Expression of Human Epidermal Growth Factor Receptor 2/neu in Carcinoma Breast with Reference to Prognostic Index

S Shivakumar[1], Medha Shankarling[2], Jasneet Kaur Sandhu[3]

[1]Professor and Head, Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka, India, [2]Assistant Professor, Department of Pathology, Srinivas Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India, [3]Tutor, Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka, India

Abstract

Introduction: In India, breast carcinoma is the second most common cause of death even though it arises in an exposed organ which is readily accessible for self-examination and diagnosis. A better understanding of various prognostic parameters has assumed a great therapeutic importance.

Aim: The aim of the study was to investigate the correlation of human epidermal growth factor receptor 2 (HER2)/neu status with important clinicopathologic prognostic parameters of carcinoma breast in particular with Nottingham prognostic index (NPI).

Methodology: Immunohistochemical techniques were used to evaluate HER2/neu in paraffin-embedded tissue specimens of 50 cases of carcinoma breast. The patients' age and the size of the tumor were noted. The histologic subtype, Nottingham modification of Scarff Bloom Richardson (NSBR) grade, lymph node staging, and NPI groups were assessed.

Results: Of the 50 cases of carcinoma breast, 17 (34%) were HER2/neu positive. The HER2/neu positivity increased with age (62.5% in patients of the 7th and 8th decades) and was statistically significant ($P = 0.05$). The HER2/neu positivity increased with tumor size, with positivity of 0% in <2 cm and 40% in >2 cm tumor but was not statistically significant ($P = 0.149$). The HER2/neu positivity was more in invasive duct carcinoma (IDC), not otherwise specified (NOS) type (100%), higher NSBR grade (94.1% in Grades 2 and 3), higher score of lymph node staging (82.4% in score 2 and 3), and in poorer NPI prognostic groups (87.2%) but were not statistically significant ($P = 0.681, 0.102, 0.139, 0.177$, respectively).

Conclusion: The HER2/neu expression was more frequent in tumor >2 cm, in IDC, NOS type, in higher NSBR grade, carcinoma breast with lymph node metastasis, and in poorer NPI prognostic groups indicating that HER2/neu may be a powerful predictor for poor prognosis.

Key words: Carcinoma breast, Human epidermal growth factor receptor 2/neu, Immunohistochemistry, Nottingham modification of Scarff Bloom Richardson grade, Nottingham prognostic index prognostic groups

INTRODUCTION

Breast carcinoma is the most common malignant tumor worldwide and is the second-leading cause of death in women due to cancer.[1] In India, breast carcinoma is the second most common cancer in women after carcinoma cervix. In India,

most of the patients present with palpable mass with 50% having lymph node metastasis at the time of their first visit.[2]

Stratification of patients after taking into consideration of various prognostic parameters has assumed a great therapeutic importance.[1] As a result, there have been outstanding advances in breast cancer management with the development of more effective treatments leading to a significant decline in breast cancer deaths and improved outcome in women living with breast disease over the last few decades.[3]

Many prognostic and predictive factors have been identified by the College of American Pathologists to guide the clinical

Corresponding Author: S Shivakumar, Department of Pathology, Mandya Institute of Medical Sciences, Mandya, India.
Phone: +91-9449622449. E-mail: shivakumar.path@gmail.com

management of women with breast cancer. The prognostic factors include invasive carcinoma or *in-situ* carcinoma, distant metastasis, lymph node metastasis, tumor size, locally advanced disease, histological grade, histological subtype, inflammatory carcinoma, estrogen receptor (ER)/progesterone receptor (PR) status, and overexpression of human epidermal growth factor receptor (HER2)/neu.[4]

In the current management guidelines, ER/PR status and overexpression of HER2/neu are the most useful predictive factors for response to specific therapeutic agents.[2]

HER2/neu, otherwise known as neu or c-erbB-2, is a product of an oncogene. Presently, immunohistochemistry (IHC) is the center stage in the demonstration of monoclonal antibodies for evaluating HER2/neu protein expression in breast carcinoma.[5] HER2/neu expression is an independent prognostic factor in patients with breast carcinoma and has greater prognostic value than most currently used prognostic factors including ER, PR status.[6] HER2/neu positive cancer patients exhibit resistance to tamoxifen but not to aromatase inhibitors or ovarian ablation.[7]

