

# Evaluation of Human Epidermal Growth Factor Receptor 2 Oncoprotein in Serum and Tissue Samples of Women with Newly Diagnosed Breast Cancer

Iman Ismael Sami Al-Mubarak<sup>1</sup>, Nazar Haddad<sup>2</sup>

## ABSTRACT:

### BACKGROUND:

The Human Epidermal Growth Factor Receptor 2 (HER2) proto-oncogene is extensively studied in breast cancer patients. Identifying patients who would benefit from HER2-targeted therapy requires assessing HER2 overexpression.

### OBJECTIVE:

This study evaluated HER2 expression in tissue through Immunohistochemistry (IHC) and in blood through Enzyme-Linked Immunosorbent Assay (ELISA), and its relationship with other tumour characteristics.

### PATIENTS AND METHODS:

This study is a comparative cross-sectional study, carried out at the Basra Oncology and Hematology Centre and Basra Hospital for Women and Children- Breast Center from February to December 2022. It involved 44 female patients with newly diagnosed primary breast cancer and 44 control group. Serum HER2 levels were studied by ELISA method. Surgical samples were investigated for the type of carcinoma, tumour size, lymph node metastasis, stage and grade of the tumour and reports of the results were obtained. Also, reports of the tissue HER2 expression were obtained.

### RESULTS:

The study showed that, the median serum HER2 in breast cancer patients was 6.90 ng/mL. The serum HER2 concentration was significantly higher among cases compared to control group ( $P= 0.030$ ). The serum HER2 was significantly higher among those who were positive for tissue HER2 ( $P= 0.04$ ) and among those with tumour metastasis ( $P= 0.038$ ).

### CONCLUSION:

This study revealed that elevated levels of serum HER2 were correlated with the IHC expression of HER2 in tissue and tumour metastasis. Also, there was a significant increase among patients with breast cancer compared with controls. For this reason, serum HER2 results holds a potential diagnostic tool with the ability to reflect the tumour burden.

**KEYWORDS:** Breast cancer, proto-oncogene, Immunohistochemistry.

<sup>1</sup>MBChB FIBMS(Chem. Path) ,Senior Doctor at Al-Sayab teaching hospital.

<sup>2</sup>MBChB FIBMS(Chem. Path) FADLM FRCP MHPE. Professor and consultant of Chemical Pathology, College of Medicine, University of Basrah.

*Iraqi Postgraduate Medical Journal, 2025; Vol. 24(4): 523-530*

*DOI: 10.52573/ipmj.2024.153131*

*Received: October 30, 2024*

*Accepted: November 15, 2024*



## INTRODUCTION:

Breast cancer is the second-leading cause of cancer death (following lung cancer) and the most prevalent malignancy in women, among women above 65 years, breast cancer is the leading cause of cancer death<sup>(1)</sup>. In Iraq, studies found an increase in the incidence rates of breast cancer among Iraqi women. It is the primary cause of mortality among Iraqi women and accounts for nearly one-third of all cancer cases reported in the nation in 2019<sup>(2)</sup>. In Basra, a study found that breast cancer was the commonest

cancer among adult females in 2017 with incidence rate found to be 60.64/100,000 adult females<sup>(3)</sup>. Clinical biomarkers that are predictive and prognostic are employed in the management of breast cancer. Estrogen receptor (ER), progesterone receptor (PR) and HER2 are immunohistochemical prognostic indicators and predictors of therapeutic response<sup>(4)</sup>. HER2 is a member of epithelial HER1, HER3 and HER4 family<sup>(5)</sup>. It is a 185 kDa transmembrane glycoprotein located at the long arm of human

