Serum Her-2/Neu a Potential Biomarker in Breast Cancer Patients: Correlation with the Clinico-Pathological Parameters

Manar Abed AL-Kreim Abed Oun*, Hedef Dhafir El-Yassin, Nada A.Al-Alwan*****

ABSTRACT:

BACKGROUND:

Breast cancer is the most common malignancy in women, and a major cause of mortality and morbidity despite the advances in diagnosis and treatment. There is evidence that changes in HER2 protein expressions are associated with breast cancer progression.

OBJECTIVE:

To investigate whether measuring this tumour marker in serum of breast cancer patients before and after treatment might also be useful markers in the diagnosis, screening and monitoring the malignant tumour progression and response to therapy.

METHODS:

Serum samples were obtained from (28) apparently healthy women (Control Group) with a mean age of 40.9 ± 7.6 years and (60) female patients complaining from primary breast cancer (Patients Group) with a mean age of 48.3 ± 8.9 years. They were divided according to their clinical end point into: Pre-Surgical Group, Post-Surgical Group and post- chemotherapy Group. Serum Her-2/nue level was measured using ELISA kits.

RESULTS:

Level of Her2/neu (3130.4 pg/ml) was significantly higher in after 6 cycles of chemotherapy group than each of control (1400.8 pg/ml), before surgery (1597 pg/ml) and after surgery (1487.4 pg/ml) ($P < 0.05$). Her2/neu is effective test only after 6 cycle chemotherapy with an accuracy of 95.2%. The best performance for Her2neu was observed at values ≥ 1464 pg/ml (sensitivity = 95% and specificity = 61%). There were significant influences of the studied personal and the pathological characteristics of the tumour upon the biomarker levels where the levels were significantly higher with the increase of tumour pathological stage and in the presence of positive status for Her2neu receptors ($P < 0.05$).

CONCLUSION:

In this study there was a statistically significant association between tissue HER-2/neu and serum HER-2 /neu levels in the extracellular domain. It could be concluded that using serum Her-2/neu in patients after six cycles chemotherapy could predict response to therapy.

KEY WORDS: serum Her-2/neu, pre-surgical, post-surgical, post-chemotherapy.

INTRODUCTION:

The HER-2/*neu* (C-*erb*B-2) gene is localized to chromosome 17q and encodes a transmembrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor (EGFR)1 or HER family [(1)]. This family of receptors is involved in cell-cell and cell-stromal communication primarily through a process known as signal transduction, in which external growth factors, or ligands, affect the transcription of various genes by phosphorylating or dephosphorylating a series of transmembrane proteins and intracellular signaling intermediates, many of which possess enzymatic activity.

The HER-2/neu oncogene is overexpressed in 25-30% of breast cancers [(2)]. Amplification and/or overexpression of HER-2 is associated with a worse clinical outcome in patients with newly diagnosed primary breast cancer [(3)].

Clinical studies have demonstrated that alterations in HER-2/ *neu* predict poor prognosis for breast cancer and are associated with features of tumor aggressiveness, such as absence of estrogen and progesterone receptors, high rate of cellular proliferation, advanced tumor stage, large tumor size, and young age at diagnosis [(4)]. Women with HER-2/

* Institute of radiation and nuclear medicin,Baghdad.
** Department of Clinical Biochemistry, College of Medicin, University of Baghdad, Baghdad.
***Director of the International Center for Cancer Research College of Medicine, University of Baghdad, Baghdad.

neu positive breast cancer have a worse prognosis than those with HER-2/neu negative cancers (50).

Serum HER-2/neu Antigen Levels as a Tumor Marker for Circulating HER-2/neu receptor protein levels that successfully predicted the presence and progression of HER-2/*neu*positive breast cancer. Eleven studies in which serum HER-2/*neu* protein levels were tested for their ability to predict response to therapy, 8 (73%) of the studies found that elevated serum HER-2/*neu* protein levels predicted therapy resistance [6,7], whereas three additional studies did not demonstrate this association [8]. Serum HER-2/*neu* levels have correlated with decreased survival and absence of clinical response to hormonal therapy in estrogen receptor (ER)-positive tumors in some studies [9], but not in others [10]. Serum HER-2/*neu* protein measurements have successfully predicted resistance to high-dose hemotherapy [7].

