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ORIGINAL ARTICLE

Correlation of Serum HER-2/neu Levels with Clinico-Pathological Variables and Tissue Immunohistochemistry in Breast Carcinoma

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ABSTRACT

Introduction: Serum levels of HER-2/neu protein is raised in primary as well as metastatic breast cancer and decreased serum HER-2/neu concentration in patients treated with HER-2/neu inhibitors like trastuzumab is a good prognostic indicator in metastatic cases. **Aims:** To evaluate the concordance of serum HER-2/neu concentration with tissue HER-2/neu immunohistochemistry and determine its relationship with various prognostic indicators like age, size of tumour, lymph node status, grade, and stage of tumour. **Methods:** The preoperative serum samples of 56 clinically and cytologically diagnosed cases of breast carcinoma were taken and postoperative mastectomy specimens were examined for the histologic type, lympho-vascular invasion, stage, and grade of the tumour. Immunohistochemical study of HER-2/neu expression and HER-2/neu serum biochemistry was studied. **Results:** Age of the patients ranged from 25-75 years. Twenty-three cases (41.1%) were premenopausal while 33 cases (58.9%) postmenopausal. Forty-seven cases (83.9%) were of the infiltrating ductal carcinoma (NOS) type. Serum positivity for HER-2/neu was noted in 29 (51.8%) cases. Serum HER-2/neu levels showed a positive correlation with increase in tumour size, grade, stage of tumour and lymph node involvement. Serum HER-2/neu positivity was seen in 20 (68.9%) IHC positive patients and 9 (33.3%) IHC negative patients, with a statistically significant correlation (p=0.005). **Conclusion:** Serum HER-2/neu levels directly correlate with prognostic factors like tumour size, grade, stage of the tumour and lymph node metastasis and can be used as a complimentary tool to tissue HER-2/neu immunohistochemistry.

KEYWORDS: Carcinoma Breast, Her-2/neu, Serum, Immunohistochemistry

INTRODUCTION

The human epidermal growth factor receptor-2 proto-oncogene plays an important role in the pathogenesis of breast cancer [1]. It is found to be amplified in 15-30% of invasive breast cancers [2]. HER-2/neu over-expression is associated with short overall survival (OS) and disease-free survival (DFS) [3].

The extracellular domain (ECD) of HER-2/neu oncoprotein sheds off from the surface of cancer cells and its levels are detected by enzyme-linked immunosorbent assays (ELISAs)[3-5]. Serum levels of HER-2/neu are raised in 0-38% of primary breast cancer, and 20-40% in metastatic cases [6,7]. A decrease in serum concentration of HER-2/neu has been reported in metastatic breast cases on trastuzumab therapy [8].

The aims of our study were to evaluate the concordance of serum HER-2/neu positivity with HER-2/neu immunohistochemistry status and to determine the association between percentage of serum HER-2/neu positivity with various prognostic factors like patient's age, tumour size, lymph node status, grade and stage of tumour.

MATERIALS AND METHODS

Our study on 56 clinically and cytologically confirmed cases of breast carcinoma was performed in the Department Pathology, Jawaharlal Nehru Medical

College, Aligarh Muslim University, Aligarh after approval from the institutional ethics committee. The inclusion criteria were patients with preoperative cytologic diagnosis of breast carcinoma. The exclusion criteria were specimens of patients diagnosed as benign breast disease or malignant breast tumours other than breast carcinomas, malignant phylloides tumours and sarcomas.

Relevant clinical history, medical records and examination were evaluated and fine needle aspiration smears were thoroughly evaluated microscopically after staining with Haematoxylin & eosin (H&E) and Papanicolaou stain (Pap) stains. The preoperative serum samples of clinically and cytologically diagnosed cases of breast carcinoma were taken and postoperative mastectomy specimens were sent for histopathologic confirmation.

