Significance of Her-2/neu protein over expression in Indian breast cancer patients

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Abstract

Background Racial disparity in presentation and outcome of breast cancer is established but cause is unexplained. Many studies show various molecular markers for racial differences in the prognosis of breast cancer. There is scarcity of data on prognostic significance of HER-2/neu in Indian breast cancer.

Aims and Objective To know incidence and prognostic significance of HER-2/neu expression in Indian patients. To correlate HER-2/neu Expression with other prognostic markers and ER/PR Receptor status.

Material and Methods 112 consecutive patients with breast cancer attending the Department of Surgical Oncology from March 1997 to March 2000 were included in this study. The

clinical data along with ER/PR status, follow up data and HER-2/neu expression examined by immuno-histochemical method was recorded.

Statistical Analysis Data was analyzed by univariate and multivariate analysis for all prognostic factors. Significance was calculated by using Chi square test and survival analysis by using Kaplan Meier survival curve.

Results The median age of 112 patients was 46.56 (± 9.55) years. HER-2/neu over expression was present in 46.37%. Significant correlation was found between HER-2/neu over expression and lymph node status, grade of tumor and ER/PR receptor status. Median follow up period of 23 months. There was significant tumour free survival advantage ($p < 0.01$) and overall survival advantage ($p < 0.001$) in patients with HER-2/neu negative expression.

Conclusion HER-2/neu oncogene over expression is higher (46.37%) among Indian patients in comparison to 25–30% shown in most western literature. HER-2/neu oncogene over expression significantly correlates with grade, lymph node involvement, ER/PR status and also affects survival.

Keywords HER 2 expression · Breast cancer in India · Prognostic markers

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Introduction

Breast cancer (BC) is a heterogeneous disease with variable biological and clinical characteristics. The racial influence in BC in terms of age at presentation, clinico-pathological

features, and outcome of treatment has been widely reported [1–3]. It has been established that BC in many Asian and African countries, affect younger females, who present themselves in advanced stage with poor prognostic features and has worse outcome when compared to their counterparts in the Western countries [4–9].

There is no doubt that the lack of awareness and early detection program contribute to advanced presentation of disease, however the biological aggressiveness in terms of tendency to affect younger females, poor differentiation and lack of steroid receptor expression remain unexplained. Abnormalities described in the structure and activity of several proto-oncogenes may contribute to the development or progression of breast cancer [10]. HER-2/neu(erbB2) is one of the proto-oncogene, a member of epidermal growth factor receptor superfamily. Its over expression and gene amplification leads to transformation of human breast epithelium to malignancy. Alterations in the proto-oncogene HER-2/neu have been extensively studied in Western and east Asian females with breast cancer and proposed as prognostic markers of potential clinical utility [11–17]. However, few studies exist on prognostic significance of these oncogenes in female breast cancer in developing countries particularly in India [18].

Material and Methods

Setting

This prospective study was carried out in the breast unit of the Department of Surgical Oncology, Banaras Hindu University between March 1997 and March 2000.

112 consecutive patients with primary breast cancer previously untreated by chemotherapy/ radiotherapy/ hormone therapy were included in this study. The clinical information, age, sex, tumor type, lymph node status, stage and therapy, overall survival was entered on the computer data base for analysis. All histology specimens were typed according to the World Health Organization classification [19] and histologically graded according to the modified Scarff-Bloom-Richardson histological grading [20]. All specimens were evaluated without knowledge of the clinical data.

Examination of HER-2/neu & ER, PR by immunohistochemical staining

Five millimeter thick sections were obtained from formalin-fixed, paraffin-embedded tumor tissues. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohols. After blocking endogenous peroxidase activity with 0.3% hydrogen peroxide in water for 5 minutes, the sections were placed in 1600 ml of antigen masking solution in boiling pressure cooker for 5 minutes for pro-