The objective of this study is to determine the concentration of Her-2/ neu tumor marker in the sera of patients diagnosed as having primary breast cancer and to correlate the findings with the clinico-pathological parameters (age, menopause, marital status, family history, stage, grade, tumor size, nodal status, estrogen receptor, progesteron receptor and Her-2/neu receptor) in those patients. In this context, the objective is to add information on biomarker validation in the development of new strategies for disease diagnosis, monitoring, and treatment.

MATERIALS AND METHODS:

The study is a descriptive case-control study; it was conducted at three main medical facilities in Baghdad: The Main Training Center for Early Detection of Breast Tumours/Oncology Teaching Hospital, Al - Elweya Center for Early Detection of Breast Tumours and Al Amal Oncology Hospital. Eighty eight Iraqi women were enrolled in the study, including 28 apparently healthy women (used as a "Control Group I") and 60 female patients complaining from primary breast cancer (PBC) - "Patients Group II" during the period ranging from November 2012 until March 2014.

Normal healthy control group included 28 healthy female volunteers of comparable age (40.9±7.6), menstrual cycle and socioeconomic status as patients. The age of the patients ranged between (30-65) years. Female with a concurrent acute illness or with a major liver, renal, heart, thyroid or other endocrine diseases were excluded.

All patients were clinically interviewed and examined using the triple assessment technique, i.e.,clinical breast examination (CBE), mammography and /or ultrasonography , and fine needle aspiration cytology (FNAC).

The collected information included all data routinely recorded on the patient's file sheet questionnaire by the examining physician: age; marital status; history of lactation, contraceptive pills and/ or hormonal therapy; and family history of breast cancer. Data on tumour size and nodal status were obtained by examination of the tissue biopsies. Abdominal ultrasound and chest X-rays were carried out to exclude metastasis, and when indicated a skeletal survey was performed.

The clinico-pathologic data were obtained from patients' pathology reports. The collected data included tumor size, tumor pathological grade, axillary lymph node involvement, vascular invasion, status of Her2/neu Receptor , Estrogen Receptor (ER) and Progesterone Receptor (PR). The clinical stage was determined by the oncologist according to the tumor-nodes-metastasis (TNM) classification system.

Patients Group II was further divided according to their clinical end point into:

I. Twenty patients who were recently detected and not yet operated upon neither receiving chemotherapy. Those were assigned as : "Pre-Surgical Group II".
II. Twenty patients who were subjected to mastectomy without receiving chemotherapy Those were assigned as: "Post-Surgical Group II".
III. Twenty patients who had adjuvant combination chemotherapy(hormonal and chemotherapy without hercaptin) for at least six cycles. Those were assigned as: "Post-Chemotherapy Group II".

Blood sampling: 10 ml of venous blood was withdrawn from normal healthy female volunteers and from patients diagnosed with PBC before treatment, after surgery and after 6 cycles of chemotherapy by ante cubital venipuncture using 21 gauge needles in the sitting position. Immediately after withdrawing, blood samples were allowed to coagulate and centrifuged for 20 minutes at 3500 rpm. The separated serum samples were divided into two tubes and stored until assayed. After thawing, each serum sample was assayed only once.

