RESEARCH COMMUNICATION

Comparison of ER, PR & HER-2/neu (C-erb B 2) Reactivity Pattern with Histologic Grade, Tumor Size and Lymph Node Status in Breast Cancer

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Abstract

Introduction: Carcinoma of the breast is the most common malignancy of women in Karachi. The current study was conducted with the objective of assessing estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu reactivity patterns of mammary cancers for correlation with histologic grade, tumor size and lymph node metastasis. Materials and methods: One hundred and fifty modified mastectomy specimens received at the section of histopathology, Aga Khan University Hospital, were selected using a non-probability sampling method. Results: Mean age of the patients was 48.3 years (95%CI 46.5, 50.2). The left breast was more commonly involved (57%). Tumor size ranged from 0.3 to 15.0 cm; 12% were ≤2.0 and 35.3% were ≥ 5.0 cm in diameter. The predominant morphology was infiltrating ductal carcinoma (85.3%). The majority of the cases presented as grade II (55.3%) lesions with tumor necrosis (70%) and lymph node involvement (71.3%). ER and PR were positive in 32.7% and 25.3% cases respectively. HER-2/neu was positive (3+) in 24.7%. ER positivity increased and HER-2/neu positivity decreased with rising age. ER and PR expression was significantly lower in HER-2/neu positive as compared with HER-2/neu negative tumors (ER 83.8% vs 69.8%; PR 91.9% vs 77.8%). In the HER-2/neu positive tumors, ER and PR expression in high grade tumors was significantly decreased compared with intermediate grade tumors (ER 5.6% vs 10.5; PR 0% vs 5.3%). ER expression in the HER-2/neu positive, large sized tumors was also significantly decreased compared with smaller tumors (ER 6.3% vs 11.8). Conclusions: ER and PR expression in breast cancers in the current study was found to be comparable to published international data, but the frequency of HER-2/neu expression was higher, possibly reflecting a young age at diagnosis. Assessment of prognostic markers for the clinical management of breast cancer patients is strongly advocated to provide best therapeutic options.

Key Words: Breast cancer - receptor status -biological behaviour - prognosis

Asian Pacific J Cancer Prev, **9**, 553-556

Introduction

Globally carcinoma breast is the most common malignancy and the leading cause of cancer death in women (Parkin et al., 2001). In Karachi it accounts for a third of the cancers in females with an age standardized incidence rate of (ASR) world of 53.8 per 100,000 population annually (Bhurgri et al., 2007).

Breast cancer survival is linked to early detection, timely appropriate treatment and genetic predisposition. Prognosis is related to a variety of clinical, pathologic and molecular features which include classical prognostic factors viz. histologic type, grade, tumor size and lymph node metastases. Estrogen and progesterone receptors (ER, PR) and more recently, HER-2/neu have with increasing importance influenced the management of the malignancy (Rampaul et al., 2001). With an established positive correlation of ER and PR with the degree of tumor differentiation, determination of ER and PR status on biopsy specimens prior to therapeutic intervention is advocated as standard practice (Mori et al., 2002).

Ovarian steroids are necessary for normal breast development. An imbalance precipitates abnormal processes like epithelial hyperplasia, intraductal and invasive carcinoma (Mori et al., 2002). Estrogen is an important mitogen exerting its activity by binding to its receptor (ER) and found in 50-80% of breast cancers. Endocrine treatments are assigned to antagonize the effects of estrogen. Therapeutic hormones like Tamoxifen competitively block ER thus antagonizing transcriptional activation of genes required for tumor growth (Yamauchi et al., 2001). The presence of hormone receptors (ER and PR) in the tumor tissue correlates well with the response to hormone therapy and chemotherapy (Barnes and Hanby, 2001). Studies have shown that 55-60% of women with ER-positive tumors respond to additive or ablative

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hormone therapy, compared with about 8% of women with ER–negative tumors. Tumors that are better differentiated are more likely to be ER and PR positive and have a relatively better prognosis (Maynard et al., 1978; Hilf et al., 1980).

PR is a surrogate marker of a functional ER and as valuable in predicting the behavior of breast carcinoma. It is expressed in 60-70% invasive breast carcinomas with a higher positivity in older age and postmenopausal women. Loss of PR by tumor cells is associated with a worse prognosis (McGuire and Clark, 1983). Patients with larger tumors, poorly differentiated morphology, increased number of axillary lymph node metastases and higher stage tumors have more chance of an ER and PR negative status (Fisher et al., 1980).

