Original Article

Association of HER-2 Expression with Age, Tumour Size, Grade, Differentiation and Lymph Node Deposit in Patients with Breast Cancer

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Abstract

Breast Cancer incidence is increasing, but its mortality has started to decrease. The existence of strong reliable prognostic and predictive factors is of utmost importance to the practicing clinician. Some factors are only prognostic such as age, tumor size, and lymph node status, while others are both prognostic and predictive such as hormone receptors and human epidermal receptor 2 (HER's-2) status. This study was done to see the association of HER-2 positivity with age, tumour size, grade, differentiation and lymph node deposit in breast cancer patient. A cross-sectional type of descriptive study was conducted among 50 patients. Patient age range is from 22 to 64 years (mean 42.64 ± 10.26 years). Tumour size ranged from 1 to 8 cm (mean 4.39 ± 1.564cm). Well differentiated tumour was 20, moderately differentiated tumour was 18 cases and poorly differentiated was 12 cases. HER-2/neu negative included 35(70%) cases and positive included 15(30%) cases. In this study it was found that the association between age groups and tumour differentiation with HER-2/neu status was statistically significant (p= <0.05).

Key words: Breast Cancer, HER-2/neu expression.

Introduction

Breast Cancer incidence is increasing, but its mortality has started to decrease. This reduction is a result of widespread screening resulting in earlier detection as well as advances in adjuvant treatment of early-stage disease.[1] It is very important to be able to select the best available therapy for an individual patient based on established predictive factors.

Therefore, the existence of strong reliable prognostic and predictive factors is of utmost importance to the practicing clinician. Prognostic factors are different from predictive factors. A prognostic factor is any measurement that correlates with disease free survival or overall survival in the absence of adjuvant therapy and that also correlates with the natural history of untreated breast cancer. A predictive factor is any measurement that correlates with the response to a given therapy. Some factors are only prognostic such as age, tumor size, and lymph node status, while others are both prognostic and predictive such as hormone receptors and human epidermal receptor 2 (HER-2) status.

Oestrogen receptor (ER) and progesterone receptor (PR) status has been used for many years to help determine a patient's suitability for endocrine therapy. More recently, testing for the human epidermal growth factor receptor 2 (HER-2 / neu) has been included in routine patient work-

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up, with recognition of its value both as a prognostic marker and, more particularly, in predicting response to trastuzumab (Herceptin TM). HER2/neu protein has garnered a great deal of interest amongst pathologists and oncologists for its potential role as a tumor marker and prognostic marker.

Materials and Methods

This study was cross-sectional type of descriptive study was conducted among

50 patients admitted in the department of Surgery, Rajshahi Medical College Hospital and diagnosed histopathologically as breast cancer from January 2010 to December 2011.

Inclusion criteria:

- Females of any age diagnosed clinically and by FNAC as having breast cancer.

Exclusion criteria:

- Patients already treated or undergone biopsy followed by chemo/radiotherapy and male patients of invasive breast cancer.

Ethical clearance:

Prior to the commencement of this study, the research protocol was approved by the Institutional Review Board (IRB) of Rajshahi Medical College, Rajshahi.

Clinical information:

All the clinical information about the patients under study was noted on structured proforma.

Sample collection:

Mastectomy specimen/lumpectomy specimens were received in the laboratory. Routine tissue processing was followed. Paraffin blocks were made and sections were taken for routine Haematoxylene and Eosin (H&E) stain and

Immunohistochemistry (IHC) stain. For IHC stain, sections were taken on sialinized slides

Examination of tissue sections:

Routinely stained sections were examined under microscope and histological diagnosis was made. All cases were classified according to WHO proposed classification (WHO 2003). In cases of invasive ductal carcinoma (NOS), grading of tumour was performed by the Bloom-Richardson grading system (Rosai 2004).

Immunohistochemistry (IHC) for HER-2/neu.

During this study, HER-2/neu reactivity were detected through immunohistochemical stain.

Assessment of IHC staining for HER-2/neu protein

HER-2/neu (c-erbB-2) over expression was measured by immunohistochemistry. Positive (3+) IHC staining was defined when more than 10% of tumour cells show strong complete membrane staining. Weakly positive (2+) IHC staining was defined when more than 10% of tumour cells show weak to moderate complete membrane staining. Negative IHC was defined when no staining was observed, or membrane staining was <10 % of the tumour cells. A faint/barely perceptible membrane staining when detected in >10 % of the tumour cells are also categorized as negative. In this study, score 2+ was interpreted as negative in the final statistical analysis as fluorescence in situ hybridization (FISH) was not performed on the weak positive cases (2+).

Analysis of data and results:

After collection of data, descriptive statistics like percentage was calculated. Analysis of data was done with the help of computer by SPSS programme.

