

Comparison of Her2/Neu Oncoprotein in Serum and Tissue Samples in Women with Breast Cancer

Rakesh Pokhrel¹, Binod Kumar Yadav^{1*}, Nisha Sharma², Vijay Kumar Sharma¹, Eans Tara Tuladhar¹, Mithileshwor Raut¹, Aseem Bhattarai¹, Raju Kumar Dubey¹, Apeksha Niraula¹, Akash Mishra¹, Uttam Budhathoki¹

Abstract

Background and objectives: Human Epidermal Growth Factor Receptor 2 (HER2/neu) is one of the most extensively studied proto-oncogenes in breast cancer patients. Accurate and timely assessment of the HER2/neu over expression is pivotal for the identification of breast cancer patients that could benefit from HER2-targeted therapy. The present study was undertaken to investigate the diagnostic utility of serum HER2/neu testing by chemiluminescent immunoassay (CLIA) in breast cancer patients and compare it with the immunohistochemistry (IHC) method of HER2/neu expression. **Methods:** Serum sample and tissue/paraffin block was collected from 52 patients with breast cancer before start of any anticancer regimen or hormonal therapy. The tissue specimens were processed in Histopathology lab. Sections were immunostained with anti –estrogen receptor (ER), anti –progesteron receptor (PR) and anti HER2/neu receptor mouse monoclonal antibodies.) Serum HER2/neu was estimated using the chemiluminiscent immunoassay using 15ng/ml as the cut off. **Results:** Out of 52 patients with breast cancer, serum HER2/neu was found elevated in 25(48.1%) patients and remaining 27(51.9%) showed normal serum HER2/neu concentrations. On IHC HER2/neu score was 3+ in 9(17.3%), 2+ in 10(19.2%), 1+ in 1(1.9%); while 32(61.5%) showed no HER2/neu expression. 31(59.6%) patients were ER positive and 28(53.8%) were PR positive. There was a significant correlation ($P<0.001$) of serum HER2 concentration with tissue expression of HER2/neu and Histological tumor grade. Serum HER2/neu levels showed a negative correlation with ER status ($P=0.047$) but no correlation with PR status. **Conclusion:** The result showed that the elevated serum HER2/neu was correlated with the IHC expression of HER2/neu in tissue and the histological grade of the tumor. Findings suggest that post initial tissue diagnosis (IHC HER2/neu), serum HER2 assay may supplement subsequent tissue tests to monitor disease status and response to therapy.

Keywords: Breast cancer- IHC- HER2/neu- ER- PR- CLIA

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Introduction

Breast cancer is the leading non dermatologic malignancy and the second most common cause of cancer death among women in the world (DeSantis et al., 2017). Up to 80% of invasive breast cancers are infiltrating ductal carcinomas (IDC). Invasive lobular carcinoma is the second most common type. Of the noninvasive in situ carcinomas, more than 80% are ductal and about 10% are lobular (Childers et al., 2017).

HER2/neu is an extensively studied proto-oncogene in human breast cancer. It is a human epidermal growth factor receptor (member of epithelial HER1, HER3 and HER4 family) (Coussens et al., 1985; Sareyeldin et al., 2019). Human epidermal growth factor receptor-2 (HER2) over expression or amplification in tumor tissue is observed in 20–30% of primary breast cancers (Larionov,

2018). Tissue HER2 status has been routinely used as an important parameter for decision making regarding anti-HER2 therapy in the neoadjuvant, adjuvant and metastatic settings (Mahtani et al., 2020). Serum HER2 extracellular domain (ECD) can be detected by the cleavage of transmembrane tissue HER2 protein mediated by a matrix metalloproteinase (Ahrwar, 2021).

IHC and fluorescent in situ hybridization (FISH) have been considered gold standard techniques for assessing HER2/neu expression, however due to several limitations of the histological methods, the serum based noninvasive HER 2neu testing has been introduced and validated (Jain, 2021).

