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Special Article

Prognostic Value of Epidermal Growth Factor Receptor in Breast Cancer: An Indian Experience.

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ABSTRACT

We studied the prognostic impact of EGFR positivity in Indian scenario and its correlation with known prognostic markers such as Estrogen receptor (ER) and HER-2/*neu* oncogene. 210 women aged less than 70 years with histopathologically proven carcinoma breast and having ambulatory general condition were included in the study. EGFR, Her–2/*neu* and ER expressions were evaluated immunohistochemically. Of the 210 patients, the EGFR, ER and HER-2/*neu* expressions were positive in 87 (41%), 139 (66%) and 60 (29%) patients respectively. EGFR had a positive correlation with systemic recurrence and inverse correlation with overall survival of the patient. In multivariate analysis it was observed that EGFR and node positivity were significant factors for overall survival and disease free survival. Our study reveals that expression of EGFR may serve as a prognostic indicator for poor survival in breast cancer patients.

INTRODUCTION:

Breast cancer is one of the leading cancer sites in females worldwide. Epidemiology data from United States suggests that 184,450 new cases and approximately 40,930 deaths are expected from breast cancer in the year 2008[1]. The data from European Union reports 269,570 new cases and 87,700 deaths each year[2]. In Indian sub-continent breast cancer is the second most common malignancy amongst females[3]. Various prognostic markers such as Estrogen Receptor (ER), Progesterone Receptor (PR), HER-2/ *neu* and Epidermal Growth Factor Receptor (EGFR) have been studied in breast cancer in order to identify patients who respond poorly to conventional treatment modalities.

Increased expression of EGFR gene has been found in a variety of tumors indicating a more aggressive disease compared to those with low or normal expressions[4,5,6]. EGFR is a M_r 170,000 membrane glycoprotein that contains ligand binding sites in its extracellular domain and tyrosine specific protein kinase activity as well as autophosphorylation sites in its cytoplasmic domains[7]. Although EGFR is present on cells derived from all three germ layers its expression in human neoplasms is variable. It can be detected in 35% of breast cancer[8] 50% of head and neck cancer[9] 56% of lung adenocarcinomas and 84% of lung squamous cell carcinomas[10]. EGFR overexpression correlates inversely with ER status and is associated with poor prognosis.

HER-2/*neu*, the other member of EGFR family has emerged as most important oncogenes in invasive breast cancer. The proto-oncogene HER-2/*neu* has been localized to chromosome 17*q* and encodes a transmembrane tyrosine kinase growth factor receptor with extensive homology to EGFR. Amplification of HER-2/*neu* occurs in 30% of early stage breast cancers, and a significant correlation between HER-2/*neu* overexpression and reduced disease free survival and overall survival of breast cancer patients has been reported[11-15].

The effect of steroid hormone on breast is mediated through a family of nuclear hormone receptors that include ER and PR. Nuclear hormone receptors operate as ligand dependent transcription factors that bind with DNA to direct changes in gene expression. ER expression and function are strongly influenced by growth factor signaling. As a result, ER expression levels correlate with distinct pattern of growth factor receptor over expression. ER negative tumors over express EGFR family members, in particular EGFR[16,17] and HER-2/*neu*[18,19]. Data suggests that EGFR and HER-2/*neu* signaling bypass the requirement of estrogen for breast cancer cell growth and drive breast cancer cells into an ER-negative, endocrine therapy resistant state[20].

EGFR deregulation occurs frequently in human breast tumors however role of EGFR in prediction of clinical outcome in breast cancer patients remains elusive.

These factors prompted us to plan a study for a deeper understanding of EGFR role in breast cancer. The primary objective of this study was to investigate the prognostic impact of EGFR positivity in Indian scenario and its correlation with known prognostic markers such as ER and HER-2/*neu* oncogene. The secondary objective was to assess the prevalence of EGFR and HER-2/*neu* positivity in Indian breast cancer patients.

MATERIALS & METHODS:

Patients

210 women aged less than 70 years with histopathologically proven carcinoma breast and having ambulatory general condition were included in the study. All the patients were required to have normal hematological and biochemical profile at the time of entry in to the study. Patients were excluded if they were pregnant or breastfeeding or had a history of previous malignancy, pelvic radiotherapy, systemic chemotherapy or metastatic disease. Patients were also excluded if they had received investigational therapy within 30 days prior to enrolment in the study. The study was conducted according to the ethical principles stated in the latest version of Helsinki Declaration, and the applicable guidelines for good clinical practice (GCP). Institutional Ethical Review Board approval and written informed consent was obtained from each patient before participation in the study.

