Her-2/neu expression and its correlation with ER status and various clinicopathological parameters

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Abstract

Introduction: Breast carcinoma is the most common malignancy in urban population in India. We conducted a one year retrospective study with an aim to study the expression of Her-2/neu gene in breast cancers and its correlation with the age of patient, ER status, axillary lymph node status, grade of tumor, stage, histological type and size of tumor.

Materials and Methods: The study was conducted in the histopathology department of The Gujarat Cancer and Research Institute, Ahmedabad on hundred random mastectomy specimens having carcinoma breast received over a period of one year. Sections were taken and stained with H&E. All tumors were histologically typed, graded and staged. Immunohistochemistry for ER, PR and Her-2/neu was applied on any one selected tumor section having an internal control as well.

Results: Out of the total 100 cases, 67% of the patients belonged to > 45 years age group. Invasive Duct Carcinoma was the most common histological type (83%). Her-2/neu expression was found in 62% of the cases. Majority of the tumors were Grade 2 (45%). Her-2/neu overexpression was observed in tumors > 5cm in size, showing ER negativity, having a higher grade and showing axillary lymph node metastasis (p<0.05). However, tumor stage, age of the patient, histological tumor type did not show any significant correlation with Her-2/neu expression (p>0.05).

Conclusion: Her-2/neu expression is common in Indian women. All high grade large tumors (>5cms) showing lymph node metastasis should undergo a Her-2/neu testing because of its prognostic and therapeutic significance.

Keywords: Breast carcinoma, Her-2/neu, ER/PR, Lymph node.

Introduction

Breast cancer is the most common cancer in women and the main cause of death in 35- 55 years old women.[1] In India, breast cancer is second to cancer of cervix among women, but is considered the leading cancer in certain metros.[2] An association has been found to exist between ER/PR status, Her-2/neu amplification or over expression and a wide variety of different clinical and pathological features including tumor size, tumor grade, stage, axillary lymph node status, age, sex, menopausal status, histological subtype etc. ER/PR and Her-2/neu immunostaining are important prognostic markers and are important ~~for~~ from treatment point of view as well. Determination of ER/PR and Her-2/neu status in biopsy specimen prior to therapeutic intervention is now advocated as a standard practice.[3]

The aim of our study was:
1. To study the expression of ER/PR and Her-2/neu in invasive breast carcinoma.
2. To correlate Her2/neu expression with various clinico-pathologic aspects.

Materials and Methods

The study was retrospectively carried out in the department of pathology, GCRI, Ahmedabad. A total of 100 Modified Radical Mastectomy (MRM) specimens received over a period of one year were included in the study. Patients having already received treatment in the form of radiotherapy or chemotherapy before MRM were excluded from the study. All the cases included had invasive epithelial malignancy. A detailed clinical history of all the patients was obtained from the case files maintained at the institute. The specimens were received in formalin, cut and fixed overnight. A detailed gross examination was carried out. Sections from tumor and lymph nodes were taken, processed and embedded in paraffin, stained with hematoxylin & eosin stain and studied under microscope to formulate a histological diagnosis comprising of the histological type of tumor, grade of tumor, lymph node status and the pathological TNM stage of tumor. All the cases diagnosed as Invasive Ductal Carcinoma, NOS were graded according to the Nottingham Histologic Score [Glandular differentiation (score 1 to 3), nuclear pleomorphism (score 1 to 3), mitotic rate (score 1 to 3)]. The other histological types were not graded. For immunohistochemistry (IHC), a section from tumor proper having internal control as well was selected. IHC was not performed on the lymph nodes. 3-4 microns thick sections were taken on Poly L-lysine coated slides. For antigen retrieval, slides were kept in citrate buffer at pH 2.5 (for ER/PR) and pH 6 (for Her-2/neu) at 100^0C in a pressure cooker for 20-25 minutes, treated with 0.6% H_2O_2 (prepared in methanol), rinsed in running tap water for 5-10 minutes. Primary and secondary antibodies were added, incubated, stained with 3'3' diaminobenzidine (DAB) and then counterstained with hematoxylin. The normal duct epithelium served as internal control for ER/PR.

