Original Article

Correlation of Her-2/neu Status With Estrogen, Progesterone Receptors and Histologic Features in Breast Carcinoma

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Keywords: *Breast carcinoma, Her-2/neu, ER/PR*

ABSTRACT

Background: Breast cancer is the commonest cancer in urban Indian females, and the second commonest cancer in the rural Indian women. One of the hallmark of the disease is expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and Her-2/neu that ultimately drives prognosis & treatment modalities.

Aims: This study is to evaluate the expression of ER and PR and Her-2/neu in Breast Carcinoma & to compare them with other prognostic parameters: histological type and grade, tumor size, patients' age and lymph node metastases.

Method: 80 cases of Invasive Breast Carcinoma received as MRM (Modified Radical Mastectomy) over a period of 6-8 months were included. Routine H&E staining and IHC analysis for ER, PR and Her-2/neu was carried out in all cases.

Result: Out of 80 cases, 69 (86%) cases were of Infiltrating Ductal Carcinoma with mean age of 53 years. ER, PR & Her-2/neu expression were seen in 56.9%, 35.5% & 21.3% respectively. ER, PR expressing tumours tend to be of lower grade I($p<0.05$). 94.1% Her-2/neu expressing tumours presented with higher tumour grade (II, III) but statistically not significant. Lymph node metastases was found to be significantly associated with PR and Her-2/neu positive status ($p=0.03$, $p=0.01$ respectively). Her-2/neu expression was inversely related to ER and PR expression ($p<0.05$).

Conclusion: Higher number of grade 1 tumors showed ER, PR positivity as compared to grade 3 tumors. Inverse relationship was observed between Her-2/neu expression and ER, PR receptor status. Her-2/neu expression was associated with higher rate of axillary metastasis but did not show any significant correlation with age, tumour size and grade.

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Introduction

With rising incidence and awareness, breast cancer is the commonest cancer in urban Indian females, and the second commonest cancer in the rural Indian women.[1] In India, Breast Cancer is second to cancer of the cervix among women, but is considered the leading cancer in certain metros such as Mumbai and Bangalore. It is estimated that approximately 80,000 cases occur annually; the age adjusted incidence rates varying between 16 and 25/100,000 population. [2] Breast cancer survival is linked to early detection, timely appropriate treatment and genetic predisposition. Prognosis is related to a variety of clinical, pathologic and molecular features which include classical prognostic factors viz. histologic type, grade, tumor size and lymph node metastases. Estrogen and progesterone receptors (ER, PR) and more recently, Her-2/neu have with increasing importance influenced the management of the malignancy. [3]

Her-2/neu, also known as c-*erb*B-2, a protooncogene located on chromosome 17, is amplified and/or the protein (Her-2) overexpressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome.[4,5] In contrast, ER is expressed in 70% to 95% of invasive lobular carcinomas and in 70% to 80% of invasive ductal carcinomas, and PR is expressed in 60% to 70% of invasive breast carcinomas.[6,7] It is known that ER and PR expression are the only predictive factors with proven usefulness in selecting patients who are likely to respond to adjuvant endocrine therapy.[8,9] Patients lacking these receptors tend to have shorter disease free survival and earlier recurrences than those expressing these receptors.[9]

The interrelationship of ER, PR, and Her-2/neu has come to have an important role in the management of breast cancer. It has been shown that patients with breast carcinoma overexpressing Her-2/neu do not respond to Tamoxifen therapy. [4]The prognostic effects of Her-2/neu expression appear to be stronger in node positive carcinomas than in node negative carcinomas.[10] Her-2/neu has gained an even greater deal of attention lately after the introduction of a humanized monoclonal antibody known as trastuzumab that can be effective in the treatment of cases in which this oncogene product is overexpressed.[11,12]

Materials and Methods

The present study was carried out in the Department of Pathology, tertiary care centre of Ahmedabad, Gujarat, India, over a period of 6-8 months. 80 cases of Invasive Breast Carcinoma received as MRM (Modified Radical Mastectomy) were included. This study has been done as per standard ethics & by maintaining confidentiality of reports. Detailed gross examination of all received MRM specimens was carried out for tumor size, location and nodal metastases. All tissues were fixed in 10% buffered formalin for 12 hours. Representative tumor tissue was submitted for processing, 4 μm thick, paraffin embedded sections were cut and stained by Hematoxylin & Eosin (H&E) for routine histological examination & diagnosis.