Treatment Plan

All patients underwent surgery followed by assessment of molecular parameters and managed according to the standard treatment protocols of the institution. Patients were followed-up every two months to monitor local recurrence, systemic recurrence and overall survival.

Assessment of Molecular Parameters

EGFR, HER–2/*neu* and ER were evaluated Immunohistochemically. The paraffin tissue sections (5 µm) were deparaffinized in xylene, dehydrated with alcohol, and rehydrated in Phosphate Buffer Saline (PBS). Endogenous peroxidase was blocked with hydrogen peroxide in PBS. Samples were exposed to protein block and incubated overnight at 4°C with primary antibody (1:100 dilution). Following day, three PBS washes were given followed by 45 minutes incubation in moist chamber at room temperature with biotin labeled conjugated secondary antibody. Positive reaction was detected by exposure to stable 3, 3'-diaminobenzidine (DAB) and slides were counterstained with Haemotoxylin. EGFR expression was correlated with the overall prognosis of the patients and also with the known prognostic markers namely ER and HER–2/*neu*. Scoring of EGFR and ER expression levels was done on the basis of method given by *Waterfield et al*[21]. Staining of less than 10% tumor tissue cells was considered as negative. Staining of 10-50%, 51-80% and >80% tumor tissue cells were scored as 1+, 2+ and 3+ (Figure 1 & 2) respectively. The expression levels of the HER-2/*neu* were evaluated using the DAKO Hercep Test[22]. The DAKO scoring system has a scale from 0 to 3+: 0, no staining or 10% or less of the tumor cells show any level of positive staining; 1+, a faint membrane staining is detected in more than 10% of the tumor cells (the cells are only stained in part of their membrane); 2+, weak to moderate staining of the entire membrane is observed in more than 10% of the tumor cells; 3+, strong staining (Figure 3) of the entire membrane in more than 10% of the tumor cells. Samples scored as 0 and 1+ were considered negative; those scored as 2+ and 3+ staining were considered as overexpression.

Figure 1: Tumor Tissue reported as EGFR "3"

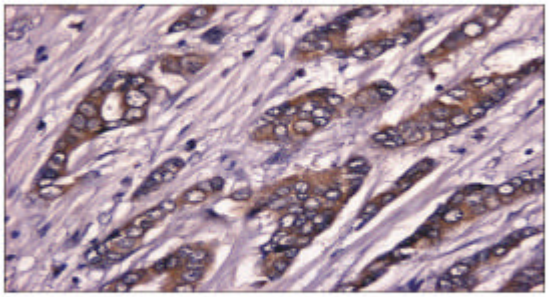


Figure 2: Tumor Tissue reported as ER "3"

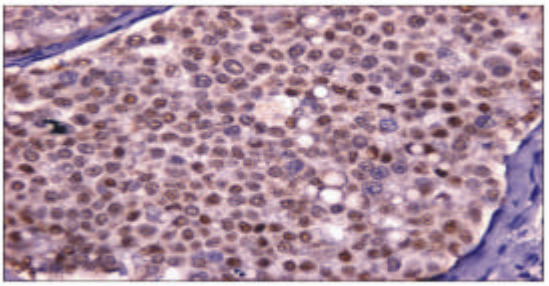
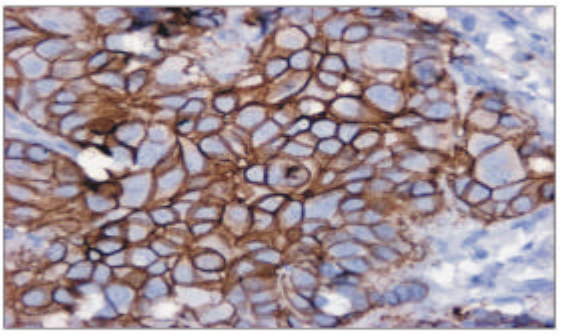


Figure 3: Tumor tissue reported as Her-2 neu "3"



Statistical Analysis

The statistical package used for analysis was SPSS version 11.0. The univariate analysis for

continuous variable was carried out using student t-test or Mann Whitney test wherever applicable. Chi-square or Fisher Exact test was applied to quantify the association between two qualitative variables. Kaplan-Meir survival analysis technique was applied to find out the independent significant risk factors separately for systemic, local recurrence and overall survival of the patients. The significance was observed with p-value < 20%.