External control for Her-2/neu was applied for each lot using a case with known Her-2/neu positivity. ER/PR stains were interpreted as positive when >1% of tumor cells showed nuclear staining as per the ASCO/CAP 2014 guidelines. Her-2/neu staining was interpreted as follows (ASCO/CAP 2014 guidelines):

0 (negative) – No staining is observed or membranous staining is observed in less than 10% of tumor cells.

1+ (negative) – A faint/ barely perceptible membranous staining is detected in more than 10% of tumor cells.

2+ (equivocal) – A weak to moderate complete membranous staining is observed in more than 10% of tumor cells.

3+ (positive) – A strong complete membranous staining is observed in >30% of tumor cells.

A score of 3+ was considered as positive in this study.

Results

Amongst the 100 cases of invasive duct carcinoma, the age of patients ranged from 30 to 70 years. Most of the patients presented with >45 years of age (67%). 62% of the cases were Her-2/neu +, 44% expressed ER and 31% expressed PR. The most common histological subtype was IDC (83%). Other histological types were Invasive lobular carcinoma (3%), medullary carcinoma (3%), metaplastic carcinoma (3%), Invasive papillary carcinoma (3%), Invasive cribriform carcinoma (2%), apocrine carcinoma (1%) and Mucinous carcinoma (2%). Majority of the cases showed lymph node metastasis (53%). Grading was done only for IDC, NOS types and majority of them presented as grade 2 (45%).Tumor size in the majority of cases was <5cm.

ER expression was significantly lower in the Her-2/neu positive as compared with Her-2/neu negative tumors (35.4% vs 57.8%). However no significant association was seen between Her-2/neu and PR. Her-2/neu expression increased with the increasing grade of tumor. However no such association was seen with the stage of tumor. Tumors were staged according to the pathological TNM staging system. Combined ER and PR positivity was inversely related to Her-2/neu expression (27.4% vs 36.8%). Her-2/neu was overexpressed in cases showing lymph node metastasis (71.6%). It was also observed that Her-2/neu expression increased with increasing size of tumor. It was positively expressed in 85.7% cases, whose tumor size was > 5cm. Table 1 and 2 show Her-2/neu expression and its correlation with various histopathological parameters. To establish a correlation between Her-2/neu and the parameters, chi square test was applied and a *p* value was obtained for each parameter. *p* value <0.05 was considered as significant.

Table 1: Relationship of Her-2/neu expression with the lymph Node status, size, grade and ER status of tumor showing statistically significant correlation. (p<0.05), [n=no. of cases]

Her-2/neu	LN +	LN −	Size<5cm	Size>5cm	Grade I	Grade II	Grade III	ER+	ER-
Positive	38	24	44	18	4	32	26	22	40
Negative	15	23	35	3	11	22	5	22	16
Total	53	47	79	21	15	54	31	44	56
Chi-square value	4.5		6.337		8.121			4.7	
p value (<0.05)	0.033		0.0118		0.0172			0.028	

Table 2: Combined table showing Her-2/neu expression with all the clinico-pathological parameters

Parameters	Her-2/neu+(% of cases) (n=62)	Her-2/neu-(% of cases) (n=38)
Age		
<45 yrs	29% (n=18)	39.4% (n=15)
>or=45 yrs	70.9% (n=44)	60.5% (n=23)
Tumor size		
<5 cm	70.9% (n=44)	92% (n=35)
>or=5 cm	29% (n=18)	7.8% (n=3)
Grade *(only for IDC, NOS),*	*(n=52)*	*(n=31)*
I	6.4% (n=4)	21% (n=8)
II	43.5% (n=27)	47.3% (n=18)
III	33.8% (n=21)	13.1% (n=5)
Histology		
Ductal	83.8% (n=52)	81.5% (n=31)
Non-ductal	16% (n=10)	18.4% (n=7)
Lymph node		
Positive	61.2% (n=38)	39.4% (n=15)
Negative	38.7% (n=24)	60.52% (n=23)
Stage		

