



EVALUATION OF THE CONCORDANCE OF SERUM HER 2/NEU LEVELS WITH IMMUNOHISTOCHEMISTRY IN TUMOUR TISSUE

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Abstract:

Background: Breast cancer is the second most common cancer in the world and it is the most frequent cancer among women with overall estimated 1.67 million new cancer cases diagnosed in 2012.

Objective: To evaluate concordance of serum HER-2/neu with immunohistochemistry in tumour tissue.

Methods: This present study included 56 patient suspected cases of malignancy of breast received in Department of Surgery and Department of Pathology in Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh during the period of May 2014 to September 2016.

Result: Most cases were in the stage 2 (50%, 25 cases), followed by 46% (23 cases) in the stage 3 and 4% in the stage 1 (2 cases). The correlation between serum HER -2 /neu and age of the patient and mental status was found to be non significant. (P = 0.864, NS) In the present study, there was also significant correlation between serum HER-2/neu and cytological grading, done by Robinson cytological grading system..(P<0.05,S) There were 69.6 %of serum HER-2/neu positive cases in stage3 as compared to 40 % in stage 2 and no case in stage 1. The correlation between serum HER-2 /neu and tumour stagewas found to be significant. (P < 0.05, S)

Conclusion: Serum HER -2/neu can be used as complimentary method besides tissue methods as it has advantages of being non invasive and provide real time assessment of HER -2/ neu level.

Keywords: Serum HER 2/neu levels, immunohistochemistry, tumour tissue

INTRODUCTION: HER-2/neu and c-erbB-2, encodes a growth factor receptor that has been found to play an important role in pathogenesis of breast cancer. ¹In 15% to 30% of invasive breast cancer cases, HER-2/neu gene is found to be either amplified or over expressed ² and HER-2/neu overexpression is found to be an independent predictor of shorter overall survival (OS) and disease-free survival (DFS). ³

HER-2/neu expression has found its application in the evaluation of prognosis and various treatment modalities like gene therapy, immunotherapy, immunotoxins and tyrosine kinase inhibitors. ^{4,5}

HER-2/neu status can be assessed by different techniques such as immunohistochemistry (IHC) and Fluorescent in situ hybridization (FISH) but each of these technique has got its own advantages and disadvantages. IHC shows variability of results due to subjectivity in interpretation of staining score, and variability is also due to antibody and staining procedure used, loss of HER-2/neu protein as a result of storage. Though FISH has a more objective scoring system and has built in control in the form of 2 copies of HER - 2/neu in non malignant cell, its disadvantages of are high cost, fluorescent microscope, inability to store slide for further review.⁶

Therefore, the current study has been under taken to evaluate concordance of serum HER-2/neu with immunohistochemistry in tumour tissue

Material and Methods: This present study included 56 patient suspected cases of malignancy of breast received in Department of Surgery and Department of Pathology in Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh during the period of May 2014 to September 2016. Relevant clinical history, medical records and examination were evaluation and fine needle aspiration was performed.

Inclusion criteria:

Patient who were preoperatively diagnosed clinically and cytologically as breast carcinoma were included in the study and preoperative serum sample were taken of these cases and postoperative mastectomy specimen was send for histopathology confirmation. Samples were investigated for the histological type of carcinoma, tumour size, lymph node metastasis, stage as well as grade of the tumour. Tissue HER2 over-expression was evaluated by immunohistochemistry (IHC) staining and HER2 levels were studied by ELISA method.

Exclusion criteria: Patient diagnosed as benign on histopathology

• **Patient with malignancies other than of breast.**

A detailed clinical history of patients was taken and medical records were evaluated for age, sex, lactational history, parity, family, socioeconomic status. Clinical examination was focussed on local examination of breast and regional lymph node spread as well as distant metastasis.

The smears thus obtained were thoroughly evaluated microscopically after staining.

HISTOPATHOLOGICAL EXAMINATION:

Mastectomy specimens with or without axillary lymphadenectomy were fixed in 10% neutral buffered formalin, (10ml of 40% formaldehyde diluted in 90ml of water). After adequate fixation/detailed gross examination was done taking into account the following parameters.

