIDUGURI

ABSTRACT

Background: Breast cancer is one of the most common cancer affecting women in Nigeria, with a very high morbidity and mortality rate if the diagnosis is delayed. It is common among women in both developed and developing countries of the world.

Objectives: This is carried out to determine the immunohistochemical and histopathological patterns of breast cancer in Maiduguri.

Methodology: One hundred and fifty two cases of female breast cancer were retrieved from the archive of Department of Histopathology, University of Maiduguri Teaching Hospital. ER, PR and HER2 expression was assessed using immunohistochemical staining.

Results:Thirty one of the 152 cases were positive for either one or two of the hormonal antigen,while 121 (79.6%) were completely negative for any of the hormonal antigen, of the 31 positive cases, oestrogenreceptors were detected in 14 (45.2%) cases,progesterone were detected in 10 (32.2%) of the cancer cases while HER 2 were detected in 7 (22.6%). The mean age of all the subjects with breast cancer is 47.6% with highest prevalence at the age range of 32 – 58.Invasive ductal carcinoma account for 88.2% of the total breast cancer followed by invasive lobular carcinoma with 4.0%.

Conclusion: From this study most cases of breast cancer in this environment are hormone receptor negative as found in most part of African continent in contrast to higher number of hormone receptor positive cases in most western and Arabian countries.

1. Introduction

Immunohistochemistry is a technique that combines anatomical, immunological and biochemical techniques to identify discrete tissue components by the interaction of target antigens with specific antibodies tagged with a visible label. Immunohistochemistry (IHC) has an expanding role in the diagnosis and management of mammary disease [1]. A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of IHC for solving everyday diagnostic problems in breast pathology [1].

The use of immunohistochemistry to further characterize breast cancer globally has introduced a new dimension to our knowledge of the disease. Breast cancer can no longer be regarded as a single entity and morphological features alone cannot completely predict the behavior of breast cancer [2]. The three immunohistochemical markers currently in routine diagnostic use in most countries are estrogen receptor (ER), progesterone receptor (PR) and Human epidermal growth factor2(Her2). These markers determine which tumours are likely to respond to hormonal therapy and Herceptin treatment [2]. It is generally acknowledged that breast cancer is a heterogeneous disease with a wide spectrum of clinical, pathologic and molecular features. The molecular classification is becoming the gold standard for complete characterization of breast cancer and the underlying technology has already generated gene-profiling models to predict outcomes [3]. Despite these remarkable achievements, in general, clinicians still rely on traditional clinic pathologic features and readily available tumor markers such as estrogen

receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). ER, PR, and HER2, routinely available in breast cancer specimens, are reliable, inexpensive, and useful for therapeutic decision making, and the results of these tests are recorded in cancer registries allowing for population-based research which make them a reasonable substitute for the more expensive molecular sub typing [4].

Breast cancer in women is a major public health problem throughout the world. It is the most common cancer among women both in developed and developing countries [5]. One out of ten of all new cancers diagnosed worldwide each year, is a cancer of the female breast [5]. It is also the principal cause of death from cancer among women globally. More than 1.38 million cases of breast cancer are diagnosed world -wide in 2008, representing 10.9 % of all cancer [5].

It is the second most common cancer now, after lung cancer, when ranked by cancer occurrence in both sexes. About 55% of the global burden is currently experienced in developed countries, but incidence rates are rapidly rising in developing countries [5].

In the National Cancer Institute, breast cancer came as number one in ranking malignant tumors constituting 17.5% of total malignancies. Females showed a vast majority of 98.35%, while only 1.65% were males [6]. Ductal carcinoma formed a majority of 85.02%, 2.04% of which were intraduct carcinomas. Hormone receptors were positive in 57.8% of cases, while Her-2/neu was positive in 44.5% of cases. Lymph nodes were positive for metastasis in 69.5% of cases [5].

Breast cancer is a heterogeneous disease whose evolution is difficult to predict.

Consequently, treatment is not as adapted as it should be. Gene expression studies have identified five molecularly distinct subtypes of breast cancer that have prognostic value across multiple treatments and can predict distinct clinical outcomes. These subtypes are termed hormone receptor(s) positive luminal A (luminal A), hormone receptor(s) positive luminal B,

luminal HER2/neu, HER2-enriched (i.e, tumors that over express ERBB2-associated genes but do not express genes that define the luminal subtype) and basal-like (triple negative) [7].These subtypes are associated with differences in clinical outcome, HER2-enriched and basal-like subtypes are hormone receptor negative and have poorer prognosis with shorter survival times than other types [8] .

In contrast, the expression of hormone receptor(s) characterizes the luminal breast cancers, with luminal B tumors having intermediate survival time & poorer outcomes than luminal A tumors having the longest survival [9].

