Evaluation of Breast Cancer Molecular Profile in Old Age Patients in Comparison to Young Age Patients

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Abstract

Background: Breast cancer is the commonest malignancy of females all over the world and the second leading cause of death due to malignancy among females. Breast cancer is regarded as a unique disease in oncology and the specific markers such as ER, PR, HER-2/neu and others are used to predict the prognosis and the treatment response to guide the therapeutic plan.

Aim of the Study: To clarify if there is any significant difference in the intrinsic molecular profile of breast cancer in old age patients in comparison to young age patients.

Material & Methods: Samples of primary carcinoma of breast, obtained from 211 female patients with breast cancer. The patients are divided into two group. Immunohistochemical analysis was done for the specimens by using Envision method for detection of estrogen receptor, progesterone receptor, Her2/neu.

Statistical analysis was done by using SPSS. In which we use frequency, percentage and mean as descriptive statistics while chi square and Yates corrected chi square as analytic statistics.

Result: We found molecular intrinsic breast cancer subtypes have a significant difference in distribution of elderly breast cancer compared to their younger counterparts where elderly patients more frequently Luminal A and Luminal B tumor subtypes and less frequently HER2+ and basal molecular subtypes.

Conclusion old age breast cancer patients have better prognosis in comparison to young age patients according to the biological profile, histopathological type and Immunohistochemical prognostic parameters.

Keywords: *Breast cancer, Molecular profile, Old age patients, Young age patients, ER, PR, HER-2/neu.*

Introduction

Breast cancer is the most common cancer in women and is the second cause of cancer death after Lung cancer in world [(1)]. In Iraq Breast cancer has become a major threat to female health, it is the leading cause of death after cardiovascular diseases among women with a cancer-related [(2)]. The large majority of breast cancers are detected during the postmenopausal years, However, breast cancer can develop at any age,from childhood to old age[(3)].The prognosis and management of BC is influenced by classic variables such as the age of patient, the tumor size, the histological type, the grade and the stage of the tumor, status of estrogen receptor (ER) , progesterone receptor (PR) and more recently Human Epidermal growth factor receptor Type- 2 (HER-2/neu) and BRCA status, cell proliferation markers such as Ki-67 and gene expression proliferation [(4,5)].

It is also established that the incidence of breast cancer increases with age. The number of elderly patients with breast cancer is increasing and the majority of females who succumb to breast cancer are >65

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years old [6]. However, older patients are more likely to present with tumors that are estrogen receptor (ER)- and progesterone receptor (PR)-positive and human epidermal growth factor receptor 2 (HER2) -negative, and these tumors are associated with improved prognosis and clinical outcomes [7]. By contrast, younger patients with triple-negative and HER2-positive breast cancers have an increased risk of relapse within 5 years of diagnosis [8]. Breast cancer that arises in young females is associated with reduced survival and higher incidence of unfavorable prognostic and predictive tumor markers [9,10].

Materials & Methods

This study was carried out during the period from January 2018 through August 2018.

Sampling of cases:

Archived and new Formalin-fixed paraffin-embedded tissue samples of 211 cases of female patients with breast carcinoma (as confirmed by H&E – stained histopathological examination) covering the period from 2017 to 2018, were included in this study. Their ages ranged between 22-85 years old with a mean age of 50.6±14.3 years old. Specimens were collected from 133 modified radical mastectomy & 78 excisional biopsy from laboratory of histopathology in Al-Sadder Teaching Medical City Hospital in Al Najaf and from some private laboratories in this governorate.

Study Group: Forty-nine case ≥ 65 years old age.

Comparative Group: one hundred sixty-two cases who were < 65 years old age (Figure 1)

Control Group:

Positive Control Slides: Parallel positive control sections were processed with each set of immunostaining. Positive controls of breast carcinoma sections which are known to express ER, PR and Her2/neu, respectively were used with each run.

Negative Controls slides: Sections untreated with primary antibody (ER, PR and Her2/neu) were considered as negative controls for each set of slides.

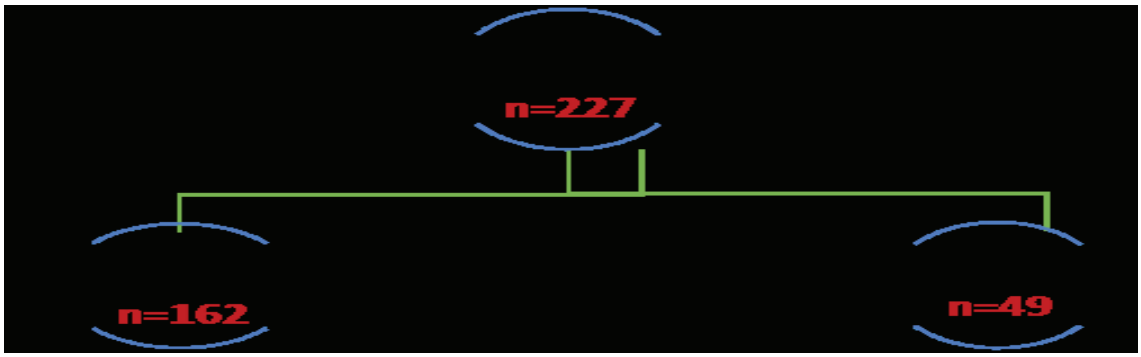

Fig. 1 Age distribution according to WHO publication [11].