Sections from the cases were reported by two pathologists independently with consensus and invasive tumors were divided into two major categories- ductal type and lobular type- acknowledging the existence of mixed and intermediate form.[13] Other morphological types like Metaplastic carcinoma, Medullary carcinoma , Papillary carcinoma, Tubulo-lobular carcinoma were also included. For invasive carcinomas, Nottingham's Modified Bloom Richardson (B-R) combined histologic system was used for grading.[13,14] (Table 1)

Final combined Bloom-Richardson (B-R) grade: Add score for tubule formation, mitoses, and nuclear pleomorphism. If B-R score is 3, 4 or 5, then B-R grade is low (I). If B-R score is 6 or 7, then B-R grade is intermediate (II). If B-R score is 8 or 9, then B-R grade is high (III).

Representative sections of tumor and normal breast tissue (as internal control) were processed for ER, PR and Her-2/neu immuno-histochemical staining. The positive control slides were prepared from breast carcinoma known to be positive for the antigen under study. For ER, PR and Her-2/neu staining, sections were taken on poly L lysine coated slides. Antigen retrieval was done by citrate buffer and the slides stained with monoclonal antibodies against estrogen and progesterone receptors by LSAB (labeled streptavidin biotin) system (BioGenex). The ER and PR results were screened and interpreted according to the published guidelines by The American Society Of Clinical Oncology & The College Of American Pathologist (ASCO/CAP).[13] This considers both the proportion and intensity of stained cells. Tumours having 1% or higher invasive cancer cells are considered positive and based on above findings final interpretation was given whether the sample is positive or negative.(Fig.1) For interpretation of Her-2/neu staining, following scoring system was used. [13]

Score 0: No staining is observed or membrane staining is observed in <10% of the tumor cells.

Score 1+: Faint/barely perceptible membrane staining is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining.

Score 2+: A weak to moderate complete membrane staining is observed in >10% of tumor cells. It is considered as weak positive.

Score 3+: Strongly Positive. A strong complete membrane staining is observed in >30% of tumor cells. Score 3+ was considered as strong positive immunostaining for Her-2/neu. (Fig. 2). The equivocal cases (score 2+) were not subjected to FISH due to funding problem.

Results were analyzed statistically and p value calculated using chi-square test.

Result

Total 80 cases of Breast Carcinoma were studied. The age of patients ranged from 26 to 85 yrs with mean age of 53.0 yrs. Most of the patients (85%) were of more than 40 yrs and majority (65%) belonged to 41- 60 yrs age group. (Table 2)

The most common histopathological pattern was Invasive Ductal Cell Carcinoma (IDC)(86.3%). Majority of tumours were of grade II (64.4%) and measured 2-5 cm in size (77.5%). 90% of tumours showed necrosis, fibrosis and calcification while 61.3% were having axillary lymph node involvement (≥1). IHC profile showed 56.9% positivity for ER, 35.5% positivity for PR and 21.3% positivity for Her-2/neu. (Table 3)

Table 4 shows correlation of age and tumour characteristics with ER, PR and Her-2/neu. Mean age of patients with strong (+3) Her-2/neu overexpression was 51 yrs which is comparatively younger (54 yrs) than those lacking Her-2/neu expression. Similarly 25% of patients ≤ 40 yrs of age were Her-2/neu positive as opposed to 20.5% of patients > 40 yrs of age. Patients with strong (+3) Her-2/neu overexpression presented with smaller tumour size (mean size 3.5cm) compared to Her-2/neu negative tumours (mean size 3.8cm). 24.2% of T2 tumours (2-5cm) were Her-2/neu positive as compared to 8.4% of T3 tumours (>5cm). Patients with strong Her-2/neu expression (94.1%) presented with higher tumour grade (II, III) compared to Her-2/neu negative patients (78.1%). In grade II and III tumours, 26.5% were strong positive for Her-2/neu as compared to 8.4% of grade I tumours. In lymph node status, 82% of Her-2/neu positive patients had lymph node metastasis (≥1) whereas only 55%of Her-2/neu negative patients had same.