Hematoxylin and eosin-stained slides of the selected cases were examined for the histologic type, microscopic extent, lympho-vascular invasion, stage, and grade of the tumour. HER-2/neu expression on tissue section was evaluated by peroxidise-antiperoxidase based immunohistochemistry (IHC) by automated technique, Ventana using HER-2/neu antibody (clone SP3, Thermo Scientific, Fremont, CA, USA) and serum levels of HER-2/neu was measured by HRP-Conjugate ELISA (BioVendor – Laboratorní medicína) method and labelled as negative and positive using cut off value of 6.8 ng /ml [6].

A semi quantitative IHC scoring system of HER-2/neu immunoexpression, based on the intensity of tumour cell membrane staining and percentage of tumour cell positivity was employed [9]. IHC 3+ score were taken as HER-2/neu positive and IHC 1+ and IHC 2+ as HER-2/neu negative in our study. Statistical analysis was carried out using SPSS software (v.18.0). Chi-square test and Pearson bivariate correlation were used to evaluate the association between serum HER-2/neu levels and tissue HER-2/neu immunoexpression; and with various prognostic factors. p value of less than 0.05 was considered to be statistically significant. Pearson correlation coefficient(r) between 0.3 and 0.5 was considered as weak correlation, 0.5-0.7 as moderate correlation and 0.7-1.0 as strong correlation.

RESULTS

Out of the total 56 cases, 34 (60.7%) cases were below 50 years and 22 (39.3%) cases beyond 50 years of age. In our study, age of the patients ranged from 25 years to 75 years, with a median age of 45 years (Table 1).

Forty-nine (98.0%) cases presented with complaints of breast lump or mass, 8 (16.0%) cases with ulceration, 6 (12.0%) cases with mastalgia, 4 (8.0%) cases each with nipple discharge and retraction and 3 (6.0%) cases with peau-d orange. Twenty-three (41.1%) cases were in premenopausal group while 33 (58.9%) cases were postmenopausal (Table 1).

Forty-seven (83.9%) cases were infiltrating ductal carcinoma (NST) type, followed by infiltrating lobular carcinoma, 5 (8.9%) cases, carcinoma with medullary features, 3 (5.4%) cases and a single (1.8%) case of invasive cribriform carcinoma (Table 1).

According to Modified Scarff-Bloom-Richardson histopathological grading, 32 (57.2%) cases were in grade 1 and 2 while 24 (42.8%) cases in grade 3. Twenty-nine (51.8%) cases were less than 5 cm in size and 27 (48.2%) were more than 5 cm in size. Thirty-one (55.4%) cases showed positive lymph nodes while 25 (44.6%) cases did not show any foci of metastasis in the lymph node. As per the American Joint Committee on cancer staging, 32 (57.2%) cases were in stage 1 and 2 while 24 (42.8%) cases in stage 3 and 4 (Table 1).

There were 50% serum HER-2/neu positive cases in patients aged below 50 years as compared to 52.4% in patients beyond 50 years of age. The correlation between serum HER-2/neu and age of the patient was non-significant and weak ($p = 0.086$; $r=0.33$). Eighteen (55.6%) postmenopausal patients showed serum HER-2/neu positivity as compared to 11(47.8%) premenopausal patients, with a statistical non-significant and weak correlation ($p = 0.586$; $r=0.49$). Serum HER-2/neu positivity increased with histological grade. Eleven (37.0%) cases in grade 1 and 2 tumours while 17 (72.7%) cases in grade 3 showed positive serum HER-2/neu levels, with a statistically significant and strong correlation ($p =0.026$; $r=0.92$). Serum HER-2/neu levels showed a positive and strong correlation with increasing tumour size. Serum HER-2/neu positivity was seen in 34.5% cases with tumour

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size less than 5 cm that increased to 70.8% in tumour size beyond 5 cm (p=0.038; r=0.89) (Table 2).

Our study showed 20 (67.9%) cases of lymph node metastasis with positive serum HER-2/neu levels as compared to 8 (31.8%) cases with negative lymph nodes, with a statistically significant and strong correlation (p =0.01; r=0.78). Percentage of serum HER-2/neu positive cases increased with stage of the disease in our study. There were 12 (41.4%) serum HER-2/neu positive cases in early stage 1 and 2 while 17(69.6%) positive cases in stage 3 and 4, with a statistically significant and slightly strong correlation (p=0.04; r=0.71) (Table 2).

