ORIGINAL ARTICLE

Correlation of human epidermal growth factor receptor 2 (HER-2/neu) receptor status with hormone receptors Oestrogen Receptor, Progesterone Receptor status and other prognostic markers in breast cancer: an experience at tertiary care hospital in Karachi

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Abstract

Objective: To determine the frequency of human epidermal growth factor receptor 2 (HER-2/neu) positivity and to correlate its status in breast cancer patients with other prognostic markers.

Methods: The comparative cross-sectional study was conducted at the Department of Histopathology, Liaquat National Hospital, Karachi, from January 1 to October 31, 2010. It included all specimens of mastectomy and lumpectomy with axillary tissue. Incisional, trucut and wedge biopsies as well as all non-epithelial tumours were excluded. All samples were processed as per standard guidelines and were evaluated by immunohistochemistry. SPSS 10 was used for statistical analysis.

Results: The age of the 100 cases in the study ranged from 20 to 82 years with a mean of 51±17.6 years. Two (2%) of the patients were males. HER-2/neu over-expression increased with increasing tumour size, grade, lymph node metastasis and with oestrogen receptor and progesterone receptor negativity. No significant correlation of HER-2/neu was seen with the age of patient and with the tumour type.

Conclusions: The expression of HER-2/neu was associated with decrease in oestrogen receptor and progresterone receptor positivity, and increase in tumour size, high tumour grade and lymph node metastasis.

Keywords: Breast carcinoma, Estrogen receptor, Progesterone receptor, HER-2/neu, C-erb B2. (JPMA 63: 854; 2013)

Introduction

The new millennium still finds breast cancer as a leading cause of cancer-related deaths in females and it is the most common cancer among women 40 to 55 years of age.[1] According to Karachi Cancer Registry data, breast cancer is the most frequent cancer in women of Karachi, accounting for one-third of the cancers in females.[2] Increase in awareness amongst women, better health education and a good screening programme can achieve a significant improvement in early diagnosis and, therefore, survival. All the developments emphasise the increasing importance of prognostic and predictive factors in the management of patients with breast cancer.

Despite improvements in conventional therapy, new approaches to the management of breast cancer are desperately needed. These are starting to emerge as an improved understanding of the biology and pathology of the disease provides new targets for anti-tumour treatment. Amongst the most well characterised of these is p185 human epidermal growth factor receptor 2 (Her2/neu), a 185kDa transmembrane protein product of HER2/neu (also known as HER2, neu and C-erbB-2) gene, which has extensive structural homology with the epidermal growth factor receptor (EGFR). This receptor is found on the surface of normal cells, but in some tumours the HER2/neu gene is over-expressed, resulting in greater than normal density of p185 HER2/neu.[3]

Over-expression of HER2/neu in human cancer was first observed during the screening of deoxyribonucleic acid (DNA) samples derived from breast cancers, about 30% of which demonstrated amplification of the proto-oncogene i.e. HER2/neu.[4]

HER2/neu is an independent prognostic factor in patients with breast carcinoma. Its over-expression predicts early metastasis, shortened disease-free period and overall survival, poor clinical outcome, early recurrence, resistance to hormonal therapy, some form of chemotherapy and early death in breast carcinoma. Therefore, its evaluation seems critical.[3]

Recently, an antibody to HER2/neuoncoprotein has been developed known as Herceptin. It has been approved by the Federal Drug Agency (FDA) as a treatment for the end-stage breast cancer patients with HER2/neu positive tumour.[5] It shows a modest but significant improvement in patients with advanced breast cancer. This benefit is restricted to patients with tumours expressing very high levels of HER2/neu, and only targets the oncoprotein.

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Immunohistochemistry (IHC) is the most commonly used method for evaluating HER2/neu protein expression on formalin fixed paraffin embedded samples of breast cancers.[3]

The purpose of the current study was to see the overall frequency of HER2/neu over-expression in breast cancer in southern Pakistani women and to correlate the positivity index with other prognostic markers like nodal status, tumour size, tumour type, tumour grade, patient's age and oestrogen receptor (ER) and progesterone receptor (PR) status.

Patients and Methods

The prospective study was conducted at Liaquat National Hospital, Karachi, from January 1 to October 30, 2012. Employing non-probability purposive sampling technique, 100 samples were collected from among the modified radical mastectomies and lumpectomies with axillary tissue. Simple mastectomies without axillary lymph node sampling, specimens showing ductal carcinoma in situ (DCIS) without invasive component, specimens with previous lump removal and then no residual tumour, specimens with previous neo-adjuvant therapy and then fibrosed tumour, specimens with marked autolytic changes and specimens having non-epithelial tumours were excluded.