Although some luminal B tumors can be identified by their expression of HER2, the major biological distinction between luminal A and B is the proliferation signature, including genes such as MKI67 (encoding Ki67), which has higher expression in luminal B tumors than in luminal A tumors. Thus, a distinction between luminal A and B tumors that is based on proliferation status among hormone receptor(s) positive luminal patients may be important to breast cancer biology and prognosis since luminal B tumors having a higher rate of tumor cell proliferation and poorer prognosis than luminal A tumors. Thus luminal A and B breast cancers appear to be distinguished by the expression of estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 proteins [10].

The Nottingham modification of the Scarff-Bloom-Richardson (NSBR) histological grading system for invasive breast cancer has been recommended by the World Health Organization (WHO) [11].

In the NSBR system, histological grading consists of three components: tubule formation, nuclear pleomorphism and mitotic count. Each of these are allocated a score of 1–3, and the final histological grade is determined according to the sum of the three components (grade 1: sum=3–

5; grade 2: sum=6–7; and grade 3: sum=8–9). Patients with the luminal A subtype were less likely to have grade 3 tumors while patients with triple negative tumors had the greatest likelihood of having grade 3. The high cost of gene expression profiling has limited its incorporation into most randomized clinical trials, and therefore, immunohistochemistry-based surrogate assay is proposed to distinguish between various breast cancer subtypes with emphasis on the role of the Ki-67 labeling index as a clinically valuable biomarker for the luminal B subtype [12].

2. Methodology

Study area

The study was carried out at the Department of Histopathology University of Maiduguri Teaching Hospital, Maiduguri.

Study design

Formalin fixed paraffin embedded sample was obtained from the archive of the Department of Histopathology, UMTH. 5 years (January 2011- December 2015) breast cancer positive cases were considered. The case to study composed of all diagnosed breast cancers one representative block was selected from each case if more than one block were retrieved from the archive.

Inclusion and Exclusion Criteria

The inclusion criteria were the breast biopsies paraffin blocks with complete patients' data during the study period. All other patients were excluded in the study including the patients with incomplete data.

Immunohistochemical method

Paraffin blocks were sectioned at four micrometer thickness, mounted on a slide and placed in the oven for 30mins. The sections were deparafffinised by passage through changes of xylene for 5 minutes each and subsequently rehydrated in descending grades of alcohol. It was then washed in buffer. The slides were incubated in hydrogen peroxide block for 10 minutes (to reduce non specific background staining due to endogenous peroxidase). They were then washed 4 times in buffer, ultra V block was applied and incubated for 5 minutes to block nonspecific background staining. primary antibody was applied for 30 minutes, then washed 4 times in buffer, primary antibody enhancer was applied and incubated for 10 minutes at room temperature, HPR polymer was applied and incubated for 15 minutes at room temperature, they were then washed 4 times in buffer and 1 drop of DAB plus chromogen substrate was added to 2mls of DAB plus substrate. It was mixed, applied to the tissue and it was finally washed 4 times in distilled water, counter stain with heamatoxylene and mount with DPX mountant [13].

Interpretation of slides

Staining intensity of immunohistochemically stained sections were semi quantitatively evaluated using the Quickscore scoring system for PR and ER and DAKO scoring system for HER2.

The proportion of positive cells (scored on a scale of 0 to 5) and staining intensity (scored on a scale of 0 to 3) were summed to produce total scores of 0 to 2 though 8.A score of 0 to 2 were regarded as negative while 3 to 8 as positive. For HER2, a zero score defines tumors with no

staining or membrane staining in less than 10% of the tumor cells, while 1+ refers to tumors with a faint membrane staining in more than 10% of the tumor cells. A weakly positive result characterized by weak to moderate complete membrane staining in more than 10% of the tumor cells is represented by a 2+ score, while a strongly positive result defined as strong complete membrane staining in more than 10% of the tumor cells is represented as 3+. Scores of 0, 1+ was classified as negative, while a score of 2+ and 3+ Was regarded as positive [14].

3. Results

The result of the study carried out to determine the immunohistochemical pattern of breast cancer in Maiduguri over the period of five years revealed a breast cancer prevalence of 13.9%.A total of one hundred and fifty two (152) cases of breast cancer specimen found over the period of the study had immunohistochemistry done on them..The result revealed only 31(20.4%) of the one hundred and fifty cases of breast cancer were positive for either one or two of the hormonal antigen while 121 (79.6%) were completely negative for any of the hormonal antigen.Of this 31 positive cases, oestrogen receptor were detected in 14(45.2%) cases,progesterone receptor were detected in 10(32.2%) of the cancer cases while HER2 were detected in 7(22.6%) of all breast cancer cases.(Table 4.1). The mean age of all subjects with brain cancer is 46.7 (53.3%) with highest prevalence of cancer at the age range of 32 -52 followed closely by 53- 67 age range having 23% prevalence (Table 4.2). The result of histopathological pattern of the breast cancer in this environment showed 134 (88.2%) were invasive ductal carcinoma followed by invasive lobular carcinoma (4. 0 %) and the other ranging from 1-2% prevalence (Table4 .3).

