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HER-2/neu OVER EXPRESSION; IMMUNOHISTOCHEMICAL DETERMINATION IN INVASIVE LOBULAR CARCINOMA OF BREAST

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ABSTRACT

O**BJECTIVE:** To determine the frequency of HER-2/neu over expression in invasive lobular carcinoma (ILC) of breast & correlate it with various standard prognostic parameters. **DESIGN:** This study was performed on formalin fixed paraffin embedded blocks of 18 diagnosed cases of invasive carcinoma of breast with classic lobular pattern. **PLACE:** Department of Pathology, Basic Medical Science Institute, Jinnah Postgraduate Medical Center, Karachi, Pakistan. **METHODS:** The paraffin block were retrieved and 5 μ m thick sections were cut and stained with H&E for the review of diagnosis and grading. The immunohistochemical staining was done on 4 μ m thick sections by using polyclonal rabbit anti HER-2 ZYMED USA, and ZYMED 2nd generation LAB-SA immunodetection system, to see the HER-2/neu over-expression. **RESULTS:** Sections containing > 50% of tumor cells exhibiting intense circumferential cell membrane staining were scored as positive. The HER-2/neu over expression was seen in 22% of cases. **CONCLUSION:** The results in this study are statistically insignificant but positive cases can get benefit from Herceptin therapy.

KEYWORD: HER-2/neu, immunohistochemistry, invasive lobular carcinoma.

INTRODUCTION

Invasive lobular carcinoma (ILC) of breast accounts for 0.7-15% of all invasive malignancies of breast^{1,2,3,4}. A palpable mass is seen in majority of cases but some tumors are hard to detect even on mammography because of the diffuse growth pattern of cellular infiltrate. Since tumor cells tend to infiltrate beyond the

palpable extent of the tumor, resection margins are more frequently tumor positive⁴ and the recurrence rate is higher than the invasive ductal carcinoma (IDC)^{5,6}. These tumors are usually multifocal, multicentric & bilateral^{7,8,9}.

The ILC has a low frequency of nodal metastasis than IDC^{3,4}. Various workers have reported that ILC has

more favorable outcome and better prognosis than IDC^{4,10}. However the variants have less favorable prognosis^{11,12}. Several studies have found no significant difference in survival of patients in ILC & IDC^{3,13}, and some studies have found worse prognosis for ILC than IDC¹⁴.

Nonetheless, it remains a challenge to predict, who are at greatest risk of poor prognosis. The discovery of the role-played by oncogenes in the genesis and progression of breast carcinoma has opened opportunities to explore their possible role as predictors of tumor behavior. Accumulating evidence indicates that abnormalities in expression of HER-2/neu gene are common in breast cancer and may be pathogenically significant^{15,16}.

The importance of HER-2/neu over-expression in the malignant process was inferred from discoveries of HER-2/neu gene amplification in some cases of human breast cancer¹⁷. The HER-2/neu oncogene has been the subject of heated debates during last decade concerning its prognostic significance for women with breast cancer¹⁸. But now HER-2/neu is thought to be an independent prognostic factor in patients with breast carcinoma¹⁹ and is predictor of shortened disease free survival, overall survival & poor clinical out-come in patients with breast carcinoma^{20,21}. More recent studies show that HER-2/neu over-expression has therapeutic implications in infiltrating breast carcinoma. The United States Food & Drug Administration has recently approved Herceptin (Trastuzumab) a monoclonal antibody against HER-2/neu which has therapeutic efficacy in HER-2/neu over expressing tumors.¹⁸

There is great discrepancy in the results of various studies regarding HER-2/neu positive over-expression in invasive lobular carcinoma of breast. Majority of studies have not found any positive over-expression²²⁻²⁵ & some have found positive over expression of HER-2/neu in ILC^{26,27}, keeping in view of all the above facts, the present study was designed to see the frequency of HER-2/neu over-expression in classic cases of invasive lobular carcinoma of breast and to correlate the positive index, with the age of patients at the time of diagnosis, nodal status, grade and size of tumor.

MATERIAL & METHODS

The present study was performed at the department of

pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan on formalin fixed paraffin embedded blocks of 18 diagnosed cases of invasive carcinoma of breast with classic lobular pattern.

5µm thick sections were cut and stained with H & E for the review of diagnosis. Grading was done by Nottingham Modification of the Bloom Richardson System.

IMMUNOHISTOCHEMICAL STAINING

Polyclonal Rabbit anti HER-2 ZYMED USA and ZYMED 2nd generation LAB-SA immunodetection system was used.

Sections were prepared for immunohistochemical staining of 4 µm thickness from representative paraffin embedded blocks. Poly L-lysine coated slides were used. The sections were allowed to dry in an oven at 56-60°C for one hour.

STEPS

Deparaffinization

Two changes of xylene for 5 minutes each.

Rehydration

Two changes each for 3 minutes in decreasing concentration of alcohol starting from absolute to 70%, rinsing with tap water at the end.