- Weight and size of the specimen, in 3 dimensions.
- Appearance of the nipple-areola and skin.
- Features seen on cut section (slices applied at intervals of 2cm) with special note on tumour location, number, size, margins, colour, consistency, presence of haemorrhage and necrosis.
- Axillary soft tissue: All lymph nodes were dissected out

Immunohistochemistry For Estrogen and HER -2/NEU Receptors:

Sections for immunohistochemical assay of EGFR2, Estrogen Receptors and progesterone receptors (4µ in thickness) were taken on poly-L-lysine coated clean glass slides and stained.

Microscopic Examination:

Microscopic evaluation was done using Olympus CH20i microscope with a high power field (40X) diameter of 0.44 mm and the corresponding field area of 0.152 mm². H&E stained histological sections were assigned for histopathological diagnosis according to WHO classification of tumour of breast(2012) .and grading was done as per the Modified Scarff Bloom Richardson grading scheme. (Elston and Ellis, 1991) and cyological grading by Robinson cytological grading system (1994).

Overall grade: Obtained by adding up scores in individual categories:

- Score 3-5: Grade I (Well differentiated or low grade)

- Score 6-7: Grade II (Moderately differentiated or intermediate grade)
- Score 8-9: Grade III (Poorly differentiated or high grade)

The scoring system of HER2-neu immunohistochemistry expression uses a semi quantitative approach based on the intensity of reaction-product and percentage of membrane positive cells giving as below (table-1). Both 2+ and 3+ were taken as tissue HER-2/neu positive, 0 and 1+ as tissue HER-2/neu negative in present study.

Final score was obtained by adding scores from the 2 categories to give a maximum score of 8. Tumours with score ≤ 2 were termed ER negative while those with score >2 were ER positive.

Statistical Analysis:

Statistical analysis was carried out using SPSS software (v.18.0). Chi-square test was used to evaluate the significance of difference between association of variables like HER-2/neu expression, and tumour grade. P-value of 0.05 or less was considered to be statistically significant. Descriptive data were tabulated as numbers and percentages. Pictorial representations in the forms of bar diagrams and pie charts were provided wherever necessary.

Results: Of the total cases, most were in the 35-50 age group, (22 cases, 44%), followed by patient in > 50 age group (42 cases, 21%) and least number of cases in <35 year age group (7 cases, 14%). In our study, the youngest patient was of 75 years while the oldest was of 25 years. Median age was found to be 45 years.

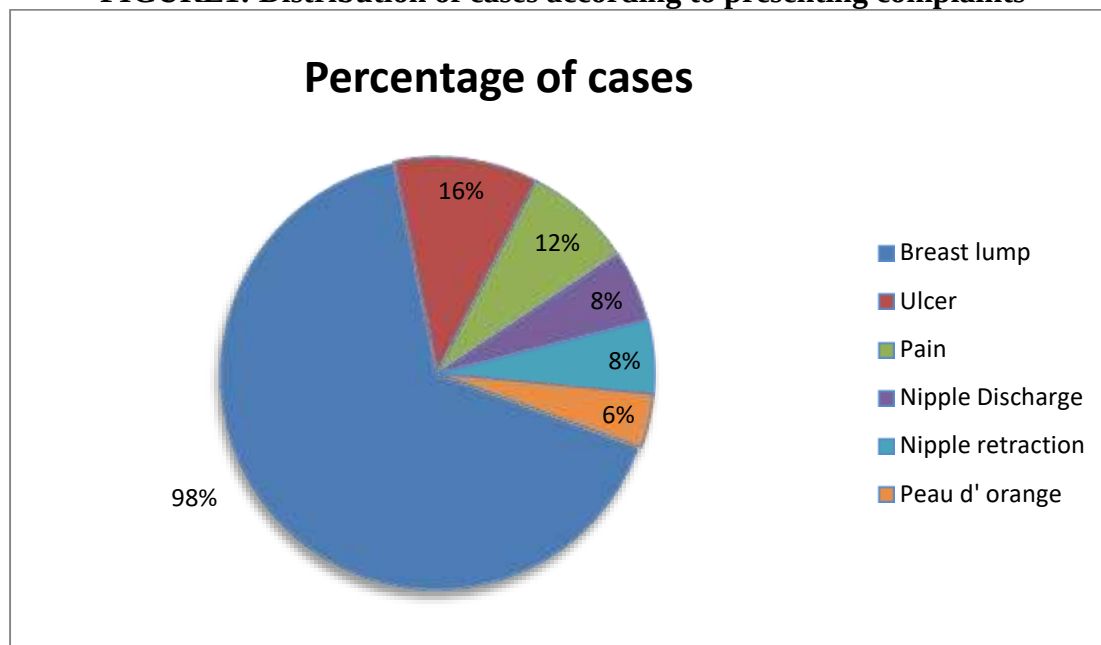
TABLE 1: Distribution of cases according to age