The aim of this study was to investigate the correlation between noninvasive serum HER2/neu assay and tissue expression of the HER2/neu by Immunohistochemistry methods.

¹Department of Biochemistry Institute of Medicine Maharajgunj, Kathmandu, Nepal. ²Department of Pathology Institute of Medicine Maharajgunj, Kathmandu, Nepal. *For Correspondence: drbinodyadaviom@gmail.com

Materials and Methods

Study design

This was a hospital based cross sectional study conducted in the department of Biochemistry and department of Pathology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal from August 2020 to January 2021. Study population included women with diagnosed breast cancer visiting to the Histopathology laboratory for Immunohistochemistry (ER, PR and HER2/neu.)

Selection of Cases

Fifty Two cases diagnosed with breast cancer on histopathological examination were included in this study. Only newly diagnosed cases of breast cancer including both primary breast cancer and metastatic breast cancer were included. Patients already on treatment were excluded. Clinicopathologic features such as age, histological type, grade, estrogen receptor, progesterone receptor and HER2/neu receptor status were all noted. Blood samples (3-5ml) were obtained from the patients at the time of diagnosis before the initiation of any therapeutic intervention.

Serum HER2 assay

Blood sample (3-5 ml) was collected by venipuncture in plain vacutainer tubes under sterile conditions. Serum was obtained after centrifuging the clotted blood sample at 1,000g for 10 min. Serum was separated and stored in aliquots at -70°C. Serum HER 2/neu concentration was measured using a 2-site chemiluminescence sandwich immunoassay (ADVIA Centaur System, Bayer Diagnostics). The cut-off value used in this study was 15.0 ng/ml as per manufacturer’s recommendation(Perrier et al., 2018).

Immunohistochemistry (IHC)

IHC staining of specimens was carried out on the tissues embedded in paraffin blocks. IHC expression of HER2/neu , ER and PR were assessed using automatic immunostainer (BioGenix), and the degree of staining was

scored by pathologist based on the method recommended by College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) (Brunelli et al., 2008).

Statistical analysis

Normally distributed continuous variables were compared using the unpaired t test. One-way analysis of variance (ANOVA) and Pearson correlation was used for ascertaining significance of continuous variables among different groups.

Results

The age range of the patients was 28-75 years with a mean age of 52.4 ± 11.7 years. Maximum number of cases were between 40-55 years (n=25, 48.1%). Majority of breast cancer patients in this study were in histological tumor grade II (n=25, 48.1%).

Comparison of Serum HER2 /neu and IHC HER2 /neu Expression

Out of the total 52 patients, IHC /HER2/neu was found to be positive in 20 (38.5%) cases. IHC result for HER2/neu were 1+ for 1(1.9%) of the patients, 2+for 10 (19.2%) and 3+ for 9 (17.3%) of patients. The tissue HER2/neu positivity by IHC was significantly associated with the elevated serum HER2/neu. Mean serum HER2/ neu level was found to be 37.89 ng/ml (range : 6.4-96.90) in IHC HER2/neu positive group, which was found to be significantly higher than in the IHC HER2/neu negative group in which mean serum HER2/neu was found to be 9.69 ng/ml (range 2.4-31.8) , (p value <0.001 Table 1).

Correlation of Serum HER2/neu with the histological tumor grade

There was a significant association between tumor grade and serum HER2/neu level. The mean value of serum HER2/neu was 25.53 ng/ml in patients belonging to tumor grade (III+IV) which is statistically higher (P<0.001) compared to the tumor stage (I+II), in which