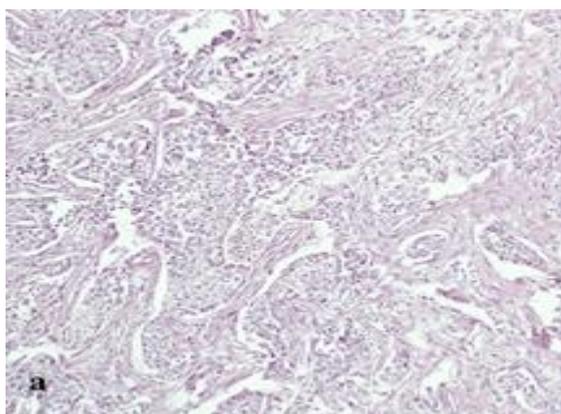|		
I	3.2% (n=2)	7.8% (n=3)
II	53.2% (n=33)	63.1% (n=24)
III	41.9% (n=26)	23.6% (n=9)
IV	1.6% (n=1)	5.2% (n=2)
ER status		
+	35.4% (n=22)	57.8% (n=22)
-	64.5% (n=40)	42.1% (n=16)
PR status		
+	27.4% (n=17)	36.8% (n=14)
-	72.5% (n=45)	63.1% (n=24)
Combined ER&PR		
ER+PR+	27.4% (n=17)	36.8% (n=14)
ER+PR-	8.0% (n=5)	21% (n=8)
ER-PR+	0% (n=0)	2.6% (n=1)
ER-PR-	64.5% (n=40)	39.4% (n=15)

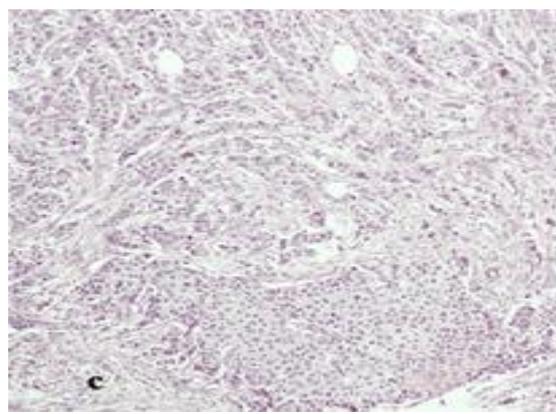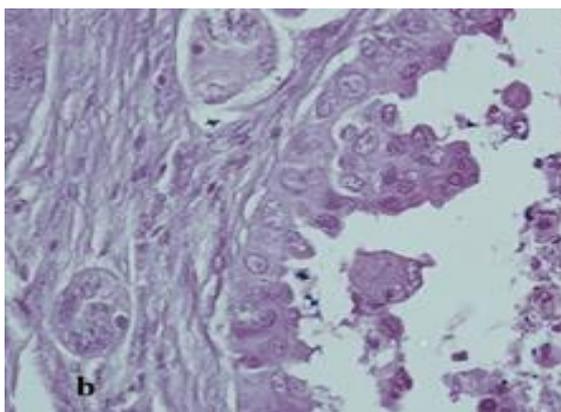**Fig. 1a: Low power view showing Invasive ductal carcinoma (grade2). (X100, H&E)**

Fig. 1b: High power view showing features of Invasive apocrine carcinoma (apocrine snouts and cytoplasmic eosinophilia) (X 400, H&E)

Fig. 1c: Low power view showing targetoid and Indian file arrangement of invasive lobular carcinoma. (X 100, H&E)

Discussion

Immunohistochemical based classification of breast cancers on both ER/PR and Her-2/neu status provides important prognostic and predictive information. Breast cancer prognosis also depends on various Clinico- pathological factors including status of lymph nodes, tumor size, tumor grade, histological type etc. Her-2/neu receptor overexpression is found to be an independent poor prognostic indicator of tumor grade, tumor size and lymph node status.[4]

In the present study, 100 patients with breast carcinoma treated with MRM were included out of which 98 were females and 2 were males. 62% of the cases were Her-2/neu positive and 38% were Her-2/neu negative. The frequency is higher in Indian women, which has been found in other studies as well.[3,5] There is a wide variation (15-35%) in Her-2/neu overexpression, our Fig. appears to be on a higher side because of more and more patients presenting with higher grade tumors.

