

Invasive Ductal Carcinoma: Correlation of Immunophenotypic Features with Age

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ABSTRACT

Objective: To evaluate the frequency of hormone receptors and Her2neu expression and their correlation with age in patients of carcinoma breast.

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Department of Histopathology, Dr. Ziauddin Hospital, Karachi, between the period 2006 - 2013.

Methodology: Estrogen receptors, progesterone receptors and Her2neu immunohistochemical staining was performed on all specimens of carcinoma breast of female patients. Allred scoring was followed using normal epithelium as internal control. Age was determined in years. Results were described as percentages.

Results: Three hundred and forty-six cases of infiltrating ductal carcinoma of breast between the age of 20 - 90 years (mean= 49 ±14 years) were studied. Seventy-nine (23%) cases were below the age of 40 years. ER +ve cases were 210 (61%), PR +ve were 190 (55%), and 78 (23%) were Her2neu +ve. Three were 12 (3%) triple positive cases and 58 (17%) triple negative cases. One hundred and seven (31%) cases were ER/PR+ve and Her2neu -ve. Most of the breast cancer cases were aged between 41 - 50 years. Estrogen receptor positivity was maximum between 41 - 60 years. Fifty percent cases of carcinoma breast below 30-year age were also ER positive.

Conclusion: A high proportion of breast cancer in young Pakistani females is alarming. Younger age harbours aggressive clinicopathologic characteristics. There is a due need for identifying high risk individuals/families including BRCA1 and BRCA2 testings, biologically driven trials devoted specifically to this group.

Key Words: *Infiltrating ductal carcinoma. Estrogen receptor. Progesterone receptors. Her2neu. Age. Immunohistochemistry status.*

INTRODUCTION

Breast cancer is the most common cancer-related deaths among North American and Western European women. It comprises 22.9% of invasive cancers in women and 16% of all female cancers. Invasive ductal carcinoma NOS type comprises the majority (70 - 80%) of all breast cancers.¹ It remains the most common malignancy in Pakistani females in both northern (26.6%) and the southern parts (20.8%) of the country.²

Tumour size, axillary lymph node metastasis, mitotic rate, hormone receptor status (ER and PR), and Her2neu status have traditionally been regarded as prognostic markers in patients with breast cancer.¹ Hormone receptor status is determined primarily to identify patients who may benefit from hormonal therapy. About 75 - 80% of invasive breast cancers are positive for ER and PR, including almost all well differentiated cancers and most moderately differentiated cancers. Apart from this, false negative results occur due to a number of

technical issues, i.e. exposure of tumour cells to heat, prolonged cold ischemic time, under or over-fixation, decalcification, which may result in loss of immunoreactivity, non-optimised antigen retrieval, type of antibody, and dark hematoxylin counter-stain obscuring faintly positive diaminobenzidine staining.³

ER/PR expressions are the most important and useful currently available predictive factors. The frequency of positivity and the level of ER/PR increase with age, reaching their highest levels in postmenopausal women.⁴ About 15 - 20% of invasive breast cancers are Her2neu positive.³ Overexpression of this gene is associated with high histological grade, occurrence of comedonecrosis, p53 mutation and is inversely associated with expression of hormone receptors. It is also related to increased disease recurrence, aggressiveness, and chemoresistance.⁴

The current study was carried out to observe the frequency of prognostic marker positivity, and their distribution in different age groups.

METHODOLOGY

This study was carried out in the Department of Histopathology, Dr. Ziauddin Hospital, Karachi. Paraffin blocks and slides of mastectomy, lumpectomy, and trucut biopsy of infiltrating ductal carcinoma NOS type of breast of females were retrieved from Histopathology

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Department between the period 2006 - 2013. Standard ethics and maintenance of confidentiality of reports were assured. Mastectomies of male patients and infiltrating ductal carcinoma of specific type were excluded from the study. Detailed gross examination of all received modified radical mastectomy specimens was carried out according to College of American Pathology guidelines. All tissues were fixed in 10% buffered formalin for 6 - 24 hours. Representative tumour tissue was submitted for routine histological diagnosis.

Immunohistochemistry stains (ER Clone SP1, PR Clone SP4, and Her2neu Clone SP3) were performed on representative blocks of paraffin embedded tissue. Four um thick sections were submitted for immunohistochemistry staining. Sections were taken on slides previously coated with Poly-L-Lysine. Antigen retrieval was done by HIER method using citrate buffer at pH 2.5 for ER/PR and pH 6 for Her2neu. Infiltrating ductal carcinoma with known ER/PR and Her2neu positivity was used as external positive controls on same slide for each case. Normal epithelial component served as internal control for ER/PR. ER, PR results were screened and interpreted as positive when more than 1% tumour cells showed positive nuclear staining (Allred scoring).

