

## Evaluation of Human Epidermal Growth Factor Receptor-2 in Iraqi Women with Breast Cancer

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### Abstract

**Background:** Breast cancer (BC) is the most common malignancy in Iraqi women in the recent years. There are many indicated marker for detection BC and determination the development of the tumor stage, one of the most important marker is human epidermal growth factor receptor-2 (HER2).

**Objective:** To evaluation of serum and tissue HER2 levels among BC women and non BC women.

**Patients and Methods:** Preoperative blood samples were collected from breast cancer women. Patients were newly diagnosing and did not receive neither radiotherapy nor chemotherapy treatment preoperatively. For the control, blood sample was collected from non-breast cancer women. All blood samples subjected to determine the serum HER-2 level.

**Results:** A total of 52 patients with breast cancer and 30 of control were included in this study. The result showed increasing significant in serum concentration of HER-2 among BC patient compared to control and tissue her-2 positive BC patient have significantly higher serum concentration of HER-2 compared to BC patients who were tissue her-2 negative ( $16.23 \pm 10.37$  ng/ml,  $10.33 \pm 5.73$  ng/ml) respectively.

**Conclusion:** Serum HER-2 level was positively associated with tissue HER-2 level, for that is an important marker for diagnosis and monitoring BC progression.

**Keywords:** Breast cancer, serum HER2, tissue HER-2.

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### Introduction

Breast cancer is the most common type of malignancy recorded in the cancer registries of almost all countries within the Eastern Mediterranean Region. In Iraq, the continuous rise in the incidence rate is associated with an obvious trend to affect premenopausal women [1]. The incidence of

breast cancer appears to be increasing worldwide [2]. Breast cancer is a multifactorial disease there are a number of risk factors that affected the probability of developing BC; several factors like (gender, age, personal history, family history, early menarche and late menopause) are not modifiable. Other

factors: postmenopausal obesity, use of postmenopausal hormones, alcohol consumption, physical activity, and smoking are modifiable risk factors [3]. Human epidermal growth factor receptor-2 is more intensively studied in BC and other solid cancers. Firstly identified by Schechter 1984 [4]. It is consisting of three principle domains the first is the extracellular ligand binding domain (ECD), the second, is the transmembrane domain (TMD) and the third, the intracellular tyrosine kinase catalytic domain (ICD) [5]. The ECD of the receptor protein can be cleaved from the cell surface by matrix metalloproteinase and then released into the blood [6]. The present study was undertaken with the aim to investigate the association of some demographic characteristics such as age, menopause state, family history and breastfeeding with the development of BC and explore the clinical utility of serum HER2/ neu estimation by ELISA in breast cancer patients and evaluate whether it could be used in place of FISH/IHC.

### Patients and Methods

This case control study included 52 female patients with BC who underwent to either mastectomy or lumpectomy, their age range (25-78) years old and 30 of non BC women. This study was performed in Baghdad Teaching Hospital – Medicine City during period from September 2017 to March 2018. The diagnosis in each case was established by clinical diagnosis and confirmed by histopathological diagnosis. Patients were newly diagnosing and did not receive neither

radiotherapy nor chemotherapy treatment preoperatively. After surgery and evaluation of sample pathology, benign cases were excluded. The result of tissue HER2 status obtained from patients' record. Five milliliters of peripheral blood were collected from all participants and put in a sterile test tube and after 30 min centrifuged at 3000g for 10 min at room temperature. Serum samples were stored at -20 C till the time of the assay. After collection of all samples, to measure the serum HER2/ECD concentration, we used to enzyme-link immunosorbent assay method (Human receptor tyrosine-protein kinase erbB-2, ELISA kit catalogue number SL2747Hu, Sun long-Biotech/China).

### Statistical analysis

The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. Continuous variables are expressed as mean± standard deviation (SD). Chi-square was used for testing the deviation from Hardy- Weinberg equilibrium as well as for comparing between categorical variables. Student t-test and Mann Whitney U test were used to compare serum levels of sHER-2 between patients and controls

### Results

Table (1) shows the demographic data of the study groups. Mean age of patients and control were  $47.63 \pm 10.34$  years and  $49.3 \pm 11.95$  years respectively with no significant difference at ( $P < 0.05$ ). Also for menopause status, there was no significance difference in the frequency of premenopausal and postmenopausal status among patients

and controls ( $P < 0.05$ ). Although patients had higher frequency for family history of BC than control (46.15 % versus 26.67%), the difference did not reach a significant level ( $P < 0.05$ ). In contrast, breastfeeding differed significantly between patients and

controls. Out of thirty healthy controls, 24 (80%) have used to feed their child by breastfeeding. On the other hand, only 24 out of 52 (46.15%) women with BC had such practice ( $P < 0.05$ ).

