Original Article

HER2 Reactivity Pattern and Its Association with Various Tumor Factors in Breast Cancer Patients

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Abstract

Background: Breast cancer prognosis and therapeutic strategies are strongly influenced by biomarker profiles, particularly HER2 reactivity, which impacts treatment selection and disease progression.

Objective: To study the HER2 reactivity pattern and identify the association of HER2 pattern with various tumor factors in patients with breast cancer.

Methods: This prospective study comprised of 150 surgically operated female cases of breast cancer admitted to the Department of Pathology for ER, PR, and HER2 testing between December 2015 and October 2017. Results: Of 150 cases, the mean age was 52 years (IQR, 41-63 years). This study showed that majority of tumors were IDC-NOS, grade 2, tumor size T2, and had negative lymph node status. HER2 negative cases were in the majority, as well as the ER/PR+ cases. Triple-negative cases were slightly higher (49 cases). When HER2 was correlated with tumor characteristics, younger patients with HER2-positive were in the majority, with the higher the tumor size is, the greater the chance for HER2 negativity. The majority of IDC cases and all ILC cases showed HER2 negativity. Both medullary cases showed HER2 positive, and one case of mucinous showed HER2 negativity with ER/PR positivity. Irrespective of ER/PR status, the majority of tumors had HER2 negativity.

Conclusion: HER2/neu positively correlates with increasing age, menstrual status, tumor size, and tumor grade. No association is found between histological type, lymph node status, and ER/PR. This study also reveals that patient with higher levels of HER2/NEU-overexpression had statistically significant lower levels of ER/PR-positive tumors. In addition, when HER2/neu is positive in tumors, along with ER and PR, it is mostly high-grade.

Keywords: Breast cancer, HER2, invasive carcinoma, triple negative

Introduction

Breast cancer is the most commonly diagnosed cancer worldwide and the second leading cause of cancer-related death among women.[1] Around 13.5% account for the new cancer cases and overall common cancer deaths.[2] Human epidermal growth factor receptor-2 (HER2) has emerged as a significant independent predictive marker in recent years. Additionally, the HER2 status has grown in importance as a prognostic indicator for evaluating patient response to Herceptin.

According to the literature, approximately 15% to 30% of breast cancers exhibit HER2 overexpression.[3] Several studies have identified HER2 overexpression in early breast cancer as a poor prognostic factor Several studies have identified HER2 overexpression in early breast cancer as a poor prognostic

factor.[4] Early and metastatic breast cancer patients now have a much better prognosis because of the discovery of trastuzumab.[5] Unfortunately, because trastuzumab is an expensive medication, most patients in developing countries cannot afford it.

This study aimed to evaluate the expression pattern of HER2 in the invasive component of breast carcinoma and to assess the association of HER2 status with various prognostic parameters, including menstrual status, tumor size, histologic grade, histologic type, regional lymph node involvement, and expression of estrogen receptor (ER) and progesterone receptor (PR). The study also aimed to explore a possible relationship between HER2 status and patient age.

Methods

This prospective study comprised 150 surgically operated female cases of breast cancer that were admitted to the Department of Pathology for ER (Estrogen Receptor), PR (Progesterone Receptor), and HER2 testing between December 2015 and October 2017 at a tertiary care Hospital in Aurangabad, Maharashtra, India.

Clinical data was obtained from the hospital records and requisition forms received in the department. Upon arrival, specimens were examined for gross features, routinely processed, and sectioned at 3–5 microns from paraffin-embedded blocks. These sections will be routinely stained with H and E and examined for invasive breast carcinoma presence. Nottingham Richardson classification was used for tumor grading and histological type.[6] The Allred scoring system was utilized to interpret ER and PR positive.[7] Reporting of ER, PR, and HER2/neu status followed the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines.[8,9] The study analyzed correlations between HER2, ER, and PR status and various histological and clinical parameters.

Ethical clearance for this study was obtained from the Mahatma Gandhi Mission's Ethics Committee for Research on Human Subjects, MGM Medical College and Hospital, Aurangabad, Maharashtra, under ethical approval number MGM-ECRHS/2017/26.