Statistical Analysis: The results were analyzed using SPSS statistical package.

Table 4.1: Frequency of distribution of breast cancer patients by age groups

Age group	Frequency	Percent
<= 22	2	1.3
23 – 37	28	18.4
38 – 52	81	53.3
53 – 67	35	23.0
68 – 82	5	3.3
83+	1	.7
Total	**152**	**100.0**

Figure 4.1; Histogram of the frequency distribution by age groups of the Patients

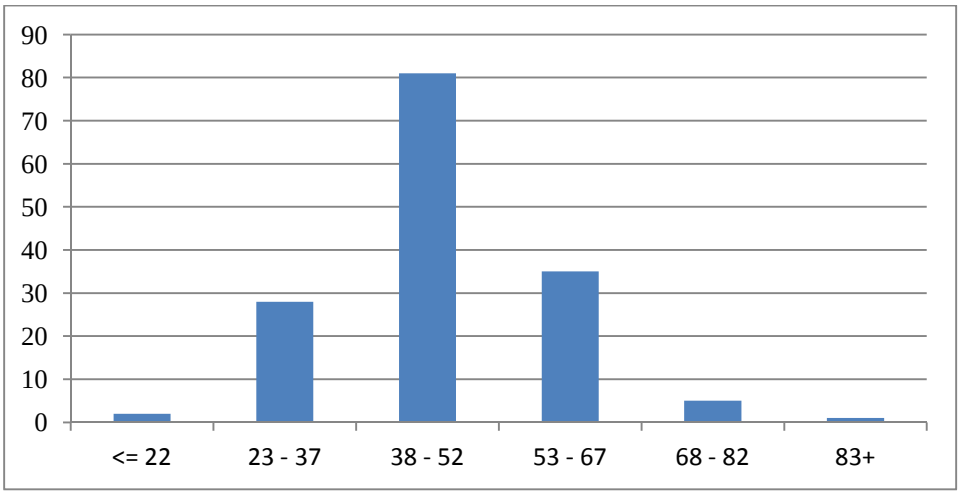

Table 4.2: Distribution of breast cancer by clinicopathological features

DIAGNOSIS	FREQUENCY	PERCENT
IDCA	134	88.2
METAPLASMIC CA	1	.7
ILCA	6	4.0
MEDULLA CA.	2	1.3
INV. PAPILLARY CA	5	3.3
ADENO CA	1	.7
APOCINE CA	1	.7
MUCINOUS CA	1	.7
CARCINOSARCOMA	1	.7
Total	**152**	**100.0**

