ORIGINAL ARTICLE

Morphological Profile and Association of HER-2/Neu with Prognostic Markers in Breast Carcinoma in Northern Pakistan

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ABSTRACT

Objective: To determine the morphological profile and association of HER-2/neu expression with histological prognostic markers and hormonal receptor expression in female breast carcinoma in Northern Pakistan.

Study Design: Cross-sectional, observational study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from January 2004 to May 2007.

Methodology: A total of 535 patients of primary operable female breast carcinoma with hormonal profile and biological status were included in the study. Patient's age, microscopic tumour size, tumour grade, lymph node status, Estrogen Receptor (ER), Progesterone Receptor (PR) and joint ER/PR status were evaluated and their association was determined with HER-2/neu expression using the x^2 test for univariate analysis.

Results: Out of 535 cases, there were 481 (89.9%) cases of infiltrating ductal carcinoma with mean age of 48 years and mean tumour size of 4.4 cm. Tumour grade II was seen in 68% cases and lymph node metastases were present in 65% cases. HER-2/neu expression was seen in 31% cases, while ER and PR expression was seen in 72.3% and 62.6% respectively. ER and PR showed inverse association with HER-2/neu while positive association was seen with lymph node metastases ($p < 0.05$). No association was seen between tumour size and tumour grade. Joint ER and PR expression also showed a higher number (73.5%) of HER-2/neu negative cases.

Conclusion: HER-2/neu, ER and PR expression is comparable to the West with inverse association with ER and PR and positive association with lymph node metastases. Since HER-2/neu expression is variable in carcinomas with different tumour characteristics, it is not possible to predict hormone receptor expression in all the cases.

Key words: *Breast carcinoma. HER-2/neu. Estrogen receptor. Progesterone receptor. Tumour marker.*

INTRODUCTION

Breast cancer is the most common carcinoma in women and accounts for 22% of all female cancers, which is more than twice the prevalence of cancer in women at any other site.[1] Prognosis and management of breast cancer are influenced by the classic variables such as histological type and grade, tumour size, lymph node status, status of hormonal receptors - Estrogen Receptor (ER) and Progesterone Receptor (PR) of the tumour and more recently, HER-2/neu status.[2,3]

HER-2/neu, also known as c-erbB-2 (HER-2), a proto-oncogene located on chromosome 17, is amplified and / or the protein (HER-2) overexpressed in 15-25% of invasive breast carcinomas and this overexpression is associated with a worse clinical outcome.[3,4]

ER is expressed in 70-80% of invasive ductal carcinomas, while PR is expressed in 60-70% of invasive breast carcinomas.[5,6] The inter-relationship between ER, PR, and HER-2 has an important role in the management of breast cancer. It has been shown that patients with breast carcinoma expressing HER-2/neu and p-53 do not respond to tamoxifen therapy.[7]

Studies on breast cancer in Pakistan have shown that there are 24-36% patients who are less than forty years of age as compared to 12% cases in the western population.[8] The tumour size in the majority of cases (56%) is greater than 5 cm at presentation and is histologically infiltrating ductal carcinoma NOS in most cases and of high grade.[9,10] The percentage of hormone receptor negative tumours is also higher than in the West.[11] The results of an earlier local study by Siddiqui *et al.* showed an association of estrogen receptor and lymph node status with HER-2/neu while no statistical significance was noted on association with other factors.[12] Moreover, at present, breast cancer patients in most centres in Pakistan are managed on the basis of routine histological markers and at the most hormone receptor status.

Only a few centers offer HER-2/neu testing facilities, which are expensive and add to the financial burden. HER-2/neu is an established prognostic marker, which may modify treatment in patients with advanced metastatic breast carcinoma and those with relapse. This study was planned, keeping in mind the possibility

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of a different biological behaviour of tumour in Northern Pakistan and develop possible guidelines for the oncologists, to advise the test in selected patients only in whom the likelihood of the HER-2/neu expression is greater.

The objective of this study was to determine the morphological profile and association of HER-2/neu expression with histological prognostic markers and hormonal receptor expression in female breast carcinoma in Northern Pakistan.

METHODOLOGY

This study was carried out at Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January 2004 to May 2007. AFIP is a referral laboratory receiving samples from the Armed Forces Hospital establishments, Northern Punjab, North-West Frontier Province and from adjoining civil and private hospitals of Rawalpindi and Islamabad region.

Female patients with breast carcinoma diagnosed on tru-cut biopsy, lumpectomy or mastectomy with or without axillary clearance who had hormone receptor assays done on their tumours were included in the study. At the same time patients who had received neo-adjuvant chemotherapy before undergoing surgery were excluded.

