

Her-2/neu Gene Overexpression in Breast Carcinoma and its Association with Clinicopathological Characteristics of the Disease

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ABSTRACT

Objective: To assess the frequency of Her-2/neu gene amplification in breast cancer and its association with clinicopathologic characteristics of the disease.

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Study was carried out at Surgical Unit I, Holy Family Hospital, Rawalpindi, from January 2005 to December 2006.

Methodology: A total of 50 patients diagnosed of having breast cancer on biopsy of breast lump were included in the study. All patients underwent mastectomy and samples were submitted to AFIP (Armed Forces Institute of Pathology, Rawalpindi) for histopathology and determination of estrogen and progesterone receptors and Her-2/neu gene amplification. Moreover, association of Her-2/neu amplification to other characteristics like tumour size, node involvement, histological grade and presence of estrogen and progesterone receptors were also studied.

Results: Out of 50 patients, 18 (36%) were positive for Her-2/neu overexpression. Among the various prognostic factors, age showed a statistically significant relation to Her-2/neu positivity ($p=0.001$). The Her-2/neu positive cancers were larger in size (mean size of 7.9 ± 3.615 and 5.4 ± 1.906 cm in Her-2/neu positive and negative cases respectively; $p=0.002$). Her-2/neu positive cases had statistically significant relation to number of involved lymph nodes (mean number of 9.9 and 4.4 lymph nodes in Her-2/neu positive and negative cases respectively; $p=0.001$). Tumour grade and nuclear grade had no statistically significant relation to Her-2/neu status ($p=0.19$ and 0.434 respectively). Both estrogen and progesterone receptors are inversely related to Her-2/neu positivity ($p=0.028$ and 0.006 respectively).

Conclusion: The pathological and clinical characteristics of Her-2/neu positive and negative subsets differ, and Her-2/neu overexpression was associated with other poor prognostic factors of age, tumour size and number of nodes involved. There was an inverse relation of Her-2/neu status and hormone receptor.

Key words: Breast cancer. Her-2/neu. Prognostic factors. Immuno-histochemistry. Hormone receptors. Lymph nodes.

INTRODUCTION

Despite advances in early detection, surgical therapy and adjuvant therapy of breast cancer, approximately 40,000 women die of the disease each year.¹ This provides the impetus for investigating the molecular biology of breast cancer and to identify molecular alterations that enhance its malignant behaviour. These findings can also be used clinically as diagnostic and predictive markers and as targets for therapy. Her-2/neu, also known as c-erb B-2, is a member of type-1 Epidermal Growth Factor Receptor (EGFR) family of receptor tyrosine kinases. Members of this family have intrinsic tyrosine kinase activity and are considered important mediators of cell growth, differentiation and

survival.² The protein product of Her-2/neu gene is a 185-kd surface membrane protein normally found in a wide variety of tissues, including epithelia of the breast, ovary, endometrium, lung, kidney, and gastrointestinal tract, and in central nervous system.³

The protein encoded by Her-2/neu is frequently overproduced in many cancers, including breast, ovarian, lung, gastric and oral cancers but in particular this protein is overproduced in 25-30% of breast cancers.⁴ Gene amplification, such that each cell contains several copies of Her-2/neu gene instead of two copies of the gene (one each on chromosome 17), has a major role in breast cancer prognosis and also predicts response to therapy including surgery, chemotherapy and endocrine therapy. Slamon and colleagues were the first to report a strong relationship between amplification of the Her-2/neu gene and a shorter Disease-Free Survival (DFS) and overall survival.⁵ Subsequent studies have confirmed this association.

The purpose of the current study was to determine the frequency of Her-2/neu overexpression in Pakistani

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subjects and to its association with other clinical characteristics of disease especially tumour size, number of nodes, histologic and nuclear grade, and status of estrogen and progesterone receptors.

