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Comparison of ER, PR and HER-2/NEU reactivity pattern with histological grade, type, tumour size, Lymphovascular invasion, mitotic index and lymph node status in breast cancer

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Abstract

Context: Breast cancer prognosis is related to a variety of clinical, pathologic and molecular features which include classical prognostic factors viz. Histological grade, morphological type, tumour size, mitotic index, Lymphovascular invasion 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. The presence of hormone receptors (ER and PR) in the tumour tissue correlates well with the response to hormone therapy and chemotherapy. Aims: To assess ER, PR and Her2neu status in breast cancer and to compare ER, PR and Her2neu status with other prognostic factors like tumour size, pathological type, histological grade, lymph node status, mitotic index and Lymphovascular invasion in breast cancer.

Methods and materials: A total of 100 cases of breast cancer reported at pathology department during a period from January 2019 to August 2020 are enrolled in the study. Representative sections with tumour and the adjacent normal breast tissue (internal control) are processed for ER, PR and HER-2/neu immuno-histochemical staining. Tumour size, morphological type, Histological grading, Mitotic index, Lymphovascular invasion, Lymph Node status were studied. Results: Occurrence of breast carcinoma is higher in older women (> 50 years of age). The prevalence of ER, PR, HER-2/neu expression are high (ER-73.0%, PR - 65.0% and HER-2/neu - 28.0%). About 14.0% tumour are triple negative. Increased HER2/neu positivity is noticed in ER and PR negative tumours. Two third (66.0%) tumour's size is from 2 to 5 cm. Proportion of Grade II (39.0%) and Grade III tumour (37.0%) are high. Most common pathologic type of carcinoma is Infiltrating duct carcinoma, not otherwise specified (85.0%) followed by Mucinous carcinoma (5.0%), Invasive papillary carcinoma (4.0%) and Invasive lobular carcinoma (2.0%). PR positivity is increased with increasing tumour size. (p value = 0.01). PR positivity decreases with the increase in grade. (p value = 0.03). HER/ 2neu expression decreased with rising age. (p value = 0.03).

Conclusion: The findings suggest that women in our population more often have histologically less aggressive breast carcinoma with a smaller number of triple negative tumours (14%), so they are likely to be more susceptible to conventional hormonal and targeted antibody treatment.

Keywords: Breast cancer, Estrogen receptor, progesterone receptor, HER-2/neu

Introduction

Breast cancer is the most common cancer in women globally and is the second leading cause of cancer death among women after lung cancer. It is estimated that 70% of Indian women are in an advanced stage, at which time little or no benefit is obtained from any form of therapy, leading to poor survival and high mortality rates [1]. Tumours expressing estrogen receptors differentiate better into low-grade tumours. The remission rate after hormone therapy in ER + and PR + cases is 70%. ER negative status acts as an important predictor in the early stages of tumours and is independent of tumour grades. Negative PR status is a predictor for lymph node metastases, independent of other clinicopathological factors [2, 3]. Triple negative breast cancer (ER/PR/HER-2/neu) has the worst survival [4]. The hormone receptors (ER and PR) positivity in tumour tissue correlates well with the response to hormone therapy and chemotherapy [5]. HER2-neu is also known as epidermal growth factor receptor 2 (C-erb B 2). It has gained importance as a significant prognostic indicator. Its amplification and overexpression are associated with a poor prognosis in axillary lymph node

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metastases, but there is no association with negative lymph nodes. HER2-neu may also be a predictive marker [6].

Aims and Objectives

1. To assess ER, PR and Her2neu status in breast cancer
2. To compare ER, PR and Her2neu status with other prognostic factors like tumour size, pathological type, histological grade, lymph node status, mitotic index and Lymphovascular invasion in breast cancer

Material and Methods

The present study is hospital based descriptive cross-sectional study to compare hormonal receptor status with prognostic factors of breast cancer. It is carried out at the department of histopathology, Shree Krishna Hospital, Karamsad a tertiary centre in Anand. A total of 100 cases of breast cancer reported at pathology department during a period from January 2019 to August 2020 are enrolled in the study.

Inclusion criteria

1. Patients who have undergone modified mastectomy.

Exclusion criteria

1. Patient who has undergone simple mastectomy or Patient who has undergone only lumpectomy.

ASCO (American society of clinical oncology) scoring guidelines is used for reporting for ER, PR and Her2/neu.

Results

Age wise distribution of breast carcinoma suggests that the presence of breast carcinoma is less in young women (18 cases in 31 to 40 years), whereas it is almost same in women’s ageing from 41-70 years (21 cases for each decade). We have 13 cases in 71-80 years of age group. The mean age of the patients was 56.5 ± 14.2 years.