Mean age for ER positive and ER negative patients were 55.5 and 49.6 yrs respectively. Among ER positive patients, 6.7% were ≤40 yrs of age as compared to 93.3% of patients >40yrs (p = 0.03). Mean age for PR positive and PR negative patients were 53.9 and 52.5 yrs respectively. 7.1% of PR positive patients were ≤40 yrs of age as opposed to 93% of patients >40yrs.These findings were similar to ER status. Mean tumour size in ER positive patients was 3.4cm versus 4.2cm in ER negative patients. In T2 tumours (2-5cm), 58% were ER positive whereas 42% of T3 tumours (>5cm) showed ER positivity. In PR positive patients, mean tumour size was 3.6cm versus 3.8cm in PR negative patients. 37% of T2tumours showed PR positivity compared to 33% of T3tumours.

Amongst grade II and III tumours, 50.8%were ER positive whereas 91.6% of grade I tumours were positive for ER (p = 0.005) i.e. lower grade tumours show more ER expression. 29.5% of grade II and III tumours were PR positive compared to 66.7% of grade I tumours (p = 0.02). Patients with PR negative status had more incidence of lymph node metastasis (66.6%) than those with PR positivity (50%)(p = 0.03). There was no statistical significant difference in lymph node metastasis detected in relation to ER status (Table 4).

Table 5 shows inverse relation between Her-2/neu expression and ER, PR. Of 17 Her-2/neu positive cases, 64.7% were ER negative and 88.2% were PR negative (p<0.05). 32.3% of ER negative and 29.4% of PR negative tumours were Her-2/neu positive. A positive correlation between ER and PR was also detected. Out of 45 ER positive cases, 28 were also PR positive whereas all 34 ER negative cases were also PR negative.

Table 1: Microscopic Grading of Breast Carcinoma: Nottingham modification of the Bloom – Richardson System.

Tubule formation	> 75% of cells arranged in tubules	score 1
	> 10 to 75% of cells arranged in tubules	score 2
	< 10%	score 3
Nuclear pleomorphism	cell nuclei are uniform in size and shape, relatively small, have dispersed chromatin patterns, and are without nucleoli	score 1
	cell nuclei are somewhat pleomorphic, have nucleoli, and are of intermediate size	score 2
	cell nuclei are relatively large, have prominent nucleoli or multiple nucleoli, coarse chromatin patterns, and vary in size and shape	score 3

Mitotic count *Via low power scanning (10x), locate the most mitotically active area of tumor and proceed to high power (40x)*	< 10 mitoses per 10 high power fields(hpf)	score 1
	≥ 10, and < 20 mitoses per 10 hpf	score 2
	≥ 20 mitoses per 10 hpf	score 3

Table 2: Distribution of patients according to age group

Age Group(yrs)	Cases(%)
21 - 30	1(1.3)
31 - 40	11(13.7)
41- 50	25(31.3)
51- 60	27(33.7)
61 - 70	10(12.5)
> 70	6(7.5)
Total	**80(100)**

Table 3: Clinicopathological features and receptor status.

Histopathological Features		No. (%)
Subtype	IDC	69(86.3)
	ILC	5(6.3)
	Medullary	4(5)
	Papillary	1(1.2)
	Metaplastic	1(1.2)
ER	Positive	45(56.9)
	Negative	34(43.1)
PR	Positive	28(35.5)
	Negative	51(64.5)
Her 2/Neu	Positive	17(21.3)
	Negative	63(78.7)
Tumor Grade	1	12(16.4)
	2	47(64.4)
	3	14(19.2)
Tumor Size	<2cm	6(7.5)
	2-5cm	62(77.5)
	>5cm	12(15)
Tumor Characteristics	Necrosis	69(92)
	Fibrosis	67(89.3)
	Calcification	71(94.7)
	Lymphocytic Infiltrate	43(57.3)
Lymphnode Status	Positive	49(61.3)
	Negative	31(38.7)
Age	≤40	12(15)
	> 40	68(85)

Table 4: Her-2/neu Status And Estrogen And Progesterone Receptor Expression In Invasive Breast Carcinoma Patients.

