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| RESEARCH ARTICLE

Physiological and Molecular Characterization of "Hormonal Receptors and "HER-2/Neu" in Breast Cancer

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| ABSTRACT
The hormonal, reproductive and demographic factors all affect the clinical course of breast cancer, which is an important health problem that remains unresolved. CAans and MVG: / Comparison of primary versus metastatic breast cancer characteristics This study aimed to evaluate the characteristics of PBC + MBC. A descriptive analytical study was performed on the patients with primary and metastatic breast cancer to identify demographic, reproductive, clinical, histopathological, and hormonal factors. The average age of the patients was 42 years. The incidence of primary breast cancer occurred at 51–55 years, whereas for metastatic disease, it was at 36–40 years. In 22.5% of primary patients, a history of AML was present and 35% had metastatic disease. Precocious menarche at 12–14 years has been described in 57% patients. The majority of patients were multiparous (75%) and 72% experienced their primiparity at or ≤25 years. Breast feeding accounted for 55.2% of primary and 47.1% of metastatic patients. The majority of patients were post-menopausal women. Incidence of axillary lymph nodes was 85% in primary and 80% in mets. Stage II was the most common (60%) followed by stage IV (26%). IDC accounted for the most common type of tumor (81%). Primary–metastatic sites, hormone receptors ER 67.8% and 54.66%, PR 66.6% and 63%; HER2Neu was significantly more positive in metastasis cases (62.7%) than in primaries (53.5%). Breast cancer in the research population is early onset, and a high proportion of the cohort developed advanced disease. Reproductive factors and hormones, molecular tumor features have the tendency to shape the course of disease, emphasizing early diagnosis and personalized screening programs.

| KEYWORDS
Breast cancer; Reproductive factors; Estrogen receptor (ER)"; Progesterone receptor (PR)", "Immunohistochemistry".

| ARTICLE INFORMATION
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1. Introduction

Breast cancer is diagnosed as the most common malignancy and the most common cause of cancer-related mortality in women worldwide, with a significant burden worldwide [1,2]. Breast cancer is still a significant public health problem despite advances in early detection, diagnosis and treatment, including the development of treatment options, particularly in low and middle-income environments with relatively late diagnosis rates and limited treatments [3,4]. Breast cancer is a complex, heterogeneous disease characterised by a variety of histopathological features, molecular profiles, and clinical behaviors [5]. The pathogenesis and development of breast cancer are driven by the interaction of genetic, hormonal, reproductive, environmental, and lifestyle factors [6]. Reproductive factors like age at menarche, age at menopause, parity, first full-term pregnancy and breastfeeding history have been studied intensively for breast cancer incidence in various studies for women [7–9]. Finally, non-reproductive factors such as family histories, use of hormonal contraceptives and infertility treatments have also been known to increase susceptibility and progression of breast cancer [10–12]. Hormonal exposure plays a crucial role in breast cancer, especially through the channels of

estrogen and progesterone which are related to the disease process [13]. Estrogen receptor (ER) and progesterone receptor (PR) status are the most well known prognostic and predictive markers used and should facilitate the choice of endocrine treatment and affect a patient's state [14,15]. ER and PR-expressing tumors have been associated with better prognosis and better receptor-positive response compared to receptor-negative tumors [16]. Another important biomarker is human epidermal growth factor receptor-2 (HER2), which has been found to be involved in cell proliferation, survival and metastasis in breast cancer [17]. Overexpression or amplification of HER2 has been linked with aggressive tumor behavior, high recurrence rate, and poor prognosis yet the recent availability of targeted therapies for anti-HER2 has greatly benefited the survival in affected patients [18,19]. As such, precise measurement of ER, PR, and HER2 expression is necessary for planning personalised treatments and for prognostic stratification [20]. Due to its trustworthiness, availability and clinical significance, immunohistochemistry (IHC) is still considered as the gold standard for investigating hormone receptor and HER2 signaling in breast cancer tissues [21]. Molecular subtypes for ER, PR, and HER2 have heterogeneity between populations and geographic regions, indicative of genetic diversity and differences from environmental to reproductive factors [22]. Breast cancer tends to onset at a younger age and at a later stage as compared to Western women in Iraq and countries in the Middle East [23]. There is very scarce regional information for the association of reproductive and nonreproductive risk factors with the hormone receptor status, most especially, in metastatic breast cancer [24]. It is important to know these associations to improve early detection strategies, facilitate therapeutic decisions and region-specific prevention and treatment [25]. Thus, the objective of this paper is to identify reproductive and non-reproductive risk factors that exist in Iraqi women who have been diagnosed with primary and metastatic breast cancer and to analyse the immunohistochemical expression of estrogen receptor, progesterone receptor and HER2, as they are important at clinical and therapeutic levels.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