The presence of HER2/neu is related to a high-grade tumor and poorer prognosis but the favorable response to monoclonal antibody therapy and disease survival.[8] Therefore, there is a growing clinical demand for analysis of the HER2/neu status of current and archived breast cancer specimens.[9]

METHODOLOGY

A total of 50 cases of mastectomy specimens of carcinoma breast with axillary clearance received in the Department of Pathology, MIMS, Mandya, during the study period from January 2010 to October 2012 were evaluated.

The study was approved by the Institutional Ethical Committee. On arrival to the department, the specimens were subjected to adequate fixation using 10% neutral buffer formalin. After examination of the specimen for gross details, representative bits were subjected to routine processing for paraffin embedding. From the paraffin-embedded blocks, 4-5 μ thick section were taken and stained with hematoxylin and eosin (H and E) stain.

According to the WHO classification system, the tumors were histologically typed. The Nottingham modification of Scarff Bloom Richardson (NSBR) grading system[10] was used for grading (Table 1) which is based on the architecture pattern, nuclear atypia, and mitotic rate of the tumor.

The combined score of architecture, nuclear atypia, and mitotic figures/10 high power field is added to get a total score. Grade 1 breast carcinomas include scores of 3-5, Grade 2 includes score of 6-7, and Grade 3 includes score of 8-9.

Each case was assessed considering important prognostic parameters such as size of tumor, histological grade, and metastasis to axillary lymph nodes to calculate the Nottingham prognostic index (NPI)[11] which is as follows: NPI = score of lymph node stage [1-3] + score of histological grade as NSBR system (1-3) + maximum diameter in centimeters ×0.2. The scoring of the lymph node is as follows: Score 1 - No lymph node metastasis; score 2 - Metastasis to 1-3 lymph nodes; and score 3 - Metastasis to >4 lymph nodes.

After calculation of the NPI score, all the 50 cases of carcinoma breast were divided into 6 prognostic groups (Table 2).[11]

Sections of 3-4 μ thickness on silane-coated slides from the representative areas of all the 50 cases were subjected to IHC study for HER2/neu. The polymer-based IHC kit of BioGenex RTU (ready to use) was used. The cell membrane of tumor cells was stained in various intensities when there was overexpression of HER2/neu protein. The HER2/neu positivity was assessed by a semi-quantitative method based on the number of tumor cells showing positivity and the staining intensity. A score of 2+ and 3+ HER2/neu expressions (Figures 1 and 2) was considered as positive for immunostaining (Table 3).

Correlation of HER2/neu expression with important prognostic parameters such as patient's age, size of tumor,

Table 1: Histological grade using NSBR system[10]

Score	Architecture (%)	Nuclear atypia	MF/HPF
1	>75 tubule formation	Mild	0-5
2	10-75 tubule formation	Moderate	6-10
3	<10 tubule formation	Severe	>10

MF/HPF: Mitotic figures/high power field, NSBR: Nottingham modification of Scarff Bloom Richardson

Table 2: NPI prognostic groups[11]

Prognostic group	NPI score
EPG	2.08-2.4
GPG	2.42-3.4
MPG I	3.42-4.4
MPG II	4.42-5.4
PPG	5.42-6.4
VPPG	6.42-6.4

EPG: Excellent prognostic group, GPG: Good prognostic group, MPG: Moderate prognostic group, PPG: Poor prognostic group, VPPG: Very poor prognostic group, NPI: Nottingham prognostic index