Key: IDCA = Invasive Ductal Carcinoma

ILCA = Invasive Lobular Carcinoma

Figure 4.2: Chart of breast cancer by clinicopathological features

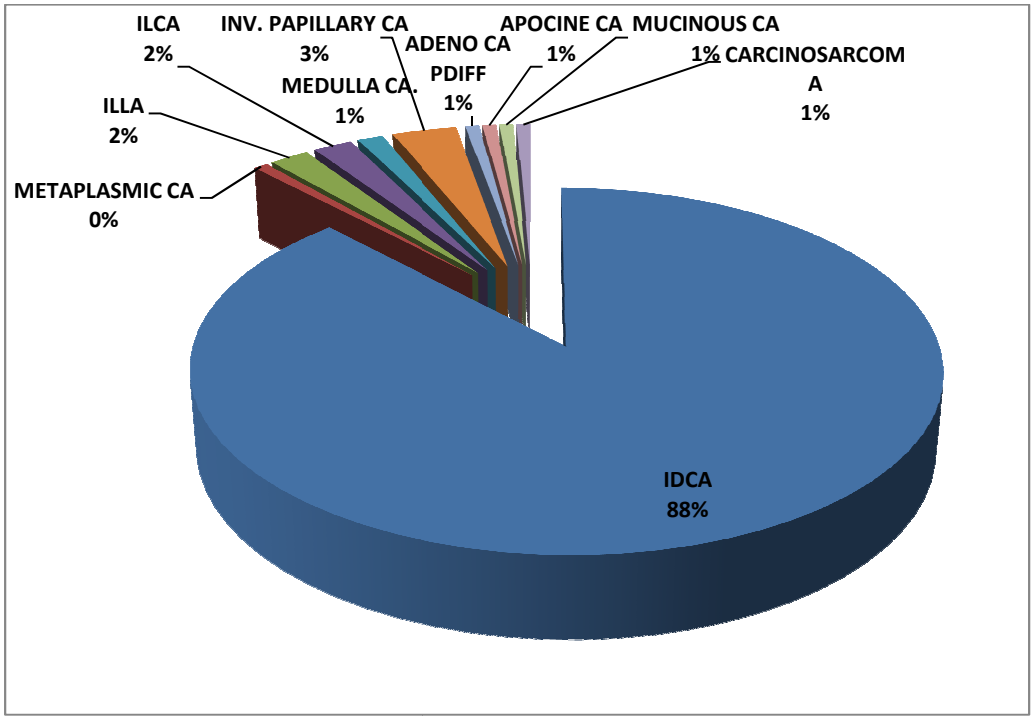

Table 4.3: Expression of ER, PR and HER2 in cases

Marker	Positive (>3)	Negative (0-2)	Total
ER	14 (45.2%)	37(72.5%)	**51**
PR	10 (32.2%)	41 (80.4%)	**51**
HER2	7 (22.6 %)	43 (86%)	**50**
Total	**31**	**121**	**152**

ER=Estrogen receptor; PR=Progesterone receptor; HER2/neu=Human epidermal growth factor receptor 2