The level of serum Her2/neu in sera was determined using a ready for use Enzyme-Linked Immunosorbent Assay(ELISA) kit for the accurate quantitative measurement of Human her2 (Abcam's , UK) according to the producer's protocol. Briefly, prepared standards and diluted samples were added to the 96 wells and incubated for 2.5 hours at room temperature or over nightat 4°C with gentle shaking. The solution of sample and standard after reaction

with the wells was discarded, washed followed by addition of diluted Biotinylated ErbB2 Detection Antibody solution and incubation for 1 hour at room temperature (18-25°C) with gentle shaking. After washing, diluted streptavidin-HRP was added and incubated for 45 minutes at room temperature with gentle shaking. After washing, TMB substrate solution was added and incubated for 30 minutes at room temperature in the dark with gentle shaking. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm. The intensity of this colored product was directly proportional to the concentration of her2 present in the samples. The absorbance was measured at 450 nm. HER2 serum concentration was determined by referring to a standard curve. The sensitivity of the assay was < 8 pg/mL.

Data processing and statistical analysis

The data processing and statistical analysis was done by the computer (SPSS system). Data was presented in simple statistical measures of percentage, mean and standard deviation.

The following statistical procedures were done: Student's t – test for comparing the significance of difference for the quantitative data, and simple linear correlation was used for determination of correlation between two quantitative parameters for the different groups. P-value was used to determine statistical significance.

RESULTS:

All the enrolled patients were females with an age range between 30 to 65 years. The mean ages for Group I and Group II were 40.9 ± 7.6 years and 48.3 ± 8.9 years respectively.The highest age group for patients range from 40-49 years.Group II were subdivided into three groups; Pre-Surgical, Post-Surgical and Post- Chemotherapy. Each group was composed of 20 patients.The majority of the studied groups were married (90.0%) (table 1), 70.0% of them were in their postmenopausal age .

Eighty five percent of Group II PBC had a negative family history of breast cancer **.**Fifty percent were diagnosed in stage II, and around 37 % in stage III. Regarding the degree of differentiation of the tumour, 53% of lumps were Grade II, and 25% were Grade III. The tumor size was larger than 2 cm in 57.6 % of patients and nodal involvement was positive in 70% of patients (table 2).

Estrogen receptor was positive in 83.3% of patients and progesterone receptor was positive in 85.% while Her2/neu receptor was positive only in 40% of patients (table 2).

The Level of Her2/neu (3130.4 pg/ml) was significantly higher in after 6 cycles of chemotherapy group than each of control (1400.8 pg/ml), before surgery (1597 pg/ml) and after surgery (1487.4 pg/ml) ,$P < 0.05$(table 3and fig 1). Her2/neu is effective test only after 6 cycle chemotherapy with an accuracy of 95.2%. The best performance for Her2neu was observed at values ≥ 1464 pg/ml (sensitivity = 95% and specificity = 61%), table 4. There were significant association of the studied personal and the pathological characteristics of the tumour upon the biomarker levels where the mean level of sHer-2/neu was significantly higher with the increase of tumour pathological stage and in the presence of positive status for Her2neu receptors, $P < 0.05$(table 7), While sHer-2/neu level showed statistically insignificant association in Pre surgical and post surgical groups(table 5,table 6).

Table 1: Distribution of the study sample according to Age, Menopausal status & Family History of BC.

	Control		Pre-Surgical		Post-Surgical		Post-Chemotherapy		All Cancer Patients	
Variable	N=28	%	N=20	%	N=20	%	N=20	%	N=60	%
Age Group										
• 30-39 year	12	42	2	10	2	10	6	30	10	17
• 40-49 year	12	42	5	25	10	50	8	40	23	38
• 50-59 year	3	11	7	35	8	40	4	20	19	32
• Over 60	1	5	6	30	0	0	2	10	8	13
Total	28	100	20	100	20	100	20	100	60	100
Marital status										
• Unmarried	5	18.0	2	10.0	2	10.0	2	10.0	6	10.0
• Married	23	82.0	18	90.0	18	90.0	18	90.0	54	90.0
Total	28	100	20	100	20	100	20	100	60	100

Menopause										
• Premenopausal	7	25.0	2	10.0	7	35.0	9	45.0	18	30.0
• Postmenopausal	21	75.0	18	90.0	13	65.0	11	55.0	42	70.0
Total	28	100	20	100	20	100	20	100	60	100
Family History										
• Positive	1	1.0	3	15.0	1	5.0	5	25.0	9	15.0
• Negative	27	99.0	17	85.0	19	95.0	15	75.0	51	85.0
Total	28	100	20	100	20	100	20	100	60	100

Table 2: Distribution of the study sample according to the Pathological Characteristics of the Tumour in Group II PBC.