chromosome 17 (17q12) <sup>(6)</sup>.HER2 is one of the main molecular targets in breast cancer pathogenesis. About 15-20% of breast cancer patients have overexpression and/or amplification of HER2, which is associated with high mortality and a poor prognosis <sup>(7)</sup>. According to the expression of the HER2, ER, PR and Ki67, a cell proliferation marker, breast tumours can be classified into many subtypes <sup>(8)</sup>. Luminal A(PR+, ER+ strong, HER2-, Low Ki67) , Luminal B (PR-, ER+ weak/ moderate, HER2+ sometimes, High Ki67) ,HER2+( PR-, ER-, HER2+), basal-like(PR-, ER-, HER2-) <sup>(9)</sup>. Identification of HER2+ tumours in women with breast cancer is critically important when choosing patients for HER2-targeted therapies such as trastuzumab <sup>(10)</sup> . The results of targeted therapy for patients with HER2-positive breast cancer appear to be improved by sensitive and accurate identification of HER2 <sup>(7)</sup>. The Food and Drug Administration (FDA) approved the serum HER-2 test in September 2000, with a normal cutoff value of 15 ng/mL. Elevated HER-2 levels are defined as those that are 15 ng/mL or higher <sup>(11)</sup>. Metalloproteases break the HER2 extracellular domain, separating it from other HER2 components and allowing it to enter the bloodstream <sup>(12)</sup> .Using (ELISA) to measure HER2 levels in serum would offer a less invasive method for evaluating HER2 status than a biopsy <sup>(13)</sup>. The aim of the study is Measurement of serum levels of HER2 in newly diagnosed breast cancer patients and compare these levels with benign breast disease and healthy individuals. Also, determine the association between serum and tissue HER2 with other pathological parameters and determine whether the currently used test (IHC) for HER2 status could be replaced by the HER2 serum assay.

### **PATIENTS AND METHODS:**

This study is a comparative cross-sectional study conducted at the Basra Oncology and Hematology Centre and Basra Hospital for Women and Children- Breast Center- Department of Pathology from February 2022 to December 2022. The total number of patients and controls in this study was 88 including 44 women with primary breast cancer and 44 controls, which included 4 patients with benign breast disease and 40 healthy individuals. All patients and controls were females with age ranging from 35-78 years. Patients with breast mass before surgical interventions and without any treatment

to be received since diagnosis (chemotherapy, hormonal or radiotherapy) were included in the study, while individuals with concomitant cancers and patients with breast cancer who had surgery or received treatment were excluded.

After the patient fulfilled the inclusion criteria, history was taken from the patient looking for the exclusion criteria by using a questionnaire formula designed for the purpose of the study. Name, age, any history of a new lump in the breast or underarm, nipple discharge other than breast milk, whether there is pain, redness or dimpling in the nipple area or breast, any family history of breast cancer, any prior treatment for breast cancer, any drug use history, and the presence of concurrent cancer were among the details provided. FNA and a diagnostic mammogram were performed to detect breast cancer. The diagnosis of breast cancer and benign breast disease was confirmed depending on the results of the histological examination of the excisional biopsy or tru-cut biopsy. The stage and grade of the breast cancer was determined also according to the results of the histological examination in addition to other imaging studies (CT scan of chest and abdomen or PET scan).

At the time of diagnosis, a volume of (3-5 mL) of whole blood was taken from the peripheral veins before starting any therapeutic intervention and labeled with the patient's name and number. The blood was drawn and allowed to clot in a sterile gel tube for 15 minutes and then centrifuged at 3000 RPM for 20 minutes at room temperature. Serum samples were stored in an Eppendorf tube at -20 °C until the time of the assay. Following collection of all samples, enzyme-linked immunosorbent assay method was used to determine the serum HER2 concentration (ELISA sandwich kit for the quantitative detection of Human epidermal growth factor receptor-2 in serum, BT LAB, Cat. No E0224Hu). Surgical samples were evaluated for the type of carcinoma, tumour size, stage and grade of the tumour, lymph node metastasis and reports of the tissue HER2 expression were obtained.

This study received approval from the Research Ethics Committee at Al-Basra Medical College on 20/3/2023 and the Ministry of Higher Education / Iraqi Board for Medical Specialties. Informed consent was obtained from all patients and healthy participants prior to blood collection.

## RECEPTOR 2 ONCOPROTEIN IN SERUM, TISSUE SAMPLES, BREAST CANCER

Statistical Package for Social Sciences (SPSS) version 26.0 for Windows 10 (IBM, USA, 2018) was used to analyze the data from this study in which for numerical variables, mean and standard deviation are utilized. The frequency and percentage of categorical variables. The Chi square test is used to compare the categorical variables while variables that were not normally distributed were expressed as mean rank, and the Mann-Whitney U test was used to compare these variables. We consider the two-tailed probability values with a  $P (\leq 0.05)$  to be statistically significant.