Table 3: Assessment of HER2/neu protein overexpression

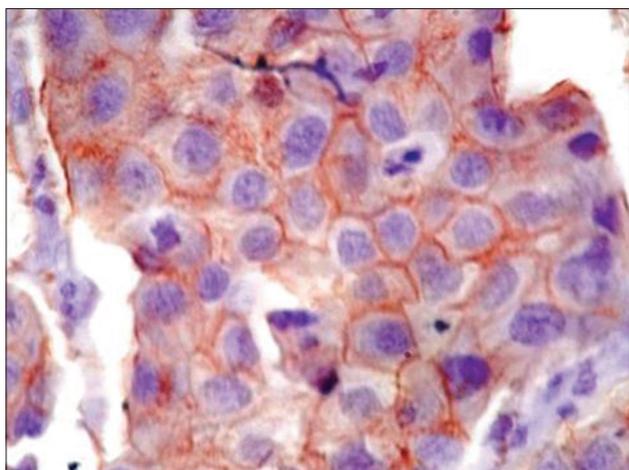| Staining pattern	Score
No or membrane staining in <10% of cells	0
Faint and part of membrane staining in >10% of cells	1
Weak to moderate complete membrane staining in >10% of cells	2
Strong complete staining in >10% of cells	3

HER2: Human epidermal growth factor receptor

Figure 1: Weak moderately complete membranous human epidermal growth factor receptor 2/neu immunostaining (score of 2+) in invasive duct carcinoma cells (DAB, ×400)
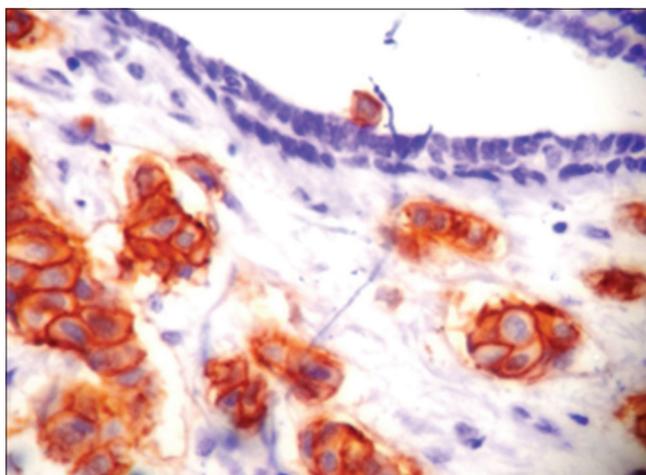

Figure 2: Strong complete membranous human epidermal growth factor receptor 2/neu immunostaining (score of 3+) in >10% of invasive duct carcinoma cells (DAB, ×400)

histologic subtype, NSBR grade of tumor, lymph node staging, and mainly with NPI groups was done.

Plan of Data Analysis

The collected data were entered in Excel sheet and analyzed using Epi Info software, and the descriptive statistics, Chi-square test, Student's *t*-test, McNemar's test, and other

Table 4: Prognostic parameters of carcinoma breast among 50 patients

Characteristics	*n* (%)
Age (years)	
Range	25-80
Mean	47
Tumor size (cm)	
<2	04 (08)
2-5	30 (60)
>5	16 (32)
Histologic type	
IDC, NOS type	44 (88)
Infiltrating lobular Ca	02 (04)
Papillary Ca	01 (02)
Atypical medullary Ca	01 (02)
Tubular Ca	01 (02)
Metaplastic Ca	01 (02)
NSBR grade	
Grade 1	12 (24)
Grade 2	25 (50)
Grade 3	13 (26)
Lymph node stage	
Score 1	20 (40)
Score 2	16 (32)
Score 3	14 (28)
NPI groups	
EPG	02 (04)
GPG	06 (12)
MPG I	08 (16)
MPG II	14 (28)
PPG	12 (24)
VPPG	08 (16)

NPI: Nottingham prognostic index, IDC: Invasive duct carcinoma, EPG: Excellent prognostic group, GPG: Good prognostic group, MPG: Moderate prognostic group, PPG: Poor prognostic group, VPPG: Very poor prognostic group, NSBR: Nottingham modification of Scarff Bloom Richardson, NOS: Not otherwise specified

applicable tests were applied for the data. The *P* < 0.05 was considered statistically significant.

RESULTS

The age of 50 patients with carcinoma breast ranged from 25 to 80 years, and the mean age was 47 years. The majority (60%) of the patients were in the 5th and 6th decades of life (Table 4).