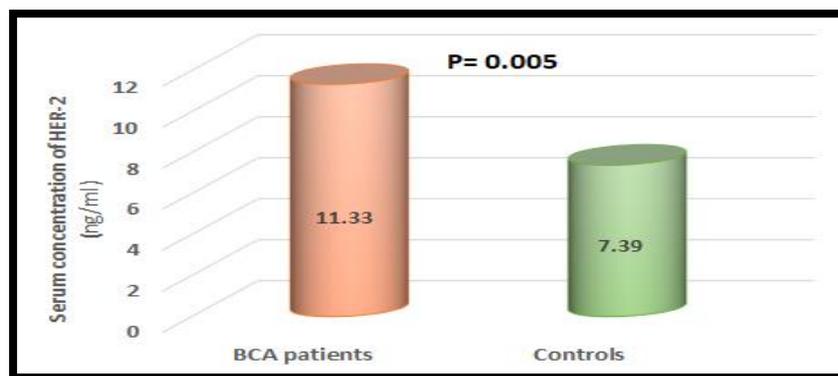
**Table (1):** Baseline characteristics of the study population.

Variables	Cases 52	Controls 30	P-value
Age (years, mean±SD)	47.63± 10.34	49.3±11.95	0.509
Menopausal status			
Premenopausal	23(44.23%)	13(43.33%)	0.937
Postmenopausal	29(55.77%)	17(56.67%)	
Family history			
No	28(53.85%)	22 (73.33%)	0.077
Yes	24 (46.15%)	8 (26.67%)	
Breastfeeding			
No	28(53.85%)	6(20%)	0.002
Yes	24 (46.15%)	24 (80%)	

\* Significant at P value < 0.05

According to the employed ELISA technique, the mean serum concentration of HER-2 in BC patients and controls were 11.33±6.97 ng/ml (range 4.4-37.2 ng/ml)

and 7.39± ng/ml (range 0-13.4 ng/ml) respectively Figure (1). Statistical analysis showed highly significant difference ( $P < 0.05$ ).



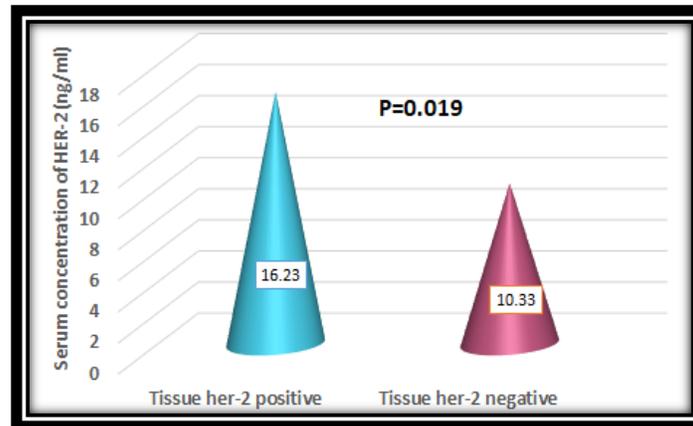
**Figure (1):** Serum concentration of HER-2 in BC patients and controls, Significant at P value < 0.05.

Out of 52 BC patients, 9 (17.31%) were tissue her-2 positive, while the other 43 (82.69%) were tissue her-2 negative. Based

on the international cut-off value for serum HER-2 positivity which is 15 ng/ml, there were a total of 9(17.31%) cases of BC who

were positive for serum HER-2, while there was none among control group positive for HER-2. Figure(2) illustrated the tissue her-2 positive BC patients had higher serum concentration of HER-2 compared to BC

patients who were tissue her-2negative( $16.23 \pm 10.37$  ng/ml versus  $10.33 \pm 5.73$  ng/ml) with a significant difference ( $P < 0.05$ ).