All samples were processed as per standard guidelines and HER-2 and ER/PR status were evaluated by IHC, using DAKO HER-2 and DAKO ER/PR System method for all patients of breast cancer on paraffin blocks of the tumour. Data was stored in SPSS 10. Categorical variables were presented as frequencies and percentages, while numerical variables were computed as Mean ± Standard Deviation. Binary logistic regression analysis was performed to calculate the significance of these factors in relation with histopathology. Statistical significance was taken at p value less than 0.05.

Results

The age of the 100 cases studied ranged from 20 to 82 years, with a mean of 51±17.6 years. Most of the patients (n58; 58%) were post-menopausal (>50 years). Regarding gender, only 2 (2%) patients were male. The left breast was slightly more commonly involved (n=56; 56%) than the right breast. The tumour size ranged from 1.2cm to 10cm with a mean of 5.6±2.8cm. Minimal numbers of recovered lymph nodes were 03 and the highest count was 55.

Of the 100 cases, 18(18%) were grade I invasive carcinomas, 45(45%) were grade II carcinomas and 37(37%) were grade III carcinomas. Besides, 85 (85%) cases were of Infiltrating ductal carcinomas (IDC) NOS,

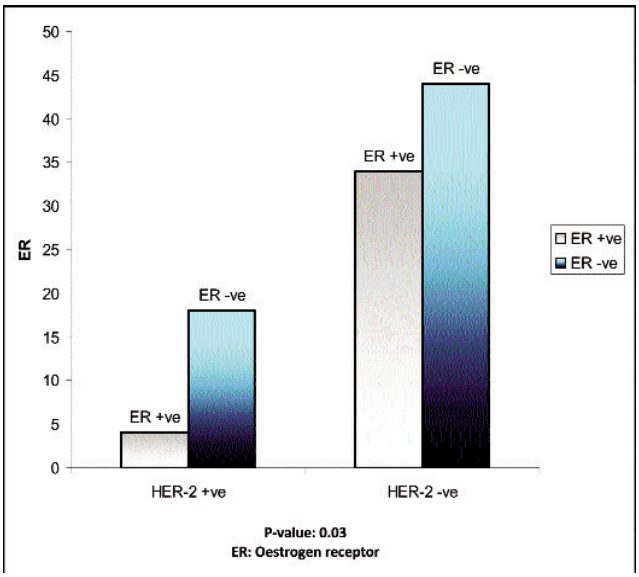

Figure-1: HER-2 with ER receptor status.

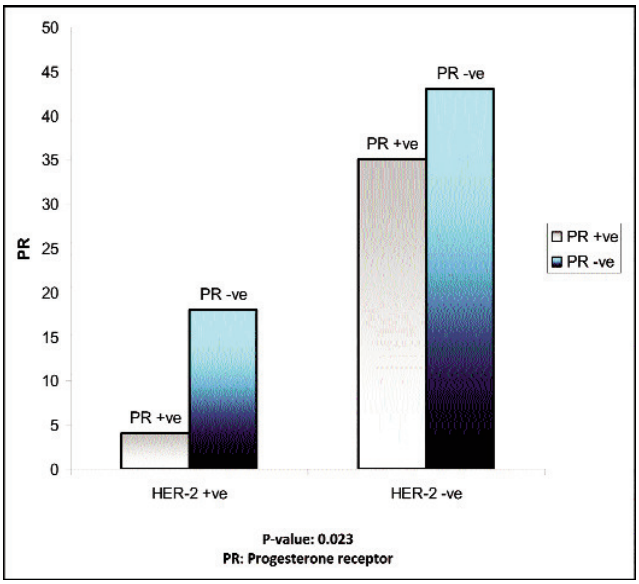

Figure-2: HER-2 with PR receptor status.

(not otherwise specified); 06 (6%) were Infiltrating lobular carcinoma (ILC); 3 (3%) were Metaplastic carcinoma (MC) and 2 (2%) were found to be Tubulolobular. There was 1 (1%) case each of Papillary, Mucinous, Signet ring, and Serous carcinomas. HER-2/neu was positive (2+ and 3+) in 22 (22%) cases, and negative in 78 (78%). ER was positive in 38 (38%) cases and negative in 62 (62%). PR was positive in 39 (39%) cases and negative in 61 (61%).