	Her 2 strong positive(+3)	Her 2 weak positive(+2)	Her 2 negative	ER positive	ER negative	PR positive	PR negative
Mean Age (yrs)	51	50.5	54	55.5	49.6	53.9	52.5
No Of Patients ≤ 40 yrs	3	2	7	3	9	2	10
No Of Patients > 40 yrs	14	8	46	42	25	26	41
Mean Size(cm)	3.5	4	3.8	3.4	4.2	3.6	3.8
No of T1 Tumors(<2cm)	1	0	5	4	1	1	4
No of T2 Tumors(2-5cm)	15	8	39	36	26	23	39
No of T3 Tumors(>5cm)	1	2	9	5	7	4	8
No Lymphnodal Metastases	3	4	24	18	13	14	17
1-3 LN Metastases	3	2	15	12	7	9	10
>3 LN Metastases	11	4	14	15	14	5	24
Tumor Grade							
1	1	1	10	11	1	8	4
2	12	6	28	27	20	16	31
3	4	3	15	4	10	2	12

Table 5: Relation of Her-2/neu expression with hormone receptor status.

Hormone Receptor	Her-2/neu Negative Patients No.(%)	Her-2/neu Positive Patients No.(%)	Chi-Square (p-value)
Estrogen Receptor Status			
Positive	39	6	0.0784
Negative	23	11	
Progesterone Receptor Status			
Positive	26	2	0.0436
Negative	36	15	

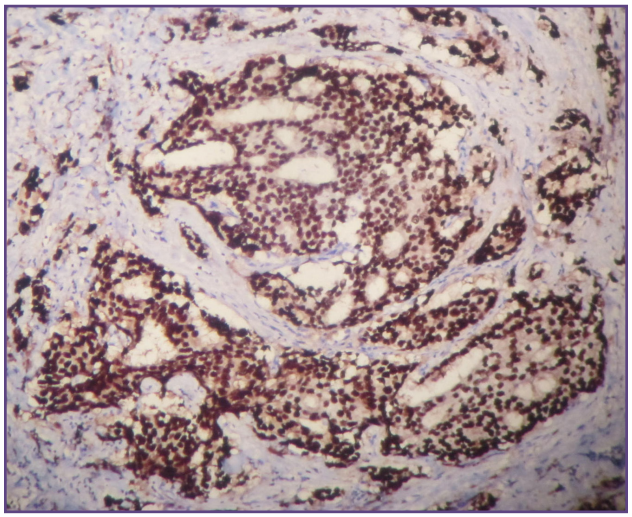

Fig. 1: ER positivity (+++) in tumour cells of Invasive Ductal Carcinoma, Breast (10x).

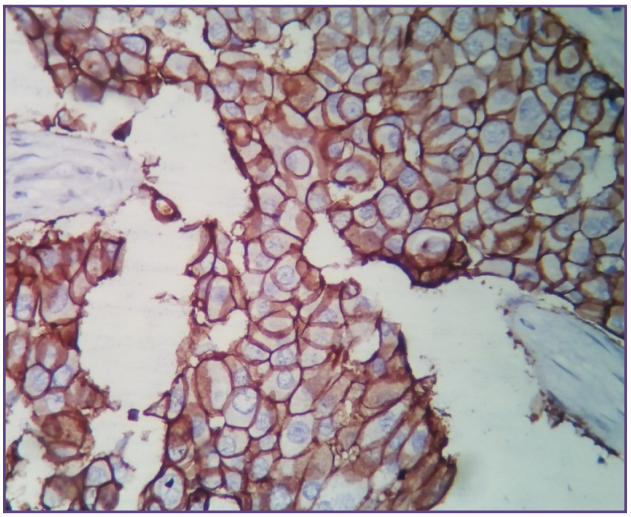

Fig. 2: Her-2/neu positivity (+++) in tumour cells of Invasive Ductal Carcinoma, Breast (40x).