HER-2/neu also known as C-erb B2 (HER-2), is a proto-oncogene located on chromosome 17. It is amplified and the protein (HER-2) overexpressed in 15-25% of invasive breast carcinoma with associated poor prognosis. HER-2/neu encodes a transmembrane glycoprotein with tyrosine kinase activity known as p185 belonging to the family of epidermal growth factor receptors (Hung and Lay, 1999). Over-expression of HER-2/neu is a good predictor of response to trastuzumab (Herceptin), but not a positive predictor of response to chemotherapy or overall survival. HER-2/neu is also an independent negative predictor of overall survival and time to relapse in patients with lymph-node-positive breast cancer (Suo et al., 2002). The expression of this protein has been associated with a poor histologic grade, spread to axillary nodes and an increase in the number of nodes involved (Slamon et al., 1987; Tsuda et al., 1991). An inverse association between HER-2/neu expression and ER and PR has been noted (Zeillinger et al., 1989).

The objectives of this study were to assess the ER, PR and HER-2/neu reactivity pattern in breast carcinomas and to correlate this reactivity pattern with histologic grade, tumor size and lymph node metastasis.

Materials and Methods

This study was carried out at the section of histopathology, Aga Khan University Hospital, a major referral center in Karachi. It receives over 40,000 surgical specimens annually from all four provinces of Pakistan. The present study included cases of breast cancer accessioned at our department during a six month period extending from 1st January 2006 to 31st August 2006. A total of 150 cases were included in the study using the non-probability sampling method. Only modified mastectomies (mastectomies with axillary lymph node dissection) were included.

Specimens were routinely processed and fixed overnight in 10% buffered formalin. They were examined grossly according to the standard guidelines, with special emphasis to the size, multifocality and lymph node sampling of the lesion. Four to five micrometer thick formalin fixed, paraffin embedded tumor sections were stained with Haematoxylin and Eosin. Histological grade was assessed according to Nottingham modification of the Bloom-Richardson system.

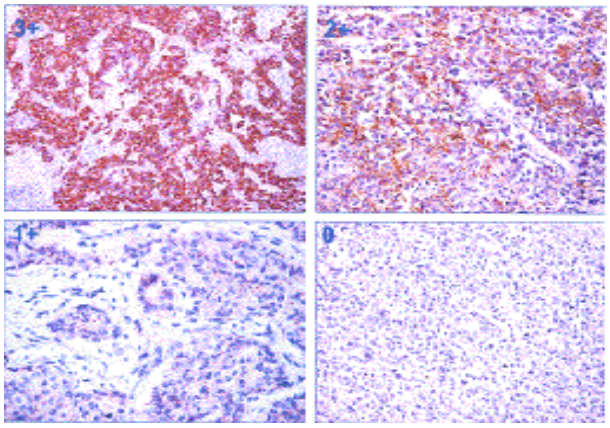

Figure 1. Immunohistochemical Staining of HER-2

Representative sections with tumor and the adjacent normal breast tissue (internal control) were processed for ER, PR and HER-2/neu immuno-histochemical staining (see Figure 1). For ER and PR staining, sections were taken on histogrip coated slides. Antigen retrieval was done by citrate buffer and the slides stained with monoclonal antibodies against estrogen and progesterone receptors by LSAB (labeled streptavidin biotin) system (ER Clone ID5 and PR Clone IA6, DAKO). For HER-2/neu staining, after antigen retrieval, slides were stained with a polyclonal antibody against HER-2/neu (DAKO) oncoprotein by envision system. HER2 score of 3+ was taken as positive. A score 3+ may be taken as "positive" as over 90% of these show gene amplification.

The data were entered and analyzed in SPSS version 16. Frequencies and percentages of categorical variables; mean and standard deviation of quantitative variables like tumor size were computed. Cases were stratified by tumor size and lymph node status. A p value of <0.05 was taken as significant, as calculated by applying correlation coefficients and multiple logistic regression.

Results

A total of 150 breast cancer cases were included in the study. The mean age was 48.3 years (95% CI 46.5, 50.2; range 25-87 years; median age 45.5 years). Most of the patients (66.0%) were ≤50 years at diagnosis. The left breast was more commonly involved (57%). Tumor size ranged from 0.3 - 15.0 cm (see Table 1). Six (4%) cases

Table 1. Distribution of Cases by Tumor Size, Necrosis, Fibrosis, Lymphocytic Infiltration and Calcification

Size	**No (%)**	**Lymphocytes**	**No (%)**
<2	18 (12.0)	Present	141 (94.0)
2-5	79 (52.7)	Absent	3 (2.0)
>5	53 (35.3)	Unknown	6 (4.0)
Necrosis	**No (%)**	**Calcification**	**No (%)**
Present	105 (70.0)	Present	33 (22.0)
Absent	39 (26.0)	Absent	114 (76.0)
Unknown	6 (4.0)	Unknown	3 (2.0)
Fibrosis	**No (%)**	**Nodal***	**No (%)**
Present	141 (94.0)	0	43 (28.7)
Absent	3 (2.0)	1-3	35 (23.3)
Unknown	6 (4.0)	>3	72 (48.0)