Age(in years)	Number of cases	Percentage of cases
<35	7	14.0%
35-49	22	44.0%
>50	21	42.0%
Total	50	100.0%

TABLE 2: Distribution of cases according to presenting complaints

Symptoms	Number of cases	Percentage of cases
Breast lump	49	98.0%
Ulcer	8	16.0%
Pain	6	12.0%
Nipple Discharge	4	8.0%
Nipple retraction	4	8.0%
Dimpling of skin (Peau d' orange)	3	6.0%

In 98 % cases (49 cases) the presenting complaint was breast lump or mass along with ulceration in 16% cases (8 cases), pain in 12% cases (6 cases), nipple discharge and retraction in 8% cases (4 cases each), Peau de orange in 6% cases (3 cases).

FIGURE1: Distribution of cases according to presenting complaints**TABLE 3: Distribution of cases according to menstrual status**

Menstrual status	Number	Percentage of cases
Premenopausal	23	46.0%
Postmenopausal	27	54.0%
Total	50	100.0%

23 cases (46% cases) were in the premenopausal group while 27 cases (54% cases) were in the postmenopausal group..

TABLE 4: Distribution of cases according to histological types of cancer

Histological types	Number of cases	Percentage of cases
Infiltrating Ductal Carcinoma (NOS)	47	94.0%
Medullary carcinoma	2	4.0%
Invasive Cribriform carcinoma	1	2.0%
Total	50	100%

94% cases (47cases) were of the Infiltrating ductal carcinoma (NOS) type. followed by medullary carcinoma (2cases, 4%) and a case of invasive cribriform carcinoma (1 case, 2%). when the grading was done by Modified Scarff- Bloom –Richardson Grading scheme out of total cases of carcinoma breast included in our study, most cases were in the grade 2 (54%,27 cases), followed by grade 3 (44%,22 cases) , and there was only one case in grade 1 (2%).

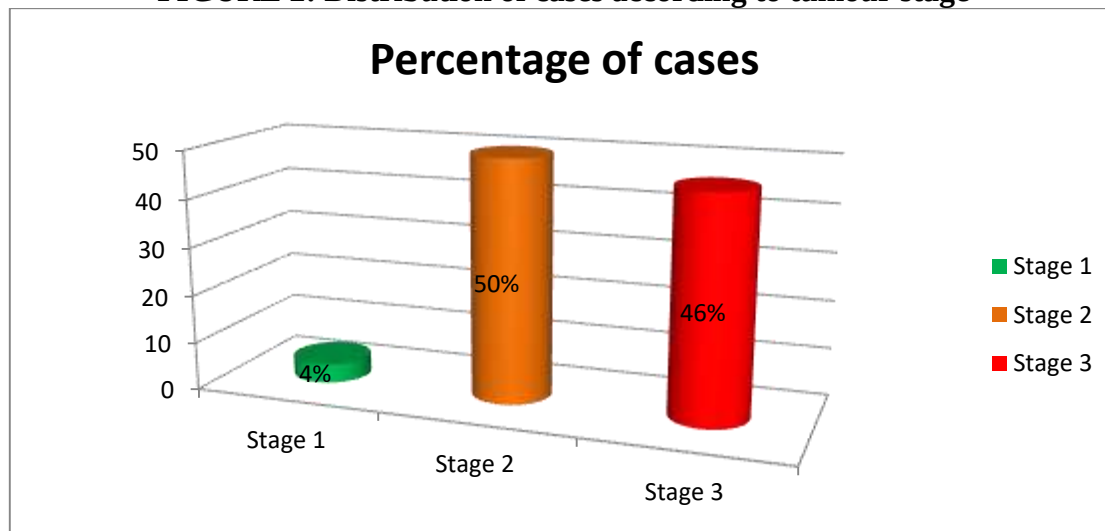
TABLE 5: Distribution of cases according to tumour stage

Tumour Stage	Number of cases	Percentage of cases
Stage 1	2	4.0%
-Stage 2	25	50.0%
Stage 3	23	46.0%
Total	50	100.0%

TABLE 5: Staging was shows distribution of cases according to stage. Staging was done by the American Joint Committee on cancer staging system, 50% of cases were in the stage 2 (25 cases),

followed by 46 % of case in stage 3 (23 cases) and 2 case in the stage 1 (4%). Majority of the cases presented in our study were in the stage 2, followed by stage 3 and least in stage 1.

