



Evaluation of HER2/Neu Receptors and Its Comparison with Pathologic Parameters in Rural Breast Cancer Patients in Eastern India

Nazir Abdul Wasim¹, Arijit Majumdar², Karabi Konar³, Prasenjit Sadhukhan⁴, Debanjan Bhattacharjee^{5*}

¹Department of Pathology, Sarat Chandra Chattopadhyay Government Medical College & Hospital, Howrah, West Bengal, India

²Department of Pathology, Deben Mahata Government Medical College & Hospital, Hatuara, Purulia, West Bengal, India

³Department of Pathology, Malda Medical College & Hospital, Malda, West Bengal, India

⁴Department of Paediatrics, Midnapore Medical College & Hospital, Paschim Medinipur, West Bengal, India

⁵Department of Pathology, Midnapore Medical College & Hospital, Paschim Medinipur, West Bengal, India

DOI: 10.21276/APALM.3329

Abstract

Background: The proto-oncogene Her-2/neu (c-erb-B2) has become an increasingly important prognostic and predictive factor in breast cancer. Overexpression/amplification of Her-2/neu has been associated with a worse outcome in patients with breast cancer. Hormone receptor expression has been reported to be low in breast cancer patients from developing countries, including India. The pattern of receptor expression in rural areas is not well studied.

Materials and Methods: Immunohistochemistry was used for the evaluation of HER-2/neu expression in the mastectomy specimens. Tumor size from the mastectomy specimen was recorded, and the number of lymph nodes was noted. Tissue processing and staining with hematoxylin and eosin were done. Post-mastectomy grading of the malignant breast lesion was done according to the Scarff-Bloom-Richardson (SBR) grading system in hematoxylin and eosin stained slides.

Results: Among the histopathological types of the tumors, IDC NOS constituted 40 cases (86.9%), invasive lobular carcinoma constituted 2 cases (4.3%), medullary carcinoma 2 cases (4.3%), invasive papillary carcinoma and mucinous carcinoma 1 case each (2.2%). The number of HER-2/neu positive cases was 28 (60.9%) and 18 cases were found negative (39.1%). The total number of ER positive and negative cases were 21 (45.7%) and 25 (54.3%), respectively. The total number of PR positive cases was 18 (39.1%) and that of negative cases was 28 (60.9%).

Conclusion: HER-2/neu overexpression is significantly associated with clinicopathological parameters such as younger age group, increased tumor size, higher tumor grades, axillary lymph node metastases, skin involvement, and higher clinical staging.

Keywords:

Breast Carcinoma, Histological Subtypes, HER-2/neu Expression, Eastern India

*Corresponding Author:

Dr Debanjan Bhattacharjee

drdebanjanbhattacharjee@patho@gmail.com

Submitted: 17-Mar-2024

Final Revision: 30-Jun-2024

Acceptance: 19-Jul-2024

Publication: 11-Aug-2024



This work is licensed under the Creative Commons Attribution 4.0 License. Published by Pacific Group of e-Journals (PaGe)

Introduction

Breast cancer is the most prevalent cancer and the foremost cause of cancer death among women across the globe. Asia accounts for 50% of all breast cancer deaths worldwide and 43% of new cases diagnosed each year. With an estimated 2.3 million new

cases in 2020, or 11.7% of all cancer cases, breast cancer in females has completely surpassed lung cancer as the leading cause of cancer incidence worldwide [1,2]. Breast cancer is the most common cancer in India, with a prevalence rate of 25.8 cases per 100,000 people. In 2020, it accounted for 13.6% of all new cancer cases and roughly 13.3 fatalities per 100,000 people [1,2]. Approximately 90,408 females died on account of breast cancer in 2020 in India [3]. The survival rate for breast cancer has also dropped 2.7 times in cases of detection at stage IV compared to stage I.