*Lymph node involvement

Table 2. ER, PR and HER-2/neu Status by Tumor Grade, Size, Lymph Node Involvement and Age

	ER positive (#49)	PR positive (#38)	HER/2neu + (#56)
Grade 1 (#10)	7 (70.0)	7 (70.0)	None
Grade 2 (#83)	40 (48.2)	30 (36.1)	19 (22.9)
Grade 3 (#57)	2 (3.5)	1 (1.75)	18 (31.6)
p-value	<0.001		
<2.0 cm (#18)	9 (50.0%)	8 (44.4)	4 (22.2)
2-5 cm (#79)	26 (32.9%)	21 (26.6)	17 (21.5)
>5.0 cm (#53)	14 (26.4%)	9 (17.0)	16 (30.2)
p-value	<0.001		
No LN (#43)	11 (25.6%)	10 (23.3)	6 (13.9)
1-3 LN (#35)	13 (37.1%)	11 (31.4)	10 (28.6)
> 3 LN (#72)	25 (34.7%)	17 (23.6)	21 (29.1)
p-value	<0.001		

Age	ER			PR			HER/2neu		
(years)	1+	2+	3+	1+	2+	3+	0-1+	2+	3+
Mean	41.1	48.0	53.0	48.7	46.3	47.2	48.1	47.9	41.1
95%CI	36-47	38-58	50-57	41-56	38-55	40-55	46-50	42-54	38-44
Range	28-49	35-77	39-75	28-60	33-77	35-69	25-87	28-77	27-49

1+ weak, 2+ intermediate, 3+strong, - negative

were multifocal.

The morphological categories were infiltrating ductal carcinoma (IDC) NOS, (not otherwise specified) 128 (85.3%) cases, metaplastic carcinoma 6 (4.0%) cases, IDC with mucinous differentiation 4 (2.7%) cases, infiltrating lobular carcinoma 3 (2.0%) cases; mixed (ductal and lobular) carcinomas, IDC micropapillary and medullary carcinoma 2 (1.3%) cases each. Other types were apocrine, papillary and pleomorphic lobular carcinoma accounting for a single case each. Ten (6.7%) cases were grade I, 83 (55.3%) were grade II and 57 (38.0%) were grade III.

Tables 2 and 3 give the ER, PR and HER-2/neu status by age, tumor grade, size and lymph node involvement. Extra-capsular involvement was observed in 76 (50.7%) cases. The minimal number of lymph nodes per case recovered was 5, maximum was 50 and the average was 16 (95% CI 15.24; 18.02). A component of DCIS was present in 58 (38.7%) cases; high grade in 19 (12.7%) and low grade in 37 (24.7%) cases.

ER and PR were positive in 49 (32.7%) and 38 (25.3%) cases respectively. HER-2/neu was positive (3+) in 37 (24.7%), 2+ in 19 (12.7%) and negative (0 and 1) in 94 (62.7%) cases. Simultaneous ER and HER-2/neu positivity was observed in 6 (4%) cases. Special sub-type of carcinomas like metaplastic carcinoma showed no HER-2 positivity, except for a case of grade II IDC with mucinous differentiation.

ER positivity increased with rising age, whereas HER/2neu positivity decreased and PR positivity did not show a clear relation. ER or PR expression correlated inversely with HER-2 over-expression (ER 8.1% vs 23.4%; PR 2.7% vs 14.9%). Vice versa ER, PR negativity increased in HER-2 over-expression (ER 83.8% vs 64.9%; PR 83.8% vs 72.3%). ER and PR expression were decreased significantly in HER-2/neu positive compared with HER-2/neu negative tumors (ER 83.8% vs 69.8%; PR 91.9% vs 77.8%).

ER positivity was observed in 70% grade I, 48.2% grade II and 3.5% grade III carcinomas (p value <0.001). Similarly PR positivity was observed in 70% grade I, 36.14% grade II and 1.75% grade III carcinomas (p value<0.001). HER-2 was positive in 1 (10%) case of grade I carcinoma, 31 (37.35%) cases of the grade II carcinoma and 24 (42.11%) cases of grade III carcinoma. In the HER-2/neu positive tumors, ER and PR expression in high grade tumors was significantly decreased compared with intermediate grade tumors (ER 5.6% vs 10.5; PR 0% vs 5.3%).