Multivariate analysis was carried out by using Cox Regression model to see the possible risk factors responsible for breast cancer. The log rank test was applied to compare survival curves in different groups. The significance of the event was observed at $p < 0.05$.

Table 1. Baseline Patient Characteristics (N=210)

Median age (range)	48 years (25-74)
Karnofsky score (%)	
90-100	159 (76%)
80	51 (24%)
Duration of lump (range)	4 months (0-60)
Menopausal status (%)	
Post menopausal	117 (56%)
Pre menopausal	42 (20%)
Peri menopausal	51 (24%)
Breast Feeding (%)	
Yes	191 (91%)
No	19 (9%)
Tumor Size (%)	
T1	17 (8%)
T2	116 (55%)
T3	45 (21%)
T4	32 (15%)
Node Involvement (%)	
N Negative	92 (44%)
N Positive (Both N1 & N2)	118 (56%)
Stage	
I	13 (6%)
IIa	59 (28%)
IIb	73 (35%)
IIIa	33 (16%)
IIIb	32 (15%)
Pathological stage	
I	21 (10%)
IIa	67 (32%)
IIb	61 (29%)
IIIa	61 (29%)
Tumor Histology (%)	
Infiltrating Ductal Carcinoma	193 (92%)
Others	17 (8%)

RESULTS:

Patient Characteristics

Baseline patient characteristics, clinco-pathological factors and time to event measures are listed in Table 1 and 2. Of the 210 patients 163 (78%) underwent modified radical mastectomy and 47 (22%) underwent breast conservation therapy. The EGFR, ER and HER-2/*neu* expressions were positive in 87 (41%), 139 (66%) and 60 (29%) patients respectively. 162 (77%) patients received loco regional treatment by radiotherapy. Adjuvant chemotherapy was given to 189 (90.0%)

Table 2. Clinicopathological Factors & Time to Event Measures (N=210)

Surgical operation (%)	
Mastectomy	163 (78%)
BCT	47 (22%)
EGFR (%)	
0	123 (59%)
1+	39 (19%)
2+	20 (9%)
3+	28 (13%)
ER (%)	
0	71 (34%)
1+	66 (31%)
2+	54 (26%)
3+	19 (9%)
HER-2/neu (%)	
0	119 (56%)
1+	31 (15%)
2+	23 (11%)
3+	37 (18%)
Adjuvant chemotherapy (%)	
None	21 (10%)
CMF	63 (30%)
CAF	93 (44%)
Taxol/Gemcitabine	33 (16%)
Adjuvant hormonal therapy (%)	
None	55 (26%)
Done	155 (74%)
Adjuvant Radiotherapy (%)	
None	48 (23%)
Done	162 (77%)
Local Recurrence (%)	
No local recurrence	201 (96%)
Local recurrence	9 (4%)
Systemic Recurrence (%)	
No systemic recurrence	153 (73%)
Systemic recurrence	57 (27%)
Status (%)	
Alive	153 (73%)
Dead	57 (27%)

Table 3. Cox Analysis of all Variables for Systemic Recurrence, Local recurrence & Overall Survival (N=210)