	Pre-Surgical		Post-Surgical		Post-chemotherapy		All Cancer Patients	
Variable	N=20	%	N=20	%	N=20	%	N=60	%
Stage								
• I	1	5.0	3	15.0	1	5.0	5	8.3
• II	10	50.0	8	40.0	12	60.0	30	50.0
• III	7	35.0	8	40.0	7	35.0	22	36.7
• IV	2	10.0	1	5.0	0	0.0	3	5.0
Total	20	100	20	100	20	100	60	100
Grade								
• I	3	15.0	5	25.0	5	25.0	13	21.7
• II	12	60.0	10	50.0	10	50.0	32	53.3
• III	5	25.0	5	25.0	5	25.0	15	25.0
Total	20	100	20	100	20	100	60	100
Tumor size								
• Up to 2 cm	2	10.0	4	21.1	5	25.0	11	18.6
• 2.1- 5 cm	11	55.0	10	52.6	13	65.0	34	57.6
• > 5 cm	7	35.0	5	26.3	2	10.0	14	23.7
Total	20	100	20	100	20	100	60	100
Nodal Status								
• Positive	19	95.0	10	50.0	13	65.0	42	70.0
• Negative	1	5.0	10	50.0	7	35.0	18	30.0
Total	20	100	20	100	20	100	60	100
Estrogen Receptor								
Positive	18	90.0	13	63.2	19	95.0	50	83.3
Negative	2	10.0	7	36.8	1	5.0	10	16.7
Total	20	100	20	100	20	100	60	100
Progesterone Receptor								
Positive	18	90.0	14	68.4	19	95.0	51	85
Negative	2	10.0	6	31.6	1	5.0	9	15
Total	20	100	20	100	20	100	60	100
Her2neu Receptor								
Positive	5	25.0	8	42.1	10	50.0	24	40
Negative	15	75.0	12	57.9	10	50.0	36	60
Total	20	100	20	100	20	100	60	100

Table 3: Descriptive statistics for Her-2/neu according to the study groups.

	Level (Pg/ml)					
Descriptive Statistics	Control	Pre-Surgical	Post-Surgical	Post-Chemotherapy	P value	Statistical of significance
Mean	1400.8	1597.0	1487.4	3130.4	< 0.05	S
Median	1360.0	1479.0	1509.0	2365.5		
SD	324.8	486.9	274.1	1997.1		
95%CI; lower	1274.8	1369.1	1359.1	2195.7		
upper	1526.7	1824.9	1615.7	4065.1		
Minimum	851	761	1086	1360		
Maximum	2243	2625	1977	9096		

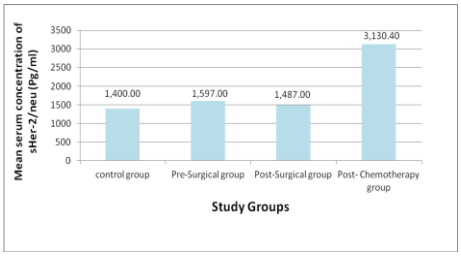

Figure 1: Mean levels of Her-2/neu in each study groups.

Table 4: Validity for serum levels of Her-2/neu at selected cut-off points for the study groups.

Variables & Study Groups	Cut-off Point*	P value	AUC	Sensitivity	Specificity
Pre-Surgical	1410.0	0.084	0.623	0.600	0.607
Post- Surgical	1410.0	0.083	0.590	0.650	0.607
Post-CT	1464.0	< 0.001	0.952	0.950	0.607

Table 5: Descriptive statistics for Her2/neu in patients with breast cancer- Pre surgical group according to the study variables.