Stratification of tumour size was performed in two groups. In HER-2 negative cases, the total number of group 1

Table-1: HER-2 with tumour size.

		Size of Tumour		Total
		<2cms	>=2cms	
HER-2 receptor status	+ve	0	22	22
	-ve	13	65	78
Total		13	87	100

P-value: 0.9.

Table-2: HER-2 with tumour grade.

		Grade of Tumour			Total
		Grade 1	Grade 2	Grade 3	
HER-2 receptor status	+ve	1	8	13	22
	-ve	17	37	24	78
Total		18	45	37	100

P-Value: 0.7.

tumours (i-e tumour size < 2cm) was 13(16.7%), and group 2 tumours were 65(83.3%). In HER-2 positive cases 22(100%) were found to lie within group 2 (i.e. tumour size equal to or more than 2cm) and none of them fell within the group 1 category (Table-1).

In terms of different grades of tumour, 22 HER-2 positive cases had 01(4.5%) grade-1; 08 (36.4%) grade-2 and 13(59.1%) grade-3 carcinomas. In HER-2 negative cases, there were 17(21.8%) grade-1, 37(47.4%) grade-2 and 24(30.8%) grade-3 tumours (Table-2).

In HER-2 positive cases, 12 (54.5%) showed lymph node involvement, while in HER-2 negative cases, there were 25(32.1%) such cases. No lymph node involvement was seen in 10(45.5%) HER-2 positive cases and in 53(67.9%) Her-2 negative cases.

In HER-2 positive cases, 14(63.6%) were in group-1 and 8(36.4%) in group-2. In HER-2 negative cases, 45(57.7%) were of group-1 and 33(42.3%) were of group-2.

As for correlation of HER-2 status with ER and PR receptor status, 4 (9.1%) HER-2 positive cases were ER and 4(9.1%) were PR positive, while 18(40.9%) were ER and PR negative each. Out of total 78 HER-2 negative cases, 34(21.8%) were ER positive, 44(28.2. %) were ER negative, 35(22.4%) were PR positive and 43(27.6%) were PR negative (Figure-1 and 2).

As for the tumour type, 20 (23.52%) of the 85 IDC cases were HER-2 positive, while 65 (76.47%) were HER-2 negative. Total 6 ILC were found and all of them were HER-2 negative. The 3 MCs were negative for HER-2neu status, while both of the tubulolobular were positive. All the other types were Her-2 negative.

Discussion

Breast cancer is the most common carcinoma in women and accounts for 22% of all female cancers, which is more than twice the prevalence of cancer in women at any other site.[6] In Pakistan, the documented frequency of breast carcinoma is 33%. Prognosis and management of breast cancer are influenced by the classic variables such as histological type and grade, tumour size, lymph node status, ER and PR status of the tumour, and more recently, HER-2 status.[7]

HER-2/neu, also known as C-erb B2 (HER-2), a proto-oncogene located on chromosome 17, is amplified and/or the protein (HER-2) over-expressed in 15% to 25% of invasive breast carcinoma and is associated with poor clinical outcome.[8] In contrast, ER is expressed in 70% to 95% of ILCs, and in 70% to 80% of IDCs. PR is expressed in 60% to70% of invasive breast carcinomas.[9] Expression of ER and/or PR generally is associated with a better outcome. Survival and response to hormone therapy are most favourable among women with tumour positive for both ER and PR, intermediate for tumours discordant on receptor status and least favourable for tumours negative for both. The interrelationship of ER, PR and HER-2 has come to have an important role in the management of breast cancer. It has been shown that patients with breast carcinoma over-expressing HER-2 don't respond to tamoxifen therapy. Though HER-2 expression is generally inversely related with ER and PR expression,[10] the precise extent of its inverse relationship and its association with classic histopathologic prognostic indicators has not been studied systematically in a large series of cases.