Many studies have focused on its pathogenesis and biological behavior. It has been documented that breast cancer is a heterogeneous disease with variable biological and clinical characteristics because of its different genetic makeup [4]. It is well known that proto-oncogenes and tumor suppressor genes are two classes of genes that play a central role in the regulation of cell growth and differentiation. Alterations in one or more of these genes appear to play an important role in the pathogenesis of most human malignancies. Hormone receptor studies such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu) are routinely done in breast carcinoma. These studies not only help in the prognosis of the tumor but also aid in deciding its treatment. The goal of assessing receptor status is to provide the right treatment to the right patient. The role of the pathologist is to accurately assess these biomarkers, and the role of the oncologist is to treat the patient with one of the several established therapies, depending on the hormone status [5].

The HER2/neu proto-oncogene is amplified and/or overexpressed in approximately 25-30% of invasive primary breast cancers [6]. An association has been found between amplification and/or overexpression of HER2/neu and a wide variety of clinical and pathological features of breast carcinoma. These include large tumor size, high grade, lack of steroid receptor expression, axillary lymph nodes metastasis, advanced stage, early relapse, and reduced overall survival [7]. There is much evidence to document the essential role of HER2/neu as a prognostic marker for the aggressiveness of breast cancer.

The aim of our study was to evaluate HER2/neu receptors and compare them with pathological parameters in rural breast cancer patients in Burdwan Medical College and Hospital, West Bengal, undergoing elective mastectomy for carcinoma of the breast.

Materials and Methods

An institution-based cross-sectional observational study was conducted at Burdwan Medical College & Hospital among adult female patients with a history of breast lump, with or without enlarged lymph nodes, from rural areas around Burdwan. All patients suspected of breast malignancy were clinically evaluated if they were older than 25 years (as breast cancer is more common beyond this age) and were included in the study. All mastectomy specimens sent to the Department of Pathology for histopathological examination were considered in the study. The study was conducted after receiving approval from the Institutional Ethical Committee, Burdwan Medical College & Hospital, West Bengal.

Parameters Studied

Immunohistochemistry was used for the evaluation of HER-2/neu expression in the mastectomy specimens. Tumor size from the mastectomy specimens was recorded, and the number of lymph nodes was noted. Tissue processing and staining with hematoxylin & eosin were done. Post-mastectomy grading of the malignant breast lesion was conducted according to the Scarff-Bloom-Richardson grading (SBR) system in hematoxylin & eosin-stained slides. Detailed clinical examination of the breast and the lump was done, and the presence of any axillary or cervical lymph nodes was also noted.

In H&E-stained histopathological sections, the following parameters were studied in cases of primary tumors: histological type, microscopic grading (Nottingham's Modification of Bloom-Richardson's grading system), degree of differentiation, and stromal characteristics. In cases of lymph nodes, the number of lymph nodes showing invasion by the tumor and the pattern of lymph node reaction was noted, whether it was follicular hyperplasia or sinus histiocytosis.

Immunostaining of the HER-2 protein was performed for all specimens. A semi-quantitative score was used to record the results of Estrogen receptor & Progesterone receptor staining. HER-2 was scored from 0 to 3 according to the criteria set by Dako [Table 1].

During analysis, scores of 0 and 1+ were taken as negative for HER-2/neu, and scores of 2+ and 3+ were taken as positive for HER-2/neu expression. Data entry was done immediately after capturing the relevant data for a given subject was complete. Logistic regression, discriminant function, and multivariate analysis by SPSS software for Windows were used for data analysis.

Table 1: Evaluation criteria for HER2 expression by IHC assay in breast cancer [10]

IHC Score	Staining Pattern	HER2 Expression
0	No staining or incomplete membrane staining which is faint or barely perceptible in $\leq 10\%$ of invasive tumor cells	Negative
1+	Incomplete membrane staining which is faint or barely perceptible in $>10\%$ of invasive tumor cells	Low expression
2+	(a) Weak to moderate membrane staining with uneven brownish yellow coloration in $>10\%$ of invasive tumor cells (b) $\leq 10\%$ of invasive tumor cells have circumferential membrane staining which is complete, intense, and has brownish coloration	Equivocal (low expression if the slide is ISH-negative, positive if it is ISH-positive.)
3+	$>10\%$ of invasive tumor cells have circumferential membrane staining which is complete, intense, and has brownish coloration	Positive

Results

Forty-six (46) formalin-fixed and paraffin-embedded surgical specimens of different malignant lesions of the breast were studied based on histopathological and immunohistochemical findings.

