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ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR STATUS WITH CA 15-3 AND HER-2/NEU VALUES IN TREATMENT OF NAÏVE FEMALE BREAST CANCER CASES IN PAKISTAN

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ABSTRACT

Estrogen Receptor has been a great tumor marker for management of breast cancer. Although the accurate role of Progesterone Receptor in cancer patients management has not been established, the pathologists' guidelines have recommended for routine measurement of Progesterone Receptor with Estrogen Receptor in breast cancer patients.
A total sample of 160 cases with primary Breast cancer was prospectively studied over a period of 5 years in KIRAN (Karachi Institute of Radiotherapy & Nuclear Medicine), Pakistan. In the present study, we have analyzed the rate of occurrence of HER-2/neu (Human epidermal growth factor receptor-2), CA 15-3(Cell surface associated Mucin-1) and Steroid Hormone Receptors in breast carcinoma patients. Summary measures were calculated for various demographic and biochemical parameters and a correlation was calculated between Tumor Markers and Steroid Hormone receptors using Kruskal Wallis H test. A statistically significant difference in Her-2/neu levels between the different hormonal receptor expression groups was found with a p value of < 0.05. Based on our present data analysis we suggest that, the combination of HR (hormone receptor) and Her -2/neu is useful for routine breast cancer diagnostics.

Keywords: Serum Her-2/neu, CA 15-3, Breast Cancer, Estrogen Receptor(ER), Progesterone Receptor(PgR), Tumor Markers

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the main cause of cancer mortality among females, representing 23% of the total cancer cases and 14% of the cancer deaths (Jemal *et al.*, 2011). Circulatory tumor marker`s presence can help in prognosis and diagnosis of various cancers. These markers are very important for monitoring cancer treatment and are helpful for follow-ups, monitoring and assessing disease progression. Owing to the diversity of breast cancer in its character or contents, individual tumor marker cannot provide all the essential details. The recent trend is to identify and established different markers that can be used in combinations. In comparison to biomarkers in the primary tumor tissue, blood tumor biomarkers project a diverse scenario and their measurements can be done repeatedly as per requirement. Currently available serum breast cancer biomarkers include CA 15-3 and serum Her-2/neu.

CA 15-3 may be the first independent prognostic serum marker in breast carcinoma (Sturgeon *et al*., 2009). Overexpression or multiplication of Her-2/neu cancer producing genes has been one of the causative factor in the progression of breast cancer into an invasive and uncontrollable disease or its spread to other organs and tissues. Recently Her-2/neu is proven to be an important biomarker and area of interest for treatment in almost 30% of breast cancer patients (Mitri *el at*., 2012). In the quest for predicting response to primary chemotherapy, molecular and biological markers are focus of attention (Aupperlee 2006; Modlich, 2005). Steroid hormone receptors (HRs)) are likely to be a predictive and prognostic marker for hormonal therapy in patients suffering from breast cancer. Therefore, it is essential to assess HR status when hormonal therapy has been chosen as mode of treatment . Estrogen receptor (ER) has been the most significant biomarker in monitoring the clinical course of the disease in breast cancer patients. Progesterone receptor (PgR) existence in tumor tissue is utilized for predicting functional Estrogen receptor and also to forecast response to disease clinical outcome and hormonal treatments (Dunnwald *et al*., 2007). In the present study we investigated the relationship of clinic-pathological biomarkers ER and PgR with serum biomarkers CA 15-3 and Her-2/neu.

MATERIALS AND METHODS

Study Settings:

Prospectively 200 women with Primary Breast Cancer who attempted to get treatment at KIRAN (Karachi Institute of Radiotherapy & Nuclear Medicine) Cancer Hospital, Karachi enrolled in this study during 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 correlate ER, PR status with CA 15-3 and 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 was made, parity, family history and educational status. ER and PR status determined from biopsy. Data were obtained from Patients 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.

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 were cross tested with CA 15-3 and Her2/neu for any significance using independent single T test and one way Anova. Different histologically defined breast cancer types and Hormonal receptor expression were taken as groups and comparisons were made for all the tumor markers using the Kruskal Wallis H Test (level of significance was taken as $p < 0.05$) followed by a series of Mann Whitney U test to see where exactly the difference was lying among groups after doing Bonferroni Adjustment.

