Correlation of Various Prognostic Parameters with Human Epidermal Growth Factor Receptor-2/Neu Status in Malignant Breast Lesions

Swati Sucharita Mohanty[1]*, Prafulla Kumar Mohanty[1], Sumit Subhadarshi Mohanty[2], Padmalaya Thakur[3], Surabhi[4]

[1]*Department of Zoology, Utkal University, India*

[2]*Department of Gastro Surgery, I.M.S and SUM Hospital, Siksha 'O' Anusandhan University, India*

[3]*Department of Obstetrics and Gynaecology, Centre for Human Reproduction, I.M.S and SUM Hospital, Siksha 'O' Anusandhan University, India*

[4]*Department of Pathology, S.C.B. Medical College and Hospital, Utkal University, India*

Abstract

The incidence of breast cancer is although low in India, but is rising. Breast cancer is the commonest cancer of urban Indian women and the second commonest in the rural women. Amplification or overexpression of Human Epidermal Growth Factor Receptor-2/neu (*HER-2/neu*) oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years, the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients. A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of immunohistochemistry for solving everyday diagnostic problems in breast pathology. The aim of this study is to determine if any correlation exists between *HER-2/neu* with respect to age, menopausal status, grade, tumor size and lymph node status in breast carcinoma. A total 108 cases have been included in this study. Mean age at diagnosis was found to be 46.29 years. It was found that *her2/neu* is overexpressed in younger age group and overexpression decreases with increase in age. *Her2/neu* negativity is observed to be maximum in postmenopausal group. *Her2/neu* is found to be overexpressed with increase in tumor size and grade. This investigation does not find any significant relationship between lymphnode status.

Keywords: Breast cancer, *Her2/neu*, age, Menopausal status, Grade, Tumor size, Lymph node

Abbreviations

HER-2/neu: Human Epidermal Growth Factor Receptor-2/neu; HP: Histopathology; IHC: Immunohistochemistry; H and E: Haematoxylin and Eosin stain; ASCO–CAP: American Society of Clinical Oncology - College of American Pathologists; IDC: Invasive Ductal Carcinoma; NST: No Special Type

Introduction

The incidence of breast cancer is low in India, but is rising. Breast cancer is the commonest cancer of urban Indian women and the second commonest in the rural women. Owing to the lack of awareness of this disease and in absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage [1]. Amplification or overexpression of Human Epidermal Growth Factor Receptor-2/neu (*HER-2/neu*) oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer [2-4]. In recent years the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients [3,5]. Immunohistochemistry (IHC) has an expanding role in the diagnosis and management of mammary disease. A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of IHC for solving everyday diagnostic problems in breast pathology [6]. The aim of this study is to determine if any correlation exists between *HER-2/neu* with respect to age, menopausal status, grade, tumor size, and lymph node status in breast carcinoma.

Methods

A total 108 cases were included in this study, out of which 95 (87.96%) cases were invasive

Table 1: Distribution of cases according to histologic types.

SI no	Histological type	Number of cases	Percentage (%)
1	Invasive ductal carcinoma (NST)	95	87.96
2	Invasive lobular carcinoma	13	12.04
	Total	108	100

ductal cancers (No special Type) and 13 (12.04%) cases were invasive lobular cancers. The investigation was conducted on histopathology (HP) and immunohistochemistry (IHC) of excised specimens of 108 females with breast carcinoma operated by trained doctors. Histopathological examination was conducted by using conventional haematoxylin and eosin stain (H and E). Immunohistochemical evaluation of human epidermal growth factor receptor 2 (*Her2/neu*) were undertaken on formalin fixed paraffin embedded tissue sections by using Novocastra's Ready to use mouse monoclonal antibody and Novolink polymer detection system. *HER-2/neu* receptors are assessed immunohistochemically and compared with patient's age, menopausal status, grade, tumor size, and lymph node status of tumor. Cancers were graded according to Elston and Ellis' [7] modification of Bloom and Richardson's [8] original classification from 1957. American Society of Clinical Oncology - College of American Pathologists (ASCO–CAP) guideline recommendations is used for scoring HER2/nue [9] which takes into account the percentage of circumferential membranous staining intensity and distribution of invasive tumor cells.