gesterone receptor (PR) immunostaining or placed in 500 ml of antigen masking solution heated in a microwave oven at 750 W for 20 minutes for estrogen receptor (ER) immunostaining. Monoclonal antibody for ER and monoclonal antibody ER at dilution of 1:50 was used. Peroxidase was demonstrated with 0.5% diaminobenzidine for 10 minutes and the sections were counterstained with Mayers hematoxylin. Positive and negative controls were performed at the same time for each section. Similar sections were also stained for HER-2/neu according to instructions of the manufacturer and using reagents included in cerb-B₂ detection kit. The reason being that it is a common observation including ours that whenever HER-2/neu is expressed, it is expressed rather homogeneously on almost all tumor cells. Likewise when it is negative, it is clearly negative therefore the common scoring system negative staining (0) had either no immunostaining of the cell membrane or faint staining involving a portion of the circumference of the cell membrane in less than 10% of the invasive cell population. Low positive staining (1+) had weak but definitive staining of the entire circumference (100%) of the cell membrane in 11% to 30% of the neoplastic cell population. Medium positive staining (2+) had strong positive staining of the entire circumference (100%) of the cell membrane in 31% to 50% of the neoplastic cell population. High positive staining (3+) had strong positive staining of the entire circumference (100%) of the cell membrane in 51% to 100% of the neoplastic cell population has not been used.

Statistical analysis

Statistical analysis of the data was done on SPSS computer statistics programme. To see the association between two variables, χ^2 – test has been applied. Z – test has been used to test the significance between two proportions. Disease Free Survival (DFS) was assessed from the day of surgery until first recurrence. DFS and Overall survival (OS) curve was calculated using the Kaplan-Meier product-limit estimate. Results were considered significant when the p value was less than 0.05. Univariate and multivariate analysis was done to examine the relation of each prognostic factor with HER-2/neu status.

Results

Clinical and pathological characteristic (Table 1)

The mean age was 46.56 ($\pm$ 9.55) years (range 25–66 years). 59 (53.7%) patients were postmenopausal. Overall only 92 patients could be staged primarily and among them 79 (85.8%) had stage III BC. Rest 20 patients presented after prior surgery outside and had no records of the treatment

Table 1 HER-2/neu over expression and clinical, histological and hormonal receptor characteristics

		HER-2/neu status				
		Positive	Negative			
Grade						
• I	32	4 (12.5%)	28 (87.5%)	I Vs II	4.16	<0.001
• II	69	39 (56.5%)	30 (43.5%)	I Vs III	4.32	<0.001
• III	11	9 (81.8%)	2 (18.2%)	II Vs II	1.59	NS
$\chi^2 = 23.12$ (p<0.001)						
Tumor size						
• <2 cm	2	-	2 (100%)	<2 Vs 2–5	1.06	NS
• 2–5 cm	30	11 (36.67%)	19 (63.33%)	<2 Vs >5	1.43	NS
• >5 cm	76	39 (51.32%)	37 (48.68%)	2–5 Vs >5	1.36	NS
$\chi^2 = 3.61$ (NS)						
Axillary lymph node status						
• Negative	32	8 (25%)	24 (75%)	Vs < +3	0.45	NS
• Positive						
<3	23	7 (30.43%)	16 (69.50%)	< 3+ Vs ≥ 3+	2.74	<0.01
≥3	56	36 (64.29%)	20 (35.11%)	- Vs ≥3+	3.55	<0.001
$\chi^2 = 16.0$ (p<0.001)						
Hormone status						
• I. ER+ PR+	13	4 (30.77%)	9 (69.23%)	I Vs II	2.31	<0.05
• II. ER– PR–	66	43(65.15%)	23 (34.85%)	I Vs III	1.21	NS
• IV. ER- PR+	15	2 (13.33%)	13 (86.66%)	I Vs IV	2.93	NS
$\chi^2 = 23.0$ (p<0.01)	18	3 (16.67%)	15 (83.33%)	II Vs III	3.63	<0.001
				II Vs IV	3.66	<0.001
				III Vs IV	0.27	NS
Recurrence						
• Present	26	22 (84.62%)	4 (15.38%)	Present Vs Absent	4.456	<0.001
• Absent	86	30 (53.57%)	56 (46.43%)			
$\chi^2 = 17.903$ (p<0.001)						
Vascular invasion						
• Present	32	22 (68.75%)	10 (31.25%)	Present Vs Absent	2.99	<0.01
• Absent	80	30 (37.50%)	50 (62.50%)			
$\chi^2 = 7.762$ (p<0.01)						
Lymphatic invasion						
• Present	34	23 (67.65%)	11 (32.35%)	Present Vs Absent	2.97	<0.01
• Absent	78	29 (37.18%)	32 (62.82%)			
$\chi^2 = 7.655$ (p<0.01)						
Age group						
• <35	8	5 (62.50%)	3 (37.5%)	<35 Vs 35-49	0.88	NS
• 35-49	61	28 (45.80%)	33 (54.10%)	<35 Vs >50	0.95	NS
• ≥50	43	19 (44.19%)	24 (63.81%)	35-49 Vs >50	1.73	NS
$\chi^2 = 5.09$ (NS)						