### RESULTS:

The study included 44 women with newly diagnosed with breast cancer. The mean age was  $50.36 \pm 9.79$  years ranging from 35-78 years. Most of them (70.5%) had a tumour size of 2-5 cm. A grade III tumour represented 68.2% (No.=30) of the cases. Stage II breast cancer were seen in 21 (47.7%). Lymph nodes involvement was seen in 28(63.6%) of women while 10 (22.7%) had a metastatic breast cancer. Table1 shows the characteristics of breast cancer patients.

**Table 1: The characteristics of the patients with breast cancer.**

Variable		No. (%)
Tumour Size / cm	< 2	12 (27.3%)
	2-5	31 (70.5%)
	> 5	1 (2.3%)
Grade	I, II	14 (31.8%)
	III	30 (68.2%)
Stage	I	6 (13.7%)
	II	21 (47.7%)
	III	7 (15.9%)
	IV	10 (22.7%)
Lymph nodes involvement	Present	28 (63.6%)
	Absent	16 (36.4%)
Metastasis	Present	10 (22.7%)
	Absent	34 (77.3%)
Total		44 (100.0%)

Each patient was investigated by receptors, progesterone receptors and tissue HER2. immunohistochemistry (IHC) for estrogen The results are demonstrated in (table 2).

**Table 2: Results of estrogen receptors, progesterone receptors and tissue HER2 among patients with breast cancer.**

Variable		No. (%)
Estrogen receptors	Positive	33 (75.0%)
	Negative	11 (25.0%)
Progesterone receptors	Positive	30 (68.2%)
	Negative	14 (31.8%)
Tissue HER2	Positive	17 (38.6%)
	Negative	27 (61.4%)
Total		44 (100.0%)

The median serum HER2 in breast cancer patients was 6.90 ng/ml ranging from 1.92 to 35.24 ng/ml. The difference in serum HER2 among different categories of the tumour characteristics was tested using Mann-Whitney U test since the serum HER2 was not normally distributed in the patients.

There was no statistically significant difference in serum HER2 with respect to tumour size, grade, stage, and lymph nodes involvement ( $P>0.05$ ). On the other hand, the serum HER2 was significantly higher among those with metastatic tumour ( $P<0.05$ ) as shown in (Table 3).

**Table 3: The difference in serum HER2 according to tumour characteristics.**

Variable	Serum HER2 in ng/ml	
	No.	Mean Rank
<b>Tumour Size</b>		
< 2	12	18.83
≥ 2	32	23.88
Mann-Whitney U = 236.0		P= 0.255
<b>Grade</b>		
II	30	24.83
III	14	17.50
Mann-Whitney U = 280.0		P= 0.078
<b>Stage</b>		
I or II	27	20.52
III or IV	17	25.65
Mann-Whitney U = 436.00		P= 0.197
<b>Lymph nodes involvement</b>		
Present	28	23.77
Absent	16	20.28
Mann-Whitney U = 259.5		P= 0.386
<b>Metastasis</b>		
Present	10	29.90
Absent	34	20.32
Mann-Whitney U = 244.0		<b>P= 0.038*</b>

The difference in serum HER2 was also tested according to presence of estrogen receptors, progesterone receptors and the tissue HER2. Serum HER2 had no statistically significant difference with respect to the positivity of

estrogen receptors and progesterone receptors (P>0.05). Contrarily, the serum HER2 was significantly higher among those who were positive for tissue HER2 (P<0.05) as shown in (Table 4).

**Table 4: The difference in serum HER2 according to estrogen receptors, progesterone receptors and Tissue HER2 categories.**

Variable	Serum HER2 in ng/ml	
	No.	Mean Rank
<b>Estrogen receptors</b>		
Positive	33	21.71
Negative	11	24.86
Mann-Whitney U = 155.5		P= 0.487
<b>Progesterone receptors</b>		
Positive	30	23.15
Negative	14	21.11
Mann-Whitney U = 229.0		P= 0.623
<b>Tissue HER2</b>		
Positive	17	27.50
Negative	27	19.35
Mann-Whitney U = 314.0		<b>P= 0.04*</b>

Another 44 healthy women were enrolled in the study with mean age of 46.84 ± 10.11 years. They were matched for age with the cases (P>0.05). The serum HER2 was also measured in the control

group. The serum HER2 concentration was markedly increased among breast cancer patients compared to the control group. (P<0.05) as illustrated in (table 5).