Differences in tissue processing and technical procedure may produce variable results. Hence, controls used are fresh autopsy or surgical specimens processed in same manner as patient's sample. The data were arranged in contingency table and were analysed by the Chi-square test (X^2) and Fisher exact test to find the correlation between these prognostic parameters and ER, PR and HER2/nue expression [10-14]. The result was considered statistically significant if p value was less than 0.05. The commercially available statistical software (PAST version 3.04 for Windows; Øyvind Hammer, Natural History Museum, University of Oslo) was used for the analysis of data. This study was conducted according to the Ethical Committee of SCBMCH and the institution took care of the entire financial burden for the completion of this prospective research study and UGC-BSR fellowship provided finance to the research scholar.

Results

Out of the 108 cases included in this study, invasive ductal carcinoma (IDC No Special Type/NST) was the most common and largest group of histological types of breast cancer, accounting for about 95 out of 108 (87.96%) cases. Besides IDC, there is also invasive lobular carcinoma (ILC) (Table 1). There were thirteen out of one hundred eight (12.04%) cases of ILC. The females were divided into three different age groups; < 41, 41-50 and > 50 (Table 2). The age of females' ranged from 22 to 67 years. Mean age at diagnosis was found to be 46.29 years. Correlating *her2/neu* with age at diagnosis, it was found that *her2/neu*+ve was the highest in 41-50 age group followed by <41 age group and >50 age group (Table 2). There were 15 out of 30 (50%) cases in 41-50 age group, 12 out of 30 (40%) cases in <41 age group and three out of 30 (10%) cases in >50 age group. *Her2/neu*–ve was highest in 41-50 age group. There were 43out of 78 (55.13%) in 41-50 age group, 24 out of 78 (30.77%) in >50 age group and 11 out of 78 (14.10%) in <41 age group.

When *her2/neu* was correlated with menopausal status, number

of *her2/neu*+ve was same in both premenopausal and postmenopausal females (Table 2). It was 15 out of 30 (50%) cases in both menopausal status. *Her2/neu*–ve was the highest in postmenopausal females, where there were fifty 58 out of 78 (74.36%) cases. 20 cases of *her2/neu*–ve out of 78 (25.64%) were in premenopausal females.

Correlation of *her2/neu* with tumor size showed that maximum number of cases of *her2/neu*+ve were in T2 stage (Table 2). There were 20 out of 30 (66.67%) cases in T2 stage, 10 out of out of 30 (33.33%) cases in T3 stage and no cases of *her2/neu*+ve was found in T1stage. Maximum number of cases of *her2/neu*–ve was in T2 stage followed by T3 stage and T2 stage. There were 49 out of 78 (62.82%) cases in T2 stage, 15 out of 78 (19.23%) cases in T3 stage and 14 out of 78 (17.95%) cases in T1 stage.

Correlating *her2/neu* with lymph node status, it was found that *her2/neu*+ve was the highest in N1 stage followed by N0 stage and N2 stage (Table 2). There were 17 out of 30 (56.67%) cases in N1 stage, 11 out of 30 (36.66%) cases in N0 stage and two out of 30 (6.67%) cases in N2 stage. *Her2/neu*-ve was maximum in N1 stage, where there were 40 out of 78 (51.28%) cases. There were 20 out of 78 (25.64%) cases in N2 stage, 18 out of 78 (23.08%) cases in N0 stage.

Correlation of *her2/neu* with histological grade showed that maximum number of cases of *her2/neu*+ve were in grade II (Table 2). There were 22 out of 30 (73.33%) cases in grade II, eight out of 30 (26.67%) cases in grade III and no case in grade I. *Her2/neu*-ve was the highest in grade II followed by grade I and grade III. There were 51 out of 78 (65.38%) cases in grade II, 15 out of 78 (19.23%) cases in grade I and 12 out of 78 (15.38%) cases in grade III.