Data entry and statistical analysis were performed using IBM SPSS Statistics for Windows, Version 21. Descriptive statistics, including means, standard deviations, and percentages, were calculated.

Results

In this study, 150 women were diagnosed with invasive primary breast carcinoma with a mean age of 52 years± 11 years (IQR: 41–63 years). Breast cancer was most commonly seen in premenopausal women (58%). These results clearly show that most of the patients having breast cancer in India are younger and in the premenopausal menstrual stage. From a radiological perspective, the right breast appeared to be significantly more impacted.

Tumor sizes were classified into three categories. The tumor size of 2–5 cm2 was represented the highest [66%]. The various histological types found in this study were Invasive Duct Carcinoma [IDC] (123 cases), Invasive lobular carcinoma [ILC] (18 cases), medullary carcinoma (1 case), mucinous carcinoma (1 case), mixed (3 cases; 2 cases IDC with medullary and 1 case IDC with apocrine differentiation) and Paget's disease of the nipple (4 cases). Most of the cases (99 cases) were Modified radical mastectomy (MRM) specimens with the majority showing no lymph node involvement. A total of 150 cases of breast cancer were graded according to Modified Bloom Richardson Histologic Grade with the majority of them grade 2. One case could not be graded as no invasion was noted. Among 150 patients, 48% and 54% expressed ER, and PR respectively. Immunohistochemistry evaluation for HER2/NEU was negative for 62% of cases whereas borderline or equivocal in 10%. The equivocal cases were advised fluorescent in-situ hybridization for confirmation. HER2/NEU was positive in 28% of cases. This study showed that the largest category of breast cancer was negative for HER2/NEU. Table 1 presents the clinicopathological characteristics of the study population.

The borderline HER2/NEU 2+ cases were not correlated as they could not be categorized in either positive or negative categories without further subjecting them to Fluorescent in-situ Hybridization (FISH). Table 2 shows the frequency of HER2 and clinicopathologic variables of the study population.

HER2-positive status was observed mostly in patients aged ≤50 years, and HER2 status was significantly associated with age (p=0.013) and menopausal status (p=0.009). In this study, it showed that, out of 150 cases, most tumors were T2 tumors, and out of these HER-2 negative cases were 63, positive were 30, and equivocal were 6 cases. A statistical significance was seen when the HER2/NEU

Table 1 Clinicopathological Characteristics of the Study Population (n 150)

Characteristic	Parameters	Number of Cases
Age	≤50 years	96
	>50 years	54
Menopausal status	Pre-menopausal	87
	Post-menopausal	63
Laterality	Right breast	77
	Left breast	72
	Bilateral	1
Tumor size	<2	9
	2–4	99
	>5	27
	Any size growing into the chest/skin	15
Histology	Invasive ductal carcinoma (IDC)	123
	Invasive lobular carcinoma (ILC)	18
	Medullary carcinoma	1
	Mucinous carcinoma	1
	Mixed	3
	Paget's disease of nipple	4
Histologic grade	Grade 1 (low)	36
	Grade 2 (moderate)	59
	Grader 3 (high)	55
Axillary Node Status	Negative (0 nodes)	73
	Positive (1-3 nodes)	59
	Positive (4-9 nodes)	6
	Positive (> 9 nodes)	12
Estrogen Receptor (ER)	Positive	72
	Negative	78
Progesterone Receptor (PR)	Positive	81
	Negative	69
HER2/Neu Receptor	Positive	42
	Negative	93
	Equivocal	15
ER/PR	ER/PR+	84
	ER/PR-	66

** ER/PR=estrogen receptor/progesterone receptor; HER2=human epidermal growth factor receptor 2

expression profiles were correlated with tumor size (p=0.001). HER-2 receptor status in the cases of invasive carcinoma (no special type), showed that the maximum cases were of HER-2 negative status. All 18 cases of invasive lobular carcinoma were found to be HER2 negative. One case of medullary was HER-2 positive and one case of mucinous was HER-