Age Distribution and Histopathological Types of Tumours: The number of invasive ductal carcinoma (IDC NOS) cases was 40 (85.9%). Of these, 7 cases (17.5%) were between 30-39 years, 18 cases (45%) were between 40-49 years, 11 cases (27.5%) were between 50-59 years, and 4 cases (10%) were obtained at 60 years or above.

Invasive lobular carcinomas were observed on 2 occasions (4.3%); 1 case was between 40-49 years of age, and the other was above 60 years of age. The number of medullary carcinoma cases was 2 (4.3%), both of which were seen in the age range between 40-49 years. Only 1 (2.2%) case of invasive papillary carcinoma was found in the age range between 50-59 years (Table 2).

Age Distribution and Histopathological Grades of the Tumours: Grading of 42 cases was done, and only 1 case (2.4%) of Grade I was seen, whereas 25 cases (59.5%) belonged to Grade II, and 16 cases (38.1%) to Grade III. The Grade I case was observed in the age range of 40-49 years. Out of 25 cases of Grade II, 4 cases (9.5%) were between 30-39 years of age, 11 cases (26.2%) were between 40-49 years, 8 cases (19.1%) were between 50-59 years, and 2 cases (4.8%) were found at 60 years or above (Table 3).

Table 2: Age distribution and histopathological types of tumours

Age (Years)	No of IDC cases	Percentage
30-39	7	17.5
40-49	18	45
50-59	11	27.5
60 and above	4	10
	Invasive Lobular Carcinoma	
30-39	0	0
40-49	1	50
50-59	0	0
60 and above	1	50
	Medullary Carcinoma	
30-39	0	0
40-49	2	100
50-59	0	0
60 and above	0	0
	Invasive Papillary Carcinoma	
30-39	0	0
40-49	0	0
50-59	1	100
60 and above	0	0
	Mucinous Carcinoma	
30-39	0	0
40-49	1	100
50-59	0	0
60 and above	0	0

Table 3: Age distribution and histopathological grades of the tumours (n= 42)

Age (Years)	Grade I	Grade II	Grade III
30-39	0	4(9.5%)	3(7.1%)
40-49	1(2.4%)	11(26.2%)	7(16.7%)
50-59	0	8(19.1%)	4(9.5%)
60 and above	0	2(4.8%)	2(4.8%)

Histopathological Types of Tumours Seen: Among the histopathological types of the tumours, IDC NOS constituted 40 cases (86.9%), invasive lobular carcinoma constituted 2 cases (4.3%), medullary carcinoma 2 cases (4.3%), and invasive papillary carcinoma and mucinous carcinoma 1 case each (2.2%) (Table 4).

Table 4: Histopathological types of tumours

Histopathological Type	No	Percentage
IDC	40	86.9
Invasive Lobular Carcinoma	2	4.3
Medullary Carcinoma	2	4.3
Invasive Papillary Carcinoma	1	2.2
Mucinous Carcinoma	1	2.2
Total	46	100

Comparison Between Immunohistochemical Findings and Different Clinicopathologic Findings

HER-2/neu and Clinicopathological Comparison: The number of HER-2/neu positive cases was 28 (60.9%), and 18 cases were found negative (39.1%). The number of positive cases in the age range of 30-39 years was 4 (8.7%), in 40-49 years it was 17 (36.9%), in 50-59 years it was 4 (8.7%), and 3 (6.5%) in the age range of 60 years or above. The number of negative cases in the age range of 30-39 years was 3 (6.5%), in 40-49 years it was 5 (10.9%), in 50-59 years it was 8 (17.4%), and 2 (4.3%) in the age range of 60 years or above. The value of χ^2 for HER-2/neu positivity in different age groups was 4.39 (at 1 df), and a significant association ($p < 0.05$) was found. This implies HER-2/neu positivity declines with increasing age (Table 5).

