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Original Article

THE ASSOCIATION OF HER-2 OVER-EXPRESSION IN RELATION TO HORMONAL RECEPTOR STATUS AND COMMON CLINICO-PATHOLOGICAL PARAMETERS IN BREAST CARCINOMA

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Abstract

Objective: To determine the association of Human Epidermal growth factor Receptor2 (HER-2) with hormonal receptor status and common clinico-pathological parameters of breast carcinoma.

Study Design: Cross-sectional, observational study.

Place and Duration of Study: Department of Surgery, Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh during the period from January 2009 to December 2009.

Methodology: A total of 100 patients of female breast carcinoma with HER-2 status, hormonal receptor profile and clinico-pathological parameters were included in this study. Patient's age, menopausal status, tumour size, lymph node status, Estrogen Receptor (ER) and Progesterone Receptor (PR) status were evaluated and their association was determined with HER-2 over expression using the chi-square test for analysis.

Results: Of these 100 cases, mean age of diagnosis was 48 years and mean tumour size 4.4 cm. Lymph node metastases were present in 50% cases. HER-2 over-expression was seen in 29% cases, while ER and PR expression was seen in 61% and 43% respectively. ER and PR showed inverse association ($p < 0.05$) with HER-2 while positive association was seen with tumour size and lymph node metastases ($p < 0.05$). No association was seen with menopausal status. Further, among 61 ER positive cases, 4 cases also HER-2 over expressed.

Conclusion: The study had indicated that Her-2 may be a powerful predictor of poor prognosis as its over-expression was strongly directly associated with tumor size and lymph node involvement and inversely associated with hormonal receptor status of breast carcinoma. ER positive cases can also be HER-2 over expressed (4%), so ER status cannot be used to select tumours for evaluation of HER-2 status. As treatment modalities of ER positive and HER-2 over expressed is different, so all the patients should be studied with both receptor and treat accordingly.

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Introduction

Among women, breast cancer is the most common cause of cancer-related death worldwide. Over 411000 deaths result from breast cancer annually, accounting for over 1.6% of female deaths from all causes. The common misconception that breast cancer is predominantly a problem of wealthy countries. The

majority of breast cancer deaths in fact occur each year in developing rather than developed countries.[1] A study in Dhaka Medical College hospital, is estimated that the incidence of breast cancer is about 17% among all cancers.[2] The annual report of National Institute of Cancer Research and Hospital (NICRH) has shown that the frequency of breast carcinoma is 12.7% in all cases of malignancy and 39.7% in malignancy affecting female.[3]

The discovery of specific monoclonal antibodies has greatly facilitated the identification of cell products or surface marker by immunohistochemistry. Immunohistochemical detection of hormone receptors (estrogen receptor-ER, progesterone receptor-PR) in breast cancer cells is of therapeutic and prognostic value. Protein products of oncogenes such as Human Epidermal growth factor Receptor-2 (HER2) in breast cancers can also be detected by immunostaining or by Fluorescence in Situ Hybridization (FISH).[4]

The efficacy of hormonal treatment (Tamoxifen) as an adjuvant therapy was first reported in 1983. It has now been shown to reduce the annual rate of recurrence by 25%, with a 17% reduction in the annual rate of death. The Tamoxifen also reduce the risks of tumors in the contra lateral breast as a preventive agent. Oral aromatase inhibitors are now licensed for treatment of recurrent disease and effective in post menopausal women. In which cases they have been shown to be superior to tamoxifen.[5] 50% to 80% breast carcinomas express ER.[6]

HER-2 over expression occurs in 20-30% of breast carcinoma.[7] This oncogene, also referred as c-erbB-2 encodes a protein with a molecular weight of 185 KD that is a trans membrane tyrosine kinase receptor belonging to the epidermal growth factor receptor family.[8] This may be partially responsible for cellular progression of neoplastic phenotype. Moreover, HER-2 may potentiate tumorgenesis by inducing tumor cell resistance to host defense mechanisms.[9] It induces resistance to tumor necrosis factor (TNF), which causes cancer cells to escape from host immune defenses[10] and appears to result in reduced sensitivity to immune effectors killing. [7,11]

An inverse relationship has been described between HER-2 over-expression (membrane staining is score 3+)[12] and the presence of estrogen receptor and progesterone receptor status in breast carcinoma.[13,14] Not all reports agree on this negative correlation.[15] HER-2 over expression is reported to be associated with a poor prognosis in breast carcinoma patients[8,16] and in some studies associated with poor response to anti-estrogen therapy. These patients are less likely to benefit from cyclophosphamide, methotrexate, fluorouracil (CMF) based chemotherapy compared with anthracycline-based chemotherapy. [8,17]