All of the chemicals and reagents included in the study material are of analytical quality. They were made up of: xylene, hematoxylin, eosin, ethanol alcohol, paraffin wax, formaldehyde solution (10% neutral buffered formalin), phosphate-buffered saline (PBS), distilled water, and DPX mounting medium. The immunohistochemical assays of the ER, PR, and HER-2/Neu were processed by commercially available kits (Dako, Denmark/North America). Table 3.1 below provides a precise list of the chemicals and their sources.

2.1.2 Equipment

The standard laboratory equipment: automatic tissue processor, paraffin dispenser, microtome, hot plate, water bath, oven, centrifuge, microscope, microwave, staining jars and slide holders (Thermo, UK). Histopathological and immunohistochemical procedures were performed using standard laboratory equipment: an automatic tissue processor, microtome, hot plate, water bath, oven, centrifuge, microscope, microwave, staining jars and slide holders (Thermo, UK). Positively charged slides, Pap pens, micropipettes, syringes, coverslips, and other consumables were obtained from certified manufacturers (Table 3.2).

2.2 Study Population

This study was done from November 2010 to May 2011 at Marjan Hospital's Oncology Unit. A total of 100 women diagnosed with breast cancer were enrolled randomly after informed consent, with no exclusion criteria other than refusal to participate. We divided patients into two groups: 80 of whom had primary breast cancer (prospective) and 20 of whom had metastatic breast cancer and were re-presenting (retrospective). Patients were 26-75 years old with a median age of 42 years..

2.3 Research Method

2.3.1 Clinical, Reproductive, and Medical History

A comprehensive reproductive and medical history was collected from each patient using a standardized case history questionnaire. Age at menarche and menopause, parity, breastfeeding history, contraceptive use, infertility treatment history, and family history of breast or ovarian cancer were obtained from all patients.

2.3.2 Fine Needle Aspiration Cytology (FNAC)

Fine needle aspiration was performed under aseptic conditions using 20–27 gauge needles. The needle was inserted into the center of the lesion, and cellular material was obtained by gentle suction. Multiple passes were performed to ensure adequate sampling. Local anesthesia was not routinely used, as FNAC is minimally invasive.

2.3.3 Tissue Fixation and Processing

Samples from excisional biopsy or mastectomy were fixed in 10% neutral buffered formalin to maintain tissue structure. Tissues were processed according to standard procedures, including dehydration with graded ethanol, clearing with xylene, and infiltration with molten paraffin wax using an automatic tissue processor. The paraffin blocks were prepared, and 5 µm-thick sections were sliced and stained with hematoxylin and eosin (H&E) for routine histological study.

2.3.4 Histopathological Examination

Sections of H&E were observed independently by two histopathologists with the help of light microscopy. Tumors were categorized based on World Health Organization (WHO) classification, graded for differentiation, and staged by the TNM system according to the American Joint Committee on Cancer (AJCC) guidelines.

