Upregulation of Telomerase Activity by HER-2/neu and Its Significance in Breast Cancer

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ABSTRACT

The protooncogene- Human epidermal growth factor receptor (HER-2/neu) is overexpressed in 20-30% of breast cancer patients. Telomerase activity provides the molecular basis for unlimited proliferate potential of the tumor. HER-2/neu and hTERT (component of telomerase) are two antigens that are particularly appealing for a broad spectrum immunotherapy. The objective of the present study was to evaluate the association between HER-2/neu status (serum and tissue) and telomerase activity with other prognostic factors in breast cancer patients.

Serum HER-2/neu levels, CEA and CA15-3 were analysed in 268 patients with breast cancer and 175 age-matched healthy controls by enzyme linked immunosorbent assay (ELISA). Immuno histochemical (IHC) method was used for tissue HER-2/neu expression in 75 samples. Telomerase activity was measured by polymerase chain reaction enzyme immuno assay (PCR-EIA) method.

Serum HER-2/neu levels were elevated in 27.2% of patients and associated with age >40 years, premenopausal group, node positive, stage and grade of disease, but not with hormone receptor status. Telomerase activity was significantly increased in 89% of the cases. Significant correlation existed between levels of serum HER-2/neu and telomerase activity (p< 0.001, r =0.49) in breast cancer patients.

Levels of serum HER-2/neu correlated with telomerase activity in the premenopausal group age >40 years and also in the early stage of the disease. Combination of these two markers may have better predictive value in the management of breast cancer.

Keywords: HER-2/neu, Telomerase activity, Prognostic factors, Signal transduction

ÖZET

Telomeraz Aktivitesinin HER-2/neu Tarafından Artırılması ve Meme Kanserindeki Önemi

Meme kanserli hastaların %20-30'unda Protoonkogen-İnsan epidermal büyüme faktör reseptörü (HER-2/neu) over ekspresedir. Telomeraz aktivitesi tümörün sınırsız büyüme potansiyalinin moleküler göstergesidir. İmmünoterapide HER-2/neu ve hTERT (telomeraz bileşeni) üzerinde çok çalışılan iki antijendir. Çalışmamızın amacı, meme kanserli hastalarda serum ve dokuda HER-2/neu ve telomeraz aktiviteleri ile prognostik faktörler arasındaki ilişkiyi değerlendirmektir. Serum HER-2/neu, CEA ve CA15-3 düzeyleri 268 meme kanserli hastada ve yaş uyarlı 175 sağlıklı kontrolde ELISA yöntemi ile ölçüldü.

Doku HER-2/neu ekspresyonu 75 hastada immünohistokimya yöntemi ile ölçüldü. Telomeraz aktivitesi polimeraz zincir reaksiyonu-enzim immünoassay yöntemi ile ölçüldü.

Hastaların %27.2'sinde serum HER-2/neu düzeyleri yükselmişti. 40 yaş üstü premenopozal grupta nod pozitiflerde, ileri evre ve yüksek grade ile korele idi. Ancak, hormon reseptör durumu ile ilişki saptanmadı. Vakaların %89'unda telomeraz aktivitesi belirgin şekilde artmıştı. Meme kanserli hastalarda HER-2/neu ve telomeraz aktivitesi arasında belirgin bir korelasyon vardı (p <0.001, r= 0.49).

Serum telomeraz aktivitesi ile HER-2/neu korelasyonu, premenopozal grupta 40 yaş üstü ve erken evre hastalarda gösterildi. Bu iki belirteçin korelasyonunun meme kanserinin tedavisinde daha iyi prediktif anlamı olabilir.