Samples were received in 10% formalin. Measurement of the tumour size was done on gross examination in cases of lumpectomy and mastectomy and classified as <2 cm, 2-5 cm and >5 cm based on TNM staging for breast carcinoma. Representative sections of the tumour and lymph nodes were submitted for paraffin embedding after formalin fixation. Three to four µm thick sections of the tissue were made and stained with Hematoxylin and Eosin (H&E) for subsequent microscopy. Histological typing of the tumour, confirmation of tumour size and detection of lymph node metastases were performed on routine H&E sections. Histological tumour grading was done using Modified Bloom and Richardson scoring system.[13] Lymph nodes were grouped as 1-3, 4-9 and >9 according to number of lymph nodes showing metastasis according to TNM staging for breast carcinoma.

Immunohistochemical determination of ER, PR and HER-2/neu was done with monoclonal antibodies using antigen-antibody Strept-avidin immunoperoxidase technique. ER and PR positivity was assessed using H-scoring system taking nuclear staining with a score >50 as positive. HER-2/neu immunohistochemical expression was assessed using DAKO scoring system taking only strong membrane staining with a score of 2+ and 3+ as positive.

Data was entered in SPSS version 11.0 and statistical analysis was done to determine frequency of the morphological prognostic parameters. Chi-square test and Fischer's exact test was used to determine association of HER-2/neu with prognostic markers. All the values were 2-tailed and a p-value of 0.05 was taken as significant.

RESULTS

During the study period from January 2004 to May 2007, a total of 9432 malignant tumours were diagnosed in both males and female patients. There were 1195 cases of breast carcinoma constituting 12.7% of all the malignancies out of which 535 fulfilled the inclusion criteria.

The mean age at presentation was 48±12.5 years ranging from 21 to 86 years. Maximum number of cases were seen in the 5th decade. There were 358 cases of mastectomy and 113 cases of lumpectomy. Axillary clearance was done in 471 cases of mastectomy and lumpectomy. Tumour size and lymph node status could not be ascertained in 64 cases of tru-cut biopsy. Infiltrating ductal carcinoma was the predominant subtype in 481 (90%) cases, while there were 23 (4.3%) cases of infiltrating lobular carcinoma. Rare subtypes i.e. medullary carcinoma, metaplastic carcinoma, mucinous carcinoma and papillary carcinoma constituted 21 (4%) of all the cases. The mean tumour size was 4.4±2.5 cm, ranging from 0.4 to 17 cm. There were 348 (65%) cases with tumour grade II, while lymph node metastasis was seen in 75% cases.

Immunohistochemically, ER expression was seen in 72.3% cases while PR was expressed in 62% cases. HER-2/neu expression was seen in 166 (31%) cases. Among the positive cases 18 (3.4%) had a DAKO score of 2+ve and 148 (27.6%) cases had a DAKO score 3+ve. Table I summarises the clinicopathological features of all 535 women with primary breast carcinoma.

ER+ve tumours overexpressed HER-2/neu in 25.8% of cases, and ER-ve tumours had HER-2/neu expression in 44.6% cases ($p < 0.001$). Similarly, women with PR+ve tumours overexpressed HER-2/neu in 26.4% of cases, whereas PR-ve tumours overexpressed HER-2/neu in 38.6% of cases ($p < 0.001$). Tumours with lymph node metastasis had 37.7% HER-2/neu expression, while HER-2/neu expression of 16.5% was seen in tumours without lymph node metastasis ($p=0.002$).

An equal expression of HER-2/neu was seen in patients of pre-menopausal (<50 years) and post-menopausal (>50 years) age groups in the range of 28-34%. Likewise, tumours of smaller (<2 cm) and larger (>5 cm) size also showed similar HER-2/neu expression in the range of 30-33%. There was no statistically significant association of HER-2/neu expression with tumour size, tumour grade, or age at diagnosis (Table II).

The evaluation of joint expression of ER and PR showed that 228 (73.8%) cases, which were both ER and PR positive, did not express HER-2/neu. Similarly, HER-2/neu expression was seen in 66 (52.8%) of those cases which were both ER and PR negative on immunohisto-

Table I: Clinicopathological features of the cases of breast carcinoma (n=535).

	N	Percentae
HER-2/neu		
0,1+ve	369	69%
2+ve	18	3.4%
3+ve	148	27.6%
ER expression		
Positive	387	72.3%
Negative	148	27.7%
PR expression		
Positive	333	62.2%
Negative	202	37.8%
Tumour grade		
Grade I	37	7%
Grade II	348	65%
Grade III	150	28%
Tumour size		
< 2 cm	81	17.2%
2.1-5 cm	259	55%
> 5 cm	131	27.8%
Lymph node metastasis		
Present	249	74.6%
Absent	85	25.4%

ER=Estrogen Receptor, PR=Progesterone Receptor

chemistry (Table III).