Our study showed that the majority of patients were > 45 years of age. The finding is in concordance with many other studies.[6,7] However, Prasad et al[8] found majority of the patients to be < 50 years of age (67.4%). We did not find any significant association

between the age of patients and Her-2/neu expression. There are studies who have reported a decline in Her-2/neu overexpression with age.[4,9]

Tumor size ranged from 1.2 cms to 10.0 cms. Majority of the tumors were <5cm (79%). We found a significant association between the tumor size and Her-2/neu expression similar to the studies conducted by Naem et al[4], Ayadi L et al,[9] Nisa A et al,[10] Bhagat et al[2] and Jain et al.[6]

Her-2/neu overexpression was found to be significantly associated with lymph node metastasis (p<0.05). Out of total 53 lymph node positive cases, 38 cases showed Her-2/neu positivity in tumor proper and only 15 were negative for Her-2/neu expression. IHC was not performed on lymph nodes showing metastasis because the study aims to correlate the expression of receptors in tumor proper with the lymph node status (positive or negative) as seen on H&E stained section irrespective of what the tumor might express on metastatic sites. Fig. 3b shows lymph node metastasis by tumor cells. Ali EM et al[7] have also stated that Her-2/neu expression is the most important defining factor for axillary lymph node metastasis. Also the ER+ /PR+/ Her-2/neu – group is the most protected group against metastasis. Some studies on the other hand have not found any association between Her-2/neu expression and lymph node metastasis.[2,5,10,11]

We found Grade II tumors to be the most frequent ones amongst all IDC, NOS cases (45%), out of which 27 cases (60%) showed Her-2/neu positivity. Similar findings were also reported by several studies.[2,6,9,10,12,13] They all found that higher grade tumors (Grade II, III) more commonly expressed Her-2/neu compared to lower grade tumors (Grade I).

The predominant histologic type was IDC (83%). Other histological subtypes were lobular (3%), medullary (3%), metaplastic (2%), invasive papillary (3%), invasive cribriform (2%), mucinous (2%) and apocrine (2%). Fig. 1, 2 and 3a, 3b show the various histological types of breast carcinoma. No significant association was found between the histologic subtypes and receptor status, which correlated with other studies[8,14,15]. Invasive duct carcinoma and cribriform carcinoma showed Her-2/neu positivity, (62.6%) and (50%) respectively. Jain et al[6] also found IDC and comedo carcinoma to show Her-2/neu overexpression compared to other subtypes. 40%, 57.9%, 74.28% and 33.3% cases belonging to stage I, II, III and IV respectively showed Her-2/neu overexpression. No significant correlation was found between the stage of tumor and Her-2/neu status. However, Jain SA et al[6] found that Her-2/neu overexpression was significantly higher in higher stage tumors compared to those, which were negative for Her-2/neu.
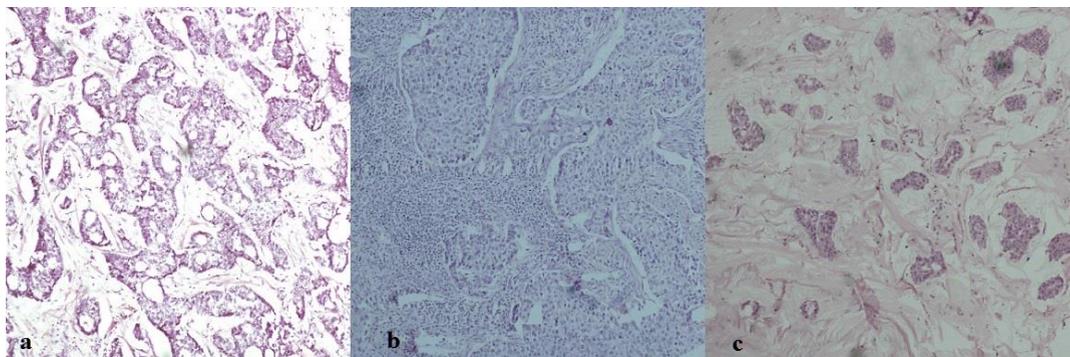