Results

Prognosis and management of breast cancer patient are influenced by some variable such as histological type, tumour differentiation (grade), tumour size, lymph node status and HER-2/neu expression pattern. A total 50 cases of invasive breast cancer were included in this study.

Table 1: Distribution of patients according to age (n= 50)

Age in years	Number of cases	Percentage (%)
21-30	6	12%
31-40	18	36%
41-50	17	34%
51-60	7	14%
61+	2	4%
Total	50	100%

Mean ±SD = 42.64 ±10.26 years.

Table 2: Distribution of tumour size (n=50)

Tumour sizes	Number	%
<2cm	6	12%
2-5cm	30	60%
>5cm	14	28%

Mean±SD of size 4.39±1.564cm.

Tumour size ranged from 1 to 8 cm with a mean±SD of size 4.39±1.564 cm. The median size was 4.45 cm
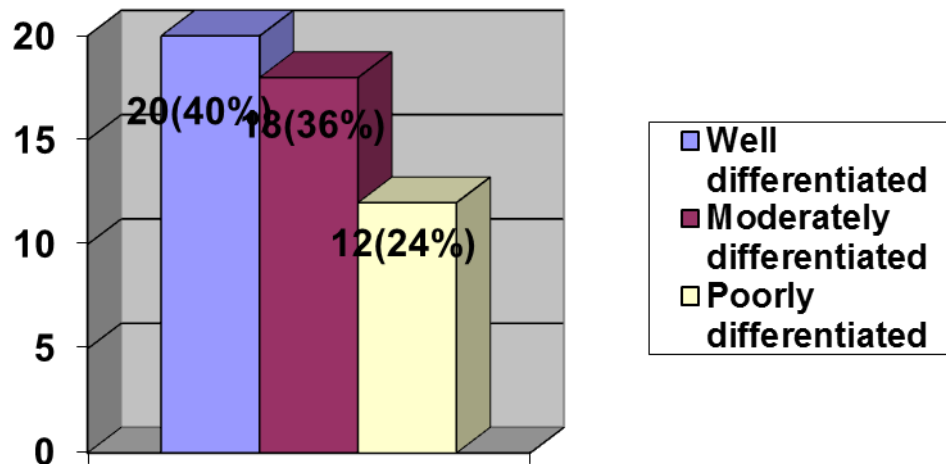
Tumour differentiation (Grade) (n=50).

Figure 1: Frequency of Tumour differentiation (n=50)

Figure 1 showed the frequency of tumour differentiation. According to Bloom Richardson Grading system, carcinoma was graded into Grade I (well differentiated), Grade II (moderately differentiated) and Grade III (poorly differentiated). Well differentiated tumour was 20(40%), moderately differentiated tumour was 18(36%) cases and poorly differentiated was 12(24%) cases.

Distribution of lymph node metastasis status (n=50)

Lymph node status was divided into five groups. These were no lymph node metastasis, metastasis within 1-3 lymph nodes, metastasis within 4-9 lymph nodes, metastasis involving ≥10 lymph nodes and lymph node status could not be assessed. Lymph node metastasis ranged from 0 to 13. Mean ± SD of lymph node metastasis was 3.58±.49. No lymph node metastasis was found in 7(14%) cases, metastasis within 1-3 lymph node was found in 14(28%) cases, metastasis within 4-9 lymph nodes was found in 17(34%) cases, metastasis to ≥10 lymph nodes was found in 4(8%) cases and lymph node status could not be assessed in 8(16%) cases.

Table 3: HER-2/neu reactivity patterns (n=50)

HER-2 positivity	Number	%
HER-2 negative	35	70%
HER-2 positive	15	30%
Total	50	100%

Table 3 showed HER-2/neu reactivity patterns. HER-2/neu reactivity pattern was determined as negative (score = 0, 1+), weakly positive (score=2+) and positive (score=3+). Weakly positive (score=2+) was considered as negative due to unavailability of FISH technique. 27(54%) cases were HER-2/neu negative (score=0, 1+), 8(16%) cases were weakly positive (score 2+) and 15(30%) cases were positive (score=3+). So negative (0, 1+, 2+) included 35(70%) cases and positive included 15(30%) cases.

Table 4: Relationship between HER-2/neu expression and age of the patients (n=50)

Age of the patients	HER-2/neu+ve		HER-2/neu-ve		Total (%)
	N	%	n	%	
≤ 45 years	11	42.31%	15	57.69%	26 (52%)
>45 years	4	16.67%	20	83.33%	24(48%)
Total	15	30%	35	70%	50(100%)

$$\chi^2 = 3.907, df=1, p= <0.05$$

The table no 4 showed that among the patients of age group ≤45 years, 11(42.31%) cases were HER-2 positive and 15 (57.69%) cases were HER-2 negative. Among the patients of age group >45 years, 4(16.67%) cases were HER-2 positive and 20(83.33%) cases were HER-2 negative. From this table it was found that the association between age groups and HER-2/neu status was statistically significant ($p= <0.05$).