Variable	Systemic Recurrence		P value	Local Recurrence		P value	Overall Survival		P value
	No	Yes		No	Yes		No	Yes	
Menopausal status									
Post menopausal	83	34	0.751	112	5	0.471	84	33	0.817
Pre menopausal	31	11		39	3		31	11	
Peri menopausal	39	12		50	1		39	12	
Breast Feeding									
Yes	137	54	0.243	183	8	0.825	138	53	0.261
No	16	3		18	1		16	3	
Tumor Size									
T1	14	3	0.726	14	3	0.016	14	3	0.749
T2	83	33		113	3		84	32	
T3	34	11		42	3		34	11	
T4	22	10		32	0		22	10	
Node Involvement									
N Negative	70	22	0.646	89	3	0.718	71	21	0.537
N Positive (Both N1 & N2)	83	35		112	6		83	35	
Stage									
I	11	2	0.843	11	2	0.086	11	2	0.859
IIa	42	17		58	1		43	16	
IIb	53	20		70	3		53	20	
IIIa	25	8		30	3		25	8	
IIIb	22	10		32	0		22	10	
Pathological stage									
I	18	3	0.288	19	2	0.595	18	3	0.735
IIa	51	16		65	2		51	16	
IIb	44	17		58	3		45	16	
IIIa	40	21		59	2		40	21	
Tumor Histology									
IDC	138	55	0.147	185	8	0.137	139	54	0.735
Others	15	2		16	1		15	2	
EGFR									
0	100	23	0.002	118	5	0.562	101	22	0.001
1+	27	12		36	3		27	12	
2+	13	7		20	0		13	7	
3+	13	15		27	1		13	15	
ER									
0	44	27	0.057	69	2	0.584	44	27	0.033
1+	50	16		62	4		50	16	
2+	45	9		51	3		46	8	
3+	14	5		19	0		14	5	
HER-2/neu									
0	92	27	0.067	115	4	0.674	93	26	0.052
1+	25	6		30	1		25	6	
2+	14	9		21	2		14	9	
3+	22	15		35	2		22	15	
Surgical operation									
Mastectomy	115	48	**0.162**	155	8	**0.407**	116	47	**0.186**
BCT	38	9		46	1		38	9	
Adjuvant chemotherapy									
None	16	5	**0.210**	19	2	**0.551**	16	5	**0.299**
CMF	40	23		61	2		41	22	
CAF	70	23		90	3		70	23	
Taxol/Gemcitabine	27	6		31	2		27	6	
Adjuvant hormonal therapy									
None	35	20	**0.073**	53	2	**0.782**	35	20	**0.058**
Done	118	37		148	7		119	36	
Adjuvant Radiotherapy									
None	39	9	**0.137**	45	3	**0.444**	39	9	**0.158**
Done	114	48		156	6		115	47	

patients, out of which CMF, CAF and Taxol/Gemcitabine based chemotherapy was given to 63 (30%), 93 (44%) and 33 (16%) patients respectively. 155 (74%) patients received adjuvant hormonal therapy by Tamoxifen. 9 (4%) patients showed local

recurrence and 57 (27%) patients showed systemic recurrence. The median time to local and systemic recurrence was 28 and 25 months respectively (range 11-90 months). At a median follow-up of 30 months 153 patients (73%) were still alive.

The univariate analysis of all variables for systemic recurrence, local recurrence and overall survival is enumerated in Table 3. EGFR had a positive correlation with systemic recurrence (p-value = 0.002) and inverse correlation with overall survival of the patient(p-value = 0.001). ER had a positive correlation with overall survival (p-value = 0.033) and inverse correlation with systemic recurrence (p-value = 0.057). HER-2/*neu* receptor had a positive correlation with systemic recurrence (p-value = 0.002) and inverse correlation with overall survival of the patient (p-value = 0.001).

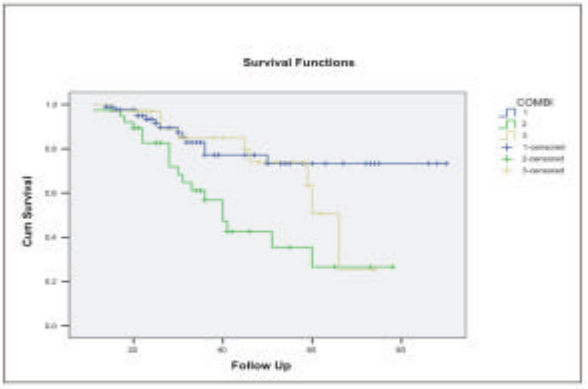
Table 4 enumerates the correlation between EGFR, ER and HER-2/*neu* expression. EGFR had positive correlation with HER-2/*neu*, p-value being highly significant (0.000) and negative correlation with ER (p-value = 0.001). ER had negative correlation with EGFR & HER-2/*neu* (p-value = 0.001 and 0.000 respectively). HER-2/*neu* had positive correlation with EGFR (p-value = 0.000) and negative correlation with ER (p-value = 0.000).

Table 4. Correlations between EGFR, ER & HER-2/*neu* (N=210)

Variable	ER (p-value)	HER-2/neu (p-value)	EGFR (p-value)
EGFR	-ve (0.001)	+ve (0.000)	--
ER	--	-ve (0.000)	-ve (0.001)
Her-2/neu	- ve (0.000)	--	+ve (0.000)

The combination data of EGFR and ER (Figure 4) reveals that EGFR positive but ER negative patients had poorer prognosis than EGFR negative but ER positive patients. While the patients which were double negative (both EGFR & ER negative) fared even worse in terms of overall survival (p-value = 0.002).
The tumors which co-over expresses EGFR and HER-2/*neu* were found to have poorer prognosis than tumors expressing single factor (p-value = 0.001).