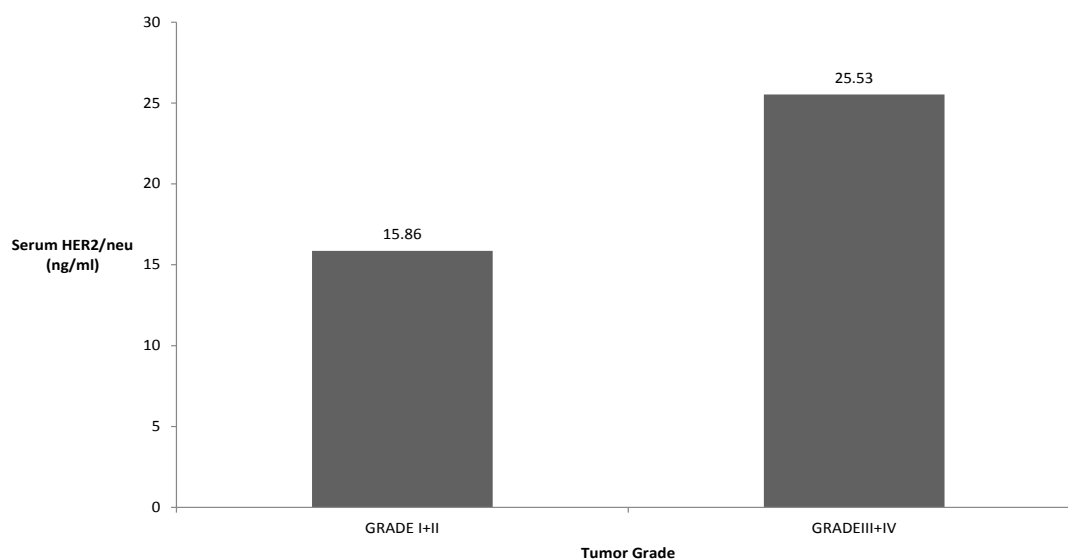


Figure 1. Comparison of Serum HER2/neu in Various Tumor Grades

Table 1. Comparison of serum HER2/neu in IHC HER2/neu Positive and Negative Groups

IHC HER2	N	Mean	Std. Deviation	Minimum	Maximum	P value
Negative	32	9.69	7.10	2.4	31.8	
Positive	20	37.89	30.02	6.4	96.9	<0.001 *
Total	52	20.54	23.63	2.4	96.9	

*P<0.05 is statistically significant

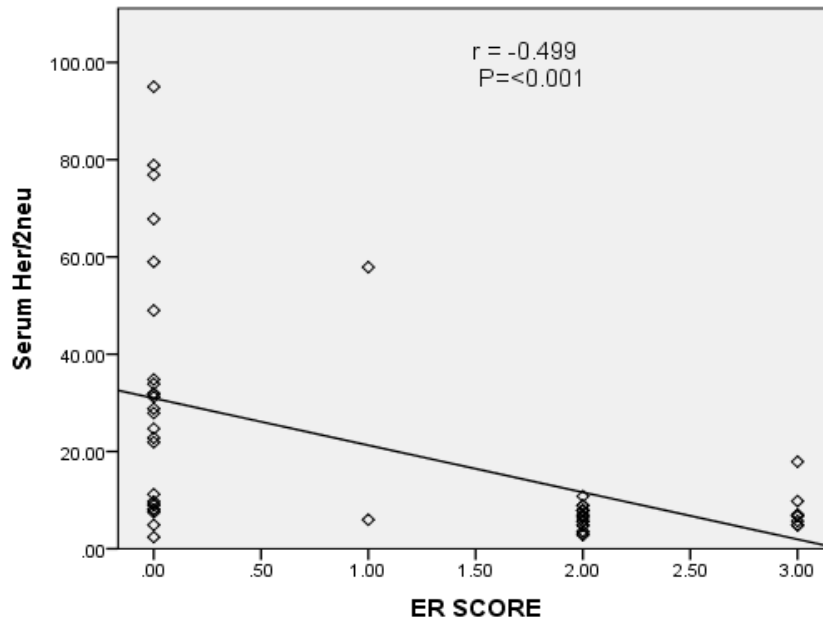


Figure 2. Correlation between Serum HER2/neu and Estrogen Receptor Score

the mean serum HER2/neu was found to be 15.86 ng/ml. (Figure 1).

however the correlation was not statistically significant (p=0.057).