LEGEND ER: estrogen receptor, PR: progesterone receptor

they had received. Axillary lymph node metastasis was found in 79 (70.54%) patients and 56(50%) of whom had >3 lymph nodes positive. 95 (83.9%) patients had invasive ductal carcinoma and 69 (61.6%) had grade II tumor. 66 patients had both ER and PR-negative breast cancer.

Modified radical/ Radical mastectomy with post operative adjuvant therapy was offered to all operable cases. 70 (62.5%) patients received chemotherapy, 50 patients (44.64%) were also given locoregional radiotherapy. Adjuvant hormonal treatment (Tamoxifen 20 mg PO, OD) was given to 84 patients (75%).

Frequency and associations with HER-2/neu expression

HER-2/neu oncogene over expression was found in 52(46.43%) patients. Expression of HER-2/neu correlated with lack of estrogen and progesterone receptors ($p < 0.01$), poor tumour differentiation ($p < 0.001$) and positive axillary lymph node metastasis ($p < 0.001$). No significant correlation was found between age and HER-2/neu over expression ($p=0.07$). Significant association was also found in term of vascular and lymphatic invasion ($p < 0.001$ each).

Survival analysis (Table-2–7; Fig. 1, 2)

The follow up period ranged from 3 months to 79 months (median, 23 months). At the time of analysis 11 patients had died (9.82%) of BC and 101 patients (90.18%) were alive. HER-2/neu positive patients had significantly lower mean OS and DFS compared to HER-2/neu negative patients [18.38(± 1.85) months vs. 28.47 (± 2.37) months and 17(± 1.77) vs. 28.17 (± 2.37) months respectively.]

Table 2 Disease free survival (DFS)

	Disease Free Survival (months)	
	Mean $\pm$ SE	Median $\pm$ SE
HER-2/neu positive (n=52)	17 $\pm$ 1.77	15 $\pm$ 1.80
HER-2/neu negative (n=60)	28.17 $\pm$ 2.37	28 $\pm$ 2.71

Table 3 Overall survival (OS)

	Overall Survival (Months)	
	Mean (months) ($\pm$ SE)	Median (months) ($\pm$ SE)
HER-2/neu positive (n=52)	18.38 $\pm$ 1.85	16 $\pm$ 4.35
HER-2/neu negative (n=60)	28.47 $\pm$ 2.33	28 $\pm$ 2.88

Table 4 Survival pattern in invasive breast cancer among favourable prognostic group

	Mean Survival (months)		
	HER-2/neu positive	HER-2 neu/ negative	
ER/PR + (46)	15.11 (14.23)	28.24 (17.31)	<0.001
Well differentiated (32)	22 (18)	27.46 (19.94)	<0.05
<5 cm cases (34)	21 (17)	28.36 (20.16)	<0.05
Axillary L.N <3/or absent (56)	16.28 (12.35)	29.04 (20.88)	<0.001

Table 5 Poor prognostic group survival effect of HER-2/neu expression

	Mean Survival (months)		
	HER-2/neu positive	HER-2 neu/ negative	
ER/PR –ve (66)	17.023 (13.11)	28.97 (21)	<0.001
Mod/Poor Differentiated (80)	16.39 (13.69)	26.43 (17.52)	<0.001
>5 cm (74)	17.04 (14.12)	26.26 (17.48)	<0.01
≥ 3 Axillary L.N + (56)	17 (13.82)	26.36 (13.8)	<0.01

Even among the patients with favorable prognostic factors, HER-2/neu over expression is significantly associated with lower DFS and OS indicating that HER-2/neu is a stronger prognostic index than others (Table 4). Similarly, we found that in poor prognostic group of patient's those who had HER-2/neu negative tumors had better prognosis (Table 5).