Interpretation and reporting of Her2neu staining was performed according to ASCO - CAP Her2 test summary of guideline 2013 recommendations.³

For data analysis, SPSS-II was used. The results were given in the text as number and percentage for qualitative/categorical variables like hormone receptor positivity to compare percentage of qualitative/categorical variables in breast cancer cases.

RESULTS

A total of 346 cases of infiltrating ductal carcinoma of breast of female patients between the age of 20 - 90 years (mean = 49 ±14 years) were studied. Among them, 210 (61%) were ER +ve, 190 (55%) were PR +ve and 78 (23%) were Her2neu +ve. Twelve (3%) were triple positive, and 58 (17%) cases were triple negative; 107 (31%) cases were ER/PR +ve and Her2neu -ve.

In 129 (37%) out of 346 cases of trucut biopsies, ER positivity was seen in 83 (64.3%), PR positivity was seen in 71 (55%), and Her2neu overexpression was seen in 41 (32%) cases. Among these, triple +ve cases were 14 (11%), and triple -ve cases were 13 (10.07%). ER/PR +ve, Her2neu -ve cases were 38 (29.4%).

Two hundred seventeen (63%) cases were mastectomy/lumpectomy specimens. Only ER positivity was seen in 109 (50.2%) cases, PR positivity in 119 (54.8%) cases, and Her2neu positivity was seen in 37 (17%) cases. Among these, 17 (8%) cases were triple +ve, 45 (20.7%) cases were triple -ve. ER/PR +ve, Her2neu -ve cases were 69 (31.7%) (Figure 1).

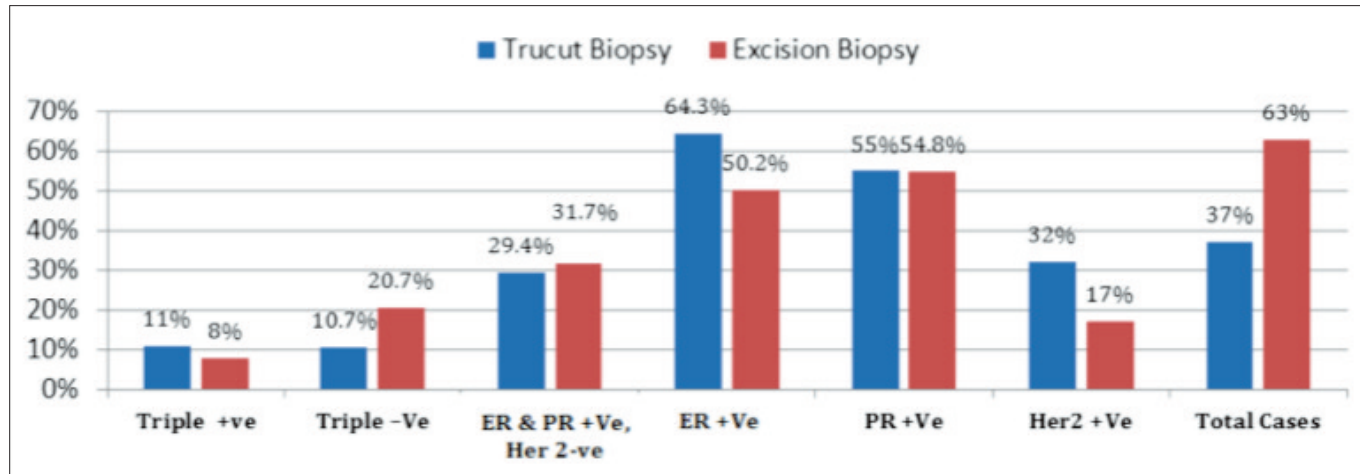


Figure 1: Frequency of ER/PR and Her2neu expression in trucut biopsy and excision specimens.

Table I: Age-wise distribution of immune receptors.

Age in years	Number of cases	ER +ve	PR +ve	Her2Neu +ve	Triple positive	Triple negative	ER, PR + Her2neu negative
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
20 - 30 (Mean: 27±3)	36 (10%)	18 (50%)	13 (36%)	6 (17%)	4 (11%)	9 (25%)	3 (8%)
31 - 40 (Mean: 37±3)	79 (23%)	37 (47%)	34 (43%)	17 (22%)	6 (8%)	22 (28%)	23 (29%)
41 - 50 (Mean: 47±3)	94 (27%)	65 (69%)	54 (57%)	27 (29%)	11 (12%)	8 (9%)	28 (30%)
51 - 60 (Mean: 56±3)	74 (21%)	51 (69%)	47 (64%)	15 (20%)	6 (8%)	9 (12%)	24 (32%)
61 - 70 (Mean: 66±3)	38 (11%)	22 (58%)	24 (63%)	11 (29%)	4 (11%)	6 (16%)	13 (34%)
71 - 80 (Mean: 75±3)	27 (8%)	15 (56%)	16 (59%)	2 (7%)	0	4 (15%)	14 (52%)
81 - 90 (Mean: 84±3)	3/ (1%)	2 (67%)	2 (67%)	0	2 (67%)	0	2 (67%)