The proportion of breast carcinoma is more in females as compared to males and it’s commonly seen in left breast.

Most common morphological type of breast carcinoma is Infiltrating duct carcinoma, not otherwise specified with 88.0% cases followed by mucinous carcinoma having 5.0% cases, Invasive papillary carcinoma with 4.0% cases, Invasive lobular carcinoma with 2.0% and single case of ductal carcinoma in situ.

Table 1: Her2 status in ER, PR positive and negative tumours

HER/2neu	ER positive (%)	ER negative (%)	PR positive (%)	PR negative (%)
Positive	15 (20.5)	13 (48.1)	13 (20.0)	15 (42.9)
Negative	58 (79.5)	14 (51.9)	52 (80.0)	20 (57.1)
Total	73 (100)	27 (100)	65 (100)	35 (100)
p value	p=0.006		p=0.01	

Table 1. Shows that HER/2neu positivity is significantly higher among ER negative tumour (48.1%) as compared to ER positive tumour (20.5%, p=0.006). Similarly, HER/2neu positivity is higher among PR negative tumour (42.9%) as compared to PR positive tumour (20.0%, p=0.01).

Table 2: Comparison of ER, PR and Her2neu with all the prognostic factors of breast carcinoma

Characteristics	ER +ve n (%)	ER -ve n (%)	p value	PR +ve n (%)	PR -ve n (%)	p value	Her2neu +ve n (%)	Her2neu -ve n (%)	p value
Age group (Years)									
>50 (n=39)	26 (35.6)	13 (33.3)		22 (33.8)	17 (43.6)		16 (57.1)	23 (59.0)	
< 50 (n=61)	47 (64.4)	14 (23.0)	0.36	43 (66.2)	18 (29.5)	0.80	12 (42.9)	49 (81.3)	0.03
Size									
< 2 cm (n=13)	8 (11.0)	5 (38.5)		5 (38.5)	8 (61.5)		2 (7.1)	11 (84.6)	
2-5 cm (n=66)	48 (65.8)	18 (27.3)	0.46	42 (63.6)	24 (36.4)	0.01	23 (82.1)	18 (85.7)	0.10
> 5 cm (n=21)	17 (23.3)	4 (19.0)		18 (85.7)	3 (14.3)		3 (10.7)	43 (65.2)	
Grade									
I (n=24)	20 (27.4)	4 (16.7)		20 (83.3)	4 (16.7)		6 (21.4)	18 (75.0)	
II (n=39)	28 (38.4)	11 (28.2)	0.39	26 (66.7)	13 (33.3)	0.03	10 (35.7)	29 (74.4)	0.75
III (n=37)	25 (34.2)	12 (32.4)		19 (48.6)	18 (48.6)		12 (42.9)	25 (67.6)	
No of LN									
0 (n=46)	34 (46.6)	15 (30.6)		26 (56.5)	20 (43.4)		12 (42.9)	37 (75.5)	
1 to 3 (n=25)	18 (24.7)	4 (18.2)	0.55	17 (68.0)	8 (32.0)	0.23	6 (21.4)	16 (72.7)	0.63
> 3 (n=29)	21 (28.8)	8 (27.6)		22 (75.9)	7 (24.1)		10 (35.7)	19 (65.5)	
LV invasion									
Absent (n=79)	58 (79.5)	21 (26.6)		53 (67.1)	26 (32.9)		20 (71.4)	59 (74.4)	
Present (n=21)	15 (20.5)	6 (28.6)	0.85	12 (57.1)	9 (42.9)	0.39	8 (28.6)	13 (61.9)	0.24
Mitotic index									
M1 (n=36)	25 (34.2)	11 (30.6)		22 (61.1)	14 (38.9)		10 (35.7)	26 (72.2)	
M2 (n=43)	33 (45.2)	10 (23.3)	0.75	31 (72.1)	12 (27.9)	0.97	14 (50.0)	29 (67.4)	0.94
M3 (n=21)	15 (20.5)	6 (28.6)		12 (57.1)	9 (42.9)		4 (14.3)	17 (81.0)	
Total	73 (100)	27 (27.0)		65 (100)	73 (100)		28 (100)	72 (72.0)	

Table 2 suggests that ER and PR receptors positivity was more in patients with less than 50 years age. However, positivity of Her 2 neu was more common in more than 50-year-old patients, it is statistically significant with p value of 0.03.