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 $\pm$ 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

Table 1. Hormone Receptor status in study group (N=160).

		Progesterone Receptor		Total
		Positive	negative	
Estrogen Receptor	positive	88	14	102
	negative	2	56	58
	Total	90	70	160

HORMONE RECEPTOR EXPRESSION

ER/ PR status was known in all Patients. ER +/PR+ were 55.0% (n= 88), ER+/PR- were 8.8% (n=14), ER-/PR+ were only 1.3% (n=2) and ER-/PR- were 35% (n=56) in this study (Table 1&2).

Measures of Tumor Markers

Pretreatment CA15-3, serum Her 2 / neu, tissue HER2 /neu and CA15-3 at 3 month follow up after treatment were measured and their summary measures were calculated among different breast cancer types and according to grades and stages of cancers (Tables: 3 & 4).

Table 2. Expression of various categories of Hormone receptors (n=160).

Hormone Receptors	Number of cases	Percent
ER +PR+	88	55.0
ER+PR-	14	8.8
ER--PR-	56	35.0
ER-PR+	2	1.3

Table 3. Measures of Tumor Markers in various Grades of Breast Cancer (N=158).

Grade	Pre treatment CA15-3 (ng/ml)	Post Treatment CA 15-3 (ng/ml)	TissueHer2	SerumHer2 (ng/ml)
I	38.92±6.38 (n=16)	23.45 ±3.75 (n=16)	2.25±1.00 (n=16)	31.9312±12.49 (n=16)
II	32.56±4.67 (n=100)	19.29±2.40 (n=100)	2.24±1.07 (n=100)	28.8676±12.80 (n=100)
III	21.71±10.560 (n=42)	25.01±3.79 (n=42)	2.19±0.96 (n=42)	25.1566±13.91 (n=42)
Total	30.32±4.29 (n=158)	21.23 ±2.10 (n=158)	2.22±1.033 (n=158)	28.1913±13.15 (n=158)

To correlate ER, PR status with CA 15-3 and Her-2/neu

We performed a Kruskal Wallis H test to see any difference between the various hormonal receptor expression groups of breast cancer with respect to both pre-treatment CA 15-3 as well as Serum Her-2 /neu levels. A statistically significant difference in Her-2/neu levels between the different hormonal receptor expression groups was found (Table 7).

There was statistically no significant difference in pre-treatment CA 15-3 level in different groups of hormonal receptor expression. π (3)=6.469, p=0.091 with mean rank of CA 15-3 as 72.89 in ER +ve/PR+ve group, 97.79 in ER +ve/PR-ve, 88.82 in ER -ve/PR-ve and 61.50 in ER -ve/PR+ve (Table 5,7 & 8).

There was a statistically significant difference in Her-2/neu levels in different groups of hormonal receptor expression. π (3)=9.567, p=0.023 with Mean Rank of Her2/neu as 83.27 in ER +ve/PR+ve group, 101.86 in ER +ve/PR-ve, 73.41 in ER -ve/PR-ve and 7.50 in ER -ve/PR+ve (Table 7 & 8).

To see which groups have the significant differences, we calculated a series of Man Whitney U test.

A Man Whitney U indicated that the pre-treatment levels of serum Her-2/neu was significantly different in ER +ve/PR-ve (Mean Rank =45.50) than ER -ve/PR-ve (Mean Rank = 33.00) U =252, p =0.04.

Table 4. Measures of Tumor Markers in various Stages of Breast Cancer.

Stage	Pre-treatment CA15-3 (ng/ml)	Post Treatment CA 15-3 (ng/ml)	Tissue Her2	Serum Her2 (ng/ml)
0	20.48±0.00 (n=2)	23.32±0.00 (n=2)	1.00±.00 (n=2)	36.72±0.00 (n=2)
I	17.43±9.64 (n=20)	15.68±10.00 (n=20)	2.00±0.91 (n=20)	24.89±14.96 (n=20)
II	32.62±5.82 (n=76)	22.36±3.69 (n=76)	2.21±1.062 (n=76)	27.51±13.14 (n=76)
III	32.95±4.94 (n=50)	22.06±2.71 (n=50)	2.44±1.03 (n=50)	30.51±11.74 (n=50)
IV	32.06±15.21 (n=8)	23.32±10.01 (n=8)	1.75±0.88 (n=8)	22.79±16.53 (n=8)
Total	30.59±4.51 (n=156)	21.47±2.188 (n=156)	2.21±1.03 (n=156)	28.01±13.14 (n=156)

Table 5. Hormone receptor expression with pre-treatment CA 15-3 levels.