Most of the cases of infiltrating ductal carcinoma, i.e. 94 (27%) were seen between 40 - 50 years of age (Table I). Estrogen receptor positivity is also maximum in patients between 41 - 60 years of age. PR positivity increased with increasing age and reaches its peak above 80 years, i.e. 2 (67%). Triple +ve cases were maximum between 81 - 90 years of age, 2 (67%). Triple -ve cases were seen mostly below the age of 40 years, i.e. 22 (28%) and no triple -ve case was seen after 80 years of age. Her2neu positivity is maximum between 41 - 70 years of age (Table I). Thirty-six (10%) cases were between 20 - 30 years of age, among which ER positivity was seen in 18 (50%) cases, triple positivity in 4 (11%) and triple negativity was seen in 9 (25%) cases. PR expression was minimum in this age group 13 (36%). No case of isolated Her2neu positivity or triple -ve phenotype was seen after 80 years of age.

DISCUSSION

Although 20% of ER and Her2 tests are inaccurate, determination of their status is still considered as standard of care for all invasive carcinomas of breast as these biomarkers are predictive of response to hormonal treatment and/or Her2neu inhibiting drugs.⁵ Accurate determination of ER/PR and Her2neu positivity in infiltrating ductal carcinoma of breast is essential for optimal choice of neo adjuvant therapies and has become mainstay requirement for the oncologist. Mean age of patient with carcinoma breast in this study is 49 ±14 years (age range 20 - 90 years) which is identical to mean age of 48.6 ±12.2 and 48.4 ±10 years as reported from Lahore⁶ and Morocco⁷, respectively. This age distribution is markedly younger than what is seen in western country like Netherlands,⁵ which reported mean age of 63 (range 36 - 91) years. This observation raises a need for risk factor evaluation in the local population or the risk factors that may be specific to our country.⁷

In this study, the age of the patients was 20 years (2 cases) which is comparable to studies from India⁸ and Iran⁹ where minimum ages were 20 and 23 years, respectively. This is in contrast with the minimum ages reported from Japan¹⁰ and Netherlands⁵ as 27 and 36 years, respectively. This calls for great awareness for self-breast examination, clinical breast examination and establishment of national breast cancer screening programmes to enhance early detection, since size of lesion at presentation is a very important prognostic factor.¹¹

One possible explanation is that consanguineous marriages are very common in Pakistan, and accordingly hereditary factors play a role. Another factor could be the increasing degree of obesity and sedentary lifestyle.⁴ Breast cancer at an early age is more likely to be associated with an increased familial risk, especially in women harbouring a germline BRCA1 mutation. In a

study from USA, women with breast cancer diagnosed before the age of 30 years, BRCA1, BRCA2 and TP53 mutations were found in about half cases who had strong family histories of breast cancer and in less than 10% of women with non-familial breast cancer.¹² Oral contraceptive use is a risk factor for the early onset of breast cancer.¹²

The current study showed estrogen receptors (ER) positivity in 61% cases while progesterone receptor (PR) positivity was seen in 55% of cases. Nearly similar results have been reported from Morocco⁷ as ER positivity in 61.6%, PR positivity in 58.4%; and in Nigeria¹¹, ER positivity in 54.2% cases while PR positivity was seen in 50% cases. Similar studies from various parts of India showed 59% and 62%, 48.2% and 37.9%, 47.6% and 48.2% of estrogen receptor expression and progesterone receptor expression, respectively.¹³⁻¹⁵ Her2neu expression in this study was 23% while studies from Morocco⁷ and India¹³⁻¹⁵ reported Her2neu positivity as 29.2%, 27.1%, 27.5% and 29.6%, respectively, which is nearer to this study.

