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To compare tissue Her-2/neu with serum Her-2/neu values in treatment naïve female breast cancer in Pakistan

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Abstract: Immunohistochemistry (IHC) of the primary breast cancer specimens are commonly used to determine / measure the Her 2/neu status of patients. Grading of immunohistochemistry (IHC) in determining HER-2/neu status of a patient is debatable because of its subjective approach. It has been depicted that 75% of the patients who do not have gene amplification by fluorescent in situ hybridization (FISH) have HER-2/neu over expression evaluated by IHC[i]. Extra cellular domain of HER-2/neu oncoprotein dropped into blood may be detected in the serum. A total sample of 160 cases with primary Breast cancer was prospectively studied over a period of 5 years in KIRAN, Pakistan. In the present study, we analyzed the rate of occurrence of HER-2/neu alterations by ELISA and IHC in breast carcinoma patients. Summary measures were calculated for various demographic and biochemical parameters. A correlation was calculated between tissue Her-2/neu and serum Her-2/neu using Pearson correlation and was found significant with a p value of 0.01. Thus showing that for routine diagnostics, the combination of IHC and ELISA is useful. Furthermore, ELISA can be used if the primary tissue sample is not available for predicting tissue HER-2 status and determination of serum HER2 ECD concentration in metastatic breast cancer may provide a useful index of tissue HER2 status, especially in light of its relatively easy and inexpensive methodology and the simplicity of sample collection.

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INTRODUCTION

Breast carcinoma is the most prevalent carcinoma afflicting females[1,2] 42% belong to the developing countries. Tumor Marker (TM) evaluation is thought to be a relatively less-expensive test and is easy to do. However, repeated assessments to monitor metastasis and prognosis involve high expenditure. Secondly, decrease in size of tumor over time can make obtaining samples a difficulty. While serum is easy to access and soluble circulating tumor markers if detected would make candidates suitable for foretelling consequence and supervising treatment course. Calculating markers is easy, objective, reproducible, and economical.

Therapy given to patients is largely becoming dependent on HER-2 alteration, thereby making its accurate assessment important. Especially in the patients selected for treatment with the recombinant humanized anti-p185 HER-2/neu antibody trastuzumab which aims to attack Her-2/neu as it is the only selection standard for this drug. Since its measurement is a crucial landmark in treatment decision-making, the method to do so in the "best" way has become the cause of controversies in this topic. When decision is to be made for care of single patient, one test does not always give all the essential details. For example, knowing IHC results, does ELISA give us any further information? Does the answer depend on whether IHC was positive or negative? In the present study, we analyzed the rate of occurrence of HER-2/neu alterations by ELISA and IHC in 160 breast carcinoma patients.

We also wanted to search the relationship of all clinico-pathological and demographic information with this tumor marker in Pakistan for the first time with a good sample size.

MATERIALS AND METHODS

Study settings

Prospectively 200 women with primary breast cancer who presented to seek treatment at KIRAN (Karachi Institute of Radiotherapy & Nuclear Medicine) Cancer Hospital, Karachi enrolled in this study from 2010-2014.

Inclusion criteria

a) Primary breast cancer patients diagnosed on the bases of fine needle aspiration / true cut biopsy and mammography.
b) Breast cancer patients with involvement of regional lymph nodes and chest wall.
c) Metastasis breast cancer patients

Exclusion criteria

Metastatic breast cancer with primary outside the breast.

Objective

To compare tissue Her-2/neu with serum Her-2/neu values in treatment naïve female breast cancer cases

Study instrument

A pre-structured Performa was designed and was pretested. A written consent was taken from all subjects prior to inclusion in the study. Demographic data recorded at the first visit included age, age at menarche, whether menopausal, age at Menopause if menopausal, age at diagnosis of cancer, number of years since diagnosis, parity, family history and educational status. Data were obtained from patient's record directly on the Immunohistochemistry (tissue Her-2/neu).

CA 15-3 and type of therapy advised by Oncologist and then serum Her2/neu was offered and done .Histopathology was noted directly from medical records. Sera from 10 healthy women served as control.

Sample collection

Pre-treatment blood samples were collected from patient in a venipuncture tube without additives or anticoagulants. Blood was allowed to clot & then centrifuged at a rate of 3000 round/min to get serum. Serum sample were stored at - 20^0C for storage until analyzed.

Data analysis

Analysis was confined to patients whose complete data on all variables was available. SPSS 17 was used for data analysis. All demographic and biochemical parameters was presented as frequencies and proportions. Serum Her2/neu was correlated with tissue her2/neu by Pearson Correlation Coefficient.

RESULTS

A total of 200 Women diagnosed as primary breast cancer were enrolled in the study but 23 patients had loss to follow-up and 17 patients were dropped due to incomplete data, thus reducing the sample size to 160 patients only. Median Age was 48.50 $\pm$ 9.95 (N=160), 67.5 % (N=108) were menopausal. Only 10 % (N=16) had a positive family history of breast cancer in first degree relatives. Mean age at Menarche was 12 years±2.0. Median number of children was 3. Mean level of CA 15-3 before any treatment was 30.96 $\pm$ 44.38 and 21.25 $\pm$ 25.94 at three month follow up after treatment. Mean level of HER-2/neu was 30.79 $\pm$ 15.2

Pretreatment Serum Her 2 /neu and tissue HER2/neu were measured and their summary measures were calculated among different breast cancer types and according to grades and stages. (Table 1, 2 and 3). Tissues and serum from 160 patients were assayed by IHC and ELISA. Pearson correlation (r value) test was used to calculate correlation between serum HER-2/neu levels and tissue HER-2/neu levels.

