

Original Research

Estrogen and Progesterone Receptors in Breast Malignancy – Their Expression and Correlation with Histologic Parameters in Southern India

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ABSTRACT

Breast carcinoma is the most common malignancy among South Indian women. Hormone receptor evaluation has become crucial for management of breast cancer. Very few studies have correlated oestrogen receptors (ERs) and progesterone receptors (PRs) with histologic parameters in Andhra Pradesh state of Southern India; therefore, the study was undertaken, and it is also to study the importance of immunohistochemistry (IHC) evaluation of ER and PR status and to evaluate and conclude the importance of modified Bloom–Richardson (MBR) grading in a routine investigation for prognosis and prediction of clinical outcomes. **Aims and Objective:** (1) To evaluate the expression of hormone receptors in breast carcinoma and (2) to correlate their expression with other histological variables. **Materials and Methods:** A prospective study was conducted for 2 years, at a tertiary care hospital in Vijayawada, Andhra Pradesh, India. In our study, 100 consecutive cases were included, which were reported by MBR system and evaluated for ER & PR receptor expression by Allred score. Statistical analysis was done by SPSS software, Student *t*-test and chi-square test. **Results:** Majority of the age group affected was below 50 years, and mean age of the study population was 46.25 years. The most common histological variant was infiltrating duct cell carcinoma (91%), and predominant histological grade was MBR grade II (47%). ER and PR positivity were noted in 56% and 53% of cases, respectively. The common phenotypic expressions were ER+ PR+ (46%) & ER– PR– (37%). ER positivity was observed in 25% of grade I, 54.54% of grade II and 20.46% of grade III lesions ($p = 0.005$), and expression of PR was seen in 25.53% of grade I, 51.06% of grade II and 23.54% of grade III lesions ($p = 0.001$). ER+ PR+ phenotypic expression was positively correlated with low histologic grade, whereas ER– PR– correlated with high histological grade ($p = 0.003$). ER+ PR+ also correlated with lymphatic spread ($p = 0.046$) and vascular invasion ($p = 0.011$). However, no correlation was observed with age, tumour size and ER & PR expression ($p > 0.05$). **Conclusion:** High nuclear pleomorphism, low tubule formation, high mitotic score & lymphovascular invasion were found more common in ER– PR– group than in ER+ & PR+ group with significant statistical correlation. ER & PR expression showed statistically significant correlation with MBR system and other pathological parameters. Hence, MBR grading should be incorporated as a routine investigation for prognosis and prediction of clinical outcome in Breast carcinoma (CA), Oestrogen receptor (ER), Immunohistochemistry (IHC), Invasive ductal carcinoma (IDC), Modified Bloom–Richardson grading (MBR), Progesterone receptor (PR) nuclear pleomorphism

KEYWORDS: Estrogen, Histologic, Immunohistochemistry, Malignancy, Oestrogen receptor, Progesterone

INTRODUCTION

Breast cancer is now the most common cancer in most cities in India, and second most common in the rural

areas. On a recent research, it was observed that, breast cancer patients of Indian origin tend to be younger, with extensive tumour spread when first diagnosed and a large proportion had high-grade

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tumours when compared with western series [1]. One of the many reasons contributing to advanced stages might be due to social stigma & lack of awareness.

Traditional prognostic factors for breast carcinoma include the tumour size, axillary lymph node status and modified Bloom–Richardson (MBR) grade. The expression of oestrogen receptor (ER) and progesterone receptor (PR) helps to evaluate patient's response to hormonal therapy and their prognosis [2–4]. Tumours that express ER and PR have better prognosis and respond better to hormonal therapy than tumours that lack them; similarly, the expression of PR in addition to ER increases the response to hormonal therapy as it implies a functioning ER pathway [5–7]. Expression of these receptors by tumours depends on factors like age, postmenopause and disease progression. Postmenopausal women with breast cancer showed better expression of receptors, than young women [8–10] and with the progression of the disease hormonal receptor expression decreased [11].

Few studies have correlated ER and PR with histologic parameters. In the present study, we evaluated the expression of ER and PR in breast carcinoma and their possible correlation with prognostic parameters like MBR grade, lymphovascular invasion and age.

MATERIALS AND METHODS

This was a prospective study done at Department of Pathology, Siddhartha Medical College, a tertiary care centre in Vijayawada, Andhra Pradesh from August 2013 to September 2015. A total number of 100 consecutive cases which were reported as breast carcinomas were included and were evaluated for ER/PR receptors expression. The specimens were grossed according to the standard protocol and were evaluated for following data (1) tumour size, (2) infiltration of the tumour to nipple and areola, (3) infiltration on to posterior margin, (4) necrosis, (5) desmoplasia, (6) calcifications and (7) lymph nodes involved. The submitted tissue specimens were fixed and done with routine tissue processing, and tissue sections were taken and stained by Haematoxylin & Eosin, and evaluated and diagnosed by MBR grading.