2.3.5 Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were immunostained using the EnVision™ detection system (Dako), a two-step polymer-based method. Five-micron sections were mounted on positively charged slides, deparaffinized in xylene, rehydrated through graded alcohols, and subjected to antigen retrieval using EnVision™ Flex target retrieval solution. Endogenous peroxidase activity was blocked, followed by incubation with primary antibodies against ER, PR, and HER-2/Neu. ER and PR were detected using monoclonal mouse anti-human antibodies, while HER-2/Neu was detected using polyclonal rabbit anti-human c-ErbB-2 antibody. Detection was achieved using horseradish peroxidase (HRP) and diaminobenzidine (DAB) as the chromogen, with hematoxylin counterstaining. Slides were then dehydrated and mounted for microscopic evaluation.

2.3.6 Interpretation and Scoring

HER-2/Neu Evaluation

HER-2/Neu immunoreactivity was assessed in accordance with the standardized HercepTest scoring system. Membranous staining intensity and distribution were evaluated in invasive tumor cells and scored as follows: score 0, complete absence of membranous staining; score 1+, faint or barely perceptible incomplete membranous staining observed in more than 10% of tumor cells; score 2+, moderate and incomplete membranous staining in more than 10% of tumor cells; and score 3+, strong, complete, circumferential membranous staining in more than 10% of tumor cells. Tumors exhibiting scores of 2+ or 3+ were considered HER-2/Neu positive.

Estrogen and Progesterone Receptor Evaluation

Estrogen receptor (ER) and progesterone receptor (PR) expression were evaluated semi-quantitatively based on the proportion and intensity of nuclear staining in tumor cells. Receptor status was classified as score 0, absence of detectable nuclear staining; score 1+, weak expression; score 2+, moderate expression; and score 3+, strong expression. Tumors showing any degree of positive nuclear staining (≥1+) were considered hormone receptor–positive.

3.Results

This investigation evaluated the demographic and reproductive features of both primary and metastatic breast cancer patients. The mean age of the enrolled cohort was 42 years. As illustrated in Figures 1 and 2, the distributions of age differed when dividing the disease into two groups, with primary breast cancer occurring most commonly in the 51–55 years age group, while metastatic breast cancer experienced the peak incidence at an earlier age, especially in the 36–40 years age group. In 22.5% of patients with primary disease and 35% of metastatic cases, a positive family history of breast cancer has been reported, as shown in Figure 3. In terms of reproductive characteristics, age distribution at menarche is shown in Figure 4, with most patients (57%) reporting menarche at 12–14 years, 40% at 15–17 years, and 3% at ≤11 years. As depicted by parity status, 75% of them were multiparous and 25% nulliparous (Figure 5). The age at first pregnancy illustrated by Figure 6 showed that most had their first pregnancy at ≤25 years; for instance, 34.7% at ≤20 years and 37.3% at 21–25 years. Figure 7 shows the distribution of the number of children where the largest proportion of patients had 3–4 children (44%) followed by 20% with 1–2 children, while lower proportions are observed in higher parity categories. Facts 8, 9 summarize child feeding practices in multiparous patients. In primary breast cancer 55.2% of patients used breastfeeding, 29.3% used artificial feeding, and 15.5% added an intermediate or mixed feeding (Figure 8). Breastfeeding was recorded for 47.1% of patients with metastatic breast cancer, 17.6% artificial feeding, and 35.3% mixed feeding (Figure 9). Menopausal status on presentation is depicted in Figure 10, and 60% of subjects were postmenopausal versus 40% that were premenopausal. Figures 11–16 together depict alternative clinical features related to disease progression. Figure 11 shows the predominance of late menopause, indicating long-term (lifetime) exposure to estrogen, an established cause of breast carcinogenesis. Similarly, findings suggested a hormonal component in tumor features, as observed in Figures 12 and 13, in which tumors had different laterality in primary/metastatic cases, a pattern familiar within clinical breast cancer spectrum studies without a definitive biological predominating pattern. Axillary lymph nodes, shown in Figures 14 and 15, were predominantly present in both primary and metastatic breast cancer, presenting advanced local disease while still underscoring the prognostic role of nodal status. The oral contraceptive pill history in Figure 16 demonstrates that a large portion of patients were exposed to hormonal contraceptives in the past leading us towards cumulative hormonal factors associated with the development of breast cancer. Together, in the final analysis, the consolidated findings of Figures 1–16 seem to support a consistent relation between the age