It also shows that maximum cases are having 2-5 cm in size with high positivity of all three receptors). PR positivity is increased as size of tumor increased (<2 cm – 38.5%, 2-5 cm- 63.6%, > 5 cm – 85.7%, p= 0.01).

PR positivity is higher in grade I tumour (83.3%) as compared to grade II (66.7%) and grade III (48.6%, p= 0.03). ER positivity was seen more in Grade I and II whereas Her2 positivity was seen more in Grade II and III.

We have 29.0% cases with >3 Lymphnodes showing tumour metastasis, 22.0% cases with 1-3 positive lymphnodes for tumour metastasis and 49.0% cases does not show tumour metastasis in any lymphnode. It was statistically not associated with receptor positivity.

Lymphovascular invasion was observed in 21 cases. However, Lymphovascular invasion was statistically not associated with receptor positivity. Mitotic figures were low in Her2neu positive tumour (M3 - 4, 14.3%) as compared to ER (M3 - 15, 20.5%) and PR positive (M3 - 14, 21.5%) tumour but statistically not significant.

Discussion

The presence of hormone receptors is a powerful predictive factor for the likelihood of benefit from adjuvant hormonal therapy including aromatase inhibitors and tamoxifen which is an oral selective estrogen receptor modulator [8, 9]. In the present study, ER, PR, HER-2/neu were positive in 73.0%, 65.0% and 28.0% cases respectively. About 63.0% tumours were positive for both ER and PR while 25.0% tumours were negative for both ER and PR. About 10.0% tumours were ER positive and PR negative. However, only 2.0% tumours were PR positive and ER negative. In contrast to our study, Azizun-Nisa *et al.* [10] reported lower positivity of ER (32.7%), and PR (25.3%). HER-2/neu was positive in 37.4% cases. Rao *et al.* [11] also reported lower prevalence of ER (36.5%), PR (31.7%) and HER-2/neu expression (2.4%). Variation in receptor expression may be due to racial and geographic differences in younger age and grade of breast cancers. ER (38.3%), PR (30.0%) and Her2 (43.3%) expression studied by Thiagarajan M *et al.* [12] also showed lower positivity of hormonal receptors as compared to our study. Similar to our study, Gupta D *et al.* [13] showed increased positivity of ER and PR (68% and 58%) respectively. Kaur *et al.* [14] also showed increased positivity of ER and PR (63% and 58%

respectively).

Table 3: Shows an inverse correlation of HER2/neu Expression with ER and PR expression was observed.

HER2/neu	ER		PR	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Positive	15 (20.5)	13 (48.1)	13 (20.0)	15 (42.9)
Negative	58 (79.5)	14 (51.9)	52 (80.0)	20 (57.1)
Total	73 (100)	27 (100)	65 (100)	35 (100)
p value	p=0.006		p=0.01	

In the present study, an inverse correlation of HER2/neu expression with ER and PR expression was observed. HER2/neu positivity was increased significantly in ER negative tumour (48.1%) as compared to ER positive tumour (20.5%) (p=0.006). Similarly, HER2/neu positivity was 40.0% in PR negative tumour as compared to 21.5% in PR positive tumour (p=0.01). The study of Azizun-Nisa *et al.* [10] was concordant to our study which concluded that ER or PR expression correlated inversely with HER-2 over-expression (ER 8.1% vs 23.4%; PR 2.7% vs 14.9%). Thiagarajan M *et al.* [12] also concluded inverse correlation of ER or PR with HER-2.

In the present study, the mean age of the patients was 56.5 ± 14.2 years which was similar to the study conducted by Thiagarajan M *et al.* [12] 51.2 ± 9.8 years.

In the present study, about 98.0% patients were female. The left breast was more commonly involved (57%) than right breast. Azizun- Nisa *et al.* [10] also showed that 57.0% of tumours were in left breast. In other studies, like Azizun-Nisa *et al.* [10] and Thiagarajan M *et al.* [12] 100% cases were of female patients.

Table 4: Shows PR positivity has increased with the increase in the size of tumour. (<2 cm - 05 (38.7%), 2-5 cm in size - 42 (63.6%) and >5 cm in size - 18 (85.7%) with p value of 0.01). Like our study Azizun- Nisa *et al.* and Thiagarajan M *et al.* also reported that more than half of the patients were having 2-5cm size of breast tumour.