Table 5: Age, tumour size and HER-2/neu expression

Age Groups	HER-2/neu expression			
	HER-2/neu Positive		HER-2/neu Negative	
30-39	4	8.7%	3	6.5%
40-49	17	36.9%	5	10.9%
50-59	4	8.7%	8	17.4%
>=60	3	6.5%	2	4.3%
Total	28	60.9%	18	39.1%
Tumour Size	HER-2/neu Positive		HER-2/neu Negative	
T1	0	0%	2	4.3%
T2	2	4.3%	8	17.4%
T3	4	8.7%	1	2.2%
T4	22	47.8%	7	15.2%

Tumour Size and HER-2/neu Expression: The number of positive cases in T1 was 0, in T2 it was 2 (4.3%), in T3 it was 4 (8.7%), and 22 (47.8%) in T4. The number of negative cases in T1 was 2 (4.3%), in T2 it was 8 (17.4%), in T3 it was 1 (2.2%), and 7 (15.2%) in T4. The value of χ^2 for HER-2/neu positivity in different tumour sizes was 11.12 (at 1 df), and a significant association ($p < 0.001$) was found. This implies HER-2/neu positivity increases with the increase in size of tumours (Table 5).

Histopathological Grades and HER-2/neu Expression (Grading of 42 Cases Done): No case was positive for HER-2/neu in Grade I, 11 cases (26.2%) were positive in Grade II, and 13 cases (30.6%) were positive in Grade III. About 1 (2.4%) case was negative for HER-2/neu in Grade I, 14 cases (33.3%) were negative in Grade II, and 3 cases (7.1%) were negative in Grade III. The value of χ^2 for HER-2/neu positivity in different histopathological grades was 4.64 (at 1 df), and a significant association ($p < 0.05$) was found. This implies HER-2/neu expression is more in higher grades (Table 6).

Table 6: Histological grade and HER-2/NEU expression

Grade	HER-2/neu expression			
	HER-2/neu Positive		HER-2/neu Negative	
I	0	0%	1	2.4%
II	11	26.2%	14	33.3%
III	13	30.9%	3	7.1%
Total	24	57.1%	18	42.9%

Histopathological Types and HER-2/neu Expression: Out of the 28 positive cases for HER-2/neu, 23 cases (50%) were positive

for IDC NOS, 2 cases (4.3%) each for invasive lobular carcinoma and medullary carcinoma, and 1 case (2.2%) for papillary carcinoma. Out of the 18 negative cases for HER-2/neu, 17 cases (35.9%) were negative for IDC NOS, and 1 case (2.2%) for mucinous carcinoma (Table 7/ Fig. 1-2).

Table 7: HP types and HER-2/NEU expression

HP Types	HER-2/neu expression			
	HER-2/neu Positive		HER-2/neu Negative	
IDC	23	50%	17	35.9%
Invasive Lobular Carcinoma	2	4.3%	0	0%
Papillary Carcinoma	1	2.2%	0	0%
Mucinous Carcinoma	0	0%	1	2.2%
Medullary Carcinoma	2	4.3%	0	0%
Total	28	60.9%	18	39.1%

Lymph Node Metastases and HER-2/neu Expression: Out of the 28 positive HER-2/neu cases, 24 cases (52.2%) showed lymph node metastases, while 4 cases (8.7%) were negative for lymph node metastases. Out of the 18 negative HER-2/neu cases, 7 cases (15.2%) showed lymph node metastases, while 11 cases (23.9%) were negative for lymph node metastases. The value of χ^2 for HER-2/neu positivity in lymph node metastases was 8.90 (at 1 df), and a significant association ($p < 0.01$) was found. This implies HER-2/neu positive cases showed more lymph node metastases (Table 8).

Table 8: Lymph node metastases and HER-2/NEU expression

Lymph Node Metastases	HER-2/neu expression			
	HER-2/neu Positive		HER-2/neu Negative	
Positive	24	52.2%	7	15.2%
Negative	4	8.7%	11	23.9%
Total	28	60.9%	18	39.1%

Clinical Staging and HER-2/neu Expression: Out of the 28 positive HER-2/neu cases, 1 case (2.2%) each was positive in stage IIA and IIB, 4 (8.7%) cases were positive in stage IIIA, and 22 (47.8%) cases in stage IIIB. Out of the 18 negative cases for HER-2/neu, 7 cases (15.2%) each were found in stage IIA and IIIB, and 2 (4.3%) cases each in stage IIB and IIIA. The value of χ^2 for HER-2/neu positivity in clinical staging was 7.4 (at 1 df), and a significant association ($p < 0.01$) was found. This implies HER-2/neu positivity increases with higher staging (Table 9).