Figure 4. Overall survival curve of Combination data EGFR-/ER+(1), EGFR+/ER- (2) and EGFR-/ ER- (3)



In multivariate analysis (Table 5) it was observed that EGFR and node positivity were significant factors (p-value = 0.000 and 0.002

Table 5. Multivariate Analysis

Variable	Overall Survival		Disease Free Survival	
	P	Relative risk (95% CI)	P	Relative risk (95% CI)
EGFR	0.000	0.354 (0.204 – 0.614)	0.000	0.357 (0.208 – 0.616)
Node Positivity	0.002	0.409 (0.231 – 0.725)	0.002	0.420 (0.237 – 0.743)
ER –ve Patients				
EGFR	0.030	0.407 (0.180 – 0.916)	0.032	0.414 (0.184 – 0.929)
Node	0.049	0.447 (0.200 – 0.999)	-	-
ER + ve Patients				
EGFR	0.000	0.231 (0.098 – 0.547)	0.003	0.322 (0.152 – 0.682)
Tumor Size	0.039	-	-	-
Node	0.008	0.311 (0.129 – 0.746)	0.016	0.369 (0.163 – 0.835)

respectively) for overall survival and disease free survival. In ER negative patients, EGFR and node positivity were significant factors (p-

value = 0.030 and 0.049 respectively) for overall survival while EGFR was significant (p-value = 0.032) for disease free survival. In ER positive patients EGFR, clinical tumor stage and node positivity were significant factors (p-value = 0.000, 0.039 and 0.008 respectively) for overall survival while EGFR and node positivity were significant (p-value = 0.003 and 0.016 respectively) for disease free survival.

DISCUSSION:

EGFR gene was identified more than two decades ago[23], however the clinical interest in the gene has gained importance recently due to the discovery of EGFR inhibitors. Our study reports several key findings that describe the novel prognostic value of EGFR thereby contributing to a deeper understanding of breast cancer. The study provides evidences showing that expression of EGFR may serve as a prognostic indicator for poor survival in breast cancer patients. *Klijn et al.*[24] summarized the findings from 57 studies with a total of 5232 patients. Only 11 out of these 57 studies performed correlation analysis out of which 55% found an inverse correlation between EGFR levels and relapse-free survival. The numbers of samples among these studies ranged from 55 to 376 per study. Our sample size of 210 cases seems to fall within the reasonable range. The current study also reveals that ER is a good prognostic indicator and HER-2/*neu* is a bad prognostic indicator for overall survival in breast cancer.

In contrast to two initial studies[25,26], showing only a tendency to a negative relationship of EGFR, at least 28 different groups[24] have reported a negative relationship between EGFR and ER levels. The current study also reports a negative correlation between EGFR and ER and a positive correlation between EGFR and HER-2/*neu*. The combination data of EGFR and ER in this study reveals that the patients which were double negative (both EGFR and ER negative) had worst prognosis in terms of overall survival.

There are contradictory reports in the literature on the prognostic significance of EGFR over expression and its relationship with known prognostics factors[8,27,28,29]. Reports on the relationship between EGFR and lymph node status are contradictory. *Sainsbury et al*[30] and *Battaglia et al*[31] observed that EGFR positivity in primary tumors is higher in patients with nodal involvement as compared to node negative patients. In addition, *Bolufer et al*[32] found that nodal involvement correlates significantly with EGFR status only in the ER positive tumor subgroup, but not in all tumors. *Sainsbury* and associates[30] and *Harris and Nicholson*[27] reported a significant positive correlation between EGFR and increasing tumor size. *Sainsbury et al*[30] indicated that by multivariate analysis EGFR status was the most important variable in predicting regression free survival and overall survival in lymph node negative patients and the second most important variable in lymph node positive patients. The present study also confirms the findings from the previous studies. Multivariate analysis showed that in ER positive patients, EGFR, clinical tumor stage and node positivity were significant factors for overall survival while EGFR and node positivity were significant for disease free survival.

CONCLUSION

From the present study EGFR, ER and HER-2/*neu* determination seems to be of great value as a prognostic indicator in breast cancer. Further research in this area should be directed to define the best treatment modality against the presence and absence of one or more of these molecular parameters in breast cancer and other tumors.

CONFLICT OF INTEREST

No conflict of interest with regards to any financial and personal relationships with other people or organizations that could inappropriately influence (bias) this work.

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