METHODOLOGY

Patients with breast cancer, visiting the Outpatient Department of Holy Family Hospital, Rawalpindi, were enrolled in this cross-sectional comparative study, through non-probability sampling from January 2005 to December 2006. Patients with breast lump proven on biopsy to be malignant were included in the study, whereas any patient with a breast lump, which was benign on biopsy was excluded from the study.

Patients had a complete physical examination along with local examination of breast mass and regional lymph nodes sufficient for clinical staging. Physicians and anaesthetist did pre-anaesthetic assessment. These patients had their routine investigations done including blood complete picture, urine analysis, random blood sugar, urea, creatinine, electrolytes, chest X-ray, electrocardiograms and ultrasound of abdomen. The patients then underwent mastectomy and axillary lymph node dissection. The mastectomy samples were submitted to Armed Forces Institute of Pathology (AFIP) for primary tumour diameter, axillary nodal status and histological grade was determined and scored as the Bloom Richardson grade.⁶ Immunohistochemistry was performed on thin sections of formalin-fixed, paraffin-embedded tissue, or on sectioned frozen specimens. It relies on use of monoclonal antibodies against epitopes of estrogen and progesterone receptors. Her-2/neu amplification was determined on paraffin-embedded tissue using a monoclonal antibody against c-erb B-2 (neu) protein, provided by Immunotec®, France. The staining is mainly located on the membrane. Her-2/neu was scored as positive when intense membrane labeling was observed in >50% of the tumour cells.

The data was stored and analyzed using SPSS 10. The patients were divided into two groups; (Her-2/neu positive vs. Her-2/neu negative subset). The association of Her-2/neu amplification to other characteristics was studied. The mean size of tumour was calculated in two groups and the two means were compared using the t-test. The Chi-square test was used to determine at the association of nodal involvement, tumour histologic grade, frequency of estrogen and progesterone receptors to the presence or absence of Her-2/neu amplification. In all the above statistics, a $p < 0.05$ is considered statistically significant.

RESULTS

The study enrolled 50 female patients confirmed on biopsy to have breast carcinoma. The age ranged from 31 to 68 years with a mean of 47.68 ± 13.58 years. Out of those 50 patients, 44 (88%) had invasive ductal carcinoma, 4 (8%) had invasive lobular carcinoma and 2

(4%) had medullary carcinoma. Eighteen (36%) patients were positive for Her-2/neu overexpression. Table I lists the characteristics of patients in the two groups i.e. Her-2/neu positive and Her-2/neu negative groups. Twenty seven (54%) patients were positive for presence of estrogen receptors and 18 (36%) patients were positive for presence of progesterone receptors.

Table I: Characteristics of patients grouped according to Her-2/neu status.

	Her-2/neu negative	Her-2/neu positive	p-value
Total number of cases	32 (64%)	18 (36%)	-
Node-positive	97%	100%	n.s
Number of nodes (mean)	4.4	9.9	0.001
Age in years (mean)	53	38.6	0.001
Tumour size in cm (mean)	5.4	7.9	0.002
Tumour grade	II (68.7%) III (31.3%)	II (50%) III (50%)	0.19
Nuclear grade	II (71.9%) III (28.1%)	II (61%) III (39%)	0.434
Estrogen receptor positive	65.6%	33.3%	0.028
Progesterone receptor positive	50%	11%	0.006