On gross examination, the size of the tumor among the 50 cases of breast carcinoma ranged from 1 to 11 cm. In 4 (8%) cases, the tumor size was <2 cm, in 30 (60%) cases the size ranged from 2 to 5 cm, and in 16 (32%) cases the size was >5 cm (Table 4).

Of the 50 cases of invasive carcinoma breast, 44 (88%) cases were invasive duct carcinoma (IDC), not otherwise specified (NOS) type, 2 (4%) cases were infiltrating lobular carcinoma, and 1 (2%) case each of tubular carcinoma, papillary carcinoma, atypical medullary carcinoma, and metaplastic carcinoma was observed (Table 4).

Table 5: Correlation of HER2/neu with prognostic parameters

Characteristics	HER2/neu negative	HER2/neu positive	P value
	n=33 (%)	*n*=17 (%)	
Age (years)			0.05
≤39	10 (30.3)	02 (11.8)	
≥40	23 (69.7)	15 (88.2)	
Size of tumor (cm)			0.149
<2	04 (12.1)	00 (00.0)	
2-5	18 (54.6)	13 (76.5)	
>5	11 (33.3)	04 (23.5)	
Histologic type			0.681
IDC, NOS type	27 (81.9)	17 (100)	
Infiltrating lobular Ca	02 (06.1)	00 (00)	
Papillary Ca	01 (03.0)	00 (00)	
Atypical medullary Ca	01 (03.0)	00 (00)	
Tubular Ca	01 (03.0)	00 (00)	
Metaplastic Ca	01 (03.0)	00 (00)	
NSBR grade			0.102
Grade 1	11 (33.3)	01 (05.9)	
Grade 2	15 (45.5)	10 (58.8)	
Grade 3	07 (21.2)	06 (35.3)	
Lymph node stage			0.139
Score 1	17 (51.5)	03 (17.6)	
Score 2	09 (27.3)	07 (41.2)	
Score 3	07 (21.2)	07 (41.2)	
NPI groups			0.177
EPG	02 (06.1)	00 (00)	
GPG	06 (18.2)	00 (00)	
MPG I	09 (27.3)	02 (11.8)	
MPG II	06 (18.2)	05 (29.4)	
PPG	05 (15.1)	07 (41.2)	
VPPG	05 (15.1)	03 (17.6)	

HER2: Human epidermal growth factor receptor, NPI: Nottingham prognostic index, NSBR: Nottingham modification of Scarff Bloom Richardson, IDC: Invasive duct carcinoma, EPG: Excellent prognostic group, GPG: Good prognostic group, MPG: Moderate prognostic group, PPG: Poor prognostic group, VPPG: Very poor prognostic group, NOS: Not otherwise specified

Among the 50 cases of carcinoma breast, as per the NSBR grading system, 12 (24%) cases were of Grade 1, 25 (50%) cases were of Grades 2, and 13 (26%) cases belonged to Grade 3 (Table 4).

When lymph node staging was done in 50 cases of carcinoma breast, 20 (40%) cases were of score 1, 16 (32%) cases were of score 2, and 14 (28%) cases were of score 3 (Table 4).

As per NPI prognostic groups, 2 (4%) cases were in EPG, 6 (12%) cases were in GPG, 8 (16%) cases were in MPG I, 14 (28%) cases were in MPG II, 12 (24%) cases were in PPG, and 8 (16%) cases were in VPPG (Table 4).

Immunohistochemistry of HER2/neu

Among the 50 cases of breast carcinoma, maximum (62.5%) HER2/neu positivity was seen in the age group of 60-80 years and least (16.7%) in the age group of 21-39 years (Table 5). A statistically significant association of HER2/neu with age was seen (P= 0.05).

All the cases with the tumor size of <2 cm were negative for HER2/neu. HER2/neu positivity was seen in 13 (43.3%) of 30 cases with tumor size between 2 and 5 cm and 4 (25%) of 16 cases with tumor size >5 cm (Table 5). No statistically significant association of HER2/neu with tumor size was present (P = 0.149).