Discussion

In this investigation, invasive ductal carcinoma (IDC No Special Type/NST) was the largest group (Table 1), accounting for 87.96% (95/108) of all the cases which is similar to the finding of Ayadi et al. [10] and Azizun-Nisa et al. [15] who found the predominant morphology to be IDC accounting about 83.8% and 85.3% respectively. Abdollahi et al. [11] also report similar results where IDC was 90.7%.

In the study group, the common age group to be affected was 41-50 years with 53.70% (58/108) cases (Table 2). The mean age at diagnosis of females was 46.29 years, unlike in the west where women aged 53-57 years are more prone to breast cancer. This is similar to the finding of National Cancer Registry Programme [16], according to which more numbers of patients are being diagnosed with breast cancer in their thirties and forties. Ayadi et al. [10] also found that women with breast carcinoma are relatively younger than in Western countries, with mean age of 51.5 years. Sofi et al. [17] also found the mean age to be 48.2 years. Bloom in 1950 in their study of prognostic markers of breast cancer noted a poorer prognosis among patients under the age of 50 years in comparison to older age group, whereas Alderson et al. [18] found age at diagnosis to be unsignificant. Di Pietro et al., Goldhirsch et al., Anders et al. and Cao et al. found similar result as that of Bloom [19-22].

In the present study, *Her2/neu* was found to be positive in 27.78% of cases (Table 2) which is comparable with the finding of Azizun-Nisa et al. [15] who found Her2 positivity in 24.7% of cases. Ivkovic-Kapicl et al. [23] reported *Her2/neu* protein overexpression in 20% of patients.

The overexpression of *Her2/neu* (Table 2) in young age group (<41 years) was found to be 40%, whereas 50% cases were in 41-50

Table 2: Correlation of various prognostic parameters with Her2/neu expression in breast carcinoma (n=108).

PROGNOSTIC PARAMETERS / Her2/neu STATUS	Her2/neu+veNO. (%)	Her2/neu-ve NO. (%)	TOTAL NO. (%)	Chi squared *p* value	Fisher's exact *p* value
A. AGE AT DIAGNOSIS (IN YEARS)					
<41	12 (40%)	11 (14.10%)	23 (21.30%)		
41-50	15 (50%)	43 (55.13%)	58 (58.70%)	0.0020202 ≈ 0.002	0.0019063 ≈ 0.002
>50	3 (10%)	24 (30.77%)	27 (25%)		
MENOPAUSAL STATUS					
PRE MENOPAUSAL	15 (50%)	20 (25.64%)	35 (32.41%)	0.015409 ≈ 0.02	0.021607 ≈ 0.02
POST MENOPAUSAL	15 (50%)	58 (74.36%)	73 (67.59%)		
B. TUMOR SIZE					
T1	0 (0%)	14 (17.95%)	14 (12.96%)		
T2	20 (66.67%)	49 (62.82%)	69 (63.89%)	0.026039 ≈ 0.03	0.026039 ≈ 0.03
T3	10 (33.33%)	15 (19.23%)	25 (23.15%)		
C. HISTOLOGICAL GRADE					
G1	0 (0%)	15 (19.24%)	15 (13.89%)		
G2	22 (73.33%)	51 (65.38%)	73 (67.59%)	0.023981 ≈ 0.02	0.010774 ≈ 0.01
G3	8 (26.67%)	12 (15.38%)	20 (18.52%)		
D. NODAL INVOLVEMENT					
N0	11 (36.66%)	18 (23.08%)	29 (26.85%)		
N1	17 (56.67%)	40 (51.28%)	57 (52.78%)	0.065921 ≈ 0.07	0.054305 ≈ 0.05
N2	2 (6.67%)	20 (25.64%)	22 (20.37%)		
TOTAL NO. (%)	30 (27.78%)	78 (72.22%)	108		

years of age group and 10% of cases in >50years. There were 55.13% of cases in 41-50 age group, <41 age group had 14.10% cases and >50 age group had 30.77% cases that were associated with *Her2/neu* negativity. Kamil et al. [24] did not find any association between age at diagnosis and Her2 status. In comparison to above study Azizun-Nisa et al. [15] reported that *Her2/neu* positivity decreased with advanced age which is consistent with present study (p-value<0.005), i.e., statistically significant.