distribution, reproductive history, menopausal status, tumor laterality, lymph node involvement, and hormonal exposure that influence the symptoms and course of primary and metastatic breast cancer.

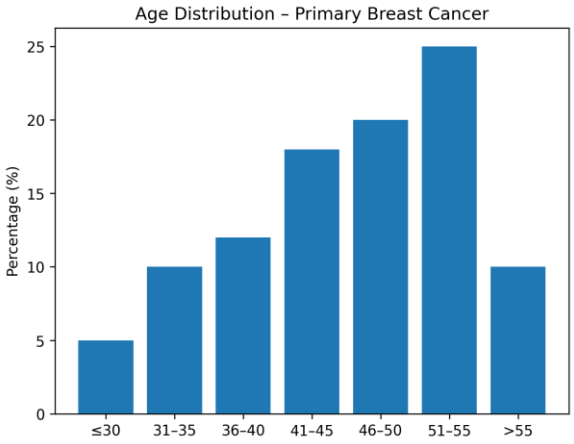


Figure 1. Age Distribution – Primary Breast Cancer

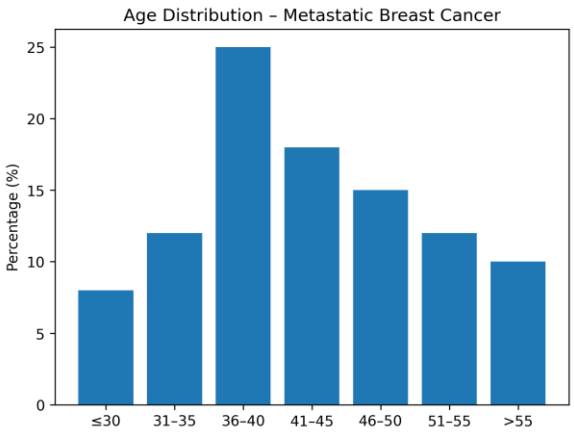