Keywords: HER-2/neu, Telomeraz aktivitesi, Prognostik faktörler, Sinyal iletimi

INTRODUCTION

Signal transduction by class I growth factor receptors absolutely require tyrosine kinase activity and tyrosine autophosphorylation.[1] In human breast cancer cells, overexpression of HER-2/neu increases basal receptor tyrosine phosphorylation to levels greater than any other cellular substrate, and the degree of HER-2 tyrosine phosphorylation generally correlates with effects on cellular transformation.[2] PKB/Akt is a serine/threonine kinase (protein kinase B) belonging to the kinase super-family of the cAMP-dependent protein kinases. Its activation is induced in the course of signal transduction by growth factors and it is involved in many cellular processes such as cell growth survival and transcription regulation.[3] PKB/Akt is activated by phosphorylation of both serine 308 and serine 473 by a kinase pathway having PI3-K as the first kinase. Akt, or protein kinase B, mediates growth factor–associated cell survival.[4] Furthermore, Akt has been implicated in regulating angiogenesis[5] and metastasis[6], two important processes in cancer development

Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences and almost universally provides the molecular basis for unlimited proliferate potential. Telomerase activity was found to be absent in most normal human somatic cells but present in over 90% of cancerous cells and in-vitro immortalized cells.[7,8] Telomerase consists of two essential components: one is the functional RNA component (in humans called hTR or hTERC)[9], which serves as a template for telomeric DNA synthesis; the other is a catalytic protein (hTERT) with reverse transcriptase activity.[10] hTR is highly expressed in all tissues regardless of telomerase activity[11], with cancer cells generally having fivefold-higher expression than normal cells.[12] The present study was therefore planned to estimate the overexpression of HER-2/neu in breast cancer patients and associate with other prognostic factors including telomerase activity.

MATERIAL AND METHODS

Selection of subjects: Patients registered in the Breast service unit of Kidwai Memorial Institute of Oncology, Bangalore, during 2004- 2008, were included in the study. Among 390 selected patients, 21 cases had benign breast disease, 4 were male patients and 97 patients were treated cases and hence excluded from the study. Remaining 268 female patients were histologically confirmed invasive breast cancer cases and included in the study. They were in the age group of 26-85 years. Age matched (± 2 yr) healthy female controls (175 cases) were arbitrarily selected from patients' relatives.

Information on patients' age, menopausal status, disease stage, estrogen receptor (ER), progesterone receptor (PR) status, clinical nodes and grade were noted from the case files. Blood samples were collected in plain tubes, centrifuged to separate the serum prior to surgery. The serum samples (0.5 ml) were stored at -20^0C until analysis (within a month).

The study protocol was approved by the Ethics committee of Kidwai Memorial Institute of Oncology, Bangalore. A written informed consent was obtained from each patient.

Serum samples were analysed for HER-2/neu, CEA and CA15.3 by ELISA. A control group comprising of 175 age matched healthy females serum was

Breast Cancer Patients (n= 268)	Sites	
	Left side n=122 (45.5%)	Right side n=146 (54.5%)
Upper left quadrant	36 (29%)	9 (6%)
Upper right quadrant	8 (7%)	53 (36%)
Lower left quadrant	63 (52%)	12 (8%)
Lower right quadrant	10 (8%)	68 (47%)
Others	5 (4%)	4 (3%)

analysed for markers. Tissue HER-2/neu expression and telomerase activity was measured in 75 samples(tumor and normal) from among 268 cases included for the study. IHC and PCR-EIA[13] were used for tissue HER-2/neuexpression and telomerase activity respectively. Analysis of variance was used to find the association of study parameters (HER-2/neu, CEA and CA15.3) with node, stage, grade and tumor size. Multivariate logistic regression analysis was used to find out any association exists between the risk factors and study parameters. The Statistical software namely SPSS 15.0, Stata 8.0, Systat 11.0 and MedCalc 9.0.1 were used for the analyses of the data.

Table 2. Comparison of prognostic factors with serum HER-2/neu overexpression in breast cancer patients