In the investigation group, 50% (15/30) cases of *Her2/neu* overexpression were both in premenopausal and postmenopausal group (Table 2). *Her2/neu* negativity was seen maximum in postmenopausal group with 74.63% (58/78). This means *her2/neu* overexpression decreases with increase in age. The correlation of *Her2/neu* status with menopausal status was found to be statistically significant (p-value<0.05). Present study (Table 2) indicates that in case of patients who were in T1 stage, 100% cases belonged to Her2 negative status. On the other side, as the size of tumor goes on increasing that is the patients with T2 & T3 stage, 66.67% and 33.33% cases showed *Her2/neu* positivity respectively (p-value <0.05). The result is consistent with that of Ivkovic-Kapicl et al. [23] and Azizun-Nisa et al. [15] who found *Her2/neu* to be strongly associated with large tumor size.

This study reports (Table. 2) 23.08% (18/78) patients with N0 stage had *Her2/neu* negative status. Similar result was found in patients with nodal involvement, that is, 51.28% and 25.64% of N1 and N2 stage showed Her2 negativity respectively. Hence the association of lymphnode status and *Her2/neu* to be statistically insignificant (p-value= 0.07). Ivkovic-Kapicl et al. [23] and Kamil et al. [24] also did not find any correlation between Her2 and lymphnode infiltration.

But Azizun-Nisa et al. [15] and Ludovini et al. [25] reported *Her2/neu* to be positively associated with lymphnode metastasis.

Conclusion

The present study was conducted on histopathology (HP) and immunohistochemistry (IHC) of excised specimens of 108 females with breast carcinoma. Out of the 108 cases included in this study, invasive ductal carcinoma (IDC No Special Type/NST) was the predominant morphology and largest group of histological types of breast cancer, with 87.96% (95/108) cases. The mean age of females was 46.29 years, unlike in the west where women aged 53-57 years are more prone to breast cancer. Age at diagnosis was found to be statistically significant to *Her2/neu*. Further, maximum number of females about 67.59% (73/108) were in the postmenopausal age group. This may be due to late presentation of patients to the clinician in India out of social inhibition. *Her2/neu* is overexpressed in 27.78% (30/108) of cases. There are 40% (12/30) *her2/neu*+ve in <41 age group and 50% (15/30) *her2/neu*+ve in 41-50 age group. This means *her2/neu* is overexpressed in younger age group. *Her2/neu* negativity is seen maximum in postmenopausal group with 74.63% (58/78). This means *her2/neu* overexpression decreases with increase in age. There are no cases of *her2/neu* overexpression in T1 stage but overexpression increases in T2 and T3 stage with 66.67% (20/30) and 33.33% (10/30) respectively. So *her2/neu* is found to be overexpressed with increase in tumor size. Likewise no cases of *her2/neu* overexpression are found to be in grade I tumor. *Her2/neu* overexpression is found to be 73.33% (22/30) and 26.67% (8/30) in high grade tumors (grade II and grade III respectively). This clearly suggests that *her2/neu* overexpression increases with increase in grade. These data are well compared with the international data. This investigation does not find any significant

relationship between lymphnode status and *Her2/neu* though some studies have demonstrated a positive correlation between them. This may be due to the different biological behaviour of the tumors belonging to different ethnic group. A significant relationship is marked between *Her2/neu* status and age at diagnosis, menopausal status, size of tumor, and grade. Therefore, it is concluded that the presence of *Her2/neu* receptors is associated with adverse prognostic factors like young age, premenopausal status, large tumor size and high grade tumors. Assessment of *Her2/neu* receptors for clinical management of breast cancer patients is strongly advocated to provide prognostic information and better therapeutic options.

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