Figure 2. Age Distribution – Metastatic Breast Cancer

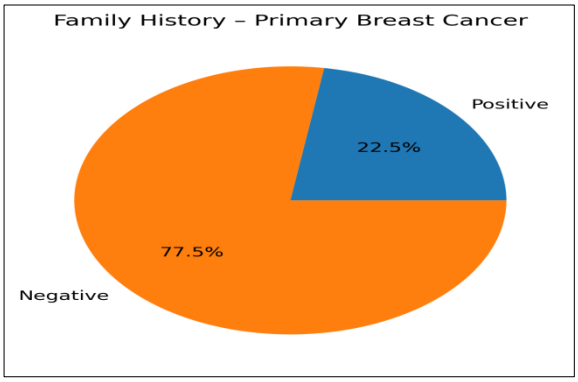


Figure 3. Family History – Primary Breast Cancer

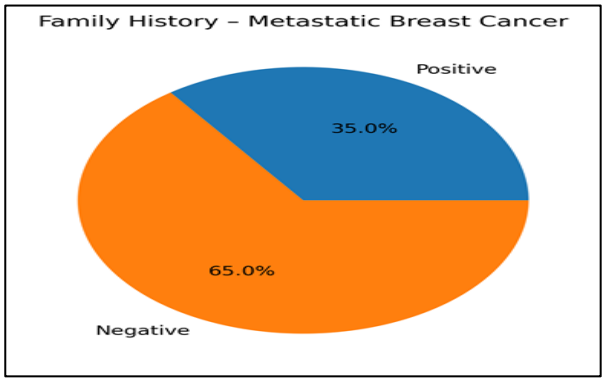


Figure 4. Family History – Metastatic Breast Cancer

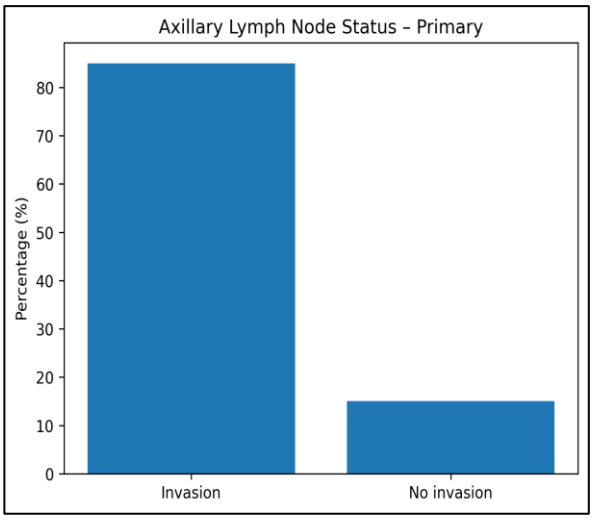