Table II: Association of HER-2/neu with prognostic markers (n=535).

	HER-2/neu		
	Positive	Negative	p-value
ER expression			
Positive	100 (25.8%)	287 (74.2%)	0.00
Negative	66 (44.6%)	82 (55.4%)	
PR expression			
Positive	88 (26.4%)	245 (73.6%)	0.009
Negative	78 (38.6%)	124 (61.4%)	
Tumour grade			
Grade I	6 (16.2%)	31 (83.8%)	0.236
Grade II	117 (33.6%)	231 (66.4%)	
Grade III	43 (28.7%)	107 (71.3%)	
Tumour size (n= 471)			
< 2 cm	25 (30.9%)	56 (69.1%)	0.986
2.1-5 cm	79 (30.5%)	180 (69.5%)	
> 5 cm	43 (32.8%)	88 (67.2%)	
Lymph node metastasis (n=334)			
Present	94 (37.7%)	155 (62.3%)	0.002
Absent	14 (16.5%)	71 (83.5%)	
Age			
< 50 years	105 (28.8%)	251 (71.2%)	0.385
> 50 years	61 (33.3%)	118 (66.7%)	

ER=Estrogen Receptor, PR=Progesterone Receptor

Table III: Frequency of joint ER and PR expression by HER-2/neu status.

	HER-2/neu		
	Positive	Negative	Total
ER+ve PR+ve	82	228	310
	26.5%	73.5%	
ER+ve PR-ve	15	62	77
	19.5%	80.5%	
ER-ve PR+ve	3	20	23
	13%	87%	
ER-ve PR-ve	66	59	125
	52.8%	47.2%	
	166	369	535

ER=Estrogen Receptor, PR=Progesterone Receptor

DISCUSSION

Breast cancer is a heterogeneous group of tumours and is the most common malignant tumour of the female population in Pakistan. It accounts for 25% of all female malignancies.[14] 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 number of axillary lymph nodes with tumour metastases, together with tumour size and grade, are morphological prognostic markers which determine long-term survival. Moreover, ER and PR expression help in decision making about anti-estrogen drug tamoxifen and aromatase inhibitors.

HER-2/neu expression is seen in 5-35% of breast carcinomas. Treatment with specific monoclonal antibody herceptin is offered for HER-2/neu positive cases. Potent inhibitory activity of the antibody herceptin against the tumour cells increases the sensitivity of these cells to undergo apoptosis following chemo-therapy subsequently.[15] HER-2/neu expression of 31% was seen in this study, which is comparable in frequency to HER-2/neu expression seen in the West.[14]

The mean age at diagnosis in this study was 48 years and the highest number of cases was seen in the 5th decade. This presentation is similar to earlier local studies by Malik *et al.*, Siddiqui *et al.* and Wahid *et al.*[9,16,17] Arryandono *et al.* from Indonesia have also suggested the mean age at diagnosis at 47 years,[18] while Western studies project the mean age at 58 years. Moreover, there are a significant number of patients who are below 50 years of age at the time of diagnosis. Wahid *et al.* showed more than 50% cases below 50 years of age,[17] while this study showed 66.5% cases below 50 years in age. No statistically significant difference in mean age of patients was seen in HER-2/neu positive and negative tumours in this study, while Almasri *et al.* reported a significant difference in the mean age of patients with HER-2/neu expression.[19]

Infiltrating ductal carcinoma remains the most common subtype in this study and other local studies by Malik *et al.*, Siddiqui *et al.* and Wahid *et al.*[9,16,17] Similarly, the Western studies and by Arryandono *et al.* reported an incidence of infiltrating ductal carcinoma upto 70% with HER-2/neu expression seen in upto 25% of the cases.[18]

Conservative nature of our society and lack of awareness play a role in the advanced stage of presentation at the time of diagnosis. Larger tumour size and higher number of lymph node metastases is seen in Pakistan. Local studies showed a tumour size of > 5 cm in majority of cases.[9,16,17] 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.[18,19] In this study, mean tumour size was 4.4 cm and only 24% cases had a tumour size larger than 5 cm, while 15% cases had tumour size less than 2 cm. No association was seen between tumour size and HER-2/neu in this study and other studies by Lal *et al.*, Almasri *et al.*, Haung *et al.* and Naqvi *et al.* showed a positive association between HER-2/neu and tumour size.[14,19-21]

Patients with increased number of lymph nodes represent a higher stage of presentation. Arryandono *et al.* reported 38.3% patients with more than 3 positive lymph nodes.[20] Earlier, Malik *et al.* had reported 74% cases with lymph node metastasis in their study out of which 30% had more than 3 positive lymph nodes.[9] In comparison, 25.4% patients did not have any lymph node involvement and 46% cases had more than 3 lymph node metastasis in this study. Arryandono *et al.*, Naqvi *et al.*, Traina *et al.* and this study demonstrated positive association between HER-2/neu expression and lymph node metastasis while no association could be established in any of the Western studies by Lal *et al.*,[16] Haung and Almasri *et al.*[19,20]

