Section: Pathology

Original Research Article

RELATION BETWEEN SERUM HER-2/NEU LEVEL WITH THE TUMOUR SIZE AND TUMOUR SIZE AND TUMOUR GRADE

Islam Barkatullah Khan[1], Aseeb Ur Rehman[1], Arif Rasul[2], Mohsin Aijaz[3]

[1]*Assistant Professor, Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, UP, India*
[2]*Assistant Professor, Department of General Surgery, Venkateswara Institute of Medical Sciences, Gajraula, UP, India*
[3]*Assistant Professor, Department of Pathology, Para Medical College, Faculty of Medicine, AMU, Aligarh, UP, India*

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Corresponding Author:
Dr. Mohsin Aijaz,
Assistant Professor, Department of Pathology, Para Medical College, Faculty of Medicine, AMU, Aligarh, UP, India
Email: maijaz051@gmail.com

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ABSTRACT

Background: Breast cancer ranks as the fifth cause of death from cancer with overall 522,000 deaths while it is the most frequent cause of cancer death in women in less developed regions (324,000 deaths, 14.3% of total). It is the second cause of cancer death in more developed regions (198,000 deaths, 15.4%) after lung cancer. The objective is to evaluate the relationship between serum HER-2/ neu and various prognostic factors such as patient age, tumour size and tumour grade.

Materials and Methods: Our study was prospective study on 56 patients in the Department of Pathology, Jawaharlal Nehru Medical College, AMU, Aligarh, during the period of May 2014 to September 2016. After taking detailed history and thorough examination, relevant clinical data was recorded and fine needle aspiration done.

Results: Most cases were in the grade 2 (54%, 27 cases), followed by grade 3 (44%, 22 cases), and there was only one case (2%) in grade 1. There were 33.3% of serum HER-2/neu positive cases with tumour size < 2 cm as compared to 34.8% in 2-5 cm tumour size and 70.8 % in tumour size > 5cm.The correlation between serum HER-2/neu and tumour size was found to significant (P <0.05, S). There were 72.7 % of serum HER-2/neu positive cases in grade 3 as compared to 37 % in grade 2 and no case in grade 1. The correlation between serum HER-2 /neu and histological grade was found to be significant. (P <0.05, S).

Conclusion: The correlation between serum HER-2/neu and tumour size was found to significant (P <0.05, S). There were 72.7 % of serum HER-2/neu positive cases in grade 3 as compared to 37 % in grade 2 and no case in grade 1. The correlation between serum HER-2 /neu and histological grade was found to be significant. (P <0.05, S).

Keywords: Correlation, Serum HER-2/neu, Tumour size, Grade.

INTRODUCTION

Breasts are a pair of modified apocrine sweat glands that form secondary sexual features in female. It is a source of nutrition to neonates apart from having immense cosmetic importance. Anatomically, breast is a complex structure comprising of ducts and stroma. These structures can give rise to benign and malignant disease, especially in females.

The incidence of breast cancer is greater in developed nation as compared to developing nation 1 In India, about 1,45,000 new cases were diagnosed in 2012, with 70,000 deaths being due to this disease entity.[1] The HER-2/neu oncoprotein is composed of three domains: one of which is the extracellular domain (ECD).[2,3] It has been found that HER-2/neu ECD may be cleaved and shed from the surface of cancer cells and its levels can be detected by enzyme-linked immunosorbent assays (ELISAs) without any significant cross-reactivity with other members of the HER receptor family.[4] It has been found that serum levels of HER-2/neu are raised in 0% and 38% in patients of primary breast cancer,[5] while it is

elevated in 20% to 40% of patients with metastatic disease.[6,7]

It is associated with resistance to endocrine therapy and chemotherapy Lipton et al,[8] HER2 ECD has shown to be associated with sensitivity to HER-2 targeted agents, such as trastuzumab. A decrease in serum HER2/neu treated with trastuzumab was predictive of response to therapy in metastatic breast cancer patients. [9] The clinical significance of circulating HER2 ECD is uncertain and the relationship of baseline HER2 ECD levels with tissue HER2 overexpression in primary tumors has been little studied. [10]

Therefore, the current study has been under taken to evaluate the relationship between serum HER-2/ neu and various prognostic factors such as patient age, tumour size and tumour grade.