Table 9: Clinical staging and HER-2/NEU expression

Staging	HER-2/neu expression			
	HER-2/neu Positive		HER-2/neu Negative	
I	0	0%	0	0%
IIA	1	2.2%	7	15.2%
IIB	1	2.2%	2	4.3%
IIIA	4	8.7%	2	4.3%
IIIB	22	47.8%	7	15.2%
IV	0	0%	0	0%
Total	28	60.9%	18	39.9%

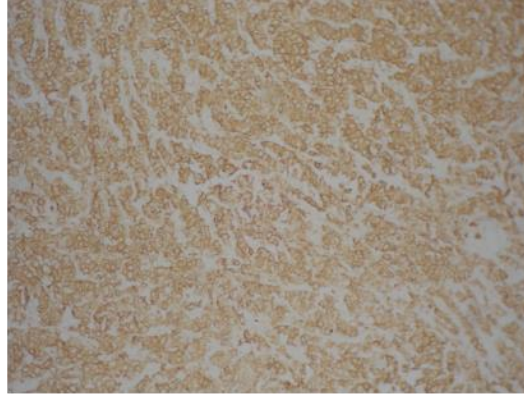


Figure 1: HER-2/neu-- 3+ (100X)

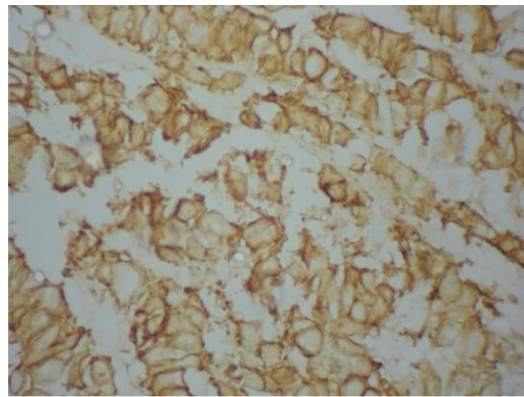


Figure 2: HER-2/neu-- 3+ (400X)

Discussion

Among the various prognostic and predictive factors of breast cancer, the most widely studied biomarker is the human epidermal growth factor receptor 2 (HER2) gene, also referred to as ERBB2 or HER2/neu, which is amplified in approximately 18-20 percent of all breast cancers. Amplification of this gene is associated with the rapid progression of the disease, increased metastatic potential, increased resistance to tamoxifen, and better response to anthracycline-based chemotherapy [11].

In the present study, 46 formalin-fixed and paraffin-embedded surgical specimens of invasive breast carcinoma were evaluated for ER, PR expression, and HER2/neu protein overexpression by applying immunohistochemical staining using antibodies for ER, PR, and HER2/neu. These were compared with clinicopathological parameters like age, tumor size, histopathological grades, lymph node metastases, skin involvement, and surgical staging.

In our study, 60.9% of cases were positive for HER2/neu, while 30.9% were negative. This result is significantly higher than those reported by many Western researchers, as reported by Marc J. et al. (1988) [12] and Yamashita et al. (2004) [13] (14.5% and 20.1%, respectively). Asughayer et al. (2006) [14] demonstrated much lower results (16.9%) in Jordanian women. Looi LM et al. (1998) [15] found HER2/neu was expressed in 20% of cases. Ariga R. in 2005 [16] observed 22% of breast cancer patients showed HER2/neu positivity. However, the study conducted by Esraa Abdul-Aal Salman Al-Dujaily in 2008 [17] revealed 67.8% HER2/neu positive cases. Saleh et al. (2007) [18] reported much higher results of HER2/neu overexpression among Kuwaiti women (87%). Panjwani P. et al. in 2010 [19] also observed that in the Indian scenario, 46.8% of cases showed 3+ reactivity of

HER2/neu, and 20.7% of cases showed 2+ reactivity of HER2/neu, while 32.5% of cases were negative for HER2/neu. The last three studies were consistent with the findings of our study. The differences in observations may be due to the differences in the sample size included in each study, or there may be geographical variation in HER2/neu expression.