Immunohistochemical material

Antibodies & Kits:

EnVision FLEX Mini Kit (High Ph,,Code K8004) which contain :DAB Chromogen with quality (1*3 ml),peroxidase-Blocking Reagent and HRP both with quality (5*20 ml),Wash Buffer (2*1 L, 20* concenterated) with PH 7.6 (+/- 0.1),Mouse (LINKER) with quality (40 ml),Target Retrieval Solution (3*30 ml,50* concenterated) with low PH (Code K8005) (LOT:20012657),Antibody diluents S0809 [12].

* DAB indicates 3,3′-diaminobenzidine.

Tissue preparations for IHC

Tissue sections of (4) microns from the formalin fixed, paraffin embedded blocks of breast tissue biopsies were cut using a microtome and mounted on the positive charged glass slides. These slides form bounds with the tissue so that specimens adhere to the slide and remain in place during the staining procedure The immunostaining

method used in the current study was En Vision immunohistochemical technique which was applied for HER-2/neu,ER and PR staining [13].

Evaluation of the Results:

The scoring of immune reactive Staining was done by calculated the percentage of immune reactive cells per total number of malignant cells. The staining intensity was evaluated by calculating the percentage of positive cells in 100 malignant cells at objective 40 total magnifications. For immunohistochemistry assessment, slide evaluation was performed using a light microscope. Slides were scanned by X10 magnification. Five cellular areas selected (The so-called hot spots) and evaluated at X40 magnification by a pathologist.

Estrogen and Progesterone Receptors Scoring System

Evaluation of ER, PR staining takes into consideration both the proportion and intensity of stained cells. The proportion score (PS) estimates the proportion of positive tumor cells and ranges from 0 to5 [12].

Her2/neu Scoring System [3,14,15]

HER-2 neu was scored on a 0 to 3 scale according to the criteria set by ASCO/CAP guidelines.

Statistical Analysis

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20. In which we use frequency, percentage and mean as descriptive statistics while chi square and Yates corrected chi square as analytic statistics.

P value ≤0.05 regarded significant.

Results

This study includes two groups according to age, where the cutoff point is 65 old age ≥65 whose constitute 23.2% of all patient and young age<65 whose consistuate76.8% of all patient, So the largest group is the young patient.

Immunohistochemical Parameters

The General Immunohistochemical Expression (Figure 2).

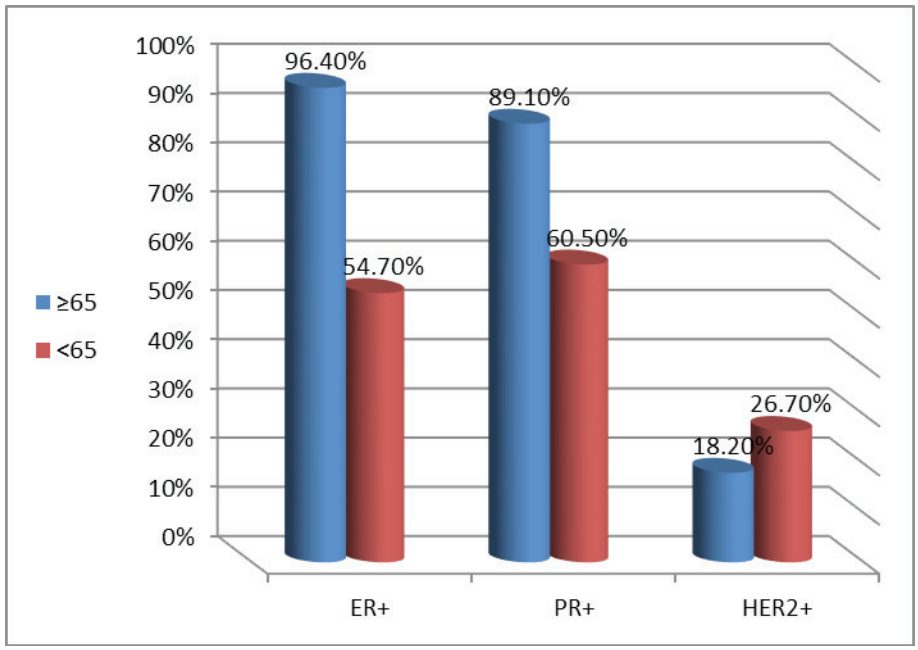

Figure 2 Frequency & percentage of ER+, PR+ & HER2+ according to the age

The biological profile of breast cancer

molecular types in patient with breast cancer as follow; luminal A 127(59.24%) cases, luminal B 18 (9.00%) cases, basal type 30 (14.22%) cases and her 2 has 36(17.54%) cases.