Among the 18 patients of Her-2/neu positive group, 6 (33.3%) were estrogen receptor positive and 2 (11%) were progesterone receptor positive. All (100%) demonstrated nodal involvement. Fifty percent were tumour grade II and 50% were tumour grade III. Sixty one percent had nuclear grade II and 39% had nuclear grade III. Among the 32 patients of Her-2/neu negative group, 21 (65.6%) were estrogen receptor positive and 16 (50%) were progesterone receptor positive. Thirty one (96.8%) demonstrated nodal involvement. Sixty eight point seven percent were tumour grade II and rest were tumour grade III. Seventy one point nine percent had nuclear grade II and the rest had nuclear grade III. Among the various prognostic factors, age showed a statistically significant association to Her-2/neu positivity (mean age of 38.6 ± 10.63 and 53 ± 14.53 years in Her-2/neu positive and negative cases respectively; $p = 0.001$). The Her-2/neu positive cancers were larger in size (mean size of 7.9 ± 3.61 cm and 5.4 ± 1.90 cm in Her-2/neu positive and negative cases respectively; $p = 0.002$). Association of node positivity to Her-2/neu status could not be assessed, as only one patient in the study was node negative and rest of the patients had axillary lymph node metastasis. However, Her-2/neu positive cases had statistically significant relationship to number of involved lymph nodes (mean number of 9.9 ± 7.20 cm and 4.4 ± 3.31 cm lymph nodes in Her-2/neu positive and negative cases respectively; $p = 0.001$). Tumour grade and nuclear grade had no statistically significant relation to Her-2/neu status ($p = 0.19$ and 0.434 respectively). Both estrogen and progesterone receptors were inversely related to Her-2/neu positivity ($p = 0.028$ and 0.006 respectively).

DISCUSSION

Breast cancer is the second most common cancer among females worldwide.⁷ Despite advances in early

detection, surgical therapy and adjuvant therapy of breast cancer, about 40,000 women die of this disease each year.¹ The Her-2/neu gene was first identified as a potent oncogene in DNA from carcinogen-induced neuroblastoma of rats.⁸ The human homologue of this cellular oncogene Her-2/neu is found to be over-produced in many cancers including breast, ovarian, lung, gastric and oral cancers.² Among the various proposed prognostic factors, Her-2/neu over-expression has been found to predict early metastasis, shortened disease-free survival, overall survival, poor clinical outcome with therapy and early recurrence in breast carcinoma.⁹

The association between amplification of the Her-2/neu gene and a shorter Disease-Free Survival (DFS) as well as overall survival, which has been confirmed by several studies.^{5,10} This subset of Her-2/neu positive breast cancer also shows resistance to certain forms of chemotherapy like CMF (cyclophosphamide, methotrexate and 5-Fluorouracil) and tamoxifen, while showing sensitivity to others like doxorubicin. Her-2/neu has been found to be associated with other poor prognostic factors including age, lymph node status, tumour size and histological grading. Trastuzumab, a humanized antibody to Her-2/neu protein is being used as a therapy for patients with advanced breast cancer.¹¹

The differences between Her-2-positive and Her-2-negative tumours in clinical behaviours can be related to their different biology. In fact, a recent biomolecular study of breast carcinomas using micro-array technology indicates that Her-2-positive tumours share some markers specific for basal epithelial cells, whereas Her-2-negative/hormone receptor-expressing tumours appear to be of luminal cell origin.¹² Naqvi *et al.* reported a frequency of Her-2/neu overexpression of 33% in lymph node positive invasive breast carcinoma in a cohort of Pakistani patients.¹³ They also found a statistically significant relation of Her-2/neu positivity with size of tumour. Siddique *et al.* had reported similar results in 1999.¹⁴ This study showed that 36% of the study population had overexpression of Her-2/neu. Most of previous studies show Her-2/neu overexpression in 25-30% of breast cancer but ranges of 14-89% have been reported. The present analysis of two series of breast carcinomas patients, considering Her-2-positive tumours as a special subset, indicates that Her-2 positivity is strongly associated with young age of patient, tumour size and number of infiltrated nodes, which are consistent with previous studies.¹⁵ This suggests that Her-2 confers a high proliferative capability to tumour cells.¹⁶

CONCLUSION

The data identified the Her-2-positive breast carcinoma as a particular subset of tumours with peculiar clinical and pathological behaviours. Thirty six percent patients were positive for Her-2/neu overexpression. Only age was found to have statistically significant association with Her-2/neu positivity and more and more young patients were having Her-2-positive breast carcinoma. The Her-2/neu positive cancers were larger in size and

having more number of involved lymph nodes as compared to patients with Her-2-negative breast carcinoma. This finding points to the need for re-evaluation of strategies from clinical trials on which clinical management of breast carcinoma is based.

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