In our study, the number of positive cases in the age range of 30-39 years was 4 (8.7%), in 40-49 years it was 17 (36.9%), in 50-59 years it was 4 (8.7%), and 3 (6.5%) in the age range of 60 years or above. The number of negative cases in the age range of 30-39 years was 3 (6.5%), in 40-49 years it was 5 (10.9%), in 50-59 years it was 8 (17.4%), and 2 (4.3%) in the age range of 60 years or above. These findings suggest that HER2/neu expression declines with age. This is in accordance with what is reported in the literature that HER2/neu expression declines with age (Eppenberger-Castori S. 2001) [20]. Kaptain et al. in 2001 [21] found that 55% of cases below 40 years and 45% of cases above 40 years of age showed HER2/neu overexpression. According to Naqvi SQH in 2007 [22], 53% of the cases below 40 years and 47% of the cases above 40 years had HER2/neu overexpression. However, Esraa Abdul-Aal Salman Al-Dujaily in 2008 [17] found HER2/neu positivity in the older age group as compared to the younger ones. So the results of HER2/neu overexpression and patients' age are consistent with some of the studies done previously.

Regarding tumor size, we found a significant association between increased tumor size and HER2/neu overexpression. Similar results are noted by Descotes et al. in 1993 [23], Looi LM in 1998 [15], Naqvi SQH in 2002 [22], Taucher in 2003 [24], Almasri et al. in 2005 [25], and Al Rashed et al. in 2007 [4]. However, Esraa Abdul-Aal Salman Al-Dujaily in 2008 [17] did not find any significant association between tumor size and HER2/neu overexpression. So, it can be said our results regarding tumor size and HER2/neu positivity are compatible with some of the studies done previously.

In our study, 42 cases were graded according to the Nottingham modification of Bloom Richardson. Only 1 case (2.4%) of Grade I was seen, whereas 25 cases (59.5%) belonged to Grade II, and 16 cases (38.1%) to Grade III. Our study revealed a significant association ($p < 0.05$) between HER2/neu overexpression and higher grades. Looi LM in 1998 [15] concluded that most of the cases showing HER2/neu positivity fell in histopathological Grade III. Al-Moundhri et al. in 2003 [26] showed that HER2/neu overexpression was higher in Grade III than Grade I breast tumors, without significant difference ($P > 0.05$). Ariga R. in 2005 [16] also found a positive correlation between high-grade tumors and HER2/neu overexpression. Al Rashed et al. in 2007 [4] showed that HER2/neu was positively stained in 4% of low-grade tumors, while 22% of high-grade tumors with a significant difference ($P < 0.05$). Saleh et al. in 2007 [18] showed that Grade II and III tumors predominantly (92.9% and 87.7%, respectively) overexpressed HER2/neu, while Grade I tumors were mostly HER2/neu negative with significant difference ($P < 0.0001$). However, Almasri et al. in 2005 [25] showed no significant difference between HER2 expression and the histological grade of breast carcinoma. So our results matched with many of the previously conducted studies.

Out of the 28 positive HER2/neu cases, 24 cases (52.2%) showed axillary lymph node metastases, while only 7 cases (15.2%) showed lymph node metastases out of the 18 HER2/neu negative cases. Therefore, we found a significant association between HER2/neu overexpression and lymph node metastases. These results agree with the study by Aziz et al. in 2001 [27], who showed a strong correlation between HER2/neu overexpression and axillary lymph node metastases. Almasri et al. in 2005 [25] revealed that 56% of HER2/neu overexpressing tumors had more than three lymph node metastases, as opposed to 42% of HER2/neu negative cases without significant difference. However, Marc J. et al. in 1988 [28] and Esraa Abdul-Aal Salman Al-Dujaily in 2008 [17] did not find any significance between HER2/neu positivity and lymph node metastases.