Higher tumour grade is associated with poor prognosis.[2] Malik *et al.* showed in their study that amongst breast carcinomas, 6% are grade I, 32% grade II and 62% grade III.[9] Similarly, Siddiqui *et al.* showed 11.3% grade I, 59.2% grade II and 29.5% grade III breast carcinomas,[10] which is in consonance with the present study where grade II tumours constituted the highest number of cases at 65%. HER-2/neu expression of 16% in grade I breast carcinomas was seen in this study, while grade II tumours had the highest expression of 26%. Rilke *et al.* in a series of 1,210 cases found HER-2/neu expression of 3.9% in grade I, 2.4% in grade II and 38.9% in grade III tumours.[23] Ayadi *et al.* found HER-2/neu expression of 14.8% in grade I and II and 27.5% in grade III carcinomas.[24] Likewise, Hoff *et al.* found HER-2/neu expression of 1, 17 and 23% respectively in grade I, II and III tumours in their study of 388 cases.[25] Western studies have demonstrated highest HER-2/neu expression in grade III breast carcinomas and negligible expression in grade I breast carcinomas.[25] No association between HER-2/neu expression and tumour grade was seen in this study, while positive association with tumour grade was seen by Lal *et al.*, Rilke *et al.* and Hoff *et al.*[14,23,25] HER-2/neu expression in grade I carcinomas is much higher in this study than seen in all the other studies in the West, which may suggest either analytical issues associated with immunohistochemical HER-2/neu testing or different biological behaviour in our population.

Earlier studies in Pakistan have shown a variable expression of ER. Fatima *et al.* reported ER expression of 40.5%,[11] while Malik *et al.* reported a much higher expression of 86%.[9] Western studies have projected an ER expression between 70-80% in invasive ductal carcinomas while ER expression of 72.3% was seen in this study. An inverse association between ER with HER-2/neu has been observed in this and other studies.[14,19,20]

PR expression was seen upto 62.2% in this study, while Fatima *et al.* have projected a PR positivity of 35%.[11] Western studies have demonstrated a PR expression of 60-70%.[25] Inverse association has been seen with PR and HER-2/neu in this study and other studies.[16,19-21,23] However, Arryandono *et al.* from Indonesia and Al-Ahwal *et al.* from Saudi Arabia did not show any association between HER-2/neu and PR.[18,26]

The analysis of conjoint expression of ER and PR seen in this study reveals the inverse association of ER and PR with HER-2/neu expression. Although ER and PR expression was significantly decreased in HER-2/neu positive tumours, a substantial proportion of ER+ve and PR+ve cases (26.5%) also expressed HER-2/neu, which makes it mandatory to perform hormone receptors and HER-2/neu in all the cases, as one can not predict expression in any particular patient. In women with an ER+ve breast cancer, HER-2/neu overexpression implies a greater likelihood of the tumour being PR-ve. Rhodes *et al.* have shown that the ER+ve PR-ve variant is a menopause related phenotype.[27] Endogenous estrogen may be too low to up-regulate PR and repress HER-2/neu by estrogen binding to ER, so that the inverse association might be restricted to postmenopausal women. Recently, it has been shown that the ER+ve PR-ve phenotype is predictive of tamoxifen resistance in postmenopausal women. ER+ve PR-ve tumours have a higher response to aromatase inhibitors and no further response beyond two years of tamoxifen use compared with the ER+ve PR+ve phenotype.[27] However, these randomised controlled studies did not associate HER-2/neu positivity with the ER+ve PR-ve phenotype, but our findings and those of others suggest this as one of the underlying mechanisms for tamoxifen resistance in such patients.[7] This is another reason to measure PR in women with an ER+ve tumour for effective treatment regimens. Moreover, there are a significant number of cases (47%) which do not show expression of ER, PR or HER-2/neu. This profile puts the emphasis on the pre-analytical issues like fixation time and antigen retrieval techniques as well as the particular tumour phenotypes and genotypes which may be prevalent in this region.

CONCLUSION

ER and PR are inversely correlated with HER-2/neu expression in breast carcinoma while positive

association is seen with the lymph node metastases. There is no association of HER-2/neu with tumour size and tumour grade. HER-2/neu expression is variable in carcinomas with different tumour characteristics. Therefore, it is not possible to predict hormone receptor expression in all cases as such and it is necessary to carry out receptor studies and HER-2/neu in all cases of breast carcinomas.

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