Stratification of tumor size was performed in 3 groups, group 1 (tumor size ≤ 2 cm), group 2 (2–5 cms) and group 3 (≥ 5 cms) in diameter. The total number of group 1 tumors was 18 [9 (50%) ER positive; 8 (44.44%) each PR and Her-2 positive]. Of the group 2 tumors 26 (32.91%) were ER positive, 21 (26.58%) were PR positive and 27 (34.18%) were HER-2 positive. Of the group 3 tumors 14 (26.42%) were ER positive, 9 (16.98%) were PR positive and 21 (39.62%) were HER-2 positive. ER expression in the HER-2/neu positive, large sized tumors was significantly decreased compared with smaller tumors (ER 6.3% vs 11.8).

Discussion

The results of our present study of breast cancer patients was 48.3 years. A third of the cases presented with large, necrotic tumors. The morphology was infiltrating ductal carcinoma with grade II or grade III disease, and lymph node involvement. These demographic findings complement other local studies which have stressed on the younger age of breast cancer cases at presentation in Karachi and the higher stage and tumor grade (Bhurgri et al., 2007). ER and PR were positive in 32.7% and 25.3% cases respectively; HER-2/neu was positive in 37.4% (3+ in 24.7% and 2+ in 12.7%) cases.

We compared our results with previously published international data. A study comprising 3,655 invasive breast cancers, conducted by the Department of Pathology, Memorial Sloan-Kattering Cancer Center, New York has reported a lower HER-2/neu (2+ or 3+) over-expression (26.89%) (Lal et al., 2005). In the study, expression of ER and PR were decreased significantly in HER-2 positive tumors in comparison with HER-2 negative tumors, however, a substantial number of HER-2 positive tumors still expressed ER or PR, an observation similar to the present study. In the Sloan-Kattering study HER-2 positivity was limited to invasive breast carcinomas of

Table 3. Correlation of HER-2/neu Status with Estrogen and Progesterone Receptor Expression

HER-2neu status	ER expression				PR expression			
	+	++	+++	-	+	++	+++	-
HER-2/neu positive (3+; #37)	5.4	3.2	8.1	83.8	5.4	-	2.7	83.8
HER-2/neu positive (2+; #19)	5.3	26.3	21.1	47.4	15.8	15.8	15.8	52.6
HER-2/neu negative (#94)	6.4	5.3	23.4	64.9	4.3	8.6	14.9	72.3

the ductal and lobular morphology, in the pleomorphic sub-types, not in the classic variety. None of the special type carcinomas like mucinous, metaplastic and adenoid cystic types showed HER-2 positivity. These findings are similar to our study except that a case of mucinous subtype of IDC in our study exhibited HER-2 positivity. In both studies HER-2 positivity was associated more strongly with higher histologic grade carcinoma. None of the grade I carcinomas were HER-2 positive in both studies; the majority of grade III tumors expressed positivity whereas a smaller component of grade II carcinomas were HER-2 positive (Lal et al., 2005).

Correlation of HER-2/neu over-expression and tumor grade was also studied by Rilke et al with a sample size of 1,210 cases. According to their study also, HER-2/neu over-expression was associated with a higher tumor grade, as observed in 3.9%, 20.4%, and 38.9% grade 1, 2, and 3 tumors respectively, whereas in our study positivity was shown in 0%, 22.89%, and 31.58%. Similarly a study conducted in Italy (Carlomagno et al., 1996) showed over-expression of HER-2/neu in 29.7% of breast cancers, significantly correlating with larger tumor size and a decreasing level of ER. Another study by Samur et al (2003) in Antalya-Turkey provided comparable results.

There haver only been few local studies for comparison. Those published had used selective parameters. Naqvi et al conducted a study at Jinnah Postgraduate Medical Centre (JPMC), Karachi on 72 cases of invasive IDC. HER-2/neu over-expression was seen in 31% of the cases with a significant relationship ($p < 0.05$) between HER-2/neu over-expression, lymph node status and tumour size. In a similar study conducted at the Liaquat National Hospital, Karachi, Fatima et al (2005) showed 55% ER and PR reactivity, while in our study ER reactivity was seen in only 32.7% of invasive breast cancers. In their study, ER positivity decreased with increasing tumor size and grade, however, no significant correlation was seen with lymph node metastasis. Similarly we found that ER positivity decreased and HER-2/neu is over-expressed with increasing tumor size and grade, although no significant correlation was seen with lymph node metastasis.

The ER, PR expression in breast cancer, in the current study is comparable to published international studies, but the frequency of HER-2neu expression is higher in the current study. This may reflect the younger age at diagnosis. Larger studies are required to study the biological behavior of breast cancer in this high risk population. The clinical importance of these prognostic markers in the management of breast cancer patients is strongly advocated in our population to improve the dismal prognosis and to provide better therapeutic options.

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