Prognostic factors	Subjects (n)	HER-2/neu Overexpression (%)	Serum HER-2/neu levels Mean ± SEM
Age in years	< 40 (62)	16 (26%)	17.82 ± 1.93^a
	>40 (206)	57 (28%)	23.12 ± 3.28^a
Menopausal status	Pre (118)	36 (31%)	19.43 ± 2.39^a
	Peri (7)	4 (57%)	$28.25 + 8.8^b$
	Post (143)	33 (23%)	17.19 ± 1.93^a
Premenopausal status	< 40 (66)	15 (22.7%)	$14.7 + 7.75^a$
	>40 (52)	21 (40%)	$25.01 + 4.9^b$
Tumor Size	<2 cm (13)	2 (15.3%)	12.44 ± 1.97^a
	2-5 cm (139)	28 (20.5%)	16.01 ± 1.58^a
	>5 cm (116)	43 (37%)	22.13 ± 2.88^b
Lymph node Involvement	No (139)	25 (17.98%)	$13.09 + 0.79^a$
	Yes (129)	48 (37.2%)	$24.31 + 2.93^b$
Stage:	Control (175)	–	$8.07 + 1.22$
Early Stage	Stage I (14)	–	9.40 ± 0.65^a
	Stage II (88)	22 (25%)	13.94 ± 0.96^a
Advanced Stage	Stage III (142)	44 (30.98%)	$19.21 \pm 1.86a$
	Stage IV (24)	7 (29%)	36.19 ± 11.73^b
Grade	Low (69)	8 (12%)	12.69 ± 0.96^a
	High (197)	65 (33%)	21.02 ± 2.02^b
ER	Negative (124)	55 (44.35%)	$26.24 + 3.05^a$
	Positive (144)	18 (12.5%)	$11.81 + 0.58^a$
PR	Negative (127)	57 (44.8%)	$25.69 + 2.96^a$
	Positive (141)	16 (11.34%)	$12.0 + 0.68^a$
Telomerase activity	Positive (67)	42 (56%)	$26.15966 + 5.18^a$
	Negative (8)	3 (4%)	$11.01563 + 1.05^b$

Non-identical superscripts were significant across prognostic factors and identical superscripts were not significant. P values obtained after square root transformation.

Table 3. Multivariate logistic regression analysis for predicting marker levels in relation to prognostic factors.

| Prognostic factors	HER-2/neu (ng/ml)			CEA (ng/ml)			CA15.3 (IU/L)		
	Logit	Adj. OR	P value	Logit	Adj. OR	P value	Logit	Adj. OR	P value
Age >40 years	1.21	3.36	0.015	-0.10	0.91	0.846	0.33	1.39	0.427
Pre-menopausal	1.00	2.37	0.007	0.07	1.08	0.836	-0.41	0.66	0.150
Node+	1.21	3.37	0.001	0.62	1.85	0.105	0.75	2.11	0.011
Advanced stage	-0.52	0.59	0.364	2.15	8.58	0.000	1.59	4.90	0.010
ER-negative	0.94	2.56	0.236	0.85	2.33	0.317	-0.32	0.72	0.639
PR-negative	0.81	2.24	0.314	-1.22	0.29	0.151	0.83	2.30	0.226
Grade 3	1.21	3.36	0.013	-0.38	0.68	0.350	-0.44	0.64	0.176
Tumor size (>5 cm)	-0.13	0.88	0.723	0.07	1.07	0.851	0.07	1.07	0.806
Telomerase activity	1.65	5.10	0.010	0.82	2.0	0.18	0.95	2.41	0.01

RESULTS

The occurrence of breast tumors was more on the right compared to the left side (Table 1). Among 268 histologically confirmed breast cancer patients, 73 patients (27.2%) were HER-2/neu positive. The cut-off value for serum HER-2/neu was <15 ng/ml as determined by Bender Med System kit protocol. Serum HER-2/neu levels were compared with tis-sue HER-2/neu expression (Figure 1) and the levels were found to be highly concordant (G= 0.885) (G= Goodman-Kruskal Gamma measure). Our study showed that serum HER-2/neu levels increased progressively with stage of the disease, in premeno-pausal patients who were above age 40 years, lymph node positive, hormone receptor status nega-tive, higher grade, larger tumor size (>5 cm) and

Table 4. Receiving operating characteristics (ROC) analysis of Telomerase activity