**Figure (1):** mean serum concentration of HER-2 in BC tissue her-2 positive and negative patients. Significant at P value  $< 0.05$

## Discussion

For age, women in control group were intentionally selected to match patients regarding age. Thus, no significant difference was found between the two groups. Otherwise age was frequently reported to be a risk factor for BC [7]. In a local study, a study carried out on 721 cases with BC and found that age group (40-49) years were more susceptible to this malignancy [8]. Globally, found the peak age in which BC more prevalent in Asian countries was between 40 and 50 years. However, this age range is still lower than those observed in most developed countries like` USA, UK with a peak age between 60 and 70(9). These variations in incidence may be due to multiple factors, including decrease in birth rate, trends of breastfeeding, increase in the

age at first pregnancy, geographic variation, racial/ethnic background, genetic variation, lifestyle and environmental factors [10] [11]. Similar to age, the menopausal status shows no significant difference between patients and controls. This result does not agree with many other international studies. In Turkey found among 346 cases, (73.1%) of women in post-menopausal situation had higher BC risk [12]. The risk of BC in women with menopause after 55 is twice the risk than women with menopause before 45 years old. Additionally, BMI may further have complicated the situation. The overweight during postmenopausal status has very important role in development BC. These variations between the current study and the other studies may be explained by the small

sample size and ethnic background of current study compared with other studies [13].

Family history was frequently recorded to be significantly associated with BC in the life time. In the current study, the result was showing no significant difference in family history between BC patients and controls. This result is not in accordance with that of other international studies. In Palestine, has investigated the association of family history of BC with incidence of this malignancy among 140 Palestinian women as well as 163 healthy controls and reported that family history was strong risk factor for BC particularly at young age [14]. In Turkey, carryout a study on 172 patients with BC and 383 controls, and showed that family history of BC were significantly association with increased BC risk among Turkish women (OR=4.67, 95% CI: 2.23- 9.76) [15]. These disparities between the current study and the other international studies may be explained by the small sample size of current study compared with other studies.

One of most important result in the current study was the inverse significant association between breastfeeding and BC. This result is in accordance with result of another international studies. In Morocco enrolled 124 BC women and 148 age matched controls and in their study. They showed that breastfeeding for more than six months was significantly associated with decreased incidence of BC [16]. It seems that the duration of breastfeeding during overall life-time has a very important role as a protective factor against BC. In a case-control study, in

Srilank a, reported that women who used breastfeeding for 24 months or more during life time had significantly lower risk of BC than those who breastfed for less than 24 months. Interestingly, there was 66.3% reduction in BC risk in women who breastfed for 12-23 months, 87.4% reduction in 24-35 months and 94% reduction in 36-47 months [17]. However, some studies reported no association between breastfeeding and BC [18] [19].

Although the mechanisms are not entirely elucidated, there are many explanations for this protection role of breastfeeding. Firstly, lactation reduces estrogen and elevates prolactin levels which results in reduced cumulative exposure to estrogen [20]. Secondly, in women who are not breastfeeding, milk pH rises (becomes alkaline) compared to that from the breastfeeding women. The epithelial cells from an alkaline environment undergo several changes, among which hyperplasia, atypia, and increased mitotic activity [21]. These changes, and may be others, can progress to BC.

The current study revealed a significantly higher serum sHER2 level BC patients than controls. In fact, elevated serum sHER-2 was frequently found in patients with BC as well as in patients with other cancers such as ovarian cancer, lung cancer, and prostate cancer [22]. In one study, about 11.4% of early patients with metastatic BC had elevated sHER-2 [23]. In another study, high levels sHER-2 was found in 18% of women

with primary BC and in 46% of those with metastatic disease [24].

Soluble HER-2 is generated in two ways: the proteolytic cleavage, known as shedding of ECD [25] and by alternative initiation of mRNA translation [26]. As a considerable percentage of women with BC have amplification/overexpression of HER2, the current result is reasonable, because there is an increase in shedding of extracellular domain of this receptor into the plasma [27].

For serum concentrations of HER2 in BC tissue her2 positive and negative patients, the result of current study revealed tissue HER2 positive BC patients significantly higher than serum concentration of HER2 BC patients who were tissue her2 negative. This result comparable with that of other previous studies. In China measured the serum HER2 level in BC patients and found that the serum HER2 level measured by dot blot assay was significantly correlated with tissue HER2 status in BC patients ( $p=0.001$ ), and also significantly correlated with HER2 level measured by ELISA ( $P=1.06 \times 10^6$ ) [28]. In Korea reported out of 2862 cases of stage I–III primary BC patients, 24 % were found to be tissue her2 positive, and only 15 % of these tissue positive patients had elevated sHER2 [29].

Several studies have demonstrated that the proteolytic cleavage of HER2 results in the release of the extracellular domain of HER2 and the production of truncated cell-associated fragments, and supported the hypothesis that the truncated HER2 protein is

associated with enhanced signalling activity and confers an adverse prognosis [30; 31].

## Conclusions

Serum HER-2 level was positively associated with tissue HER-2 level, for that is an important marker for diagnosis and monitoring BC progression.

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