In our study, 29 (51.8%) cases showed serum positivity for HER-2/neu while 27 (48.2%) cases were serologically negative. We found positive serum HER-2/neu in 20 (68.9%) IHC positive and 9 (33.3%) IHC negative patients, with a statistically significant and strong correlation (p=0.005; r=0.83) (Table 3).

Table 1 Distribution of cases according to patient's characteristics: (n=56)

Patient's characteristics	Number of cases	Percentage (%)
Age:		
≤ 50 years	34	60.7
> 50 years	22	39.3
Presenting complaints:		
Breast lump	49	87.5
Ulcer	8	14.2
Pain	6	10.7
Nipple discharge	4	7.1
Nipple retraction	4	7.1
Dimpling of skin (Peau d' orange)	3	5.4
Menopausal status:		
Premenopausal	23	41.1
Postmenopausal	33	58.9
Histopathological type:		
Infiltrating ductal carcinoma	47	83.9
Infiltrating lobular carcinoma	5	8.9
Invasive carcinoma with medullary features	3	5.4
Invasive cribriform carcinoma	1	1.8
Histopathological grade:		
1 and 2	32	57.2
3	24	42.8
Tumour size:		
≤ 5 cm	29	51.8
>5 cm	27	48.2
Lymph node status:		
Negative	25	44.6
Positive	31	55.4
Tumour stage:		
1 and 2	32	57.2
3 and 4	24	42.8

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Table 2 Relationship of serum HER-2/neu with different prognostic factors: (n=56)

Prognostic factors	No of cases	Serum HER-2/ neu negative (<6.8 ng /ml)[6]	Serum HER-2/ neu positive (>6.8 ng /ml)[6]	Statistical correlation
Age				
≤ 50 Yrs	34	17(50%)	17(50%)	p=0.086
>50 Yrs	22	10(47.6%)	12(52.4%)	r=0.33
Menopausal status				
Premenopausal	23	12(52.2%)	11(47.8%)	p=0.586
Postmenopausal	33	15(44.4%)	18(55.6%)	r=0.49
Histopathological grade				
1 and 2	32	21(63%)	11(37%)	p=0.026
3	24	7(27.3%)	17(72.7%)	r=0.92
Tumour size				
≤ 5cm	29	19(65.5%)	10(34.5%)	p=0.038
>5cm	27	8(29.2%)	19(70.8%)	r=0.89
Lymph node status				
Negative	25	17(68.2%)	8(31.8%)	p=0.01
Positive	31	11(32.1%)	20 (67.9%)	r=0.78
Tumour stage				
1 and 2	32	20(58.6%)	12(41.4%)	p=0.04
3 and 4	24	7(30.4%)	17(69.6%)	r=0.71

Table 3 Relationship of serum HER-2/neu with tissue HER-2/neu status: (n=56)

Tissue HER- 2/ neu	No of cases	Serum HER-2 /neu negative (<6.8 ng /ml)[5]	Serum HER-2 /neu positive (>6.8 ng /ml)[5]	Statistical correlation
Negative (1+/2+)	27(100%)	18(66.7%)	9(33.3%)	p=0.005 r=0.83
Positive (3+)	29(100%)	9(31.1%)	20(68.9%)	
Total	**56(100%)**	**27(48.2%)**	**29(51.8%)**	

DISCUSSION

The present study on 56 patients of breast carcinoma was performed to ascertain the clinical utility of serum levels of HER-2/neu: an alternative to tissue HER-2/neu. It has been reported that about 15-30% of invasive breast cancers show HER-2/neu over-expression [2,9]. It is a useful prognostic marker to assess treatment response by overall survival (OS) and disease-free survival (DFS) [2,10-13].