Table 5: Relationship between HER-2/neu expression and tumour differentiation (n=50).

Tumour differentiation	HER-2/neu+ve		HER-2/neu-ve		Total (%)
	n	%	N	%	
Well differentiated	3	15.00%	17	85.00%	20(40%)
Moderately differentiated	5	27.78%	13	72.22%	18(36%)
Poorly differentiated	7	58.33%	5	41.67%	12(24%)
Total	15	30%	35	70%	50(100%)

$$\chi^2 = 6.77,\ df=2,\ p= <0.05$$

Table 6: Relationship between HER-2/neu expression and tumour size (n=50).

Tumour size	HER-2/neu+ve		HER-2/neu-ve		Total (%)
	n	%	n	%	
<2 cm	2	33.33%	4	66.66%	6(12%)
2-5 cm	11	36.67%	19	63.33%	30(60%)
>5 cm	2	14.29%	12	85.71%	14(28%)
Total	15	30%	35	70%	50(100%)

$$\chi^2 = 2.313,\ df=2,\ p= >0.05$$

Tumour< 2 cm in diameter & tumour 2-5 cm showed more number of HER-2/neu positivity than that of tumour> 5 cm in diameter. But this correlation between the expression of HER-2/neu and tumour size was not statistically significant (p=>0.05)

Table 7 Relationship between HER-2/neu expression and lymph node status (n=50).

Lymph node status	HER-2 +ve		HER-2 -ve		Total (%)
	n	%	n	%	
Node positive	12	34.29%	23	65.71%	35(70%)
Node negative	2	28.57%	5	71.43%	7(14%)
Not assessed	1	12.50%	7	87.50%	8(16%)
Total	15	30%	35	70%	50(100%)

$$\chi^2 = 1.480,\ df=2,\ p= >0.05$$

In this table the relationship between HER-2/neu expression and lymph node status was not statistically significant (*p= >0.05)*

Discussion

This cross-sectional study was performed to see the correlation HER-2/neu expression with age, tumour size, grade, differentiation and lymph node deposit in invasive breast carcinoma. A total 50 cases of invasive breast carcinoma were included in this study. In this study, age distribution ranged from 22 to 64 years with a mean±SD of age 42.64±10.26 years. In this study carcinoma was graded into well differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (grade 3).Well differentiated tumour was 20(40%), moderately differentiated tumour was 18(36%) cases and poorly differentiated tumour was 12(24%). Ayadi et al[2] described in his study that grade 1 tumour was 11%, grade 2 was

63.2% and grade 3 tumour was 25.8%. Taucher et al[3] mentioned grade 1 18%, grade 2 was 32.3% grade 3 was 40.5% and grade 3 9.2%. Huang et al[4] showed Grade 1-2 60.8% and grade 3 was 39.2%. Vang et al[5] mentioned grade 1 26.2%, grade 2 30.8% and grade 3 was 43.0%. So the result of this study is consistent with most of the previous studies.

Almasri & Hamad[6] and Farzamiet al[7] found that younger age groups (≤ 50 years age) are more likely to have HER-2/neu over expression than patients older than 50 years. Ratnatunga & Liyanapathirana[8] also found HER-2/neu overexpression more among the younger (≤ 45 years) group than the older (>45 years) group. Taucheret al[3] described age group of <35 years and >35 years. All of them found significant correlation (*p*<0.05) between HER-2/neu over expression and patient's age. But Ayadi et al[2] did not find significant correlation between HER-2/neu over expression and patient's age (women older or younger than 50 years). In the present study younger age group (≤ 45 years) showed 42.31% over expression of HER-2/neu and older age group (>45 years) showed 16.67 % HER-2/neu over expression which is statistically significant (*p*= <0.05).

In this study 30% cases are HER-2/neu positive and 70% are HER-2/neu negative when considering 3+ score as positive and score 0, 1+, 2+ as negative. Arafah[9] 35.3%, Hossain[10] 32%, Slamon et al[11] 28%, Benohr et al[12] 26.6%, Jacobs et al[13] 24%, Almasri & Hamad[6] 24% cases found HER-2/neu positive which correlated well with this study. But Taucheret al[3], Ayadi et al[2], Huang et al[4], Vang et al[5], Prati et al[14] found HER-2/neu over expression in 17.3%, 18.1%, 10.9%, 13.0%, 19.1% cases respectively. So it was found that HER-2/neu positivity ranges from 13.0% to 35.3%.