Antigen retrieval

Heat induced epitope retrieval (HIER) method was used. Slides were left in phosphate buffer saline (PBS) for 30 minutes at room temperature.

Serum blocking agent

Applied over the encircled area, kept for 10 minutes, and then solution was drained off.

Primary antibody

Primary antibody was applied and left for 60 minutes. Slides were rinsed with PBS buffer.

Secondary antibody

Biotinylated secondary antibody applied and left for 20 minutes and slides were rinsed with PBS buffer.

Enzyme conjugate

Application of streptavidin alkaline phosphatase for 20 minutes. Slides were drained and rinsed with PBS buffer.

Substrate chromogen solution (Fast red)

Applied over the section and left for 10 minutes. Slides rinsed with PB buffer.

Counter stain

Mayer’s hematoxylin was used for counter stain.

MOUNTING

Section were mounted with Faramount aqueous base mounting medium.

INTERPRETATION OF RESULTS

The tumor was interpreted as positive for overexpression of HER-2/neu protein product when > 50% of the tumor cells gave circumferential intense fast red membrane staining, which was identified with a 10 x and confirmed with 40 x objectives. Cytoplasmic staining without membranous staining was considered as negative for HER-2/neu overexpression.

STATISTICAL ANALYSIS

The computer package “Microsoft Excel” was used for data feeding and “EPI-INFO” was used for statistical analysis. The results were given in the text as number and percentage for qualitative variables. To compare proportion / percentage between groups by Chi-square test. In all statistical analysis, only p-values <0.05 are considered significant.

RESULTS

In this study, 18 cases of classic invasive lobular carcinoma were subjected to immunohistochemical staining for HER-2/neu over-expression.

Out of these, 4 (22%) exhibited intense circumferential cell membrane staining with additional focal cytoplasmic staining in > 50% of tumor cells and these cases scored as positive for HER-2/neu over-expression (Fig-1).

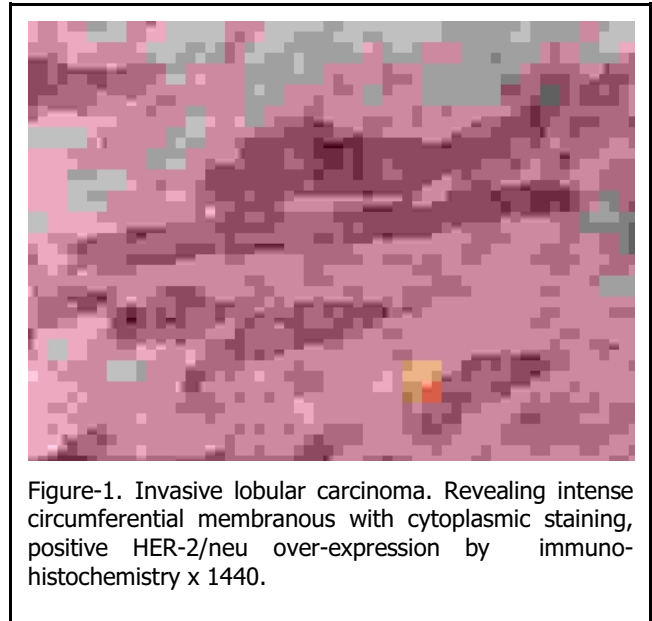


Table-I. HER-2 Over-expression compared to lymph node status

Lymph node status	No of patients	HER-2 positive over-expression	%age
-	5	-	-
1-3	7	3	43
>3	6	1	17

Compared lymph node status P value was >0.05 (insignificant)

The various prognostic factors including lymph node status, grade, size of tumor and age of patient were evaluated (table I-IV) but none of them achieved statistical significance.

Table-II. HER-2 Over-expression compared to grade of tumor

Circle	No of patients	HER-2 positive over-expression	%age
I	7	1	14
II	6	1	17
III	5	2	40

Comparing grade tumor P value was >0.05 (insignificant)

Table-III. HER-2 Over-expression compared to size of tumor

Size (cm)	No of patients	HER-2 positive over-expression	%age
≤ 2.0	5	1	20
2.1 - 5.0	5	1	20
≥ 5.1	8	2	25

Comparing size tumor P value was >0.05 (insignificant)

Table-IV. HER-2 Over-expression compared to age of patient

Age (yrs)	No of patients	HER-2 positive over-expression	%age
≤35	1	-	-
36 - 50	11	2	18
≥51	6	2	33

Comparing age patient, P value was >0.05 (insignificant)

DISCUSSION

Compared to the invasive ductal carcinoma a vary little work has been done on invasive lobular carcinoma of breast. Most of the researchers were unable to find any positive over expression in ILC²²⁻²⁵

In this study, we found 22% positive over expression of HER-2/neu, confirming the results of those previous studies which found the positive over-expression of HER-2/neu oncoprotein^{26,27}.

CONCLUSION

Although, we could not find any statistically significant result, but the data of this study shows that by the help of HER-2/neu oncoprotein as a marker high risk patients can be detected.

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