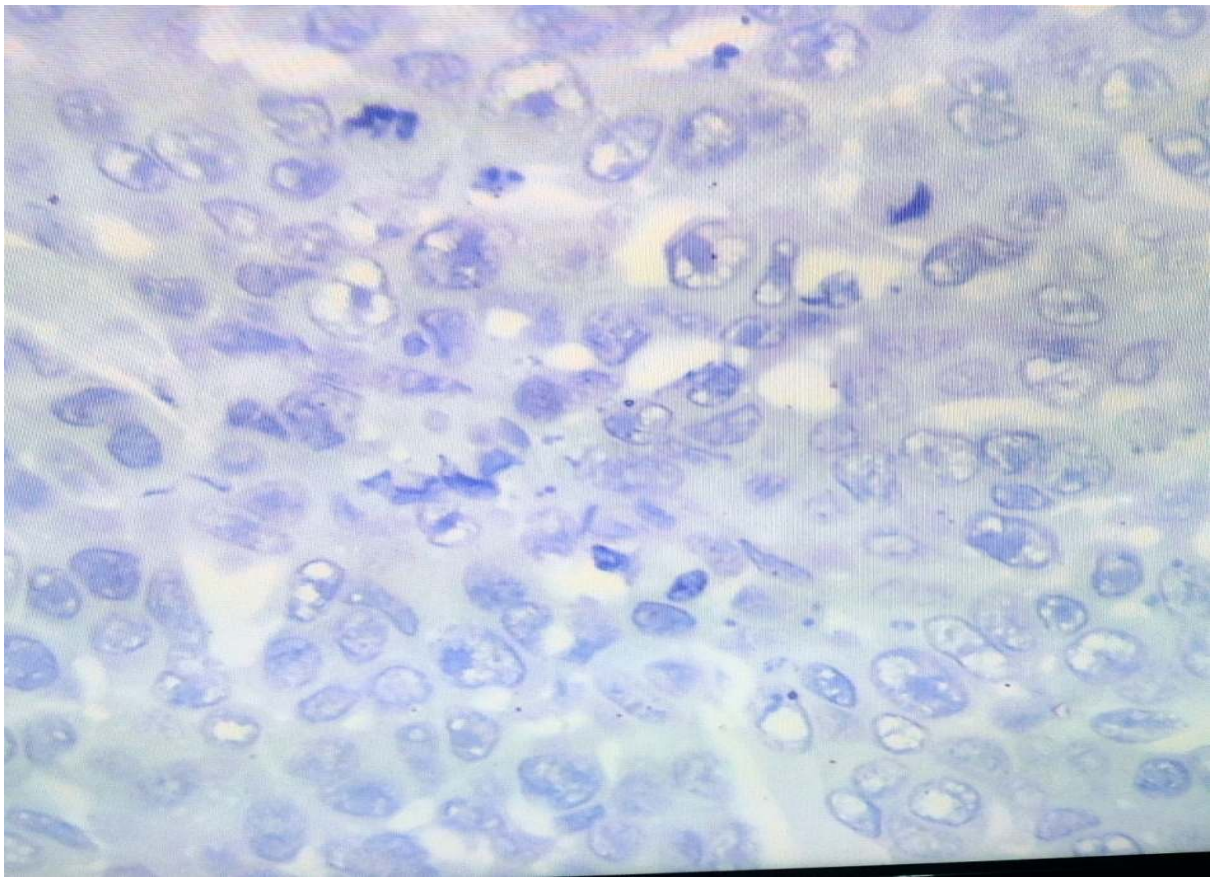

Fig4.3 Photomicrograph of IDC showing negative membrane staining for HER2 X 100

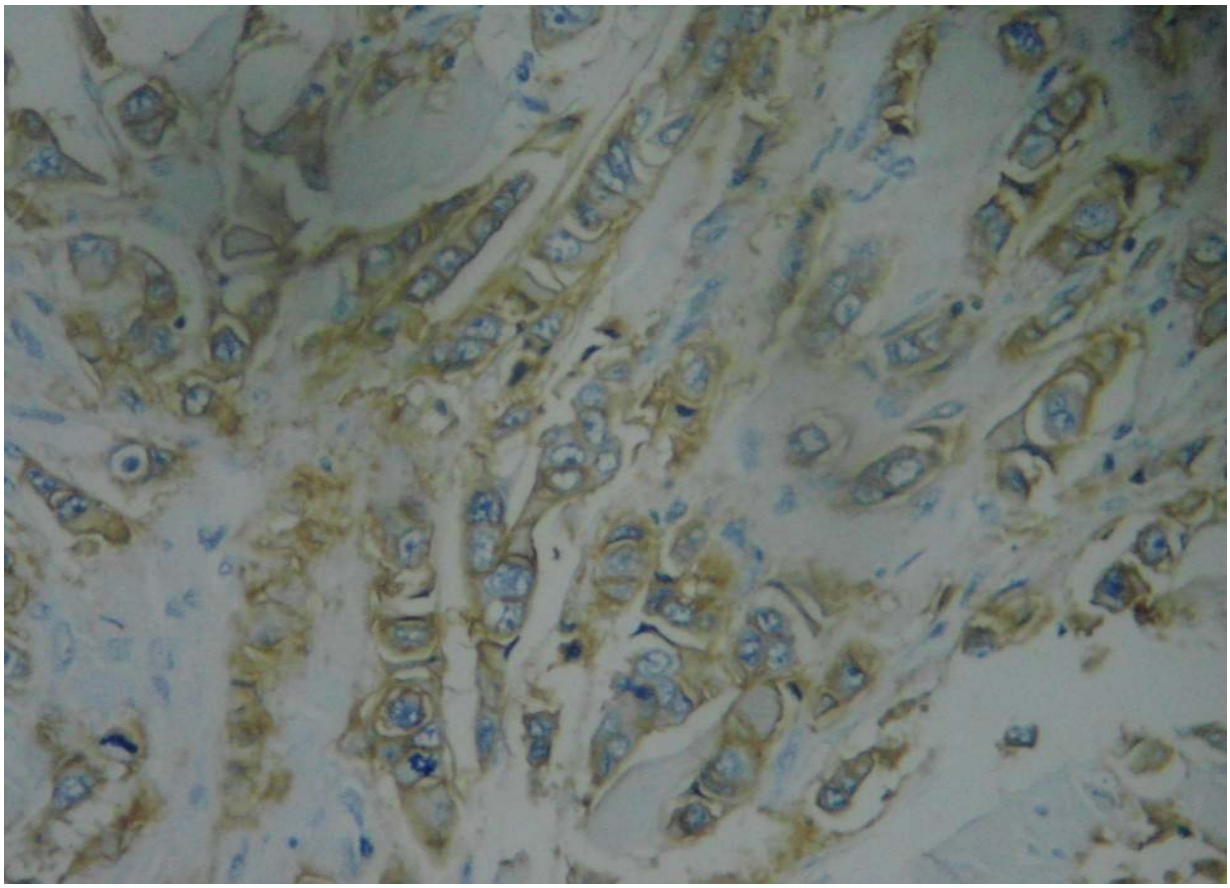

Fig 4.4 Photomicrograph of IDC showing positive membrane staining for HER2 X 100

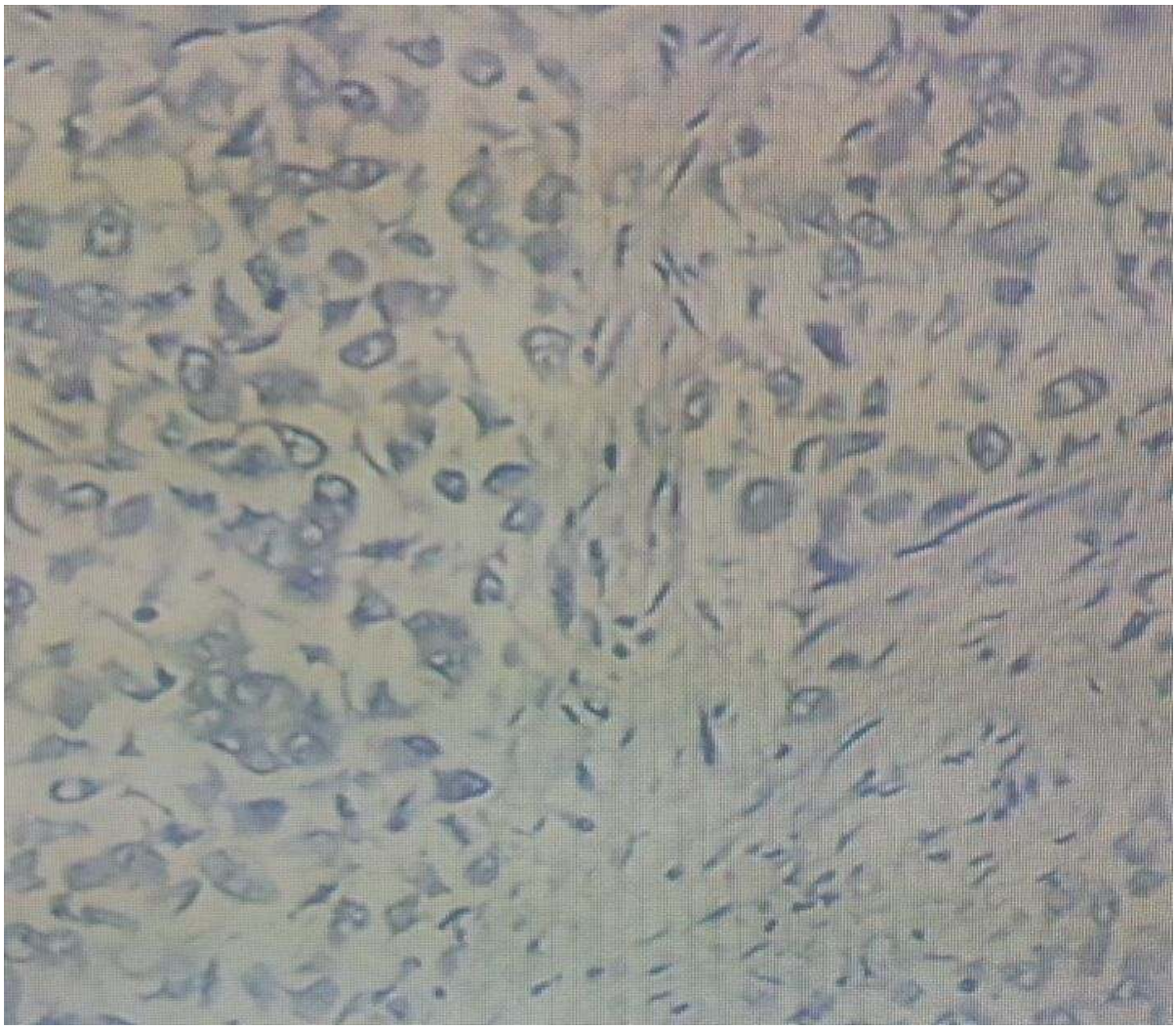

Fig 4.5 photomicrograph of IDC showing negative nuclei staining for ER X100

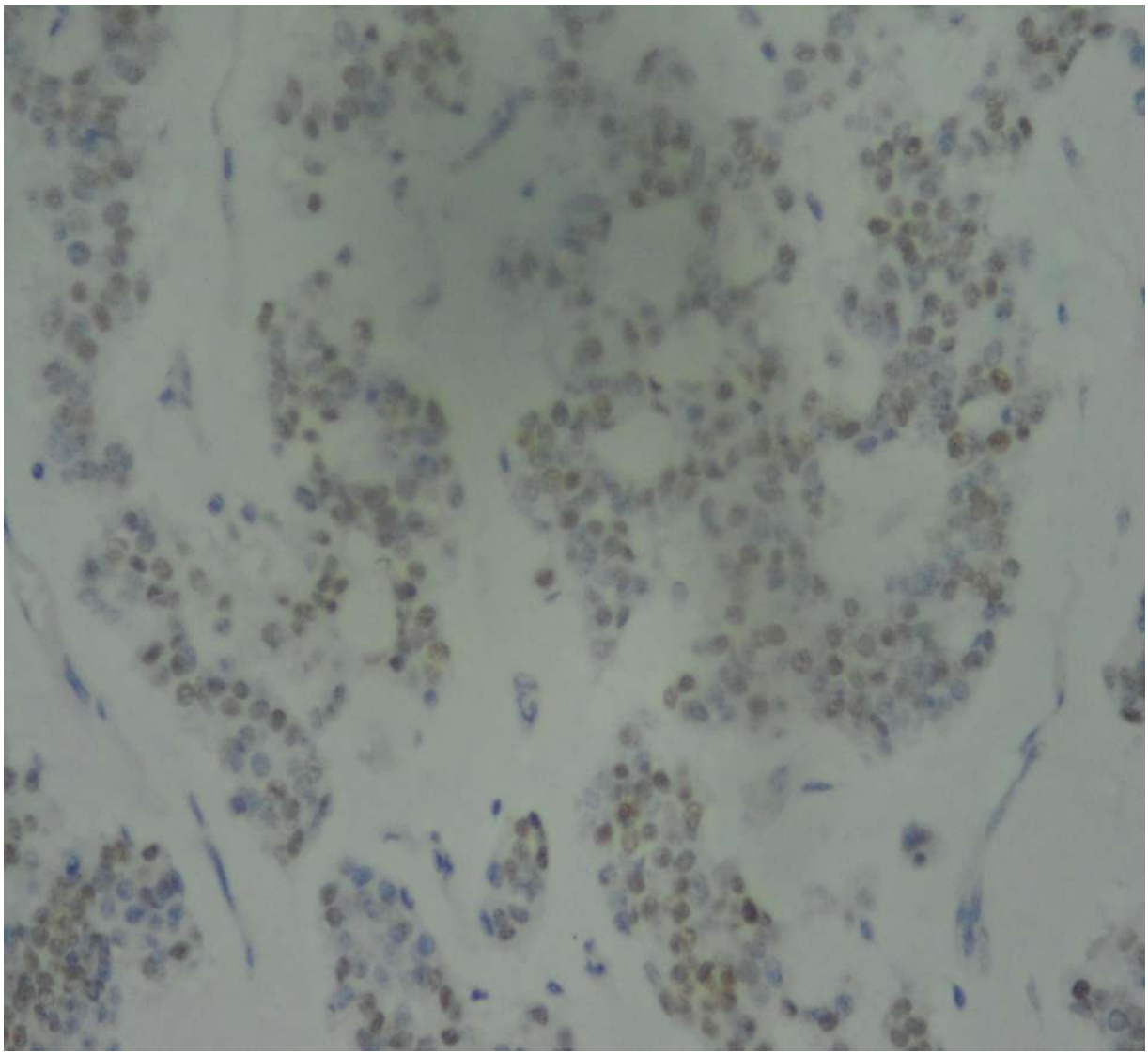

Fig 4.6 Photomicrograph of IDC showing positive nuclei staining for ER X100

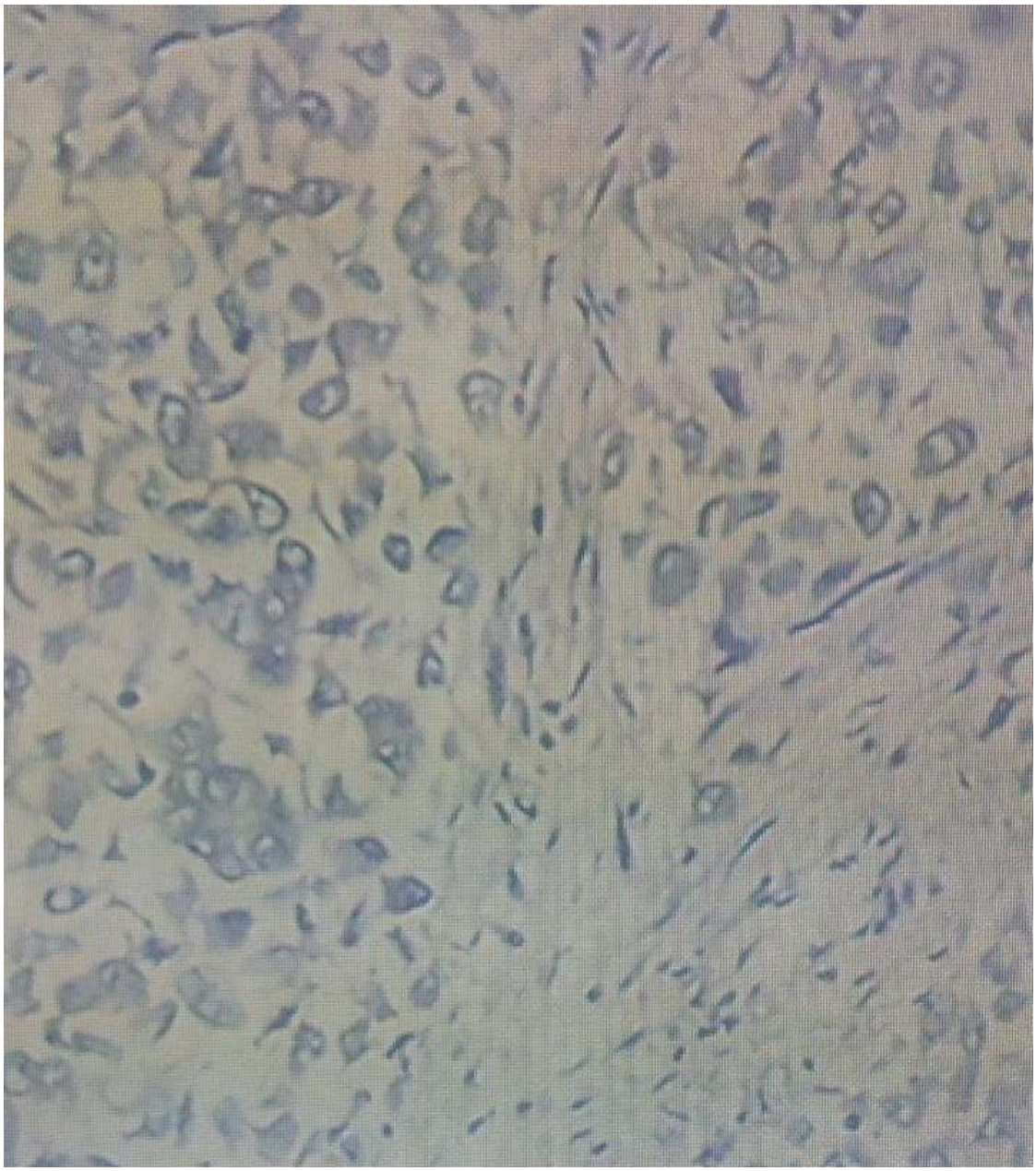

Fig 4.7 photomicrograph of IDC showing negative nuclei staining for PR X 100

4. Discussion

Immunohistochemistry based classification of both ER, PR, and HER2 status provide prognostic and therapeutic information not achivable from either alone. The use of IHC in breast cancer has become an integral part of a complete and comprehensive histopathology report, in terms of prognosis and prediction of response to treatment, in addition to histological grade and tumor sub types, hormone marker ER, PR and HER2 has become the mainstay requirement for the oncologist in the developed world, assessment for hormonal receptors expression