Old age group patient has molecular types as follow; luminal A 37(78.2%) cases, luminal B 9 (16.4%) cases, basal type 2 (3.6%) cases and her 2 has 1(3.6%) case.

young age group patient has molecular types as follow; luminal A 90(56.4%) cases, luminal B 9 (6.4%) cases, basal type 28 (16.3%) cases and her 2/neu has 35 (20.9%) cases.

Clinicopathological analysis Tumor size (Figure 3).

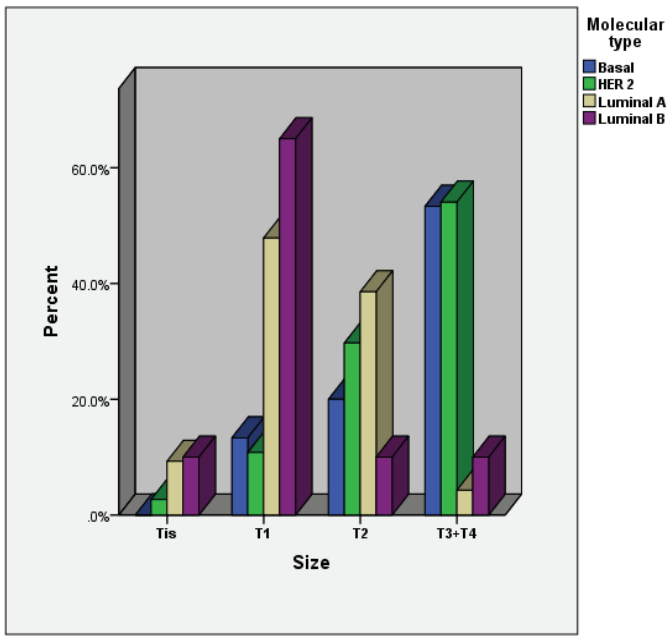

Figure 3 Distribution of molecular types according to size of tumor.

Histopathological types (Figure 4).

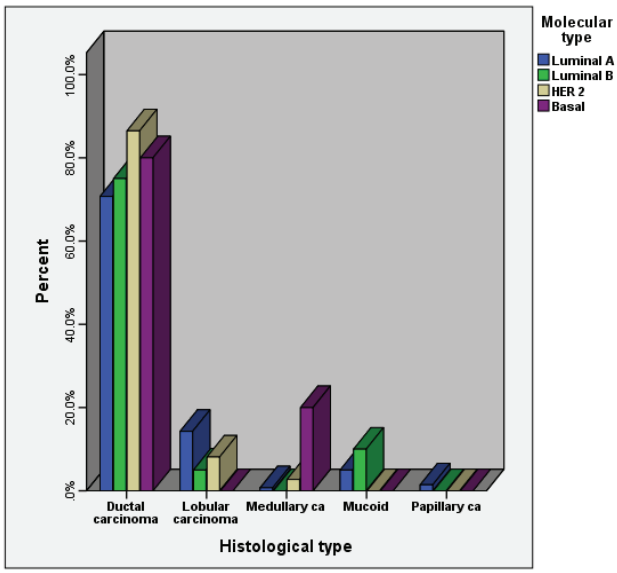

Figure 4 shows significant association between biological profile and histological type.

Stage of Axillary Lymph Node Involvement (Figure 5).