**Table 5: The difference in serum HER2 in the two study groups.**

Study Group	Serum HER2 in ng/ml	
	No.	Mean Rank
Cases	44	50.41
Controls	44	38.59
Mann-Whitney U = 708.0		<b>P= 0.030*</b>

A cut-off value of 15 ng/ml was chosen to differentiate those with positive serum HER2 concentration and those with negative serum

HER2 concentration. There was a significant variation in the serum HER2 concentration between the two study groups ( $P < 0.05$ ), as demonstrated in (table 6).

**Table 6: The association of serum HER2 concentration in the two study groups according to the FDA cutoff value.**

Study Group	Serum HER2 in ng/ml	
	< 15	≥ 15
	No. (%)	No. (%)
Cases	31 (70.45%)	13 (29.55%)
Controls	39 (88.63%)	5 (11.37%)
Pearson Chi-square = 4.470		<b>P= 0.034*</b>

Table 7 illustrates that among the patients with serum HER2 levels over 15 ng/ml, six (46.1%)

tested positive for tissue HER2 and had metastatic tumours.

**Table 7: Characteristics of patients with serum HER2 concentration ≥ 15 ng/ml.**

Tissue HER-2	Serum HER2 in ng/ml	
	No.	%
	No. (%)	
Positive	6	46.1
Negative	7	53.9
<b>Metastasis</b>		
Absent	7	53.9
Present	6	46.1

**DISCUSSION:**

Breast cancer is a serious health issue worldwide. A worse clinical prognosis and an aggressive type of the disease are linked to HER2 overexpression. A potential approach has been the use of serum HER2 as a biomarker for cancer recurrence and to assess the disease status in individuals with HER2 overexpression. (8).

In the clinical setting, the primary methods for determining HER2 status are IHC and FISH (14). Both tests, however, have been proven to have some disadvantages, including the need for intrusive patient testing, the absence of real-time follow-up and their dependence on tumour biopsy or surgical removal. As a result, they are unsuitable for monitoring the progression of the disease in patients with breast cancer. Imaging techniques frequently utilized in early diagnosis

and tracking therapeutic response, in addition to histopathology testing. However, they also have drawbacks such as radiation exposure and the difficulty in distinguishing small lesions. Although serum HER2 testing has not been utilized in clinical settings for the past ten years, it might be a useful biomarker for monitoring metastasis and recurrence, as shown by several studies (6).

The aim of the current study is to assess serum levels of HER2 in individuals with recent diagnosis of breast cancer and comparing these levels with benign breast disease and healthy individuals. Also, to investigate whether the HER2 serum assay could substitute the standard test (IHC) for HER2 status.

In our current study, elevated serum HER2 levels were identified in 13 women with breast cancer (29.55%). Additionally, those with metastatic tumours had considerably greater serum HER2 levels (P value= 0.038) as shown in table (3). These findings are in line with those obtained by Shukla et al. and Tchou et al. who observed that increased HER2 serum levels in breast cancer were highly linked to distant metastases<sup>(12,19)</sup>. This can be explained by the fact that there is less HER2 ECD in the body fluids of patients with stage I or II breast cancer because the tumour tissue is localized and very few HER2 proteins reach the body fluid. Patients with tumour growth, invasion of surrounding tissues, and local or distant lymphatic metastasis have HER2 ECD levels in their body fluids that gradually rise. This leads to an abnormal elevation of serum HER2 in stage III and IV patients and a strong correlation between serum HER2 and tissue HER2.<sup>(15)</sup>

The correlation between tissue HER2 positivity by IHC and elevated serum HER2 was significant (P value= 0.04) as shown in Table (4). These findings agree with those obtained by Mokhtari et al. and Witzel et al. who observed a strong relationship between IHC HER2 and serum HER2.<sup>(16,17)</sup>

Nevertheless, some earlier research did not discover a significant relationship between serum HER2 and IHC expression of the HER2<sup>(18)</sup>. The most significant cause of disagreement is the classification system for HER2 positivity. Patients may be misclassified as HER2 negative due to a primary tumour with lower than the proposed percentage of HER2 positive cells and, consequently, will not be classified as HER2 positive. Other causes for this discrepancy are heterogeneity of breast cancers and the conversion of HER2 status. It is proposed that HER2 "negative" tumours may contain a small number of cancer cells that overexpress HER2. Due to the growth advantage of these HER2 shedding cells, their percentage may rise as the disease progresses, causing the tumour to switch from being HER2 negative to positive<sup>(14)</sup>.