Table 2 Frequency of HER2 Status by Clinicopathologic Variables

Variables	HER2 Positive		HER2 Negative		HER2 Equivocal		p-value
	(n=42)	%	(n=93)	%	(n=15)	%	
Age							
≤50 years	32	76	50	54	14	93	0.013
>50 years	10	24	43	46	1	7	
Menopausal Status							
Pre-menopausal	30	71	44	47	13	87	0.009
Post-menopausal	12	29	49	53	2	13	
Tumor size							
< 2cm	1	3	2	2	6	40	
2–5 cm	29	69	64	69	6	40	0.001
>5cm	3	7	24	26	0	0	
Any	9	21	3	3	3	20	
Histologic Type							
IDL	36	86	73	79	14	93	
ILC	0	0	18	19	0	0	0.324
Others	6	14	2	2	1	7	
Tumor Grade							
Grade 1	10	24	21	23	5	33	
Grade 2	23	56	29	31	6	40	0.007
Grade 3	8	20	43	46	4	27	
Lymph Node Status							
0 Nodes	25	60	45	48	3	20	
1-3 Nodes	17	40	39	42	3	20	0.526
4-9 Nodes	0	0	0	0	6	40	
>9 Nodes	0	0	9	10	3	20	
ER Status							
ER Positive	18	43	45	48	9	60	0.551
ER Negative	24	57	48	52	6	40	
PR Status							
PR Positive	24	57	47	51	10	67	0.476
PR Negative	18	43	46	59	5	33	

**HER2 = human epidermal growth factor receptor 2, ER = estrogen receptor, PR = progesterone receptor, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma

2 negative. Three out of four cases of Paget's disease were found to be HER-2 positive and one with HER-2 equivocal status. In the mixed carcinoma cases, both cases of IDC with medullary showed HER-2 status positive, and the case with IDC with apocrine showed HER-2 status negative. HER2 status was statistically not significantly associated with histologic type (p = 0.324).

The majority of HER2 negative cases were seen in grade 3 and HER2 positive cases were seen in grade 2 tumors. One case of Paget's disease of nipple with ductal carcinoma in situ did not show invasion and could not be graded.

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Table 3 Hormone Receptor Status by Histologic Type

Histologic Type	ER/PR+(Luminal A/B)	HER2 Equivocal	HER2-rich	Triple-Negative
Invasive Ductal Carcinoma (IDC)	67	3	14	39
Invasive Lobular Carcinoma (ILC)	12	0	0	6
Medullary Carcinoma	1	0	0	0
Mucinous Carcinoma	1	0	0	0
Paget's Disease	0	1	0	3
Mixed	2	0	0	1

However, the HER2 positivity was closely related to grade (p = 0.007). The majority of HER2 negative receptors were seen when lymph node status was negative (no nodes seen). No significant association was noted (p=0.526).

Association of HER2 with ER (p = 0.551) and PR status (p = 0.476) did not show much difference and was not statistically significant. HER2/NEU profiles were correlated with ER, PR expression profiles, it derived into an inverse correlation (p value < 0.05). The HER2/NEU positive tumors have less chance of having expression of ER PR.

Hormone receptor status with histologic types showed that 67 cases of IDC were ER/PR+, 3 cases were equivocal, 14 cases were HER2 rich (ER-/PR-) and 39 cases were triple negative. In the case of ILC, 12 cases were ER/PR positive, and 6 cases were triple negative. All the cases of medullary and 1 case of mucinous showed ER/PR positive, however, both the cases of medullary were HER2 positive and 1 case of mucinous was HER2 negative. In the case of 4 Paget's disease, 1 case was excluded for IHC as there was no invasion, 1 case showed HER2 equivocal and 3 cases showed Triple negative. In total,49 cases of triple-negative breast cancer were seen in this study. Table 3 shows hormone receptor status with histologic type.

Discussion

This study found that 28% of breast cancer cases were HER2-positive and showed statistically significant associations with age, menstrual status, tumor size, and tumor grade. No significant associations were observed with histologic type or lymph node status. An inverse association was seen between HER2 and hormone receptors ER/PR.