Hanna et al. suggested the prognostic significance of serum HER-2/neu as well as tissue HER-2/neu over-expression [7]. Serum levels of HER-2/neu can be detected by a dynamic, quantitative ELISA test and can be performed when primary tumour sample is not available [14,15]. Twenty-nine (51.8%) breast cancer cases in our study showed positive levels of serum HER-2/neu and 48.2% cases were serologically negative with a cut off of 6.8ng/ml [6]. However, Hanna et al, Reix et al and Tchou et al have reported 9.5%,

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18.0% and 31.0% serum HER-2/neu positive breast cancer cases respectively [7,8,16].

The majority of our patients (60.7%) were below 50 years of age. Dong and Chung have also reported preponderance of patients below 50 years of age [17]. In our study, 50% cases below 50-year age group were serum HER-2/neu positive as compared to 52.4% beyond 50 years of age, with no significant statistical correlation, which suggests that serum HER-2/neu positivity is not related to age of the patient. Lam et al. also found no statistical correlation in serum positivity in younger patients less than 34 years as compared to beyond 50 years of age [18].

In our study 55.6% of postmenopausal patients showed serum HER-2/neu positivity as compared to 47.8% of premenopausal, with a statistical non-significant and weak correlation ($p = 0.586$; $r=0.49$). Lee et al. also found this relationship to be non-significant ($p = 0.25$) [19]. We can infer that menopausal status is not related with serum HER-2/neu levels.

Serum HER-2/neu positivity increased with histological grade, with 11 (37.0%) positive cases in grade 1 and 2 tumour as compared to 17 (72.7%) serum HER-2/neu positive cases in grade 3, which showed a statistically significant and strong correlation ($p=0.026$; $r=0.92$), a finding consistent with Lam et al. and Lee et al [18,19]. However, Farzadnia et al. have stated no statistical significance between serum HER-2/neu positive cases and histological grade of tumour ($p = 0.076$) [20].

Serum HER-2/neu positivity in our study was seen in 34.5% patients with tumour size less than 5 cm, which increased to 70.8% in patients with tumour size more than 5 cm, a finding concordant to studies by Lam et al [18]. It can be postulated that serum HER-2/neu levels was associated with size of the tumour.

Our study showed 20 cases (67.9%) of lymph node metastasis with positive serum HER-2/neu levels, a finding concordant to reports by Lam et al. and Lee et al [18,19]. However, Farzadnia et al. found no significant correlation between lymph node status and positive serum HER-2/neu levels ($p =0.297$) [20]. Serum HER-2/neu level is elevated in advanced disease as compared to the primary breast carcinoma with a positive correlation to tissue over-expression [21,22].

Percentage of serum HER-2/neu positive cases increased with stage of the disease in our study. There were 41.4% positive cases in early stage 1 and 2 disease and 69.6% in stage 3 and 4 disease, with a statistically significant and slightly strong correlation ($p=0.04$; $r=0.71$). This points towards an assertion that the percentage of serum HER-2/neu positive cases increases with stage of the disease. Our findings were similar to Lam et al. and Lee et al., but discordant with the study by Farzadnia et al., who reported no statistical significance between serum HER-2/neu positivity and stage ($p =0.865$) [18-20].

We found positive serum HER-2/neu in 68.9% of tissue IHC positive patients and 33.3% of IHC negative patients, with a statistically significant and strong correlation ($p=0.005$; $r=0.83$). Lee et al. have reported 87.1% cases with increased HER-2/neu serum levels in 38.1% tissue IHC positive breast cancer cases while only 3.3% in IHC negative cases [19]. Eric et al. have reported a statistical significant correlation but Alicezah et al. did not find any association between abnormal serum HER-2/neu levels and IHC expression [21,22]. The reason for this variability in the results could be the different number of subjects with varied characteristics in their study and the use of different techniques with different cut off values.

CONCLUSION

Serum HER-2/neu levels has a positive correlation with size, grade, stage of tumour and lymph node metastasis and can be used as a complimentary bedside method to tissue immunohistochemistry.

Conflict of Interest

Authors declare none

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Authors Contributions

IB collected the data, SSA performed the research, KA wrote the paper, and TM did the clinical inputs.

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