The study did not reveal any statistically significant difference in serum HER2 levels compared to the positivity of estrogen receptors and progesterone receptors expression in the tissue (P value >0.05) as shown in Table (4), which is in line with the results obtained by Imoto et al.<sup>(20)</sup>. Also, there was no apparent correlation between the various age groups and serum levels of HER2. Another study discovered

no connection between the age of breast cancer patients and their serum HER2 level.<sup>(21)</sup> On the other hand, Thriveni et al. revealed that the patients' ages and their serum HER2 level had a strong association<sup>(22)</sup>. The reason for this phenomenon is the variation in the frequency of ER and PR negative tumours among different age groups of patients<sup>(23)</sup>.

Our study found that serum HER2 has no significant correlation with other pathological parameters. (P value > 0.05). These findings agree with those obtained by Farzadnia et al. who found that there is no apparent association between serum levels of HER2 and pathological parameters<sup>(24)</sup>. While Shukla et al. discovered a significant relationship between serum HER2 concentration and most of the pathological parameter<sup>(12)</sup>. These findings suggest that serum HER2 ECD level may not be a more reliable indicator of the severity of the disease than tissue HER2 receptor<sup>(23)</sup>.

the control group (P value= 0.030) as illustrated in table (5). These results are consistent with those achieved by Shukla et al. in a study conducted in New Delhi, India, from November 2011 to March 2013<sup>(12)</sup>. This may be due to the high levels of HER2 ECD in the serum of many breast cancer patients as the extracellular portion of the receptor is released from the surface of tumour cells into the circulation by metalloproteases<sup>(14)</sup>.

### CONCLUSION:

This study showed that serum HER2 levels were significantly increased in patients with breast cancer compared with controls. Also, serum levels of HER2 were found to be correlated with the IHC expression of HER2 in tissue and increased in metastatic tumours.

For this reason, quantitative assessment of the non-invasive serum-based HER2 evaluation may have diagnostic significance for breast cancer early detection and assist in determining the optimal course of treatment for patients with breast cancer.

**Conflict of interest:** The authors declare that they have no conflict of interest.

### Author contributions:

Dr. Iman Ismail Sami Al-Mubarak: Conceptualization; Investigation; Writing original research draft; Data Curation.

Dr. Nazar Samir Haddad: Formal analysis, data curation, conceptualization, writing review and editing, and resource.