MATERIALS 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 was 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.

Histopatholog1cal 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 mm^2. 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 cytological 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

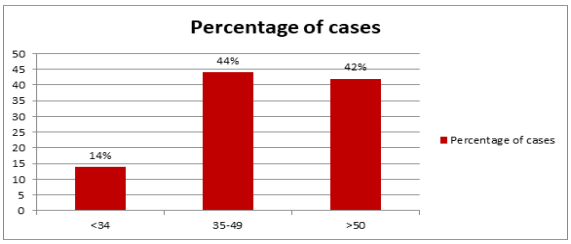

Figure 1: Distribution of cases according to age

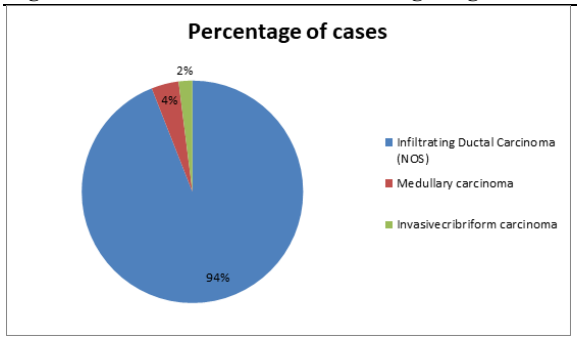

Figure 2: Distribution of cases according to histological types of cancer

Most cases 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 (7cases,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.

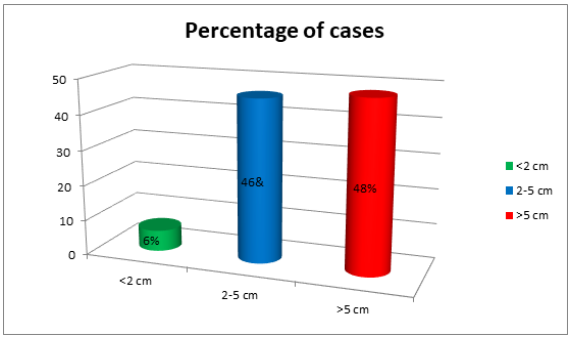

Figure 3: Distribution of cases according to tumour size

Table 1: 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 (8cases), pain in 12% cases (6 cases), nipple discharge and retraction in 8% cases (4 cases each), Paeu de orange in 6% cases (3 cases). 23 cases (46% cases) were in the premenopausal group while 27 cases (54% cases) were in the postmenopausal group. 94% cases (47 cases) were of the Infiltrating ductal carcinoma (NOS) type, followed by medullary carcinoma (2 cases, 4%) and a case of invasive cribriform carcinoma (1 case, 2%).

Table 2: Distribution of cases according to tumour size

Tumour size	Number	Percentage of cases
<2 cm	3	6.0%
2-5 cm	23	46.0%
>5 cm	24	48.0%
Total	50	100.0%

[Table 2] shows most of the cases in present study were in the range >5 cm (48%, 24 cases), followed by patient with tumour size 2- 5 cm (46%, 23 cases), least number of cases with size less than 2 cm (6%, 3 cases).

Table 3: Distribution of cases according to histological grade (Modified Scarff Bloom Richardson Grading scheme)

Histological Grade	Number of cases	Percentage
Grade 1	1	2.0%
Grade2	27	54.0%
Grade 3	22	44.0%
Total	50	100.0%

Table 4: Relationship of serum HER-2 /neu with tumour size

Tumour size	Number	Serum HER- 2/neu negative	Serum HER-2 /neu positive	P value
<2cm	3(100%)	2(66.7%)	1(33.3%)	0.038,S
2-5cm	23(100%)	15(65.2%)	8(34.8%)	
>5cm	24(100%)	7(29.2%)	17(70.8%)	
Total	50(100%)	24(48%)	26(52%)	

[Table 4] shows relationship of serum HER -2/neu with tumour size serum HER -2/neu positivity increased with increase in tumour size from 33.3% cases in patients with less than 2 cm tumour size, followed by 35 % cases in 2-5 cm size group and most patients with > 5 cm size with 70.8 % cases. Statistical analysis showed significant correlation between these variables. ($\chi 2 = 6.56$, P <0.05, S).