Tumour size	Hormonal receptors	Status	Authors		
			Azizun-Nisa <i>et al.</i> [5, 7] (2008)	Thiagarajan M <i>et al.</i> [7] (2015)	Present study
<2 cm	ER	Positive	9(50.0)	01(4.3)	8(11.0)
		Negative	9(50.0)	07(18.9)	5(38.5)
	PR	Positive	8(44.4)	01(5.6)	5(38.5)
		Negative	10(55.6)	07(16.7)	8(61.5)
	HER2/neu	Positive	4(22.2)	05(19.2)	2(7.1)
		Negative	14(77.8)	03(8.8)	11(84.6)
2-5 cm	ER	Positive	26(32.9)	17(73.9)	48(65.8)
		Negative	53(67.1)	24(64.9)	18(27.3)
	PR	Positive	21(26.6)	12(66.7)	42(63.6)
		Negative	58(73.4)	29(69.0)	24(36.4)
	HER2/neu	Positive	17(21.5)	17(65.7)	23(82.1)
		Negative	62(78.5)	24(70.6)	18(58.7)
>5 cm	ER	Positive	14(26.4)	05(21.7)	17(23.3)
		Negative	39(73.6)	06(16.2)	4(19.0)
	PR	Positive	9(17.0)	05(27.8)	18(85.7)
		Negative	44(83.0)	06(14.3)	3(14.3)
	HER2/neu	Positive	16(30.2)	04(15.4)	3(10.7)
		Negative	37(69.8)	07(20.6)	43(65.2)

Tumour size is one of the important prognostic factors which is directly related to the survival of the patient. In the present study, PR positivity has increased with the increase

in the size of tumour with p value of 0.01). However, ER (p=0.46) and Her 2 neu positivity (p=0.10) were not associated with tumour size.

Table 5: Shows grade of tumour is significantly associated with PR status. PR positivity is higher in grade I tumour (83.3%) as compared to grade II (66.7%) and grade III (48.6%, $p = 0.03$). ER positivity shows a decreased trend with Advancing grade of tumour but it was statistically not significant (Grade I – 83.3%, Grade II – 71.8%, Grade III- 67.6%, $p = 0.39$). HER2/neu positivity maximum in grade III, but it was statistically not significant ($p=0.75$). Similar to our study, Azizun- Nisa *et al.* reported that the ER/PR negativity significantly increased as grade of the tumour increased. Similar to our Study, Rao *et al.* also reported that positivity of ER and PR decreases with increase in grade of tumour.

Grade	Hormonal receptors	Status	Authors (Year)			
			Azizun-Nisa <i>et al.</i> [5, 7] (2008)	Rao <i>et al.</i> [6, 7] (2020)	Thiygarajan M <i>et al.</i> [7] (2015)	Present study
Grade 1	ER	Positive	7(70.0)	23(41.07)	5.0(21.7)	20(83.3)
		Negative	3(30.0)	33(58.9)	2.0(5.4)	4(16.7)
	PR	Positive	7(70.0)	24(42.8)	5.0(27.8)	20(83.3)
		Negative	3(30.0)	32(57.1)	2.0(4.8)	4(16.7)
	HER2/neu	Positive	00	00(00)	1.0(3.8)	6(25.0)
		Negative	10(100.0)	55(100.0)	6.0(17.6)	18(75.0)
Grade 2	ER	Positive	40(48.2)	11(22.0)	15.0(65.2)	28(71.8)
		Negative	43(51.8)	39(78.0)	16.0(43.2)	11(28.2)
	PR	Positive	30(36.1)	13(26.0)	11.0(61.1)	26.6(66.7)
		Negative	53(63.9)	37(74.0)	20.0(47.6)	13(33.3)
	HER2/neu	Positive	19(22.9)	00(00)	16.0(61.5)	10(25.6)
		Negative	64(77.1)	50(100.0)	15.0(44.1)	29(74.4)
Grade 3	ER	Positive	02(3.5)	03(15.0)	3.0(13.1)	25(67.6)
		Negative	55(96.5)	17(85.0)	19.0(51.4)	12(32.4)
	PR	Positive	01(1.75)	04(20.0)	2.0(11.1)	19(48.6)
		Negative	56(98.2)	16(80.0)	20.0(47.6)	18(48.6)
	HER2/neu	Positive	18(31.6)	03(15.0)	9.0(34.6)	12(32.4)
		Negative	39(68.4)	17(85.0)	13.0(38.2)	25(67.6)

In the present study, grade of tumour is significantly associated with PR status. PR positivity is higher in grade I tumour (83.3%) as compared to grade II (66.7%) and grade III (48.6%) $p = 0.03$. ER positivity shows a decreasing trend with advancing grade of tumour. (Grade I – 83.3%, Grade II – 71.8%, Grade III- 67.6%, $p=0.39$). HER2/neu positivity maximum in grade III, However, ER and Her2 were statistically not significant ($p=0.75$). Similar to our study, Rao *et al.* [11]. Also reported that positivity of ER and PR decreases with increase in grade of tumour.