The HER2/neu was expressed in 17 (34%) of 50 cases of carcinoma breast, and all the positive cases were IDC and NOS type. All the other histologic subtypes were negative for HER2/neu (Table 5). No statistically significant association of HER2/neu with histologic type was present (P = 0.681).

While correlating the overexpression of HER2/neu with NSBR histologic grading, the percentage of HER2/neu positivity increased with grade and was 8.3% (1 of 12 cases) in Grade 1, 40% (10 of 25 cases) in Grade 2, and 46.2% (6 of 13 cases) in Grade 3. No statistically significant association of HER2/neu with NSBR histologic grading was present (P = 0.102).

The percentage of HER2/neu positivity increased with lymph node staging. HER2/neu positivity in the primary breast tumor was seen in 15% (3 of 20 cases) of score 1 lymph node staging, 43.7% (7 of 16 cases) of score 2, and 50% (7 of 14 cases) of score 3 lymph node staging. However, no statistically significant association of HER2/neu with lymph node staging was seen P= 0.139.

Correlation of HER2/neu positivity with NPI showed that all cases belonging to EPG and GPG were negative for HER2/neu. The positivity for HER2/neu was seen in 2 (18.2%) cases of MPG I, 5 (45.5%) cases of MPG II, 7 (58.3%) cases of PPG, and 3 (37.5%) cases of VPPG. No statistically significant association of HER2/neu with NPI was present (P = 0.177).

DISCUSSION

It is tragic that carcinoma breast is the second most common cause of death in women even though these neoplasms arise in an exposed organ, which is readily accessible for self-examination and diagnosis. There are outstanding advances in understanding breast cancer, and various prognostic factors are defined which can increase the survival rate.

Prognostic information is important in counseling patients about the likely outcome of their disease, choosing appropriate treatment, and the design of clinical trials. Prognostic factors fall into two groups - Those related to the extent of carcinoma (tumor burden or stage) and those related to the underlying biology of cancer.

The present study investigated the correlation of expression of HER2/neu with important clinicopathologic prognostic parameters of carcinoma breast.

In the present study, the mean age of 50 patients with carcinoma breast was 47.2 years and was similar to studies of Ayadi *et al.*,[12] (51.5 years), Azizun-Nisa *et al.*,[13] (48.3 years), Moradi-Marjaneh *et al.*,[14] (47.4 years), and Al-Moundhri *et al.*,[15] (49.6 years). The HER2/neu expression increased with age and positivity of 16.7% was seen in 21-39 year age group patients who increased to 62.5% in patients of 60-80 year age group. Similar results were seen in a study done by Al-Moundhri *et al.*,[15] where 12 out of 13 patients above 40 years of age were positive for HER2/neu. This may be related to the accumulation of somatic mutations with age.[16]

The results of HER2/neu positivity were relatively different in diverse studies. We found HER2/neu expression of 34% in the present study. Literature reports positive results ranging from 15 to 93.4%.[12-14,17-19] Possible sources of this variation may be attributed to different tissue fixation procedures, properties of different antibodies, scoring methods applied for HER2/neu positivity, the different stains used for staining, and microwave procedure of the tissue during antigen retrieval of IHC staining process (Table 6).

Tumor size is one of the most useful predictors of behavior in breast carcinoma. None of tumors <2 cm were positive for HER2/neu and 36.9% of tumors >2 cm showed HER2/neu positivity. Similarly, other studies[17,14,18-20] also showed a higher rate of HER2/neu expression in larger tumor with the statistically significant association (Table 7).

In the present study, all the HER2/neu positive cases were seen in infiltrating duct carcinoma, NOS type. Similarly, in the study by Saleh and Abdeen[19] and Naeem *et al.*,[20] 71.7% and 90.9% of infiltrating duct carcinoma, NOS type cases showed HER2/neu positivity. This suggests that HER2/neu positivity is common in infiltrating duct carcinoma, NOS type. In contrast, Ayadi *et al.*,[12] reported 16.8% and 25% HER2/neu positivity in ductal and non-ductal carcinomas, respectively.

An increasing percentage of HER2/neu positivity was seen with increasing NSBR histologic grade in studies done by Moradi-Marjaneh *et al.*,[14] Ivkovic[17] Lovekin *et al.*,[18] Saleh and Abdeen,[19] Naeem *et al.*,[20] and also in the present study (Table 8).