| Telomerase activity absorbance	Sensitivity	Specificity	% of correct classification	LR+	LR–
0.12	100.00%	93.33%	96.67%	15.00	0
0.13	97.33%	93.33%	95.33%	14.60	0.03
0.14	96.00%	93.33%	94.67%	14.40	0.04
0.16	93.33%	93.33%	93.33%	14.00	0.07
0.17	93.33%	96.00%	94.67%	23.33	0.07
0.18	93.33%	97.33%	95.33%	35.00	0.07
0.2	93.33%	98.67%	96.00%	70.00	0.07
0.35	**93.33%**	**100.00%**	**96.67%**	**>70.00**	**0.07**
0.38	92.00%	100.00%	96.00%	>70.00	0.08
0.41	89.33%	100.00%	94.67%	>70.00	0.11
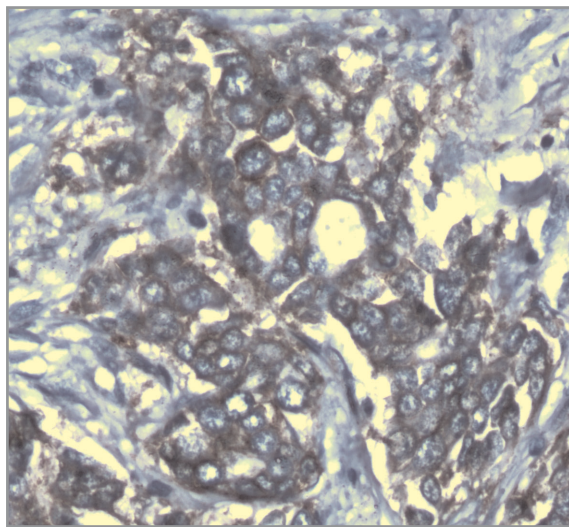
HER-2 POSITIVE
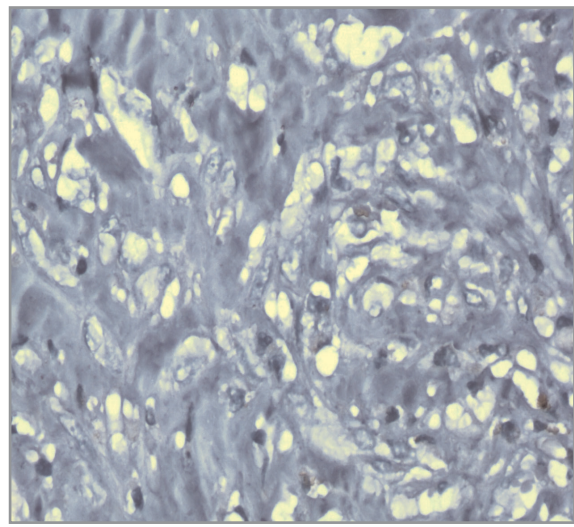
HER-2 NEGATIVE

Figure 1. Tissue HER-2/neu Status (IHC)
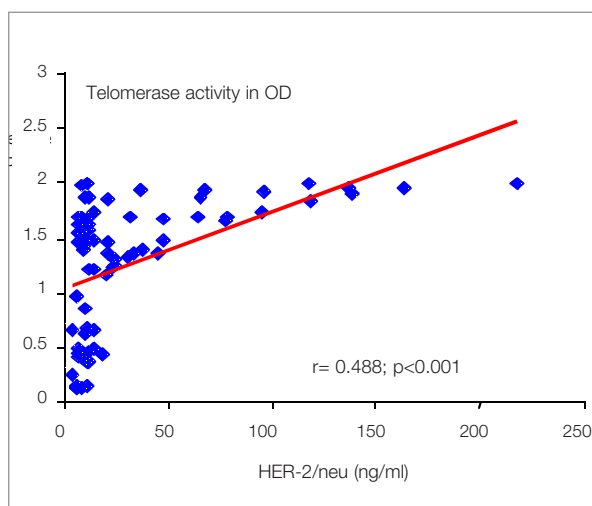
Figure 2. Pearson correlation of Telomerase activity with HER-2/neu in early stage.
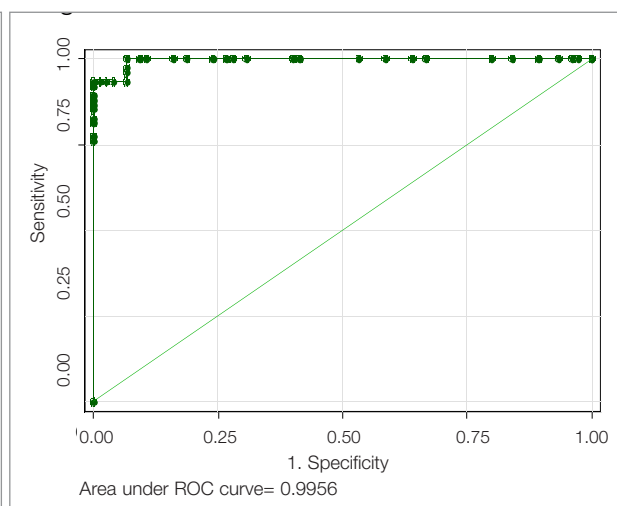
Figure 3. Receiving operating Characteristics (ROC) analysis of Telomerase activity

expression of telomerase activity (Table 2). Percentage of expression HER-2/neu was higher in premenopausal patients (40%) compared to postmenopausal (23%) in the age group of >40 years.