HER-2/neu oncogene has been the subject of heated debate during the last decade as a prognostic factor. It's over expression is thought to be an independent prognostic factor in breast carcinoma cases and though it is predictor of shortened disease-free interval and short survival with poor outcome in patients, Herceptin is a hope for improving prognosis of such cases which are otherwise refractory to other kinds of treatment. For example treatment with Herceptin, the monoclonal antibody against HER-2/neu protein, may prolong the survival of patients with metastatic disease.[11]

We compared our results with a number of international studies in view of a proposition that breast carcinogenesis is a remarkably different process in the subcontinent compared to the western population. A large study comprising 3655 invasive breast cancers was performed in the Department of Pathology, Memorial Sloan-Kettering Cancer Centre, New York.[12] HER-2 was over-

expressed (IHC score of 2+ or 3+) in 26.89% of tumours, while in our study, HER-2 was over-expressed in 22% of the tumours. In the other study, the expression of ER or PR was decreased significantly in HER-2 positive tumours in comparison with HER-2 negative tumours. However, a substantial number of HER-2 positive tumours still expressed ER or PR. Similarly, in our study only 9.1% tumours exhibited simultaneous ER, PR and HER-2 positivity. In the other study, HER-2 positivity was limited to invasive breast carcinomas of the ductal type and among lobular carcinomas, HER-2 positivity was observed only in the pleomorphic type and not in the classic type tumours. None of the special type carcinomas like mucinous, metaplastic and adenoid cystic types showed HER-2 positivity. In contrast, our study experienced HER-2 positivity in 2 special type carcinomas i.e. in signet ring type and one of the 2 tubulolobular type.

In the New York study, HER-2 positivity was associated more strongly with higher histological grade in primary and metastatic carcinomas, which is the dissimilar finding observed in our study. None of the grade-I primary (including tubular) carcinomas was HER-2 positive in the other study. In contrast 1 out of 18 grade-I tumours, i.e. 5%, in our study were HER-2 positive. Vast majority of HER-2 positive tumours in the other study were grade-3 tumours; a similar finding seen in the present study. Their data further demonstrated that only a minority of HER-2 positive tumours were grade-2, while in the current study 36% of grade-2 tumours were HER-2 positive, and 59% of grade-3 tumours were HER-2 positive.

Correlation of HER-2 over-expression and tumour grade was also studied by one study on 1,210 cases.[13] HER-2 over-expression was observed in 3.9%, 20.4%, and 38.9% in tumours of grades 1, 2, and 3 respectively. This is in contrast to our study in which HER-2 positivity was shown in 5%, 36%, and 59% in tumours of grades 1, 2 and 3 respectively.

Another study done at Italy[14] showed over-expression of HER2/neu in 29.7% of breast cancers and this was significantly correlated with a larger tumour size and with a decreasing level of ER.

Another study in Antalya, Turkey[15] was done on 169 breast cancer cases, with results that are comparable with those of the current study.

A study done in Cairo, Egypt, also showed more or less similar results as in the present study. In their study, 40 cases were HER-2 positive in which 47.5% were ER positive, 52.5% were ER negative, 65% showed PR positivity and 35% showed PR negativity. Lymph node metastasis was seen in 65% of cases with HER-2 positive status.[16]

Considering the local literature, a number of studies were performed to see the prognostic factors in breast carcinoma in the local population.[17-22] One study took 72 diagnosed IDC cases.[23] HER2/neu over-expression was seen in 31% of cases and a significant relationship ($p<0.05$) was found between HER2/neu over-expression and lymph node status. Our study observed it in 22% of cases. In another study[24] HER2/neu over-expression was observed in 33% of invasive breast cancers. Another study on 150 breast cancer cases showed 55% of cases exhibiting ER and PR reactivity,[1] while in our study ER reactivity was seen in only 38% of invasive breast cancers. In the other study, ER positivity decreased with increasing tumour size and grade, but no significant correlation was seen with lymph node metastasis. Similarly, we found that ER and PR positivity decreased as HER2/neu was over-expressed, and significant correlation was found with lymph node metastasis and HER-2/neu positivity. In a Bangladeshi study, higher number of breast tumour cases showed positivity with ER and PR; 69.0% of the specimens were ER positive, 72.3% were PR positive, and 28.4% tested positive for HER-2/neu.[25]

There were also few limitations of the current study. As all the cases that were biopsied during the study period, fulfilling the inclusion criteria were included, no definitive sampling technique was applied. Although sample size was large, but this limitation may have affected the results. Secondly, the groups were simply divided into HER-2/neu positive and HER-2/neu negative groups. No statistical test for grouping was applied.

Conclusion

The expression of HER-2/neu is associated with decrease in ER and PR positivity; increase in tumour size; high tumour grade; lymph node metastasis. No significant correlation was found between HER-2 expression and tumour type or age of the patient. More intense strategic measures for diagnosis and therapeutic purposes are needed in southern Pakistani patients.

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