In this study, tumour size ranged from 1 to 8 cm with a mean±SD of size 4.39±1.564cm. The median size was 4.45 cm. Among those less than 2 cm tumour was 6(12%) cases, 2-5 cm tumour was 30(60%) cases and more than 5 cm tumour was 14(28%) cases. Huang et al[4] described tumour

size less than 2 cm was 52.90% and more than 2 cm tumour was 47.10%, Ayadi et al[2] described tumour size <5 cm was 76% and >5 cm was 23.9%. So in this study tumour size is more than previous study. This may be due to late service seeking approach in our country due to different causes such as poor health knowledge, economic status, social ignorance and deprivation, no national programme to generate knowledge, poor Electronic media/ TV/paper work. Tumours of <2 cm size are 33.33%, 2-5 cm tumours are 36.67% and tumours of >5 cm size are 14.29% HER-2/neu positive. The correlation between tumour size and HER-2/neu positive are not statistically significant (*p*= >0.05). Huang et al[4], Ayadi et al[2] and Farzamiet al[7] (2009) also found no significant association between tumour size and HER-2/neu reactivity. Huang et al[4] showed that tumour size <20 mm were 10% HER-2 positive and tumours>20 mm were 11.4% positive (p=0.617), Ayadi et al[2] and Farzamiet al[7] found p=0.104 and p=0.497 that is no significant correlation. But Taucher et al[3] found inversely associated relationship between tumour size and HER-2/neu status with *p*=0.0076. Almasri & Hamad[6] described in their study that tumours with strong HER-2 expression tended to be larger than those lacking expression with mean size of 4.7cm and 4cm respectively.Among tumour size more than 5cm, 35% were HER-2 positive and tumour of 2-5 cm size was 22% positive .

Among the 20 well differentiated tumours 3(15%) showed positive reactivity and 17(85%) tumours showed negative reactivity for HER-2/neu. In case of moderately differentiated tumours 5(27.78%) cases showed positive reactivity, 13(72.22%) tumours showed negative reactivity HER-2/neu. Among 12 cases of poorly differentiated tumours 7(58.33%) cases showed positive reactivity and and 5(41.67%) tumours showed negative reactivity for HER-2/neu. The association between HER-2/neu expression and tumour differentiation was statistically significant (*p*= <0.05). Taucher et al[3], Huang et al[4] also found positive association between HER-2/neu expression and poor tumour differentiation which was statistically significant and Ayadi et al[2] found marginal significance

between HER-2 overexpression and histologic grade; only 14.8% of grade 1-2 carcinoma were overexpressed compared to 27.5% with grade 3 carcinoma (p=0.072). Arafah[9] and Hossain[10] did not find significant association between tumour differentiation and HER-2/neu reactivity that is p value > 0.25 and p=0.504 respectively.

In this study among 35 patients of lymph node metastasis 12 (34.29%) showed positive reactivity and 23 (65.71%) showed negative reactivity for HER-2/neu. Of 7 patients with no lymph node metastasis 2(28.57%) showed positive reactivity and 5(71.43%) showed negative reactivity for HER-2/neu. The relationship between HER-2/neu expression and lymph node status was not statistically significant (*p=* >0.05). Similar findings were also reported by other authors as Taucher et al[3] in their study among 923 patients 441 had lymph node metastasis and among them 81(18.4%) had HER-2/neu positive tumours and patients without lymph node metastasis (n=482) 78(16%) had HER-2/neu positive tumours. No significant correlation between lymph node status and HER-2/neu status was found (p=0.38). Prati et al[14] among 73 cases with positive lymph node found that 16(21.92%) cases were HER-2 positive and 57(78.08%) were HER-2 negative. Among 80 lymph node negative cases, 12(15%) were HER-2 positive and 68(85%) were HER-2 negative. There was no significant correlation between lymph node metastasis and HER-2 status. Huang et al[4] in his study found that among lymph node positive cases 12.1% were HER-2 positive and among lymph node negative cases 10% were HER-2 positive which was not statistically significant(p=0.692). But Borg et al[15] found statistically significant relationship between HER-2 amplification and the number of positive nodes (p=0.044) and Ayadi et al[2] found strong association between HER-2/neu expression and lymph node involvement (p=0.000).

Conclusion

Analysis of HER-2/neu status in breast cancer is important because it provide valuable prognostic, predictive and therapeutic information. In this study HER-2/neu overexpression was evaluated by immunohistochemistry in 50 patients with infiltrating breast carcinomas and was positive in 15 cases (30%). In this study it was revealed that HER-2/neu expression significantly correlates with high histological grade and age of the patient.

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