Figure 5. Axillary Lymph Node Status – Primary Breast Cancer

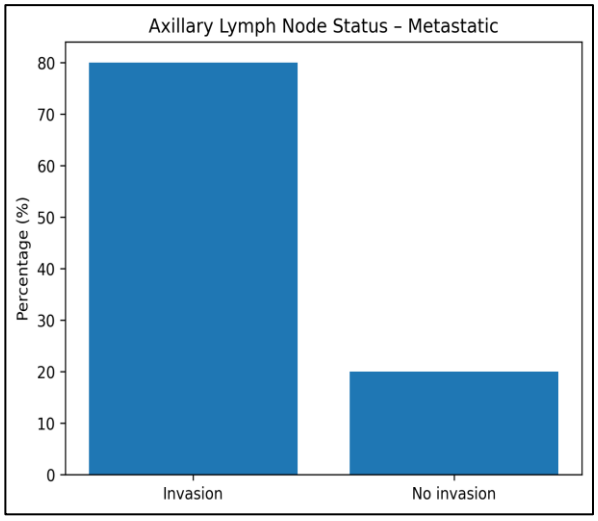


Figure 6. Axillary Lymph Node Status – Metastatic Breast Cancer

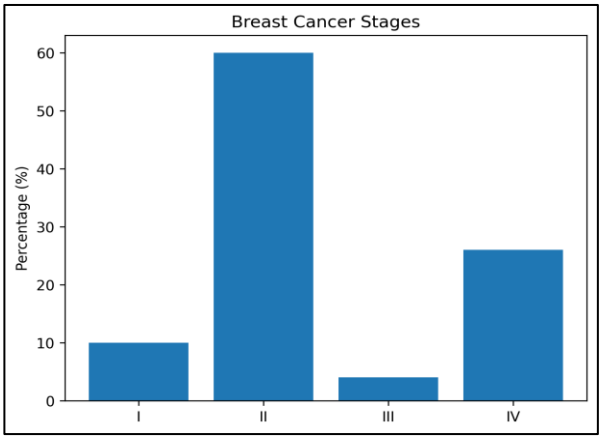


Figure 7. Breast Cancer Stages

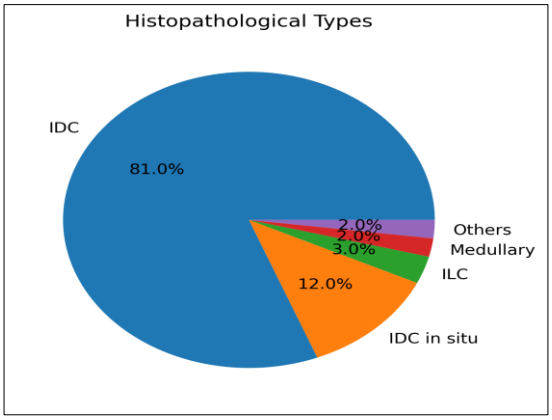


Figure 8. Histopathological Types of Breast Cancer