Variables	Mean	SD	N	P value	Statistical of significance
Age Group				>0.05	N.S
30-45 year	1339.0	29.7	2		
46-65 year	1625.7	506.1	18		
Marital status				>0.05	N.S
Unmarried	1374.5	60.1	2		
Married	1621.7	508.2	18		
Menopause				>0.05	N.S
Premenopausal	1339.0	29.7	2		
Postmenopausal	1625.7	506.1	18		
Family History				>0.05	N.S
Negative	1612.8	514.4	17		
Positive	1507.3	347.4	3		
Stage				>0.05	N.S
I	2351.0	.	1		
II	1506.3	363.9	10		
III	1511.9	495.9	7		
IV	1971.5	924.2	2		
Grade				>0.05	N.S
I	1799.0	514.8	3		

II	1528.8	307.9	12		
III	1639.6	828.1	5		
Tumor Size				>0.05	N.S
Up to 2 cm	2032.5	450.4	2		
2.1- 5 cm	1510.0	287.2	11		
> 5 cm	1609.3	708.5	7		
Nodal Status				>0.05	N.S
Negative	2351.0	.	1		
Positive	1557.3	465.8	19		
Estrogen R				>0.05	N.S
Negative	1247.0	687.3	2		
Positive	1635.9	470.2	18		
Progesterone R				>0.05	N.S
Negative	1247.0	687.3	2		
Positive	1635.9	470.2	18		
Her2neu R				>0.05	N.S
Negative	1529.1	364.2	15		
Positive	1800.6	769.8	5		

Table 6: Descriptive statistics for Her2/neu in patients with breast cancer- Post surgical group according to study variables.

Variables	Mean	SD	N	P value	Statistical of significance
Age Group				>0.05	N.S
30-45 year	1609.6	271.8	8		
46-65 year	1405.9	254.3	12		
Marital status				>0.05	N.S
Unmarried	1585.5	130.8	2		
Married	1476.5	285.8	18		
Menopause				>0.05	N.S
Premenopausal	1589.4	287.0	7		
Postmenopausal	1432.5	261.6	13		
Family History				>0.05	N.S
Negative	1473.5	274.3	19		
Positive	1751.0	.	1		
Stage				>0.05	N.S
I	1452.7	245.5	3		
II	1611.0	238.3	8		
III	1372.1	310.7	8		
IV	1525.0	.	1		
Grade				>0.05	N.S
I	1507.6	217.5	5		
II	1439.2	294.4	10		
III	1563.6	317.7	5		
Tumor Size				>0.05	N.S
Up to 2 cm	1506.8	326.6	4		
2.1- 5 cm	1500.9	268.4	10		
> 5 cm	1497.0	311.2	5		
Nodal Status				>0.05	N.S
Negative	1554.5	206.5	10		
Positive	1420.3	325.5	10		
Estrogen R				>0.05	N.S
Negative	1553.3	252.1	7		
Positive	1482.4	278.7	13		
Progesterone R				>0.05	N.S
Negative	1613.8	213.3	6		

Positive	1459.9	278.9	14		
Her2neu R				>0.05	N.S
Negative	1502.2	301.4	12		
Positive	1517.2	223.1	8		

Table 7: Descriptive statistics for Her2/neu in patients with breast cancer-Post-Chemotherapy group according to the study variables.