The HER2-positivity rate in this study (28%) is consistent with previous Asian data, such as the study by Mudduwa et al.[10] in Sri Lanka, which reported 19.1% HER2-positivity among breast carcinoma cases. However, the frequency of HER2/NEU positivity varies among Indian studies.

The mean age of patients included in this study was 52 years. The results are in concordance with the study conducted by Mudduwa et al.[10] in which the mean age was 52.5 years and 85.7% of the patients were more than 40 years. Similar to other studies, (Gullick et al.[11]; Amirifard et al.[12]) a statistically significant correlation between age and HER2-neu overexpression was seen in this study. Premenopausal women overexpressed more HER2 than postmenopausal women. Similar results were observed by several authors.

HER-2 receptor negativity was seen in the majority of T2 tumors and this was in concordance with the study done by Bergeron et al.[13] in which the majority of HER-2 receptor negativity was present in 31 out of 139 (31.2%) stage II lesions.

HER-2 receptor status in the cases of invasive carcinoma (no special type), showed that the maximum cases were of HER-2 negative status. This was in discordance with the study done by Bergeron et al.[13] which showed 68 out of 185 cases of invasive carcinoma to have HER2 overexpression.

HER-2 neu expression was correlated in high-grade tumors, whereas grade I tumors were expressing higher ER&PR, which was comparable to a study done by Onitilo et al.[14] In this study, the majority of HER2-negative cases were seen in grade 3 tumors. This finding was also seen in the study done by Arafah et al.[15] in which the majority of HER2 negative cases were seen in the grade 3 tumor The majority of HER2 negative cases were

seen in negative lymph node status. This was also in concordance with the study done by Englander et al.[15] in which around 72 patients were seen to have HER2 negative status in negative lymph node status.

This study shows that most of the patients who had Her-2/neu protein over-expression were ER-/PR- and this was in concordance with the study done by Ariga et al.[17] Notably, a relatively high number of triple-negative breast cancer (TNBC) cases (n=49) was identified in this cohort. While higher than commonly reported in literature, this finding was in agreement with observations by Ahmed et al.[18]

The proportions of ER-positive and ER-negative, cases did not differ much. Also, PR positive and PR negative cases also did not differ much, although both ER and PR negative cases were slightly higher than ER/PR positive cases. Also, the proportion of ER+/PR+/HER2+ and ER-/PR-/HER2 cases did not differ much, although ER+/- and PR+/- with HER2 negative cases were much higher. And this was in concordance with the study done by Onitilo et al.[14] in which there were 65% of HER2 negative tumors.

Out of 150 malignant cases, an intriguing finding was that: only two cases had ER-/PR+. This was a controversial finding in terms of clinical significance. Some reports claim that this subtype represents a distinct, clinically useful entity, while others pose this to be a technical artefact and not very clinically significant. PR has no independent prognostic value in patients with ER-.[19] PR expression is a respective marker of a functional ER pathway. Hence patients with ER(-)/PR(+) subtype can still have survival benefits from hormone therapy. This phenotype has been described by Shen et al.[20]

The majority of invasive carcinoma (NST), were ER/PR+. This contradicted the findings of Devi et al. study.[21] The literature states that medullary cancer expresses no HER2/neu; nevertheless, two medullary instances expressed ER, PR and HER2/neu, which is consistent with the findings of Devi et al. study.[21] Due to funding limitations, the cases of HER2 equivocal could not be confirmed with Fluorescent in-situ Hybridization (FISH).

In conclusion, immunohistochemical analysis of ER, PR, and HER2 receptors is widely accessible and cost-effective, providing critical prognostic, predictive, and therapeutic guidance. Despite heterogeneity in hormone receptor expression patterns, routine testing for HER2 along with ER and PR status is essential for optimal clinical management. Further research should be carried out to understand the various trends and relationships between different variables associated with breast cancer for better prognosis of breast cancer patients in order to obtain better clinical outcomes with improved survival.

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