status is required to determine patient eligiabity for hormonal therapy. However, in the developing countries clinicians administer hormonal therapy without any knowledge of their patient receptors status.ER, PR and HER2 expression status is not routinely determined in the developing countries because of limited resources and relatively high cost of testing.

The result of the immunohistochemical pattern of breast cancer in this study revealed that ER was positive in 45.2%, PR was positive in 32.2 % while HER 2 was positive in 22% cases.

This is a little slightly lower than the report carried out in Ibadan by [1] that show 65.1% ER positively, 54.7% PR positively and 79.7% HER 2 negative. But inline with the report of Nwotor*et al*.,2014 with ER positive in 54.2% cases while PR was seen in 50% with HER 2 present 1n 31%. Recently [15] reported a similar study in Abuja with ER positive in 46.3% and PR positive in 42.6%.

In Ile-Ife a studied carried out by [16] reported ER positively in 34.6% PR positively in 25% and HER 2 positivity in 38.2% which is also in line with this study.

In Ghana,it was reported an ER, PR and HER2 receptor positivity of 32.1%, 25.6% and 22.5% respectively, recently in AI Khobor Saudi Arabia (S.A) the rate of positive hormone receptor and

HER2 in breast cancer using IHC were 69.2%, 61.5 % and 25.1% for ER, PR and HER2 respectively.In China ER was positive in 53%, PR was positivein 51.5% and HER 2 in 46.2% [17]. In the Arabian countries, the frequency of the IHC positive hormone receptor and HER2 show great variation, Runnak and colleagues in 2012 investigated 514 cases of breast cancer in Iraq females of different origin, Arabic and Kurdish, they found that 73% were ER positive, 64.2% where PR positive only 20.4% of breast cancer cases were HER2 positive.The low rate of IHC staining positive for ER, PR and HER22 in Maiduguri is in harmony and fall in the same range of other populations in Nigeria [18] and Ghana on the other hand the rate of positivity in ER, PR and HER2 in Iraq, Egypt and USA [19, 20]. Shows high rate of positivity.

Alternatively contributing factor to those finding could be biological and lifestyle aspect.

The mean age of all subject in the study was 46.7 years, this is similar to mean age of 49.7 years, 48.1 years and 47.5 years reported in Nigeria, Senegal and India respectively but less than mean age of 55-58 years reported in Western countries like USA [21].

This might be as a result of good screening programme in this developed countries and also presence of good diagnostic facility that will enable early diagnosis and treatment.

The majority of breast cancer in this study were Invasive ductal carcinoma with 88.2%.

5. Conclusion

From this study, it can be concluded that most cases of breast cancers are hormone receptor negative as found in most part of the African continent in contrast to highest number of hormone receptor positive cases of breast cancer in most Western and Arabian countries. The prevalence of hormone receptors positive breast cancer stand at 20.4% with ER accounting four 45.2% of

the hormone receptor positive cases while PR positive account for 32.2% and HER 22.6%.The mean age of the subject is 46.7. The histopathological pattern of breast cancer in this study revealed that 88.2% of all breast cancer are invasive ductal carcinoma.

Consent Disclaimer:

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

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