For patients in whom amplified HER-2 can be detected and is over expressed, Trastuzumab (Herceptin) is the first new 'biological therapy' to enter clinical use. This therapy may be most effective in node positive patients with invasive breast cancer whose disease is relatively insensitive to chemotherapy or endocrine therapy.[18] Other agents currently available include Bevacizumab, a vascular growth factor receptor inhibitor and Lapitinab, a combined growth factor receptor inhibitor.[5]

Assessment of HER-2 receptor status with ER, PR status is essential for selection of accurate therapy. Only hormone receptor positive cases can be benefited by Tamoxifen. HER-2 receptor may be a marker of Tamoxifen resistance. If Tamoxifen is given in both hormone receptor and HER-2 receptor positive cases, then Tamoxifen may stimulate the tumor cell growth rather than regression.[15, 19]

Besides several prognostic factors like tumor size, histological grade, steroid hormone receptor status and lymph node status which are significant in the management of breast cancer; HER-2 status might also serve as an additional parameter.

Receptor status has definite role on prognosis of breast cancer. Receptor status can change conventional treatment plan to more acceptable and better prognostic hormonal therapy and biological targeted therapy. So, this should be done on routine practice in all breast carcinoma specimens. The aim of this study is to determine the association of HER-2 over expression in relation to estrogen and progesterone receptor status as well as conventional common clinico-pathological parameters of breast carcinoma.

Materials & Methods

This is an observational cross-sectional study. The study was carried out in the Department of Surgery, SSMC Mitford Hospital, Dhaka during the period from January 2009 to December 2009.

Study population were female patients with breast carcinoma diagnosed by FNAC, admitted and operated in Sir Salimullah Medical College Mitford hospital and other hospitals and clinics in Dhaka city

during the mentioned period. 100 patients of breast carcinoma which was selected conveniently.

Included cases were female patients with cytologically diagnosed malignant breast lump. Excluded cases were patients with benign breast lump and patients who have already received chemotherapy or radiotherapy.

Informed written consent was taken from each patient and their privacy and confidentiality was maintained. Each admitted patient with cytologically malignant breast lump was evaluated by taking history, physical examination and investigations. Patients were operated under general anaesthesia. Operation note including procedure and all findings were recorded in prescribed data collection sheet. The resected specimens were preserved in 10% buffered neutral formaldehyde solution (40% formaldehyde-100ml, Distilled water-900ml, Sodium dihydrogen phosphate monohydrate-4gm, Disodium hydrogen phosphate anhydrous- 6.5gm [20]) at room temperature not more than 24-48 hours in a suitable container previously labeled.[7,19]

Tumor tissues as well as dissected lymph nodes were examined macroscopically and microscopically. Histology were assessed for tumor subtypes, invasiveness, histological grades and lymph node status. Carcinoma was classified on the basis of the H & E stained slides.

Immunohistochemical staining:

The status of HER-2, estrogen and progesterone receptors will be determined by immunohistochemistry on paraffin-embedded sections 5 µm thick. Immunostaining will be performed with Supersensitive polymer HRP IHC detection system-Biogenex. Antibodies used – RabMAbs SP1, Labvision; RabMAbs SP2, Labvision for hormone receptors and C-er-B2-CB11, Novacastra for HER-2 receptors. Sections will be scored semi-quantitatively. Immunohistochemistry of all cases are done from Metropolis Health Services (India) Ltd, Mumbai, India with the collaboration of Roche Bangladesh Limited.

For estrogen and progesterone receptors 'Allred score' is applied. First, an intensity score is assigned, which represents the average intensity of positive tumor cells (score 0= none; score 1= weak; score 2= moderate & score 3= strong). Next a proportion score is assigned, which represents the estimated proportion of positive stained tumor cells (score 0= none; score 1=1%; score 2= 1-10%; score 3 = 10-30%; score 4 = 30- 60% & score 5= 60-100%). Then both scores are added to obtain total score, which range from 0 to 8. Score 0 to 2 is negative; Score 3 and above is positive. [21, 22]

For HER-2 over expression score 0= no staining or membrane staining in less than 10% of cells. Score 1+ = a faint/ barely perceptible membrane staining in more than 10% of cells. Cells are only stained in part of their membrane. Score 2+ =weak to moderate complete membrane staining in more than 10% cells. Score 3+ = strong complete membrane staining in more than 10% of cells. Score 0 and 1+ is negative, score 2+ is weakly positive, score 3+ is strongly positive (over expressed). [12]

Data management and analysis:

Relevant informations of all cases were recorded in pre designed data collection sheet. The HER-2 over-expression in breast cancer tissue was studied statistically in relation to prognostic factors including: estrogen and progesterone receptor status as well as conventional common clinico-pathologic parameters. To evaluate the statistical significant in the present study, Chi-square test was applied; in the present study a p-value of <0.05 was considered significant. Statistical analysis was performed using SPSS software, version-16. Findings were focused with the tables.