Fig. 2a, 2b and 2c: Low power view of invasive cribriform carcinoma, medullary carcinoma and mucinous carcinoma of breast respectively. (X100, H&E)
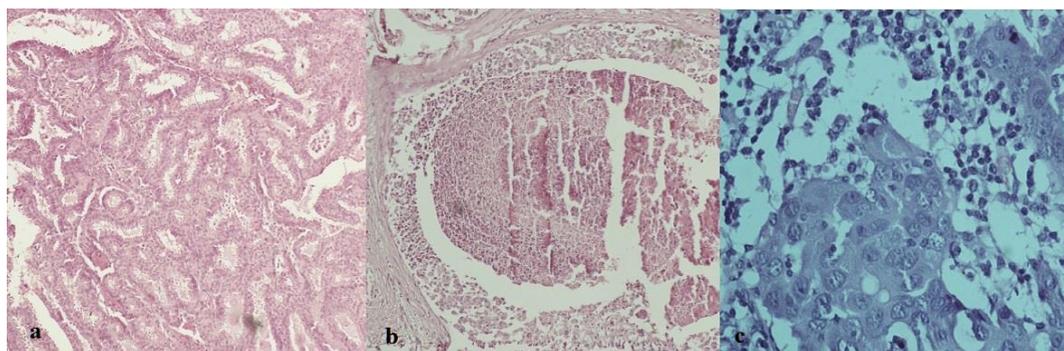

Fig. 3a and 3b: Low power view showing solid variant of invasive papillary carcinoma and comedocarcinoma respectively. (X100, H&E)
Fig. 3c: High power view showing tumor nests in a lymph node. (X 100, H&E)

ER expression was inversely related to Her-2/neu expression. Only 22 out of the 62 cases expressing Her-2/neu were found to be positive for ER as well (35.4%). This finding is in concordance with several other studies.[2,6,10,16,17] who also have found an inverse relationship between Her-2/neu and steroid receptor level. Fig. 4 shows ER, PR and Her-2/neu positive expression in tumor. This association has been related to the fact that estrogens and its receptors are required to suppress Her-2 which leads to lower or absent hormone receptors in women with Her-2/neu positive breast cancers. This is probably why women who express Her-2/neu may be resistant to tamoxifen.[18] 39.4% cases were triple negative in our study i.e. lacking ER, PR and Her-2/neu expression. Moses Embroise et al have documented 25% of triple negative cases in their study.[19] Studies have shown that ER/PR-, Her-2+ estimates were similar to triple negative values in terms of survival[20] and had the worst overall survival and disease free ratio. In ER/PR+, Her-2 negative group, chemotherapy offered significant overall and disease free survival advantages. It was shown that these groups of tumors benefit less from taxanes.[21]
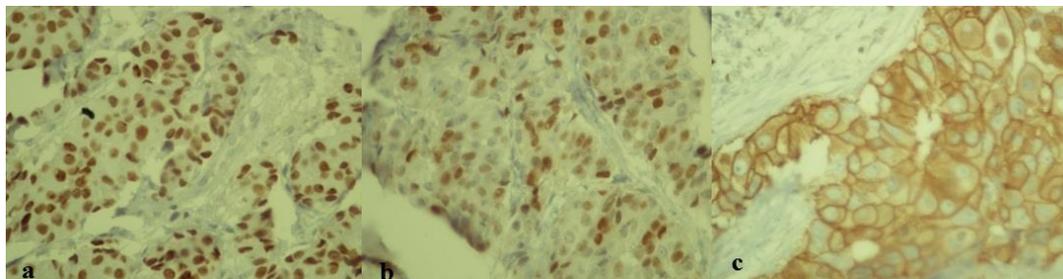

Fig. 4a, 4b and 4c: high power view showing nuclear positivity for ER, PR and strong membranous positivity (3+) for Her-2/neu immunostain respectively. (X 400, IHC)

Most of the cases included in this study were lost to follow up, many of whom refuse to take Her-2/neu receptor targeted therapy (herceptin) due to economic reasons.

To conclude, it can be said that Her-2/neu overexpression has been found to have a significant correlation with the size of tumor, grade of tumor and the axillary lymph node status. Its expression is inversely related to the ER expression. Thus, large tumors with a higher grade, positive lymph nodes and a negative ER expression should be examined for Her-2/neu expression by immunohistochemistry because of therapeutic and prognostic implications and should be advocated as a routine practice in reporting of all types of breast carcinomas.

Acknowledgement

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