Discussion

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior. Clinical and pathological variables such as tumor size, histologic grade, histologic type, lymph node metastases, vascular space invasion, tumor cell proliferation, tumor necrosis, extent of ductal carcinoma *in situ*, age, and pregnancy may help in predicting prognosis and the need for adjuvant therapy. [15] The presence of hormone receptors (ER and PR) in the tumor tissue correlates well with the response to hormone therapy and chemotherapy. [16] Tumors that are better differentiated are more likely to be ER and PR positive and have a relatively better prognosis.[17] The overall survival was significantly better for patients without Her-2/neu receptor markers compared to patients with Her-2/neu overexpression .[18]

Present study comprised of 80 cases of primary breast carcinoma. Infiltrating ductal carcinoma(86%) was the most common histological type which is comparable to Azizun N et al (85.3%)[19] and Nidal MA et al (84%).[20] The mean age at diagnosis in present study was 53.0 yrs comparable to studies done by Azizun N et al,[19] Nidal MA et al [20] and Pathak TB et al.[21] The left breast was more commonly involved (55%).[19] T2 tumours comprised the majority of cases in our study as seen in other studies with frequency ranging from50% to 65%.[19,20] Tumor grade is one of the prognostic factors in the breast cancer, tumors expressing higher grade tend to have poor prognosis. In this study, grade II tumors constitute the highest number of cases 64.4% followed by grade III and grade I. This is similar to studies done by Azizun N et al [19] and Pathak TB et al.[21]

Our study showed 56.9% of cases with positive ER status while 35.5% PR positive status. The study by Nidal MA et al [20] showed 53.0% and Fatima S et al [22] showed 55% positivity for ER, Bhagat VM et al [23] reported 37.9% positivity for PR.

In this study, we found that 21.3% cases were Her-2/neu positive. Although there is a wide variation in Her-2/neu overexpression and amplification, our figure appears to be within the commonly accepted range of 20% to 30% reported in literature.[19,20,23,24]

In our study significant association was found between age and ER status (p=0.03). The mean age of ER positive patients was 6 years more than ER negative patients. These findings are

in agreement with other reports in the literature, which show an association between ER expression in breast carcinoma patients and older age at the time of diagnosis. [20,25] In literature, Her-2/neu expression tend to decline with age as opposed to ER expression which tends to increase with age.[26] In present study also Her-2/neu positive patients were younger than those lacking expression; mean age being 3 yrs less than Her-2/neu negative patients.

In present study, significant association was found between tumour grade and ER, PR expression (p=0.005, p=0.01 respectively). This is in accordance with studies done by Azizun N et al [19]

and Fatima S et al[22] which also reported decreased expression of ER and PR in the higher grade III tumours. However no significant correlation could be established between Her-2/neu and the grade. There was no significant association found between tumour size and Her-2/neu, ER and PR expression.

Axillary lymph node metastases (≥1) was found in 82% of Her-2/neu overexpressing tumours compared to 55% of Her-2/neu negative tumours(p=0.01). A direct relationship between lymph node metastases and Her-2/neu expression was also reported by Hoff ER et al.[27]

A significant inverse relation between Her-2/neu overexpression and ER, PR expression was found in present study (p=0.04 and p= 0.02 respectively). Similar results were found in studies done by Azizun N et al,[19] Bhagat VM et al,[23] Arafah M,[28] MS Al-Ahwal.[29] A strong correlation between ER and PR expression was also noted in our study.

Conclusion

Breast cancers usually presented in 4th to 6th decade of life with mean age being 53 years. IDC was the most common histopathological type. No significant association of Her-2/neu, PR expression was found with age and tumour size. Better differentiated grade I tumours showed higher ER, PR positivity compared to grade III tumours in contrast, Her-2/neu expression increases with increasing grade of tumour. Her-2/neu expressing tumours were significantly associated with axillary lymph node involvement. Inverse relationship was observed between Her-2/neu expression and ER, PR status. As ER, PR and Her-2/neu are important prognostically as well as for the selection of best therapy for better survival of breast cancer patients, their assessment is strongly advocated in each case.

Funding

None

Competing Interests

None Declared

Reference

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