FIGURE 2: Distribution of cases according to tumour stage



56 % (28 cases) of cases showed lymph node metastasis while 44 % (22 cases) of cases did not show lymph node metastasis.

TABLE 6 : Distribution of cases according to serum HER -2/neu level

Serum HER-2/neu level	Number	Percentage of cases
Positive (>6.8 ng/ml)	26	52.0%
Negative(<6.8 ng/ml)	24	48.0%
Total	50	100.0%

52% (26 cases) showed serum positivity while 48% (24 cases) were serologically negative when using cut off 6.8 ng /ml.

There were 55% serum HER -2/neu positive in 35-49 year age group, followed by 52% of cases in >50 year age group and least serum HER-2/neu positivity in <34 year age group (43%). Statistical analysis showed that this correlation was not significant. ($\chi^2=0.293$, $P = 0.864$, NS).

Table 7: Distribution of cases according to cytological grade (Robinson cytological Grading System):

Robinson cytological Grade	Number of cases	Percentage of cases
Grade 1	7	14
Grade 2	22	44
Grade 3	21	42
Total	50	100

Distribution of cases breast carcinoma according to Robinson cytological Grading System Majority of cases were of grade 2 tumour 22 cases (44%), followed by grade 3 (42%), and the least population of patients were in the grade 1 (14%).

There were 42.9% serum HER-2/neu positive cases with < 34 age as compared to 54.5% in 35-49 age group and 52.4% in > 50 age group. The correlation between serum HER -2 /neu and age of the patient was found to be non significant. ($P = 0.864$, NS)

Serum HER -2 /neu positivity was seen in 55.6% of postmenopausal patient, slightly higher proportion of cases than who are premenopausal. (47.8%) but the statistical correlation was non significant ($\chi^2=0.297$, $P=0.586$, NS). Relative percentage of cases with serum HER --2 /neu

positivity increased with stage, no case in grade 1, followed by 40% of cases in grade 2, 69.6% in grade 3. This correlation was found to be statistically significant ($\chi^2=6.45$, $P<0.05$, S)

Table 8: Relationship of serum HER-2 /neu with cytological grade

Cytological grade	Number	Serum HER-2 neu negative	Serum HER-2 /neu positive	P value
Grade 1	7(100%)	6(85.7%)	1(15.3%)	0.007,S
Grade 2	22(100%)	13(59.1%)	9(40.9%)	
Grade 3	21(100%)	5(23.8%)	16(76.2%)	
Total	50(100%)	24(48%)	26(52%)	

The relative percentage of serum HER --2 /neu positive cases increased with histological grade, 15.3% case in grade 1, followed by 40.9% of cases in grade 2, 76.2% in grade 3. The correlation was found to be statistically significant ($\chi^2= 9.9$, $P <0.05$, S)

Discussion: In present study, there were relatively more percentage of serum HER-2/neu positive cases in the 35-50 age group (54.5%) than in cases with >50 years of age (52.4%) and least in <34 year age group (42.9%). No trend was established with age, the correlation was found to be statistically non significant ($P = 0.864$, NS). Similarly, no correlation was found in the previous study⁷ who reported relatively more serum positive cases in age less than 34 (10/172), followed by cases in 50 year age group (61/1180) and least in 35-59 year age group (55/1510), there was no clear cut correlation between these variables, hence statistical correlation was non significant ($P = 0.100$, NS).