Results

The 100 cases included in the present study showed an age incidence which ranged from 27ys-75ys. Thirty eight cases (38%) were pre-menopausal and the other sixty two cases (62%) were post-menopausal. Thirty two cases (32%) had a tumor size <2cm, forty six cases (46%) had a tumor size 2-5 cm, while twenty two case (22%) had a tumor size >5cm. Fifty cases (50%) presented with lymph node metastasis.

The study finding reveals that the mean age is 44.72 years and standard deviation is ±11.3. The highest numbers of patients 65% are age group 30 -49 years. In this study, more women of breast carcinoma are late child bearing and around menopausal age group.

This study shows that highest number of the patients 81% conceived two to three times. It also revealed that 15% conceived four times or more. In this study, more women of breast carcinoma are multipera.

In this study, both lymph node positive and lymph node negative cases are in same proportion.

29% of breast carcinoma cases have shown HER-2 over expression where as 71% of cases has not shown HER-2 over expression. 61 of breast carcinoma cases are ER positive where as 39 of cases are ER negative on immunostaining. 43 of breast carcinoma cases are PR positive where as 57 of cases are PR negative on immunostaining.

Her-2 over-expression in relation to ER status:

ER was positive in sixty one cases (61%) and among them fifty seven cases (57%) did not show HER-2 over expression. On the other side thirty nine cases (39%) were ER negative, out of them twenty five cases (25%) were HER-2 over expressed. Statistically there is a highly significant inverse association between HER-2 over expression and ER positive immunostaining.

Among 61 ER positive cases, 4 cases also HER-2 overexpressed. So, ER status cannot be used to select tumours for evaluation of HER-2 status, and ER and PR positivity does not preclude a positive HER-2 status.

Her-2 over-expression in relation to PR status:

As regard the PR immune-staining 43 cases (43%) were PR positive, among them forty one cases (41%) did not show HER-2 over expression. Statistically there was also significant association between HER-2 over expression and PR negative immunostaining. So, there is also inverse relation between HER 2 over expression and progesterone receptor status.

HER-2 over-expression in relation to Tumor size:

Most of the HER-2 over expressed cases had a tumor diameter >5 cm. Statistically there is a significant association between HER-2 over expression and the increased size of tumor.

HER-2 over-expression in relation to Lymph nodes status:

Most of the HER-2 over expression cases had lymph node invasion. Statistically there is a significant association between HER-2 over expression and the lymph node invasion. so, there is direct relation between HER 2 over expression and lymph node invasion.

HER-2 over-expression in relation to Menopausal status:

There is no association between HER-2 over expression and Menopausal status. Most of the ER positive cases are post menopausal. There is a significant association between ER positive and menopausal status.

Table-I

Clinical and pathological criteria of patients

Clinicopathological variable	Frequency	Percentage
Menopausal status		
Pre menopausal	38	38
Postmenopausal	62	62
Tumor size		
<2cm	32	32
2-5cm	46	46
>5cm	22	22
Stage of tumour		
Stage I	31	31
Stage II	29	29
Stage III	40	40
Lymph node status		
Positive	50	50
Negative	50	50
Estrogen receptor status		
ER +ve	61	61
ER –ve	39	39
Progesterone receptor status		
PR +ve	43	43
PR –ve	57	57
HER-2 receptor status		
HER-2 overexpressed	29	29
HER-2 not ovrexpresed	71	71