An increased expression of HER2/neu in cases of carcinoma breast with lymph node metastases was seen in studies by Azizun-Nisa *et al.*,[13] (89.3%), Moradi-Marjaneh *et al.*,[14] (76.9%), Ivkovic[17] (52.2%), and Naeem *et al.*,[20] (91.0%). Similarly, the present study also showed an 85% of HER2/neu positivity in cases of carcinoma with lymph node metastasis suggesting that HER2/neu positivity increases the risk of metastasis.

Lovekin *et al.*,[18] in 1991, found that HER2/neu positivity is associated with poorer prognosis while following up 782 patients of carcinoma breast and concluded that HER2/neu is an independent predictive factor for the shorter survival rate in invasive carcinoma of breast. The NPI is a well-established and widely used method of predicting survival of operable primary breast carcinoma. The NPI is compiled from tumor grade, tumor size, and lymph node status of the primary tumor. No studies correlating the NPI and HER2/neu status with the best of our knowledge have been done so far. In the present study, most of the positive cases were seen in very poor (37.5%),

Table 6: Incidence of HER2/neu expression in various studies

Study	Incidence positive/total (%)
Ayadi *et al.*[12]	28/155 (18.1)
Azizun-Nisa *et al.*[13]	56/150 (37.4)
Moradi-Marjaneh *et al.*[14]	165/319 (51.7)
Tatjana *et al.*[17]	23/120 (20.0)
Lovekin *et al.*[18]	70/480 (15.0)
Saleh and Abdeen[19]	155/166 (93.4)
Present study	17/50 (33.0)

HER2: Human epidermal growth factor receptor

Table 7: Correlation of HER2/neu positivity with tumor size

Study	Tumor size			P value
	<2 cm	2-5 cm	>5 cm	
Moradi-Marjaneh *et al.*[14]	46.0	46.0	62.0	0.016
Tatjana *et al.*[17]	0.0	18.1	47.6	<0.0001
Lovekin *et al.*[18]	3.0	11.0	21.0	<0.0001
Saleh and Abdeen.[19]	5.9	92.9	87.7	<0.005
Naeem *et al.*[20]	0.0	18.2	81.8	>0.05
Present study	8.3	40.0	46.2	0.102

HER2: Human epidermal growth factor receptor

Table 8: Correlation of HER2/neu positivity with NSBR histologic grades

Study	NSBR histologic grades (%)			P value
	Grade 1	Grade 2	Grade 3	
Moradi-Marjaneh *et al.*[14]	46.0	46.0	62.0	0.016
Tatjana *et al.*[17]	0.0	18.1	47.6	<0.0001
Lovekin *et al.*[18]	3.0	11.0	21.0	<0.0001
Saleh and Abdeen[19]	5.9	92.9	87.7	<0.005
Naeem *et al.*[20]	0.0	18.2	81.8	>0.05
Present study	8.3	40.0	46.2	0.102

HER2: Human epidermal growth factor receptor, NSBR: Nottingham modification of Scarff Bloom Richardson

poor (58.3%), and moderate prognostic Group II (45.5%). None of the cases in excellent and good prognostic groups were positive for HER2/neu.

CONCLUSION

The aim of this study was to determine the frequency of expression of HER2/neu with various prognostic indices in carcinoma breast, particularly to NPI, which has been aptly brought out in this study. The HER2/neu positivity rate was high in patients of the 5th and 6th decades and was statistically significant.

Although there is no statistical association of HER2/neu with tumor size, histologic subtype, NSBR histologic grade, lymph node status, and NPI, its expression was more frequent in cases with tumor size >2 cm, infiltrating duct carcinoma, NOS type, higher NSBR grade, carcinoma breast with lymph node metastases, and higher NPI score indicating that HER2/neu may be a powerful predictor for poor prognosis.

Further studies have to be done to determine the significance of correlation of HER2/neu with NPI and to find if this correlation has an advantage over NPI alone in predicting the survival rate of breast carcinoma patients.

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