	Hormone Receptor		Statistic
Pre treatment CA15-3	ER +PR+	Mean	23.353±21.228
	ER+PR-	Mean	66.744±80.174
	ER--PR-	Mean	34.527±54.989

p>0.05 is insignificant

Table 6. Hormone receptor expression with serum Her-2/neu.

	Hormone Receptor		Statistic
Serum Her-2/neu	ER +PR+	Mean	31.547±15.63
	ER+PR-	Mean	37.171±13.174*
	ER--PR-	Mean	28.746±14.541*

*p< 0.05 is significant

Table 7. Ranked scores between pre-treatment CA 15-3 and serum Her-2 /neu (Kruskal Wallis Test Description).

Ranks

	Hormone Receptor	Number	Mean Rank
Pre-treatment CA15-3	ER +PR+	88	72.89
	ER+PR-	14	97.79
	ER--PR-	56	88.82
	ER-PR+	2	61.50
	Total	160	
Serum Her-2/neu	ER +PR+	88	83.27
	ER+PR-	14	101.86
	ER--PR-	56	73.41
	ER-PR+	2	7.50
	Total	160	

It also showed considerable significance in between ER-ve/PR+ve and other hormonal types but we discarded the results considering a very small group of only 2 patients in this group.

Table 8. Chi-square test and degree of freedom of pre-treatment CA15-3 &Her -2/ neu groups.

Test Statistics [a, b]

	Pre-treatment CA15-3	SerumHer2
Chi-Square	6.469	9.567
Df	3	3
Asymp. Sig.	.091	.023*

a. Kruskal Wallis Test

b. Grouping Variable: Hormone Receptor

* p value is significant at 0.05 in various groups with respect to Her-2/neu Values

DISCUSSION

Hormone receptor is a histopathological parameter evaluated for a correlation with tumor markers(Canizares *et al*., 2001; Gion *et al.*, 1991; Gion *et al.*, 1999). This correlation is also observed in our study. Earlier studies were unable to establish significant correlation of CA 15-3 and Her-2/neu with hormone receptor status as Faheem et al. (2012) reported an inverse relationship between hormone receptor expression and Her-2/neu. Only Gion *et al*. (1999) found a high number of patients with CA 15-3 positivity in a group with ER+/PR- tumors compared to those with ER- /PR+ tumors. Manar Atoum *et al.* (2012) reports that CA15-3 was increased among ER+/PR+ and ER+/PR-ve. In present study CA15-3 level and estrogen receptors status are strongly correlated and this result is consistent with observations reported by Bensouda *et al.* (2009) (Table 4).

In the current study, there was a statistically significant difference in Her-2/neu level in different groups of hormonal receptor expression. π (3)=9.567, p=0.02 with mean rank of Her-2/neu as 83.27 in ER +ve / PR+ve group, 101.86 in ER +ve / PR-ve, 73.41 in ER –ve / PR-ve and 7.50 in ER –ve / PR+ve. In other words, Her-2/neu positivity was detected in 55% ER+/PR+ and 8% ER+/PR- cancer patient's respectively. It was also found in 35.1 %, ER -ve/PR-ve and 1% ER -ve/PR+ve (Table 7 & 8).

In this study association of both markers, CA 15-3 and Her-2/neu studied with clinical and pathological parameters (Table3 &4). An insignificant correlation was found between both markers. Same the results were of Sohail *et a*l. (2002). However another study mentioned that there is no association (Park *et al*., 2008).

Limitations

This study has used a relatively short clinical follow-up period for a disease which is prone to delayed recurrences. Thus evidences confirming the ability of markers to predict overall relapse rates must await continuing long term studies of tumor outcome.

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