Immunohistochemical evaluation for ER & PR was done on the representative tumour sections by the HRP horseradish peroxidase –polymer method. Protein retrieval was done by microwave technique at a pH of 6 to 6.8, and following antibody clones were used – Mouse Anti-Human ER alpha Clone 1D5 and Mouse Anti-Human PR clone PgR636. The normal epithelial component present in the tissue section served internal control for ER/PR. Well-preserved & better stained areas of the sections were assessed. The ER & PR positive cells take up nuclear stain and it's considered positive when >10% of cells show the stain [12]. Allred scoring system evaluates two main features those are proportion and the intensity of stained cells which are then added to get a total score; a minimum score of 3 was considered as positive expression [13]. ER and PR have four phenotypic expressions. ER+/PR-, ER-PR+, ER-PR- The association between categorical variables was tested by using chi-square, the comparison of continuous variables between groups was done by Student's *t*-test. A *p* value (two-tailed) of less than 0.05 was considered statistically significant.

RESULTS

In our study of 100 consecutive confirmed cases of breast carcinoma, age group of the subjects ranged from 28 to 82 years, most effected age group was 41–50 years with 31 cases (31%) with a mean of 46.25 years, young women with breast cancer (<40 years) were 34 cases (34%). The most common symptom was breast lump seen in 87 cases (87%) & on gross examination, majority 80 cases (80%) had tumour size of 2.0–5.0 sq. cm. A little less than half, that is 47 cases (47%) belong to MBR grade II, 41 cases (41%) were grade I and 12 cases (12%) grade III. Other histological features include 27 cases (27%) had necrosis, 28 cases (28%) had lymphovascular invasion, pagetoid spread in 3 cases (3%). Infiltrating duct cell carcinoma was most common (91%); other variants include DCIS (*Ductal carcinoma in situ*) (4%), carcinoma with medullary features (2%), carcinoma with neuroendocrine features (1%), malignant phyllodes

(1%), mucinous carcinoma (1%). However, lymph nodes were negative in maximum cases (48%) in comparison to 33% positive cases.

Expression of ER+ was seen in 56 cases (56%), and 53 cases (53%) showed PR positivity. Most common phenotype was ER+ & PR+ 46 cases (46%), ER- & PR- 37 cases (37%), ER+ & PR- 10 cases (10%) and ER- & PR+ 7 cases (7%). Majority of infiltrating duct cell carcinoma expressed ER+ PR+, mucinous Ca, neuroendocrine carcinoma & DCIS showed ER+PR+, whereas malignant Phyllodes, medullary carcinoma and papillary insitu showed ER- PR- (Table 1).

ER positivity was observed in 25% grade I, 54.54% grade II and 20.46% grade III carcinomas (p value <0.005), whereas PR positivity was observed in 25.53% grade I, 51.06% grade II and 23.54% grade III carcinomas (p value <0.005). ER and PR expression in high grade tumours was significantly decreased when

compared with intermediate grade tumours. A significant correlation (p value <0.05) was observed with nuclear pleomorphism, mitotic count and lymphovascular invasion (Tables 2–4). ER and PR expressions were not associated with patients' age, menopausal status, tumour size, lymph node and necrosis (p value >0.05) (Figure 1).

Table showing correlation of oestrogen and progesterone expression with age:

Table 1: Showing the observation and results of various histopathological and clinical variables

Showing Correlation of Age and Expression	Age ≤ 50	Age > 50	<i>p</i> Value
Total No. of cases (<i>n</i>)	65	35	–
ER+	39(60%)	17(48.58%)	0.272
ER–	26(40%)	18(51.42%)	
PR+	36(55.38%)	17(48.58%)	0.515
PR–	29(44.62%)	18(51.42%)	