Out of the 28 positive HER2/neu cases, 1 case (2.2%) each was positive in stage IIA and IIB, 4 (8.7%) cases were positive in stage IIIA, and 22 (47.8%) cases in stage IIIB. Out of the 18 negative cases for HER2/neu, 7 cases (15.2%) each were found in

stage IIA and IIIB, and 2 (4.3%) cases each in stage IIB and IIIA. Hence, we found a significant association between HER2/neu overexpression and staging. Our finding is supported by the study of Almasri et al. in 2005 [25], who found a significant difference between HER2/neu overexpression and staging. In contrast, Aziz et al. in 2001 [27] and Esraa Abdul-Aal Salman Al-Dujaily in 2008 [17] did not find any association between HER2/neu overexpression and staging.

In our study, out of the 28 positive HER2/neu cases, 22 cases (47.8%) showed skin involvement, while 6 cases (13%) did not have skin involvement. Out of the 18 negative HER2/neu cases, 22 cases (47.8%) showed skin involvement, while 6 cases (13%) did not have skin involvement. So, there was a significant association between skin involvement and HER2/neu overexpression. Unfortunately, we could not find any literature that agrees or disagrees with our last finding.

Conclusion

Among the histopathological types of the tumours, IDC NOS constituted 40 cases (86.9%), invasive lobular carcinoma constituted 2 cases (4.3%), medullary carcinoma 2 cases (4.3%), invasive papillary carcinoma and mucinous carcinoma 1 case each (2.2%). The number of HER-2/neu positive cases was 28 (60.9%), and 18 cases were found negative (39.1%). The total number of ER positive and negative cases were 21 (45.7%) and 25 (54.3%), respectively. The total number of PR positive cases was 18 (39.1%) and that of negative cases was 28 (60.9%). This indicates the majority of the tumours did not have hormonal receptor expression. We found a significant association between age distribution and HER-2/neu overexpression, which implies HER-2/neu overexpression decreases with increasing age. However, we could not find any significant association between age distribution and hormonal receptor positivity. Regarding tumour size and immunohistochemical association, we noticed a significant association between HER-2/neu positivity and tumour size, indicating that larger tumours or tumours fixed to the chest wall or having skin fixity had more HER-2/neu overexpression. Higher tumour grades had more HER-2/neu overexpression, and there was a significant association between the two. We observed a significant association between axillary lymph node metastases and HER-2/neu expression, indicating that HER-2/neu positive tumours have more incidences of lymph node metastases. A statistically significant association between skin involvement and HER-2/neu overexpression was seen, which means tumours having skin involvement have higher incidences of HER-2/neu positivity. There was a significant association between clinical staging and HER-2/neu overexpression, implying that HER-2/neu positivity increases with higher clinical staging.

Funding: *This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*

Competing Interests: *The authors have no conflict of interest to declare.*

Statement of Ethical Approval for the Study: *Ethics approval has been taken from IEC, Burdwan Medical College & Hospital, West Bengal.*

Statement of Informed Consent: *Informed consent was obtained from all participants before collecting the data.*