Correlation of Serum Her2/neu with ER and PR IHC Expression

There was a significant negative correlation (r= -0.499) between serum HER2/neu and ER status in breast cancer (p= <0.05) Figure 2. There was negative correlation between serum HER2/neu and PR status (r= -0.22)

Predictive Significance of Serum HER2/neu for Tissue HER2/neu Status

To study the potential of serum HER2/neu to predict the tissue HER2/neu status, receiver operating characteristic (ROC) curve was plotted. At the cut-off value of HER2/neu level of 16.2 ng/ml, the sensitivity and specificity

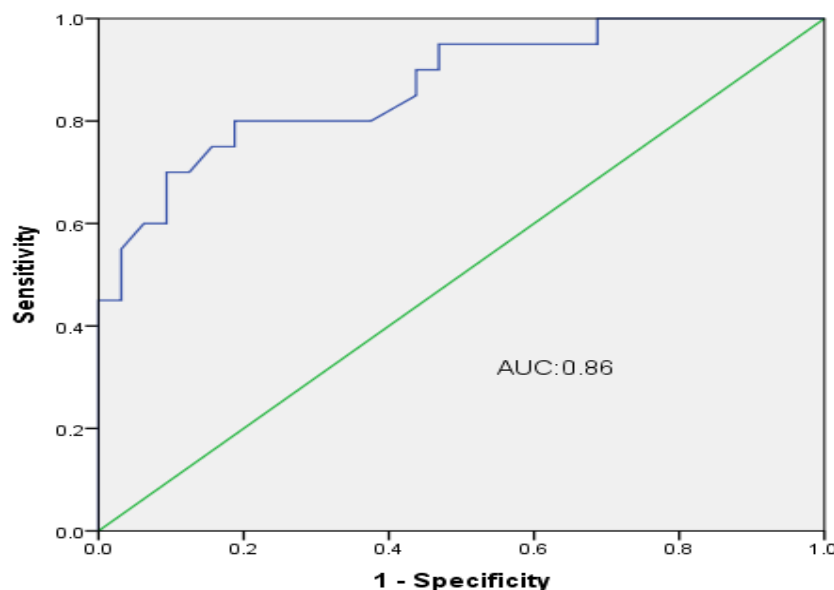


Figure 3. ROC Curve Plot of Serum HER2/neu for Tissue HER2/neu Status

were 75% and 85% respectively. Increasing the cut-off to 25.7 ng/ml the sensitivity dropped to 60% and specificity increased to 90.6%. While considering 16.2 ng/ml as cut-off the ROC Curve showed an area under the curve value of 0.86(95% CI: 0.76 - 0.97, $P < 0.005$, Figure 3).

Discussion

HER2 is a 185 kD integral membrane protein. Extracellular domain of HER2 after being broken by metalloproteases, gets detached from other parts of HER2 and enters the blood circulation (Pavithran, 2021). Currently, IHC, in which the HER2 ECD is immunostained, and FISH in which amplified HER2 DNA is fluorescently labeled (Chen et al., 2019) are the commonly used techniques for assessment of the expression of HER2/neu (Mokuyasu and Suzuki, 2017). Serum HER2 levels have been reported to correlate with tissue HER2 status (Shi et al., 2017; Mokhtari and Khosravi, 2021) which is similar to our result in which there is a significant correlation between serum HER2/neu and IHC HER2 status. However some studies did not find significant association between serum HER2/neu and IHC expression of the HER2/neu (Kalantari et al., 2019). False evaluation of the tissue HER2 status of the primary tumor (e.g., due to testing inaccuracies or the intratumoral heterogeneity of HER2 expression) could explain this discrepancy. Patients with negative tumor HER2 status at the time of biopsy may become HER2-positive as they progress to metastatic disease. This could explain discrepancies between negative tissue testing and increased serum HER2 concentrations in metastatic breast cancer. Moreover, reduced metalloprotease activity could lead to decreased serum HER2 concentrations (Marchiò et al., 2021).