Using logistic regression analysis, it was found (Table 3) that prognostic factors like age >40 years, node positive, grade 3 of disease, telomerase activity were significantly associated prognostic factor with serum HER-2/neu levels. Hormone receptor status was inversely associated with HER-2/neu levels as shown by adjusted odds ratio (OR> 2.2). Serum CEA level was significant in stage 4 of the disease. Serum CA 15.3 level was significant in node positive cases, stage 4 of the disease and in patients expressing telomerase activity. HER-2/neu levels was an independent prognostic factor only with stage of the disease. Significant correlation(r= 0.49) was observed between HER-2/neu levels and telomerase activity in stage 2 of the disease (Figure 2).

Table 4 shows shaded line as cut off point for telomerase activity. A value of 0.35 was taken as cut off with a sensitivity of 93.33%, specificity of 100%

and highest likelihood positive ratio of >70.00. Area under ROC curve was 0.9956 (Figure 3). This is considered as excellent under classification of ROC curve.

Telomerase activity was expressed in 67 cases (89%) of tumor samples. (Absorbance value more than 0.35 was considered as positive). Mean and SEM of serum HER-2/neu levels was significantly elevated (26.16 ± 5.18) in cases with telomerase activity.

DISCUSSION

In breast cancer, tumors on the right side were more compared to the left side.Lower outer quadrant was the major site to develop tumors. Study by Pritchard KI et al.[14] showed that highest percentage (58.8%) of breast cancer belonged to 40-49 yrs age group. In this study, we found that highest overexpression of HER-2/neu was in the age group of age > 40 years and premenopausal patient. Though perimenopausal group showed highest percentage (57%) of serum HER-2/neu levels, the sample size being too small, was not considered for discussion.

HER-2/neu overexpression is an indication of poor prognostication. This finding combined with telomerase positivity may give better prediction for management of the disease. ELISA is an alternative method to IHC for detecting HER-2/neu overexpression in clinical practice.[15]

High levels of serum HER-2/neu were associated with ER and PR negativity and high histoprognostic grade.[16] Similar findings have been observed in our study by using multivariate logistic regression analysis even though ER, PR, stage of disease and tumor size were independent prognostic factors. ER and PR status were inversely related to HER-2/neu status.

Serum HER-2/neu level is useful when the primary tumor tissue is not available for IHC or fluorescent in situ hybridization (FISH) assays. The PCR-EIA method offers a rapid, quantitative, nonisotopic assay for the determination of telomerase activity. Expression of telomerase activity in human cancers and not in normal somatic cells is suggestive of this enzyme as a good target for anticancer drugs. Study by Hosseini-Asl S et al. focuses on regulation of telomerase in breast cancer. There is an association between hTR and the prognostic factors in human breast cancer.[17]

Telomerase activity has been detected in approximately 90% of tumor samples and enzyme is an ideal target for developing broadly effective anticancer drugs.[18] In cooperation with several oncogenes, telomerase expression results in direct tumorigenic conversion of normal human epithelial cells and fibroblasts. The specific role of telomerase in this process is that it provides an unlimited replicative potential. Study by Yang H et al. showed that HER-2 induces telomerase activity and mRNA levels and promoter activity of hTERT in human mammary epithelial cells. These effects were largely abrogated by PI3K inhibitor or expression of PTEN and dominant negative AKT2.[19]

Thus, certain signal transduction pathways (Akt) mediated by HER-2/neu receptor activates association of telomerase component. This concept may help to develop drugs for inhibition. In conclusion, telomerase component hTR and hTERT need to be studied in detail along with Her-2/neu signal transduction mediators.

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