Variables	Mean	SD	N	P value	Statistical of significance
Age Group				>0.05	N.S
30-45 year	3513.8	2404.0	11		
46-65 year	2661.8	1341.3	9		
Marital status				>0.05	N.S
Unmarried	3491.5	1081.2	2		
Married	3090.3	2090.9	18		
Menopause				>0.05	N.S
Premenopausal	3716.3	2625.4	9		
Postmenopausal	2651.0	1225.6	11		
Family History				>0.05	N.S
Negative	3178.7	1979.3	15		
Positive	2985.4	2279.9	5		
Stage				<0.05	S
I	2141	.	1		
II	2238.3	428.9	12		
III	4912.6	2544.2	7		
IV	---	---	0		
Grade				>0.05	N.S
I	2389.2	823.5	5		
II	3282.3	1846.1	10		
III	3567.8	3100.7	5		
Tumor Size				>0.05	N.S
Up to 2 cm	2910.8	1676.3	5		
2.1- 5 cm	2811.5	1496.3	13		
> 5 cm	5752.0	4729.1	2		
Nodal Status				>0.05	N.S
Negative	3053.7	1418.7	7		
Positive	3171.7	2302.9	13		
Estrogen R				>0.05	N.S
Negative	3160.0	.	1		
Positive	3128.8	2051.8	19		
Progesterone R				>0.05	N.S
Negative	3160.0	.	1		
Positive	3128.8	2051.8	19		
Her2neu R				<0.05	S
Negative	1966.1	298.5	10		
Positive	4294.7	2306.1	10		

DISCUSSION:

The present study showed that breast cancer occurs most frequently during the fourth and fifth decades of women's life followed by the sixth/ These results were compatible to what has been reported in other studies, conducted in Iraq; documenting that the malignant breast lesions occur mostly during the fourth decade followed by the sixth [11,12]. In addition the current study showed that the mean age of breast cancer patients was 48.3 years. This is in agreement with the result published by many studies in Iraq and other Arabian countries in addition to Islamic republic of Iran [13].

The Iraqi cancer board (2004) registered the mean age of Iraqi women, diagnosed with breast cancer was 49 years; close to what was reported from Iran (48 years); and comparable to those figures displayed

by local investigators including the Iraqi National Breast Cancer Research Center[14].

On the other hand, American and European studies usually record significantly higher ages for their breast cancer patients at the time of presentations reaching 66 years [15]. In South Africa it has been observed that breast cancer occurs at earlier ages in Blacks (mean 49 years) than in Whites [16]. In general the age-standardized incidence of breast cancer is lower in developing countries than in developed countries, and incidence rates vary widely between and within countries [17]. No significant relationship was noted between menopausal status and the frequency of breast cancer. In this report it has been authorized that the increased incidence of breast cancer in women aged below 50 years could be due to active ovarian function and estrogen hormone secretion while the increased incidence in those aged above 50 years could attribute to an imbalance in the adrenal estrogen [18].

The need for accurate detection of the Her-2 alteration in breast cancer patients is of utmost importance because therapeutic decisions for patients are increasingly dependent on this information. The current study showed that the serum level of Her-2/*neu* was significantly higher in breast cancer patients after six cycle chemotherapy than in the normal healthy controls. There was no statistical significant difference among both Pre and Post surgery groups, It has been observed that an increase in serum Her-2/*neu* protein levels after chemotherapy predicted therapy resistance [19].

Breast cancer cells overexpressing Her-2/*neu* are intrinsically resistant to DNA-damaging agents and altered apoptosis responses [20]. Apoptosis is a predominant mechanism by which cancer chemotherapeutic agents kill cells [21]. The failure of cancer cells to detect chemotherapeutic agent-induced damage and to activate apoptosis may lead to multidrug resistance.

The results of the present study showed a significant correlation between serum Her-2/*neu* level and tumor pathological stage. The results of this study are in line with the results of [22] who reported that the soluble c-erb B-2 fragments in serum correlates with disease stage and predicts more shortened survival in patients with early-stage and advanced breast cancer. Athough, all of the patients were free of any cancer recurrence or metastasis, 10 out of the 20 (50%) breast cancer patients with positive Her2/neu showed elevated serum Her2neu levels in post-chemotherapy group. The present results are in accordance with the result of [23] who found significant (P <0.001) association between tissue Her-2/*neu* and serum Her-2/*neu* in extracellular domain (ECD) levels.

CONCLUSION:

It could be concluded that using serum Her-2/neu in patients after six cycles chemotherapy could predict response to therapy.

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