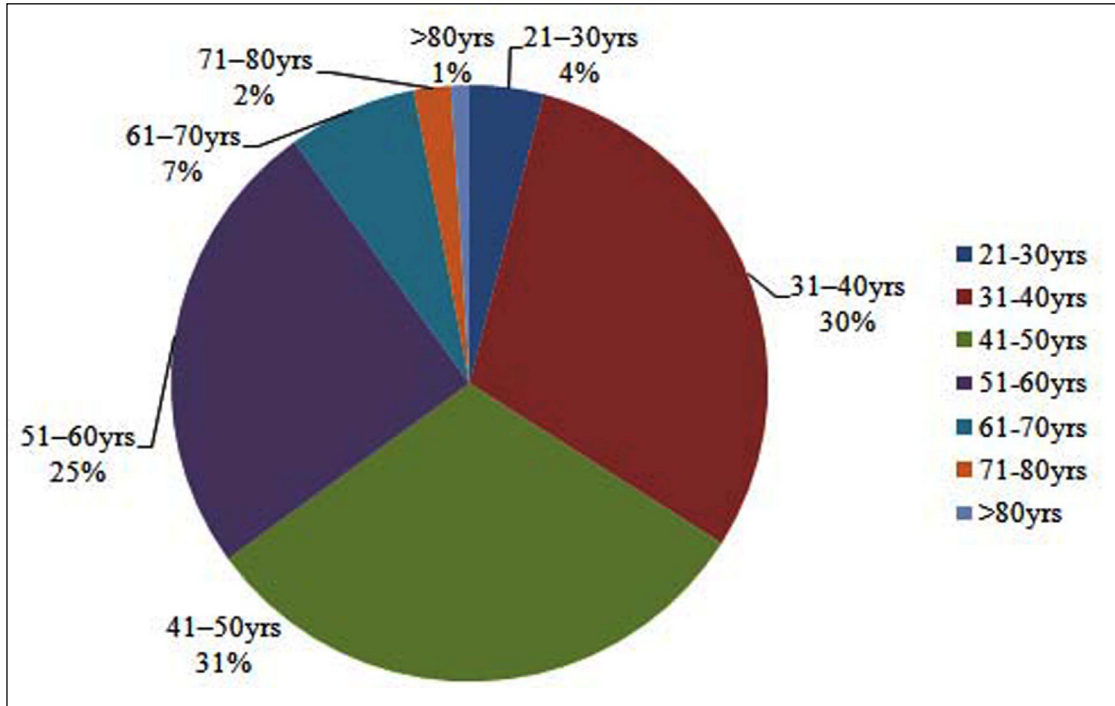


Figure 1: Age in year

Table 2: Showing correlation between ER PR and MBR grading

Variable	ER Status		PR Status	
	Negative (n = 44)	Positive (n = 56)	Negative (n = 47)	Positive (n = 53)
MBR Grading				
I	11 (25%)	30 (53.57%)	12 (25.53%)	29 (54.73%)
II	24 (54.54%)	23 (41.07%)	24 (51.06%)	23 (43.39%)
III	9 (20.46%)	3 (5.36%)	11 (23.40%)	1 (1.88%)
p value	0.005		0.001	

Table 3: Showing phenotypic expression with histological variables

		ER+ PR+ (n = 46%)	ER+ PR- (n = 10%)	ER- PR- (n = 37%)	ER- PR+ (n = 7%)
Age	≤50	32	6	23	3
	>50	14	4	14	4
MBR grade	I (n = 41%)	27	3	9	2
	II (n = 47%)	18	5	19	5
	III (n = 12%)	1	2	9	0
Invasive duct cell carcinoma (n = 91%)		42	10	33	6
Medullary carcinoma (n = 2%)		0	0	1	1
Neuroendocrine carcinoma (n = 1%)		1	0	0	0
Mucinous carcinoma (n = 1%)		1	0	0	0
Malignant phyllodes (n = 1%)		0	0	1	0
Ductal carcinoma insitu (n = 4%)		2	0	2	0

DISCUSSION

In the present study, majority of the affected cases were <50 years which was a decade earlier when compared with western case reports [14]. Infiltrating duct cell carcinoma (NOS) was most common 91%; other variants were 2 cases (40%) Medullary carcinoma, 1 case (20%) Mucinous carcinoma, 1 case (20%) Carcinoma with neuroendocrine features, and 1 case (20%) was Malignant Phyllodes; similar findings were reported in Shushan Shweta *et al.* [15]. In the study, majority of 47 cases (47%) were reported as grade II of MBR staging, 41 cases (41%) as grade I and 12 cases (12%) as grade III; the findings were similar to Asian, European case reports [16,17]. Lymph node metastases was negative in 48 cases (48%),

positive in 33 cases (33%), not available in 19 cases (19%), and the observations were similar to Onitilo *et al.* [18], which showed majority of the cases with no metastases to regional lymph nodes, and also 7.8% cases were no lymph nodes available.

ER expression was positive in 56 cases (56%) and negative in 44 cases (44%). Positive PR expression was seen in 53 cases (53%) and negative in 47 cases (47%). The observations were similar to other Indian and European studies [15,16].