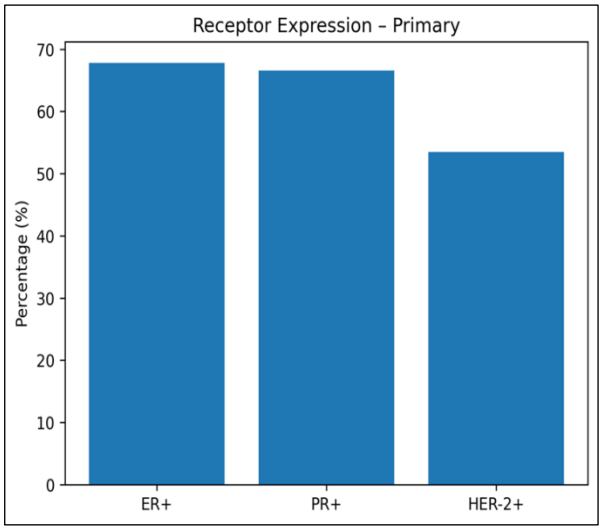


Figure 9. Receptor Expression – Primary Breast Cancer

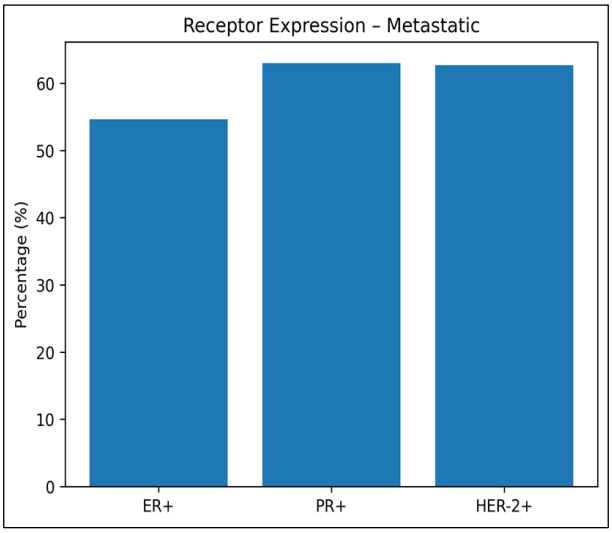
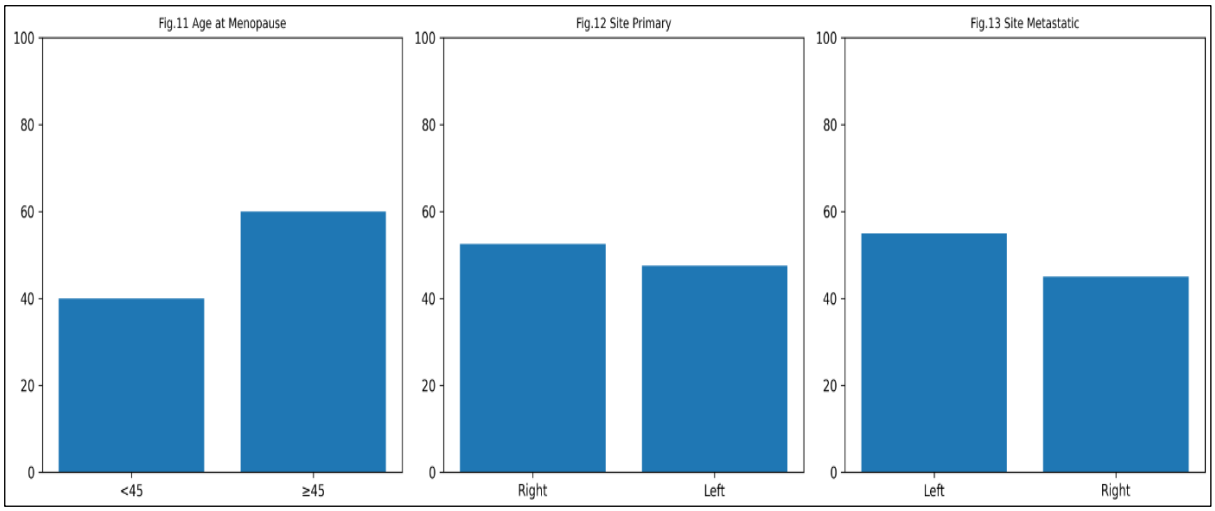
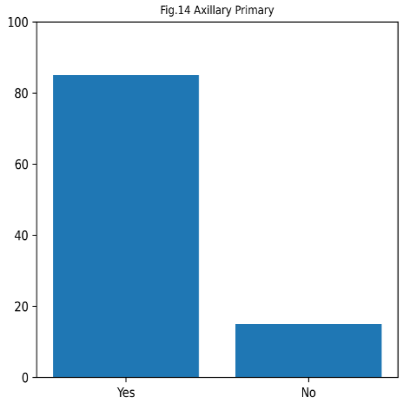
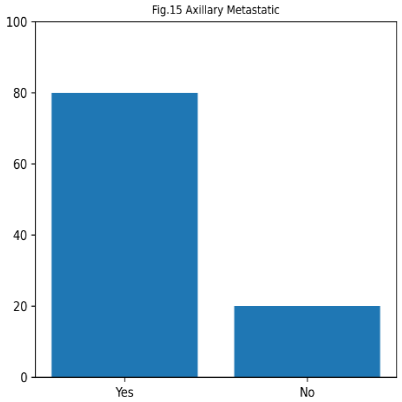
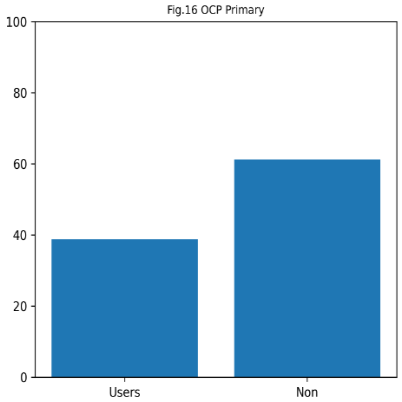