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. *Int J Cancer*. 2021 Apr 5.
2. Park JE. Cancer. In: Park JE, editor. *Park's Textbook of Preventive and Social Medicine*. Banarsidas Bhanot Publishers; 2019. p. 433-434.
3. GLOBOCAN. GLOBOCAN, The Global Cancer Observatory, Fact Sheet, India. [June; 2023]. 2020. Available from: <https://www.uicc.org/news/globocan-2020-new-global-cancer-data> [Accessed 2023 Sep 11].
4. Rashed MM, Ragab NM, Galal MK. The association of HER-2/NEU over-expression in relation to P53 nuclear accumulation, hormonal receptor status and common clinico-pathological prognostic parameters in a series of Egyptian women with invasive ductal carcinoma. *Eur J Gen Med*. 2007;4(2):73-9.
5. Mohamad IB, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. *Med J Dr D.Y. Patil Univ*. 2016 Nov-Dec;9(6):674-9.
6. Coussens L, Yang-Feng TL, Lioa YC, Chen Eea. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*. 1985;230:1132-9.
7. Anwar N, Mokhtar N, Mourad M, et al. *cerbB2* and its relation to lymph node positivity: Short term follow-up in Egyptian cancer patients. *CMB*. 1994;1:325-9.
8. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer*. 1957;11:359-77.
9. Nottingham or Bloom-Richardson (BR) Score/Grade. Available from: <https://staging.seer.cancer.gov/cs/input/02.05.50/breast/ssf7/?version=/tnm/home/1.7/> [Accessed 2023 Sep 11].
10. Che Y, Ren F, Zhang X, Cui L, Wu H, Zhao Z. Immunohistochemical HER2 Recognition and Analysis of Breast Cancer Based on Deep Learning. *Diagnostics (Basel)*. 2023 Jan 10;13(2):263.
11. Walker RA. Use and assessment of diagnostic and predictive markers in breast pathology. *Curr Diagn Pathol*. 2007;13:126-34.
12. van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, Nusse R. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med*. 1988 Nov 10;319(19):1239-45.
13. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, et al. Breast and Endocrine Surgery, Nagoya City University Hospital, Nagoya, Japan. *Breast Cancer Res*. 2004;10:186/738.
14. Sughayer MA, Al-Khawaja MM, Massarweh S, Al-Masri M. Prevalence of hormone receptors and HER2/neu in breast cancer cases in Jordan. *Pathol Oncol Res*. 2006;12(2):83-6.
15. Looi LM, Cheah PL. C-erbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast correlates to high histologic grade and loss of estrogen receptor protein. *Malays J Pathol*. 1998;20:19-23.
16. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P, et al. Correlation of her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. *Breast J*. 2005;11:278-80.
17. Al-Dujaily ES. Pathological Study of Breast Cancer By Application of Epidermal Growth Factor Receptor Type II(HER-2/neu) (Immunohistochemical study) [thesis]. 2008.
18. Saleh F, Abdeen S. Pathobiological features of breast tumours in the State of Kuwait: a comprehensive analysis. *J Carcinog*. 2007 Sep 24;6:12.
19. Panjwani P, Epari S, Karpate A, Shirsat H, Rajsekharan P, Basak R, et al. Assessment of HER-2/neu status in breast cancer using fluorescence in situ hybridization & immunohistochemistry: Experience of a tertiary cancer referral centre in India. *Indian J Med Res*. 2010 Sep;132:287-94.
20. Eppenberger-Castori S, Kueng W, Benz C, Caduff R, Varga Z, Bannwart F, et al. Prognostic and predictive significance of ErbB-2 breast tumor levels measured by enzyme immunoassay. *J Clin Oncol*. 2001 Feb 1;19(3):645-56.
21. Kaptain S, Tan LK, Chen B. Her-2/neu and breast cancer. *Diagn Mol Pathol*. 2001 Sep;10(3):139-52.
22. Naqvi SQH, Jamal Q, Mahmood RK, Zaidi SMH, Abbas F. Significance of HER-2/neu oncoprotein overexpression on node positive invasive breast cancer. *J Coll Phys Surg Pak*. 2002;12:534-7.
23. Descotes F, Adessi GL. Correlation study between HER-2/neu amplification and prognostic factors in an unselected population. *Anticancer Res*. 1993;13:119-24.
24. Taucher S, Rudas M, Mader RM, Gnant M, Dubsy P, Bachleitner T, et al. Do we need HER-2/neu testing for all patients with primary breast carcinoma? *Cancer*. 2003;98:547-53.

25. Almasri NM, Al Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. *Breast Cancer Res.* 2005;7:1200.
26. Al-Moundhri M, Nirmala V, Al-Mawaly K, Ganguly S, Burney I, Rizvi A, et al. Significance of p53, Bcl-2, and HER-2/neu protein expression in Omani Arab females with breast cancer. *Pathol Oncol Res.* 2003;9(4):226-31.
27. Aziz SA, Pervez S, Khan S, et al. Significance of immunohistochemical c-erbB2 product localization pattern for prognosis in human breast cancer. *Pathol Oncol Res.* 2001;7.
28. Marc J, Johannes LP, Wolter JM, Van DV, et al. Neu-protein overexpression in breast cancer: Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med.* 1988;319:1239-45.