The phenotypes obtained were ER+ & PR+ 46 cases (46%), ER- & PR- 37 cases (37%), ER+ & PR- 10 cases (10%) and ER- & PR+ in 7 cases (7%). According to WHO breast tumours, each combination

Table 4: Showing expression ER and PR phenotypes in correlation with variables

Variables	ER & PR Status			p Value
	ER+PR+ (n = 46)	ER- PR- (n = 37)	ER+ PR- & ER- PR+ (n = 17)	
Tubule formation				
>75%	9 (19.56%)	3 (8.11%)	1 (5.88%)	0.173
10-75%	24 (52.17%)	15 (40.54%)	9 (52.94%)	
<10%	13 (28.27%)	19 (51.35%)	7 (41.18%)	
Nuclear pleomorphism				
Minimal variation in size and shape of nuclei	17 (36.96%)	7 (18.92%)	4 (23.53%)	0.011
Moderate variation in size and shape of nuclei	25 (54.35%)	16 (43.24%)	11 (64.71%)	
Severe variation in size and shape of nuclei	4 (8.69%)	14 (37.84%)	2 (11.76%)	
Mitosis				
0-5	28 (60.87%)	10 (27.02%)	6 (35.29%)	0.021
6-10	18 (39.13%)	25 (67.57%)	10 (58.82%)	
>11	0	2 (5.41%)	1 (5.89%)	
MBR grading				
I	27 (58.69%)	9 (24.32%)	5 (29.42%)	0.003
II	18 (39.14%)	19 (51.35%)	10 (58.82%)	
III	1 (2.17%)	9 (24.33%)	2 (11.76%)	
Histological variables				
Necrosis (n = 27)	10 (37.03%)	12 (44.44%)	5 (18.53%)	0.535
Pagetoid spread (n = 3)	1 (33.34%)	0	2 (66.67%)	0.504
Lymphatic spread (n = 18)	4 (22.23%)	11 (61.11%)	3 (16.66%)	0.046
Vascular invasion (n = 20)	4 (20%)	13 (65%)	3 (15%)	0.011

is associated with significant different rates of response to hormonal therapy. The phenotype ER+ & PR+ have better response, whereas ER- & PR- tumours have poor response to therapy, and it was suggested that single hormone receptor positive tumours have worse clinicopathological features than double positive or double negative hormone receptor status in breast malignancies [19]. The findings were similar to Potemski *et al.* [20], Kim *et al.* [21] and Dunnwald *et al.* [22].

Although many studies have observed that the hormone receptors expression increases with that of patient's

age [23-26]; however, in our study, no such association was obtained which might be due to increased prevalence of higher grade tumours and late diagnosis in our population leading to more cases without ER/PR expression. Our study showed no correlation between ER/PR expression and menopausal status of the patient which was in contrast to many previous studies [24].

A significant correlation was found between MBR grade and hormone receptor expression which was similar to observation of various authors [23,25-28]. The expression of both ER and PR were analysed

along with the three components of the MBR grade, which nuclear pleomorphism and mitotic count had statistically significant correlation for both ER & PR expression ($p = 0.005$).

The majority of the cases were grouped in the phenotype ER+ & PR+, with 27 cases (58.69%) in grade I, 18 (39.13%) in grade II and 1 (2.17%) in grade III. Among ER- & PR- group 19 cases (51.35%) in grade II, and 9 cases (24.32%) belonged to grade I and grade III. In the present study, among grade-I tumours, 27 cases (58.69%) were ER+ & PR+ and only 9 cases (24.32%) were ER- PR-. Among grade-III tumours, only one case was ER+ & PR+, whereas nine cases (24.32%) were ER- & PR-. Grade II had majority of the cases out of which 18 cases (39.13%) were ER+ & PR+ & 19 cases (51.35%) were ER- PR-. In a study by Dunwald *et al.* [30], among grade-I tumours 81.1% were ER+ & PR+, and only 4.15% were ER- PR-, whereas among grade-III tumours, 39.1% were ER- PR- and only 44.45% were ER+ PR+.

In the present study, a significant correlation was seen with nuclear pleomorphism, mitosis, MBR grading and hormone receptors ($p < 0.005$) correlation. However, there was no significant correlation with lymphnode involvement which is in agreement with most of the other series [27,29].

A significant correlation of ER & PR expression was observed with lymphovascular invasion. The presence of definite vascular invasion is suggestive of prognostic significance in long-term survival of patients and in predicting local recurrences after conservative surgery.

CONCLUSION

ER- PR- status correlated with traditional histological parameters like high histologic grade, lymphatic and vascular invasion with statistically significant correlation. Determination of hormone receptors ER and PR, MBR grade taken together offers valuable prognostic information for clinical management of patients with breast carcinoma.

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