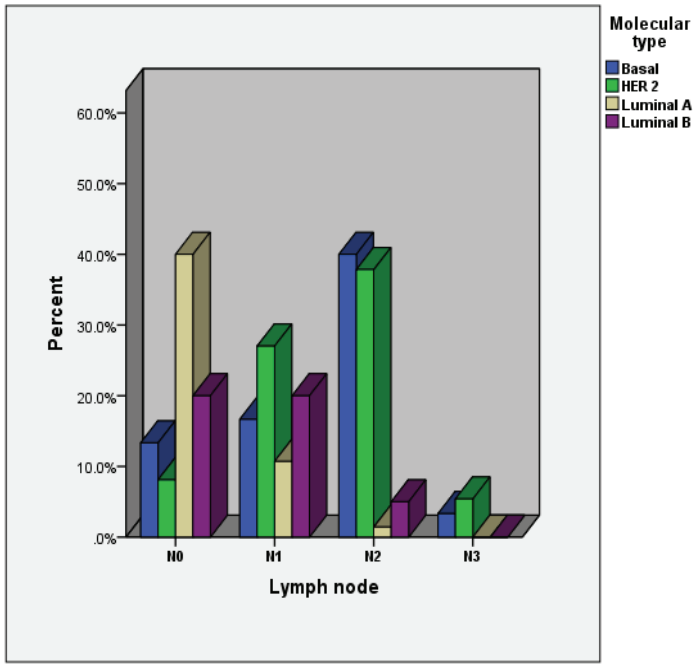

Figure 5 Distribution of molecular types according to lymph node involvement.

Discussion

We used in this study IHC surrogates, which we validated against gene expression determined molecular subtypes, to identify breast tumor molecular subtypes in a 211 breast cancer patients. In the present study of patients, the distribution of particular molecular subtypes was127 luminal A, 18 luminal B,36 HER2+ and 30 basal like. This agree with Ihemelandu et al. [16] who classified breast cancer into four molecular subtypes (basal cell-like, HER2/neu, luminal A and luminal B) and analyzed the prevalence and clinicopathological associations for these molecular subtypes, the luminal A type was the most prevalent 140 case. this agree with Xiaofeng Dai et al [17], and disagree wih Changhong et al. [18] who found, Luminal B is the major molecular subtype of breast cancer patients This is different to other research reports and may reflect the genetic background of racial differences and may also be due to the detection method used and the standards used for judging the cause of the disease. this agree with, and disagree wih TNBC lacks expression of ER, PR, and HER2 receptors, accounts for 10–20% of all reported breast cancer, and largely overlaps with the basal intrinsic subtype Madhuri K et al. [19]. We demonstrated that the distribution of molecular subtypes between elderly and young patients were statistically significantly different.

De Kruijf et al. [20] showed that the molecular intrinsic breast cancer subtypes have a different distribution in elderly breast cancer compared to their younger counterparts, where elderly patients more frequently had less aggressive Luminal A and Luminal B tumor subtypes.

where we defined elderly breast cancer patients as patients aged 65 years or older according to World Health Organization definition [21]. With this cut-off point, elderly breast cancer patients showed more often Luminal A and Luminal B molecular subtypes less often Her2/neu, basal molecular subtypes. This agree with Perou et al. [22], Carey et al. [23] and others [24,25,26]. while disagree to Danae Pracella et al., who found no specific age distribution related to molecular subtype of breast cancer [27]. De Kruijf et al. [20] also identified a statistically significant association (P=0.02) between patient age and tumor molecular subtype with luminal tumors being more frequently identified in elderly patients with basal like type tumors were younger at the time of diagnosis and had larger, more undifferentiated tumors this agree with Dent et al. [19], Markata et al.[28],who found that young females exhibited tumors with HER2+ and basal

molecular subtypes also findings agreed with result of Reina Haque et al.[29] which showed that Her2 enriched subtypes occur mainly in premenopausal women. ,and Park *et al* [30], While some preliminary studies suggested that the distribution of histologic subtypes is different in young women with a higher prevalence of triple negative and HER2+ disease, a clear molecular characterization of BC in these patients is lacking[31,32]. Though triple the high frequency of basal-like tumors among young women could be an explanation for the biological aggressiveness that has been associated with breast cancer diagnosed in young women when compared to breast cancer diagnosed in older women this agree with , In our study, the distribution of the molecular intrinsic breast tumor subtypes in this older breast cancer population showed a higher prevalence of the assumed more indolent Luminal A tumor and a relatively low prevalence of the more aggressive Basal molecular tumor subtype but higher in a younger breast cancer this agree with Carey et al. [33].

These results imply that the chance of getting a more aggressive molecular tumor subtype decreases with increasing age, which is in accordance with the observation of milder tumor characteristics in the older breast cancer population. This agree with Charla C. et al. [11]. Our results support the premise that breast cancer clinical behavior is significantly affected by patient age. We suggest that competing risks of death in elderly patients, ER driven differences and micro-environmental changes in biology are underlying these age-dependent variations in patient prognosis.

Conclusion

1-Old age patient with breast carcinoma have lower histopathological grade and stage of axillary lymph node involvements than young patients with more proportion of ER and PR positive cases.

2 –Old age breast carcinoma patients have better prognosis in comparison to young age patients according to the biological profile, histopathological and immunohistochemical prognostic parameters.

3-The pattern of molecular type of breast cancer patient was statistically significant correlate with age of patients, size of the tumor mass, histopathological typing, grading and lymph node status.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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