Relationship of serum HER-2 /neu with menstrual status: In present study, 23 cases (46% cases) were in the premenopausal group while 27 cases (54% cases) were in the postmenopausal group. Serum HER-2/neu positivity was seen in 55.6% of postmenopausal cases, slightly higher proportion of cases than who were premenopausal (47.8%). The relationship of serum HER-2/neu with menstrual status was found to be statistically non significant ($P = 0.586$, NS).⁸ Ludovini et al (2008) also found this relationship to be non significant ($P = 0.25$, NS). Imoto et al (1991) found this to be even more unrelated ($P = 0.912$, NS). In summary, in most of the studies menstrual status was not significantly related.⁹

Relationship of serum HER -2 /neu with histological grade: In our study, histological grading of breast carcinoma patient was done modified Scarff - Bloom- Richardson system (Elston and Ellis, 1991) in present study there were 54% cases in grade 2, followed by 44% cases in grade 3, least number of patient in grade 1. The relative percentage of serum HER-2/neu positive cases increased with histological grade. No case in grade 1, followed by 37 % of cases in grade 2, 72.7% in grade 3. There existed a significant correlation between serum HER-2/neu and histological grade ($P <0.05$, S), as in studies done by Ludovini et al (2008), ($P = 0.003$, S), Imoto et al (1999) ($P = 0.004$, S)^{8,9}.

Relationship of serum HER-2/neu with stage: In present study, staging was done by American Joint Committee on cancer staging system for breast cancer and found that majority of cases were in the stage 2 (50%), followed by stage 3 (46%). The relative percentage of cases with serum HER-2/neu positivity increased with stage, no case in grade 1, followed by 40% of cases in grade 2, 69.6% in grade 3, the correlation was found to be statistically significant ($P < 0.05$, S) and also in studies done by Ludovini et al (2008)⁸, ($P = 0.008$), Imoto et al (1999)⁹, ($P = 0.023$, S). However, in studies done by Farzadnia et al (2010), the correlation was non significant ($P = 0.865$, NS)¹⁰

Relationship of serum HER-2/ neu with lymph node status: In our study, lymph node metastasis was seen in 56 % (28 cases) cases lymph node metastasis while 44 % (22 cases) of cases did not show lymph node metastasis., we found abnormal serum level in 67.9 % lymph node positive patient as compared to 31.8 % in lymph node negative patient. This correlation was found to be statistically significant ($P <0.05$, S) and also in various studies, Ludovini et al, 2008. ($P = 0.035$, S), Imoto et al (1999) ($P = 0.047$, S). However, Farzadnia et al (2010) found no correlation ($P = 0.297$, NS).^{8,9,10}

Relationship of serum HER -2/neu with estrogen receptor expression

In the present study, serum HER -2 /neu was positive in 63% cases with ER receptor negativity while 27% in ER receptor positive cases, we found correlation of serum HER-2 /neu with estrogen receptor expression and found this correlation to be significant ($P < 0.05$, S). In the study done by **Ludovini et al (2008)** also found this correlation to be significant ($P = 0.016$, S). However, **Imoto et al (1999)** found this correlation to be non significant. ($P = 0.258$, NS).^{8,9}

Relationship of serum HER-2/neu with progesterone receptor expression

Serum HER -2 /neu positivity was 61.5% in patients with PR receptor negativity while 18.2 % in PR receptor positive patient. Relationship with progesterone was found to be significant in present study ($P < 0.05$, S). **Ludovini et al (2008)** ($P = 0.007$, S). also found this correlation to be significant, where as **Imoto et al, 1999** ($P = 0.152$, NS), found this correlation to be non significant.^{8,9}

Relationship of serum HER-2/neu with tissue HER-2/neu: In our study, we found abnormal serum level in 72 % of IHC positive patient and 32 % of IHC negative patient. This correlation was found to be statistically significant ($P = 0.005$, S). We compared our results with previous studies. Study done by **Liue et al** including 2682 reported 126 (4.4%) had elevated serum HER-2/neu level and 692 patients (24.2%) showed overexpression in tissue, with a concordance of 78.7%. Abnormal HER-2/neu serum levels were found in 15.0 % (104/692) IHC + subjects, in contrast to only 1% (22 /2168) in IHC- patients ($P < 0.001$, S).⁷ Similarly, **Ludovini et al, 2008** studied 256 stage I - III patients and found a tissue/serum relationship of 87.1 % . Abnormal HER-2/neu serum levels were found in 38.1 % (16/42) IHC + subjects (3 + or 2 + FISH +) in contrast to only 3.3 % (7 /214) in IHC-patients ($P < 0.0001$, S).⁸

Conclusion: Serum HER -2/neu can be used as complimentary method besides tissue methods as it has advantages of being non invasive and provide real time assessment of HER -2/ neu level.

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