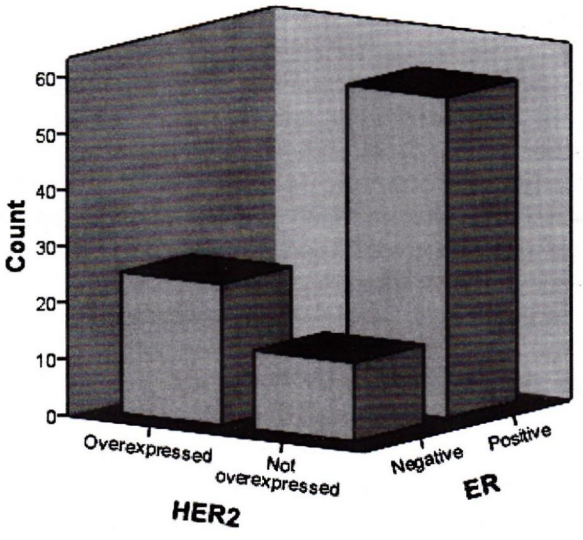

Fig.-1: *Distribution of patients accordingly to HER-2 and ER status.*

Table 12

HER-2 overexpression in relation to Hormonal receptor status, tumour size, lymph nodes and menopausal status

Variable	HER2 overex		HER2 no overx		Total		Chi-square	p-value
	n	%	n	%	n	%		
1. Estrogen recep.								
ER+ve	4	4	57	57	61	61		
ER-ve	25	25	14	14	39	39	38.261	0.01
Total	29	29	71	71	100	100		
2. Progesterone recep.								
PR+ve	2	2	41	41	43	43		
PR-ve	27	27	30	30	57	57	21.722	0.01
Total	29	29	71	71	100	100		
3. Tumour size								
<2cm	0	0	32	32	32	32		
2-5cm	13	13	33	33	46	46	33.513	0.01
>5cm	16	16	6	6	22	22		
Total	29	29	71	71	100	100		
4. Lymph node								
+ve	26	26	24	24	50	50		
-ve	3	3	47	47	50	50	25.692	0.01
Total	29	29	71	71	100	100		
5. Menopause								
Pre	12	12	26	26	38	38		
Post	17	17	45	45	62	62	0.198	>0.5
Total	29	29	71	71	100	100		

Discussion

Breast carcinoma is the commonest malignancy among women of Bangladesh. The aggressive biological behavior of invasive and metastatic cancer is considered to be the most insidious and life threatening aspect for breast cancer patients. It is mostly the result of changes in many molecular characteristics of tumor cells.[23] The biology of breast cancer remains poorly understood as knowledge about individual prognostic factors provides limited information.[24] A wide variety of morphology-based and molecular-based breast cancer prognostic factors and tumor markers have been studied. An expanded understanding of the biology of breast cancer has led to the identification of the HER-2 receptor as an important growth factor.[16] Clinical studies have demonstrated that overexpressions in HER-2 predict poor prognosis for breast cancer. These are associated with features of tumor aggressiveness, such as absence of estrogen and progesterone receptors, high rate of cellular proliferation, advanced tumor stage, large tumor size, and young age at diagnosis.[25]

The prognostic variables provide substantial information, which is useful in guiding the oncologist in determining the choice of treatment for the individual patient. At present, it is agreed that the axillary lymph node metastases, tumour size and grade, are morphological prognostic markers which determine long-term survival. Moreover, ER and PR status help in decision making about anti-estrogen drug tamoxifen and aromatase inhibitors. [26]

The mean age at diagnosis in this study was 44.72 years and the highest number of cases was seen in the 5th decade. This presentation is similar to earlier local studies by Quddus.[27] Arryandono et al. from Indonesia have shown the mean age at diagnosis at 47 years,[28] while Sharif et al from Pakistan shown the mean age at 48 years.[26] Wahid et al. showed more than 50% cases and Sharif et al. showed more than 66.5% cases below 50 years of age, [26,29] while this study showed 73% cases below 50 years in age.

In the present study, mean tumour size was 3.25 cm and 22% cases had a tumour size larger than 5 cm, while only 32% cases had tumour size less than 2 cm. Local studies showed a tumour size of 2-5 cm in majority of cases.[27] In Muslim countries like Indonesia and Jordan, the mean tumour size reported between 2-5 cm by Arryandono et al. and 5 cm by Almasri et al. respectively.[28,30] Larger tumour size and higher number of lymph node metastases is seen in Bangladesh. Conservative nature of our society and lack of awareness play a role in the advanced stage of presentation at the time of diagnosis.

HER-2 overexpression is seen in 5%-35% of breast carcinomas. Treatment with specific monoclonal antibody trastuzumab is offered for HER-2 overexpressed cases. Potent inhibitory activity of the antibody trastuzumab against the tumour cells increases the sensitivity of these cells to undergo apoptosis.[31] HER-2 over expression of 29% was seen in this study, which is comparable in frequency to HER-2 overexpression seen 29% in India by Gennari et al,[32] 31% in Pakistan by Sharif et al [26] and also 31% in West by Lal et al [33]. Study in this subcontinent and also from West is almost similar to our findings.