HER-2/neu over expression remained strong and independent prognostic factor for breast cancer after Cox multivariate analysis (Table 6, 7).

Discussion

Breast cancer is a heterogeneous in nature. Several biological features such as tumor size, axillary lymph node status, tumor grade and ER and PR content provides accurate prognostic prediction and are useful to guide the selection of treatment of the individual patient. At present it is agreed by common consensus that axillary lymph node status is the most reliable predictor of survival and tumor size and hormone ER/PR receptor status provide a second line of prognostic predictors of clinical importance.

Fig. 1 Kaplan-meier survival curve for prediction of tumor free survival

Fig. 2 Kaplan-meier survival curve for prediction of overall survival

Table 6 Independent variables related to Disease Free Survival (Cox multivariate analysis)

Variable	Coefficient	SE	P-value	Hazard Ratio
Axillary LN positive (≥ 3)	0.022	0.233	0.925	1.022
Tumour size (>5 cm)	0.034	0.220	0.076	1.035
Mod/ Poor Differentiated	0.088	0.259	0.735	0.916
HER-2 / neu +ve	0.833	0.250	0.001	0.435
ER/PR –ve	0.134	0.222	0.546	1.143

Table 7 Independent variables related to overall survival (Cox multivariate analysis)

Variable	Coefficient	SE	P-value	Hazard Ratio
Axillary LN positive (≥ 3)	0.057	0.234	0.808	1.059
Tumour size (>5 cm)	0.097	0.220	0.660	1.102
Mod/Poor Differentiated	0.058	0.260	0.824	0.944
HER-2 / neu +ve	0.773	0.251	0.002	0.461
ER/PR –ve	0.150	0.222	0.392	1.209

Breast carcinoma is characterized by a multitude of genetic abnormalities involving both oncogenes and tumor suppressor genes. The significance of amplified oncogene in breast cancer has been the subject of debate. HER-2/neu(C-erb-B-2) is the gene known to show the highest frequency of amplification, and has in several reports been associated with breast carcinoma progression and clinically aggressive behaviour of BC [22–30]. There was inverse relationship in between HER-2/neu over expression and ER/PR receptor status (table 4), similar observation also revealed by other studies [26, 27].

This study re-enforces similar results from other studies that HER-2/neu over expression is a strong prognostic marker and predictor of survival in Breast Cancer [29]. This is also supported by survival analysis that shows significant survival advantage for HER-2/neu negative patients in comparison to HER-2/neu positive patients.

The frequency of HER-2/neu over expression in the cohort of patients reported in our study (46.37%) is higher than the most studies in western countries showing 20–35% positivity among invasive breast cancer [10]. This indicates poor prognosis of our patients and this is again supported by lower hormone receptor expression on our patients which is 25–27% in comparison >50% in most western literature [26]. One possibility of difference in incidence of HER-2/neu could be because 1+ staining detected in immunohistochemical technique is regarded as negative by FISH technique, but many series are showing similar low incidence despite of not grading the stain. Our finding is helpful in the way of application of HERCEPTIN in Indian patients. Herceptin is antiHER-2/neu antibody that is effective in cases with HER-2/neu over expression and improves survival of patients. Its efficacy has been proven in western set up. Due to cost factor it is still not affordable by Indian patients. Tissue microarrays (TMAs) as current

medical research tools significantly lower the costs of immunohistochemical examinations (IHC) and fluorescence in situ hybridization (FISH) while enabling high levels of standardization and reliability [28].

Conclusion

We have described the clinicopathological variables and immunohistochemical expression of HER-2/neu in Indian females with non-metastatic breast cancer. A large number of our breast cancer patients had lymph nodes positivity, higher grade, hormone receptor negative tumors with HER-2/ neu over expression and poor Overall Survival and Disease Free Survival. The results suggest that Her-2/neu is associated with significant prognostic value independent of other clinical factors such as tumor size, histological grade and lymph node status. It was also found that HER-2/neu expression is inversely related with hormone receptor positivity.

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