Triple negative cases comprised 17% in our study, which is comparable to figures reported from Lahore,⁶ West Virginia,¹⁵ and India,⁸ where they found 16.6%, 18.9% and 16.5% of triple negative cases, respectively. Studies from Iran, and India showed 21.6%, 25.8%, and 22.7% triple negative cases, respectively.^{9,14,15} Triple negative breast cancers have recently attracted a lot of attention because of their aggressive pathologic features and poor clinical outcome.^{15,17} These are more likely to be occult on mammography, ultrasound imaging and they have shorter median time to death (4.2 versus 6 years), higher propensity for distant recurrences (33.9% versus 20.4%) and shorter mean time to local (2.8 versus 4.2 years) and distant recurrences (2.6 versus 5.0 years) compared to those with other breast cancers. Patients younger than 50 years of age diagnosed with triple negative breast cancer are now routinely recommended to undergo genetic counselling and BRCA mutation testing regardless of whether they have a family history of breast/ovarian cancer, and some centres refer triple negative breast cancer patients for genetic counselling at any age. Other risk factors include high waist to hip circumference ratio, higher parity and lower duration of breast feeding, nulliparity and late age first child birth.¹⁷

In this study, triple positive cases were 3% which is in contrast with figures reported from USA,¹⁸ Iran⁹ and Romania¹⁹ as 10.2%, 20.2% and 45.5%, respectively; which is markedly different from those results. The best survival rate was observed in the female patients with ER+/PR+ and Her2+ phenotype.¹⁹ This difference in triple positive cases may be either due to inherent difference in tumour or due to less sample size in current study. Most cases of carcinoma breast were reported between 41 - 50 years (94%), which is comparable with

study from Nigeria¹¹ which reported maximum number of cases between 40 - 49 years of ages. This is in contrast with study reported from Australia²⁰ where most of patients were between 50 - 60 years, and USA where peak age for breast cancer is between 60 - 70 years.²¹

In current study, 10% cases were below 30 years and 23% cases were below the age of 40 years which is in stark contrast to international reports of 7%. Survival rates in this group is worse as compared to older women, and younger age has been observed to be an independent predictor of adverse outcome.

Breast cancer at an early age is more likely to be associated with an increased familial risk, especially in women harbouring a germline BRCA1 mutation. In a study of women with breast cancer diagnosed before the age 30 years, BRCA1, BRCA2 and TP53 mutations were found in about half, who had strong family histories of breast cancer; and less than 10% in non-familial breast cancer. Patients with familial PTEN mutation (Cowden syndrome) also have increased risk for early breast cancer.¹²

In this study, most of the patients with triple -ve breast cancer (28%) were between the ages of 31 - 40 years. Figures reported from India are 41.8% triple -ve cases in patients less than 35 years and 16.5% triple -ve cases (above 40 years), respectively.^{8,22} A study from Iran reported 21.6% triple negative cases (mean age 47 years).⁹ This observation is different from studies from Iran and India, where very young patients (< 35 years) have higher chance of having breast carcinoma with triple negative phenotype compared to older patients.²² Differences in percentage of triple negative cases may be due to small sample size in current study. Triple negative breast cancers occur more frequently in African-American women, oral contraceptive use for more than one year, BRCA1 mutation carriers, women in low socioeconomic group and in those patients having limited access to medical assistance.²³

Triple negative subtype has the worst overall survival and worst disease-free survival when compared with others, i.e. ER/PR+, and Her2- subtype. ER/PR+, Her2- and triple positive have better prognostic, therapeutic connotations. Studies have shown that hormone receptor positive tumours are responsive to adjuvant hormonal or chemotherapeutic regimens and give a better survival advantage.⁸ ER/PR+, Her2+ have better response to chemotherapy. Women with ER+ breast cancer typically receive endocrine therapy (tamoxifen) and women with Her2+ breast cancer may receive anti-Her2 (trastuzumab and lapatinib). Targeted therapies for triple negative breast cancer are lacking and this continues to direct the focus of ongoing research.¹⁸

In this study, maximum number of cases of estrogen receptor positivity, i.e. 69%, was seen between 41 - 60 years of age. A study from India¹³ reported highest ER

positivity (59%) in the age group of > 65 years which is higher than the present results. PR positivity increases with age and reaches its peak above 80 years of age which is in contrast to study from India¹³ where PR expression did not vary with age.

These results were compared with a number of international and local studies in view of proposition that breast carcinogenesis is remarkably a different process in the subcontinent compared to the western population.²⁰

In current study, 10% of cases were below 30 years of age which is in contrast with Western reports of 1% and 0.43% in UK before this age.¹² Nearly identical ER positivity was noted, i.e. 50% below the age of 30 years and 58% above the age of 60 years which is quite different from local and international literature. According to available literature, tumours below the age of 30 years are mostly ER/PR -ve.²⁵

CONCLUSION

A high proportion of breast cancer cases were seen in young age, which has found to be an independent predictor of adverse outcome and worst survival rates. There is a due need of identifying high risk individuals/families including BRCA1 and BRCA2 testings, and biologically driven trials devoted specifically to this group in our country.

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