Significant association was seen between large tumour size and HER-2 over expression in this study. Other studies by Lal et al., Almasri et al., Haung et al. and Naqvi et al. showed a positive association between tumour size and HER-2 over expression. [7,30,33,34,35] But Sharif et al. was found No association between tumour size and HER-2 over expression. [26]

50% cases show lymph node metastasis in this study. Local studies by Quddus reported 64% cases with lymphnode metastasis.[27] In comparison, Malik et al. had reported 74% cases with lymph node metastasis in their study.[36] Arryandono et al, Naqvi et al, Ray et al, Climent et al, Traina et al [28,35,37,38,39] and this study demonstrated positive association between HER-2 over expression and lymph node metastasis while no association could be established by Lal et al, Almasri et al and Haung et al. [30,33,34]

ER positivity of 61% was seen in this study. In Pakistan Sharif et al reported ER positivity of 72.3% and Malik et al reported ER expression of 86%.[26,36] Western studies have projected an ER expression between 70-80% in breast carcinoma. An inverse association between ER with HER-2 has been observed in this and other studies. [7,30,33,34] In this study ER expression is less than other study. This may be due to our limitation of small sample size which may be overcome by population based study.

PR expression was seen 43% in this study, while Rashed et al. have PR expression of 42% in Egypt and Fatima et al. have PR expression of 35% in Pakistan. [7,40] Western studies have demonstrated a PR expression of 60-70%.[41] Inverse association has been seen with PR and HER-2 over expression in this study and other studies.[7,30-35,42,43] However, Arryandono et al. from Indonesia and Al-Ahwal et al. from Saudi Arabia did not show any association between HER-2 and PR.[28,44] Pattern of progesterone receptor expression is different in this study from study of west. It is almost similar to study in Pakistan and Egypt. This may be due to different pattern of expression in this area, which may demand further study.

There was no significant association between Her-2 over expression and menopausal status. Same finding was reported in Rashed et al study.[7] In Ray et al study; HER-2 over expression was significantly higher among post-menopausal in comparison with pre-menopausal women.[37]

This study reveals the inverse association of ER & PR with HER-2 over expression. Although ER & PR expression was significantly decreased in HER-2 over expression of tumour but a substantial proportion of ER positive & PR positive cases also over expressed HER-2. This makes it mandatory to perform hormone receptors study and HER-2 study in all the cases.

There was a significant association between HER-2 over-expression and the increased size of tumor. There was also a significant association between HER-2 over-expression with the lymph node invasion. On the other hand HER-2 over-expression showed an inverse significant association with ER and PR status. Meanwhile there wasn't any significant association of HER-2 over-expression with the menopausal status. Further, ER status cannot be used to select tumours for evaluation of HER-2 status.

In the present study HER-2 over expression is in 29% cases and ER positive in 69% cases. Both receptors positive is in 4% cases. Protocol for ER positive patient is Tamoxifen or aromatase inhibitor. Who are both receptors positive, tamoxifen may not response them. So these patients should avoid tamoxifen and receive monoclonal antibody or even anthracycline based chemotherapy.

In our country more patients are present in advanced stage with large tumour size. These patients are likely to be HER-2 over expressed. Special attention should be paid for this HER-2 over expressed patients.

In conclusion this study indicated that Her-2 may be a powerful predictor of poor prognosis. It's over expression was strongly associated with absence of hormonal receptor positivity and unfavorable clinico-pathological parameters like tumor size and lymph node involvement of breast carcinoma. As 4% of the patients have both ER positive and HER-2 over expressed, so ER status cannot be used to select tumours for evaluation of HER-2 status. In our routine practice we use Tamoxifen for carcinoma breast patients, as we know the majority of breast carcinoma patients are ER positive. But in our study we found that ER positive is 69% cases, HER-2 over expressed is 29% cases and in 4% cases are both ER positive & HER-2 over expressed. If we use Tamoxifen in case of HER-2 over expressed patient, then it may do no benefit rather harm to the patient. We should not use Tamoxifen randomly for all patients and we should not use Tamoxifen to the entire patients who have ER positive. As 4% of the patients have both ER positive and HER-2 over expressed, so for the management of breast carcinoma we should go also for HER-2 study in our patients. Otherwise we can mistake for the management of these patients.

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