## REFERENCES:

1. Chmielowski B, Territo MC, editors. Manual of clinical oncology. 8th edition. Philadelphia: Lippincott Williams & Wilkins; 2017.
2. Al-Hashimi M. Trends in Breast Cancer Incidence in Iraq During the Period 2000-2019. *Asian Pac J Cancer Prev.* 2021;22(12):3889–96.
3. Abood RA, Abdahmed KA, Mazyed SS. Epidemiology of Different Types of Cancers Reported in Basra, Iraq. *Sultan Qaboos Univ Med J SQUMJ.* 2020;20(3):295.
4. Chand P, Garg A, Singla V, Rani N. Evaluation of Immunohistochemical Profile of Breast Cancer for Prognostics and Therapeutic Use. *Niger J Surg Off Publ Niger Surg Res Soc.* 2018;24(2):100–6.
5. Pokhrel R, Yadav B, Sharma N, Sharma V, Tuladhar E, Raut M, et al. Comparison of Her2/Neu Oncoprotein in Serum and Tissue Samples in Women with Breast Cancer. *Asian Pac J Cancer Prev.* 2022;23(2):429–33.
6. Zhang P, Xiao J, Ruan Y, Zhang Z, Zhang X. Monitoring Value of Serum HER2 as a Predictive Biomarker in Patients with Metastatic Breast Cancer. *Cancer Manag Res.* 2020 ;12:4667–75.
7. Ahirwar R. Recent advances in nanomaterials-based electrochemical immunosensors and aptasensors for HER2 assessment in breast cancer. *Mikrochim Acta.* 2021;188(10):317.
8. Perrier A, Gligorov J, Lefèvre G, Boissan M. The extracellular domain of Her2 in serum as a biomarker of breast cancer. *Lab Invest.* 2018;98(6):696–707.
9. Monica Roychowdhury. Molecular subtypes [Internet]. [cited 2022 Dec 26]. Available from: <https://www.pathologyoutlines.com/topic/breastmolecularsubtypes.html>
10. Duffy MJ. Predictive Markers in Breast and Other Cancers: A Review. *Clin Chem.* 2005 ;51(3):494–503.
11. Clinical Relevance of Serum HER2 and Circulating Tumour Cell Detection in Metastatic Breast Cancer Patients. *Anticancer Res [Internet].* 2017 ;[cited 2024 Jul 25];37(6). Available from: <http://ar.iiajournals.org/content/37/6/3117.abstract>
12. Shukla S, Singh BK, Pathania OP, Jain M. Evaluation of HER2/neu oncoprotein in serum & tissue samples of women with breast cancer. *Indian J Med Res.* 2016;143(Suppl 1):S52–8.
13. Albagoush SA, Limaiem F. HER2. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022;[cited 2022 Nov 14]:1–8. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK537134/>
14. Lam L, McAndrew N, Yee M, Fu T, Tchou JC, Zhang H. Challenges in the clinical utility of the serum test for HER2 ECD. *Biochim Biophys Acta.* 2012;1826(1):199–208.
15. Zhang P, Xiao J, Ruan Y, Zhang Z, Zhang X. <p>Monitoring Value of Serum HER2 as a Predictive Biomarker in Patients with Metastatic Breast Cancer</p>. *Cancer Manag Res.* 2020;12:4667–75.
16. Witzel I, Loibl S, von Minckwitz G, Mundhenke C, Huober J, Hanusch C, et al. Monitoring serum HER2 levels during neoadjuvant trastuzumab treatment within the GeparQuattro trial. *Breast Cancer Res Treat.* 2010;123(2):437–45.
17. Mokhtari M, Khosravi MH. Utility of Serum HER-2/ neu in Prediction of Tissue HER-2 /neu Status of Primary Breast Cancer. *Middle East J Cancer.* 2021;12(4):483–90.
18. Quaranta M, Daniele A, Coviello M, Savonarola A, Abbate I, Venneri MT, et al. c-erbB-2 protein level in tissue and sera of breast cancer patients: a possibly useful clinical correlation. *Tumori.* 2006;92(4):311–17.
19. Tchou J, Lam L, Li YR, Edwards C, Ky B, Zhang H. Monitoring serum HER2 levels in breast cancer patients. *SpringerPlus.* 2015;4:237.
20. Imoto S, Wada N, Hasebe T, Ochiai A, Kitoh T. Serum c-erbB-2 protein is a useful marker for monitoring tumour recurrence of the breast. *Int J Cancer.* 2007;120(2):357–61.
21. Ryu DW, Lee CH. Impact of Serum HER2 Levels on Survival and Its Correlation with Clinicopathological Parameters in Women with Breast Cancer. *J Breast Cancer.* 2012 ;15(1):71–78.
22. K. T, Deshmane V, Bapsy PP, Krishnamoorthy L, Ramaswamy G. Clinical utility of serum human epidermal receptor-2/neu detection in breast cancer patients. *Indian J Med Res.* 2007 ;125(2):137.
23. Tsai Shin-Cheh, Huei-Tzu Chien, Chen Hsiu-Pei. Relationships between serum HER2 ECD, TIMP-1 and clinical outcomes in Taiwanese breast cancer | *World Journal of Surgical*

Oncology | Full Text [Internet]. 2012;[cited 2023 May 16]. Available from: <https://wjso.biomedcentral.com/articles/10.1186/1477-7819-10-42>

24. Farzadnia M, Meibodi NT, Shandiz FH, Mahmoudi M, Bahar MM, Memar B, et al. Evaluation of HER2/neu oncoprotein in serum and tissue samples of women with breast cancer: correlation with clinicopathological parameters. *Breast Edinb Scotl.* 2010;19(6):489–92.