Figure 10. Receptor Expression – Metastatic Breast Cancer









4. Discussion

The purpose of the present study is to provide a complete investigation of the demographic, reproductive, clinical, and hormonal characteristics of patients with both primary and metastatic breast cancers. To these, we throw light on some interdependent factors that seem likely to influence how the disease expresses and advances. The mean age of patients in our cohort, as we describe it, was 42 years, which is relatively younger than that reported for many Western populations. These findings are supported by earlier reports showing that in the developing world, breast cancer cases seem to occur at earlier ages than in developed countries (due to differences in genetic predisposition, pattern of reproduction, environmental exposures and screening practices). There is a clear difference between primary vs metastatic breast cancer in age distribution, with metastatic cases occurring early in a younger age group. Based on this, one might suspect that breast cancer in younger women has the capability of having a more aggressive biological course with earlier metastatic spread. Earlier and more frequent reports of increased tumor grade, late stage at diagnosis and worse prognosis have also been described in international studies of younger breast cancer patients. Family history was found to be more common in metastatic versus primary case than in the primary disease, indicating a stronger correlation of this population being more likely to have genetic susceptibility for aggressive disease than overt disease progression by tumorigenesis. Genetic susceptibility has long been shown in early-onset and advanced disease at diagnosis to be associated with genetic predisposition, including inherited mutations in DNA repair mechanisms. Reproductive factors were also found to be significant predictors of breast cancer risk in this cohort. Early menarche predominance represents long-term exposure to endogenous estrogen, one of the most known factors for breast cancer risk. Despite the fact that almost all women in the present study were multiparous and experienced early first pregnancy—things traditionally believed to confer protection—the existence of breast cancer indicates that parity's protective capacity can be influenced by other prevailing factors, such as early hormonal exposure, less breastfeeding, and genetic risk. The number of children in the population is distributed according to population fertility patterns, and thus does not outweigh the cumulative effect of hormonal exposure throughout the reproductive course. Breastfeeding practices displayed clear differences between primary and metastatic breast cancer. Reduced breastfeeding rate and increased mixed feeding among metastatic cases may indicate the loss of the protective effect of long-term breastfeeding. Breastfeeding is reported to lower lifetime exposure to estrogen and stimulate terminal breast tissue differentiation and hence can decrease the risk of malignant transformation. Reduced or stopped breastfeeding may thus lead to more aggressive disease behavior. Menopausal status was pivotal for the clinical presentation of breast cancer. The preponderance of postmenopausal patients illustrates the cumulative impact of prolonged exposure to estrogen on breast tissue. Post-menopausal women, estrogen is still generated from a process of peripheral aromatization in adipose tissue after menopause to continue to stimulate the hormone release, providing the hormone activation of breast tissue. This mechanism is well established in postmenopausal breast cancer and is also consistent with the tumor features seen in the current study. There were modest differences for tumor laterality between primary and metastatic disease, and this was not a clinical event that revealed any biological dominance, as was previously reported internationally. In contrast, axillary lymph node involvement became prominent in both groups, indicative of advanced local disease at presentation. Lymph node status is a strong prognostic factor in breast cancer and is strongly associated with outcome and prognosis as well as stage of disease. The elevated nodal involvement in the current study may have been due to the predominance of stage II and stage IV disease, with delayed detection and limited access to early detection programs. The hormonal exposure as a result of oral contraceptive pill use revealed that overall use was comparable between the primary and metastatic populations, however, the time of prolonged use is greater among the metastatic population. Such observations lend favor to the idea that long-term exogenous hormonal exposure may play a more prominent role in tumor progression and aggressiveness than cancer initiation itself. The current study results generally reveal that breast cancer progression from primary to metastatic disease is mediated by a multifactorial interplay of age, reproductive history, menopausal stage, hormonal exposure, and clinical presentation. The earlier presentation of the new disease, the involvement of the lymph nodes in metastatic cancer, and the state at diagnosis, which are at an advanced stage, necessitating earlier diagnosis, require more education, earlier screening

and personalized risk assessments, especially in younger and at-risk women. The data provide key findings that provide insight into breast cancer response in the studied population in terms of behavior and highlight the need for reproductive and hormonal factors to be included in breast cancer prevention and treatment planning approaches

5. Conclusion

This study also demonstrates that breast cancer presentations take place at a relatively young age in the population investigated and that advanced features are highly common. These findings highlight the significance of demographic and reproductive characteristics (early menarche, menopausal status, breastfeeding, and hormonal exposure) in breast cancer incidence and progression. Such a high metastatic rate occurs at a younger age, in addition to the increase in axillary lymph node involvement plus the advanced stage of tumor, indicating a more aggressive disease burden and delayed diagnosis. Hormonal receptor expression differences in primary versus metastatic breast cancer further indicate an interest in a more aggressive cancer molecular profiles trajectory in the disease course. These findings indicate the necessity for early surveillance, increased knowledge and individual risk surveillance in younger and more susceptible women. Including reproductive and hormonal factors in breast cancer screening/prevention interventions may produce earlier detection of breast cancer and better patient outcomes.

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