IHC: Immunohistochemical analysis using rabbit monoclonal antibody detected 48 cases with HER-2/neu overexpression (42 as 2+, and 48 as 3+) and 70 cases showed under expression (50 as 1+ and 20 as negative).

Table 1: Summary measures of Tissue and Serum Her-2/Neu in Various Grades of Breast Cancer (n=158)

Grade	TissueHer2	SerumHer2
I	2.25±1.00 (n=16)	31.93*±1249.85 (n=16)
II	2.24±1.07 (n=100)	28.86*±1280.08 (n=100)
III	2.19±0.96 (n=42)	25.15*±1391.29 (n=42)

Table 2: Summary measures of Tissue and Serum Her-2/Neu in Various Stages of Breast Cancer (n=156)

Stage	Tissue Her2	Serum Her2
0	1.00±.00 (n=2)	36.72±0.00(n=2)
I	2.00±0.91 (n=20)	24.89±1496.80 (n=20)
II	2.21±1.062 (n=76)	27.51±1314.24 (n=76)
III	2.44±1.03 (n=50)	30.51±1174.22 (n=50)
IV	1.75±0.88 (n=8)	22.79±1653.89 (n=8)

Table 3: Summary measures of Tissue and Serum Her-2/Neu in Various Histopathological subtypes of Breast Cancer (n=160)

Histopathology	TissueHer2	SerumHer2
Infiltrating Ductal Carcinoma	2.25±0.98 (n=121)	29.63±1281.81 (n=121)
Invasive Ductal Carcinoma	2.250±1.125 (n=16)	18.32±1677.36 (n=16)
Poorly Differntiated Carcinoma	1.40±.894 (n=5)	29.68±984.15 (n=5)
Ductal carcinoma in Situ	3.00±1.30 (n=8)	35.53±463.20 (n=8)
Mucinous Carcinoma	1.40±0.89 (n=5)	18.66±694.73 (n=5)
Infiltrating Lobular Carcinoma	2.20±.836 (n=5)	26.61±610.47 (n=5)

Table 4 : Tissue Her2 , Serum Her2 Cross-tabulation

			Serum Her2 Category		Total
			Negative	Positive	
Tissue Her2 Categories	Negative	Count	31	81	112
		% of Total	19.4%	50.6%	70.0%
	Positive	Count	5	43	48
		% of Total	3.1%	26.9%	30.0%
Total		Count	36	124	160
		% of Total	22.5%	77.5%	100.0%

Table 5: Correlation of Tissue Her-2/Neu and Serum Her-2/Neu.

		Tissue Her2	**Serum Her2**
Tissue Her2	Pearson Correlation	1	.189
	Sig. (2-tailed)		.016*
	N	160	160
Serum Her2	Pearson Correlation	.189	1
	Sig. (2-tailed)	.016	
	N	160	160
*****Correlation is significant at the 0.05 level (2-tailed).			

ELISA method using anti-sp185HER-2 human monoclonal antibody detected 124 cases with HER-2/neu overexpression (Table 4). Baseline concentrations of serum HER-2/neu were elevated (that is, = 15 ng/ml) in 77.5% (N=124) of the 160 evaluable patients and 22.5% (N=36) cases were HER-2 negative (HER-2/neu<15ng/ml). The Mean and standard deviation of serum Her-2/neu positive group was 34.77+1.096 ng/ml and for HER-2/neu negative group was 17.09+2.792 ng/ml.

Serum HER-2 was>15ng/ml in 77.5 % (n=124) of the patients, including 43 from the tissue HER-2/neu positive group (n=48) and 81 from the tissue HER-2/neu negative group (n=112 i-e 1+, 2+ and Negative). Serum HER-2 was <15ng/ml in 22.5 % of the patients, including 31 from the tissue HER-2/neu negative group (n=112) and 5 from the tissue HER-2/neu positive group (n=48).A statistically significant correlation (P<0.05, r=0.18,) was observed between serum HER-2/neu and tissue HER-2/neu. (Table 5)

DISCUSSION

ELISA method seems to be promising because it has some advantages like it is quantitative, repeatable and objective. Before chemotherapy, blood can be collected in the clinic. It has this advantage too that before making any decision for chemotherapy, HER-2/neu status may be observed in real-time from the blood. In this study, to evaluate concentrations of shed HER-2/neu receptor, ELISA assay was used.

In recent publications, the direct relation of circulating HER-2/neu antigen concentrations with the HER-2/neu over expression, tumor burden and receptor activation is established. The serum antigen concentrations correlated with over expression of the HER-2/neu protein and increased tumor size is observed in MDA MB-361 xenografts of nude mice[2]. An important correlation is found among serum HER-2/*neu* protein levels and metastasis, disease reappearance, or less survival in sixteen studies (73% involving 3458 patients) out of twenty one studies on 4088 patients[3,4] On 379 patients, 2 studies showed no any relation between serum levels with prognosis[5,6] Of the 11studies in which serum HER-2/*neu* protein levels were estimated to evaluate their potential as a predictive factor for response to the treatment[7,8]. Eight studies reported raised serum HER-2/*neu* protein levels predicted failure of response to treatment. But remaining three studies did not show such connection[9].

According to our study, there is a statistically significant (p<0.05) correlation of tissue Her2/neu with the levels of serum Her2/neu with the help of ELISA, the detection of HER-2/neu protein over expression showed a positive correlation (r = 0.19) with result of the IHC used for tissue Her 2/neu. In an early study 378 patients in a crucial important Herceptin metastatic breast cancer trial showed the positive correlation of 74% patients with increased serum HER- 2/neu who had positive HER-2/neu by IHC (84% correlation with 3+IHC, and 44% by 2+ IHC)[9] Kong et al and Rani James et al also support it[9,10].

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