Our study found negative correlation between serum HER2 neu and estrogen receptor (ER) expression in the tissue which is consistent with other studies (Shukla et al., 2016). However few studies did not find any association between raised serum HER2/neu and estrogen receptor status (Arana Echarri et al., 2021). In our study there was no significant correlation between serum level of HER2/neu and different age group. Various other studies also did not find a significant association between serum HER2/neu level and age of the patients with breast cancer (Goksel et al., 2007; Ryu and Lee, 2012). However study by Pallud et al (Pallud et al., 2005) and Thiriveni et al. (Thiriveni et al., 2007) found a significant positive association between serum HER2/neu level and age.

We found a significant positive correlation between serum HER2/neu and Histological grade of the tumor. The similar association between serum HER2/neu and tumor grade was observed in a study by Shukla et al in India in which the serum HER2/neu was found to be correlated positively with tumor size, clinical stage, nodal status, histological grade, distant metastasis, tissue HER2 status and negatively associated with estrogen receptor status (Shukla et al., 2016). The study by Ludovini et al also found significant association between serum HER2/neu concentration and most of the clinicopathological

parameters mentioned above (Ludovini et al., 2008).

In the present study, 4 of the 52 cases were negative for HER2/neu expression on IHC but had raised serum HER2 levels while 3 were positive on IHC and negative on CLIA. Lack of tissue immunostaining may be related to the sensitivity of the method, tissue fixation playing an important role. The issue can be resolved to some extent by employing FISH analysis for ascertaining tissue HER2 status. However, it has been postulated that even a few HER2/neu positive cells within an essentially HER2 negative tumor can shed sufficiently detectable amount of HER2/neu protein in the serum which might explain the discrepancy seen in 3 cases in our study which were positive for IHC HER2/neu expression but were negative on CLIA (Zhang et al., 2020).

Optimum cut-off value of serum HER2/neu for predicting tissue HER2/neu status was found to be 16.02 ng/ml in our study which is close to the cut-off value 15.0 ng/ml as recommended by Food and Drug Administration (FDA) (Tsé et al., 2012). This cut off has been found to be between ranges 8.83-21.6 in different studies (Zhang et al., 2018; Shamshirian et al., 2020; Mokhtari and Khosravi, 2021).

In conclusion, our study has found a strong correlation between the serum HER2/neu and tissue HER2/neu status. Which opens a new dimension to apply the non-invasive serum based HER2/neu evaluation to guide the best possible therapeutic intervention in breast cancer patients. In our study there was a significant positive correlation between the serum HER2/neu status and histological tumor grade hence serum HER2/neu results holds a potential diagnostic tool to reflect the tumor burden and disease progression. In addition to its diagnostic and prognostic potentials, serum HER2 ECD levels have other promising clinical values. As serum HER2 ECD can be measured rapidly and repeatedly, monitoring HER2 ECD levels at different times might yield clinically meaningful data.

Limitations

Due to the limited timeframe of the study the serum HER2/neu level could not be compared before and after the initiation of the herceptin therapy.

A follow up study couldn't be performed for evaluating the difference in serum HER2/neu level, after the clinical improvement in the course of disease.

The equivocal results of IHC HER2/neu could not be confirmed with the further methods such as FISH.

Author Contribution Statement

RP, BKY, NS and BKS conceived the design of the study. RP, UB and AM performed the acquisition of data. RP, BKY and ET analyzed and interpreted the data. RP, UB, AN, RD and AM drafted the manuscript. RP, BKY, MT and AB performed the critical revision for important intellectual content. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

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Approval of the Study

This study has been approved for Master's level thesis by Department of Biochemistry, Institute of Medicine, Kathmandu Nepal.

Ethical Approval

The study has given ethical clearance by the institutional review committee, institute of medicine and the letter has been submitted along with the manuscript submission.

Conflict of Interest

None of the authors have any conflict of interest to report.

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