Table 5 Relationship of serum HER-2 /neu with histological grade

Histological grade	Number	Serum HER-2 neu negative	Serum HER-2 /neu positive	P value
Grade 1	1(100%)	1(100%)	0(0%)	0.026,S
Grade 2	27(100%)	17(63%)	10(37%)	
Grade 3	22(100%)	6(27.3%)	16(72.7%)	
Total	50(100%)	24(48%)	26(52%)	

[Table 5] shows relationship of serum HER-2/neu with histological grade. 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 The correlation was found to be statistically significant ($\chi 2 = 7.29$, P <0.05, S)

DISCUSSION

Majority of the patients of breast cancer presented with breast lump (98% cases,49 cases) followed by ulceration (16% cases,8 cases), pain (12%, 6 cases) nipple discharge (8%, 4 cases) nipple retraction (8%, 4 cases) and peau d' orange (6% cases, 3 cases).

In the present study, the majority of the patient were in the 35-50 age group constituting 44% of cases (22 cases). This finding was similar to the findings by Clemmensen et al (1979), who found most of the cases in the 35-50-year age group. Breast cancer is uncommon below age of 35 with increasing incidence between 35 yr to 50 yr. Bimodal trend has been found with dip in incidence at the time of menopause. a secondary rise at the age of 65, However we did not appreciate any dip near menopause.

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 study done by Liu et al (2014).[11] 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).

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 menstural status was found to be statistically non-significant (P =0.586, NS). Ludovini et al,[12] (2008) also found this relationship to be non-significant (P = 0.25, NS). Imoto et al. [13] (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.

Relationship of serum HER-2/neu with size: In the present study, majority of cases were with size more than 5 cm (48%, 24 cases), followed by patient with tumour size 2- 5 cm (46%, 23cases), least number of cases with size less than 2 cm (6%, 3cases). Serum positivity varied from 33.3% cases in patients with less than 2 cm tumour size, followed by 34.8 % cases in 2-5 cm size group and most patients with > 5 cm size with 70.8 % cases and this correlation was found to be statistically significant (P <0.05, S) and also in studies other studies. [13] However in studies done by Ludovini et al (2008). [12] (P = 0.61, NS) and Farzadnia et al (2010). (P = 0.862, NS) the correlation was non-significant. [14]

Relationship of serum HER-2 /neu with cytological grade of the tumour: We tried to find correlation of serum HER-2/neu with cytological grade. Cytological grading was done by Robinson cytological grading system. Serum positivity was more in cases of Grade 3 (76.2%), followed 40.9% in Grade 2 and 15.3% in grade 1and this relation was statistically significant (P value <0.05, S)

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). However, in studies done by Farzadnia et al (2010) the correlation was non-significant. (P = 0.076, NS). [12-14]

CONCLUSION

Histological grading was done by modified Scarff - Bloom- Richardson scheme and most cases were in the grade 2 (54%, 27 cases), followed by grade 3 (44%, 22 cases), and there was only one case (2%) in grade 1. There were 33.3% of serum HER-2/neu positive cases with tumour size < 2 cm as compared

to 34.8% in 2-5 cm tumour size and 70.8 % in tumour size > 5cm.The correlation between serum HER-2/neu and tumour size was found to significant (P <0.05, S). There were 72.7 % of serum HER-2/neu positive cases in grade 3 as compared to 37 % in grade 2 and no case in grade 1. The correlation between serum HER-2 /neu and histological grade was found to be significant. (P <0.05, S)

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