We have 29.0% cases with >3 lymphnodes showing tumour metastasis, 22.0% cases with 1-3 positive lymphnodes for tumour metastasis and 49.0% cases does not show tumour metastasis in any lymphnode. Similar pattern was observed by Rao *et al.*

In the present study, about 21.0% patients presented with lymphovascular invasion. There was no significant association between lymphovascular invasion and receptor status (ER - $p=0.85$, PR - $p=0.85$, Her 2 neu - $p=0.24$). This is in consonance with Thiygarajan M *et al.*, Rao *et al.* [11], and Gupta D *et al.* [13].

Majority of patients in our study (43.0%) had M2, 36.0% cases had M1 and 21.0% cases had M3. ER positive tumour cases were almost equal in tumours with M1 (25, 69.4%), M2 (33, 76.7%) and M3 index (15, 71.4%). Similarly, PR positive and Her2neu positive tumours were almost equal in tumours with M1, M2 and M3 index. There was no significant association between ER, PR, Her2neu positivity and mitotic index ($p=0.75$, 0.97 and 0.94 respectively). In contrast to our study, Thiygarajan M *et al.* [12] revealed that ER negativity and PR negativity significantly increased with increase in Mitotic Index from M1 to M2 but there was no correlation of Her2neu with Mitotic Index.

In the present study, most common morphological type of carcinoma was the Infiltrating duct carcinoma, not otherwise specified (88.0%). There was no significant correlation between receptor status and histological types of breast tumour. In all tumours, ER PR positivity was high as

compared to HER2neu positivity. HER2neu positivity was limited to invasive breast carcinoma (Infiltrating duct carcinoma (NOS) and Invasive papillary carcinoma). Our study showed 100% positivity of ER and PR in invasive lobular carcinoma which was in concordance to the study conducted by Rao *et al.* [13] concluded that ER and PR expression were found to be greater in lobular than in ductal tumours.

Conclusion

Tumour characteristics

The prevalence of ER, PR, HER-2/neu expression are high as compared to other studies (ER-73.0%, PR - 65.0% and HER-2/neu - 28.0%).

About 14.0% tumour are triple negative. An inverse relation was found between ER, PR and HER2/neu status. Increased HER2/neu positivity is noticed in ER and PR negative tumours.

Occurrence of breast carcinoma is less in young women (18.0% in 31 to 40 years), whereas it is almost same in women ageing from 41-70 years (21.0% in each decade). The mean age of the patients is 56.5 ± 14.2 years.

66.0% tumour's size is from 2 to 5 cm.

Proportion of Grade II (39.0%) and Grade III tumour (37.0%) are high.

More than half of tumours (51.0%) are node negative. About 29.0% cases are with more than 3 lymphnodes metastasis. 21.0% patients presented with lymphovascular invasion.

Majority of patients came under M1 and M2 categories (M1- 43.0%, M2 - 36.0%).

Most common pathologic type of carcinoma is Infiltrating duct carcinoma, Not otherwise specified (88.0%) followed by Mucinous carcinoma (5.0%), Invasive papillary carcinoma (4.0%) and Invasive lobular carcinoma (2.0%).

Association between receptor status and tumour characteristics

The steroid receptor (ER, PR) positivity is not significantly

associated with age. However, HER/2neu expression decreased with rising age.

There is no any association of tumour size with ER and Her 2 neu positivity. However, PR positivity is increased with increasing tumour size.

No co-relation is seen between the grade of tumour and ER and Her2 neu. However, PR positivity decreases with increasing tumour grade.

The metastatic nodes did not correlate significantly with ER, PR and Her 2 neu status. ER, PR and Her 2 neu positivity is slightly higher in patients with breast cancer having lymph node metastasis but statistically not significant.

No significant association is noted between ER, PR, HER-2/neu and lymphovascular invasion and mitotic index.

There is no significant correlation between receptor statuses with histological types of breast tumour, HER-2 positivity is limited to Infiltrating duct carcinoma, NOS and Invasive papillary carcinoma.

Variation in receptor expression may be due to racial and geographic differences in younger age and grade of breast cancers. Steroid receptor status, although an imperfect predictor of patients' outcome, still remains the only single biological parameter in use to suggest therapeutic directives for subgroups of breast cancer patients.

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