ORIGINAL RESEARCH

Evaluation of Expression of Estrogen and Progesterone Receptors and Correlation with Clinical Parameters in Breast Carcinoma

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ABSTRACT

Introduction: The presence of estrogen and progesterone receptor in breast cancer not only predicts response to hormone therapy but is also a powerful independent prognostic factor. Hence, the present study was undertaken to find expression of ER and PR in breast carcinoma and to correlate expression of this tumor markers with clinical parameters.

Materials and Method: The present study was carried out on 50 cases of breast cancer received as lumpectomy or mastectomy specimens. The tissue was formalin fixed and paraffin embedded. All the cases were subjected to immunohistochemistry for ER and PR expression. After protein blocking, the slides were incubated overnight with the available ER and PR primary antibodies and they were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy. Positive and negative controls were run with every batch of the IHC. Quick score method of assessment was used to assess the range of immunostaining.

Results: Estrogen receptor positivity was seen in 32% cases. Progesterone receptor positivity was seen in 38% cases.ER PR positivity was seen in 26% cases, ER+PR- in 6%, ER- PR+ in 12% whereas ER PR negativity was seen in 56% cases.

Conclusion: Carcinomas of breast should be subjected to immunohistochemistry to find out the status of hormone receptors i.e. ER, PR and other biomarkers for recommending required hormonal therapy.

Keywords: Biomarkers, Prognosis, Immunohistochemistry, Hormonal Therapy

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INTRODUCTION

Breast Cancer is the most common malignancy in women. It is the commonest cause of death in developed countries in middle aged women and is becoming frequent in developing countries as well. Worldwide mortality rates from breast cancer have increased during the past 60 years in every country. In Indian women breast cancer is not only frequent but also it occurs a decade earlier.[1,2]

With the newer understanding of the disease pattern, tumor grading, immunohistochemistry and genetic study, various other factors have become more important than size of tumor and clinical staging. These are called prognostic factors. Knowledge of these prognostic factors is very important as they help in deciding the type of surgical management required and whether adjuvant therapy is required or not and what type of drug can give best results in a particular case. Hormonal receptor status is used to select adjuvant treatment.[3] The presence of estrogen receptor in breast cancer not only predicts response to hormone therapy but is also a powerful independent prognostic factor. In the Western literature Estrogen Receptor positivity varies between 50-70% but in Indian literature the positivity is 30- 50%.[4] Recent studies also suggest that assessment of progesterone receptor are equally or more valuable than those of ER in predicting the disease-free interval in patients with breast cancer. Western data showed progesterone receptor positivity of 57.74% but in Indian literature the positivity is 41.5%.[5,6] Hence measurement of ER and PR in tumor tissue is very important in patients with breast cancer. Thus the present study was undertaken to find expression of ER and PR in breast carcinoma and to correlate expression of this tumor markers with clinical parameters.

MATERIALS AND METHOD

The present study was carried out on 50 cases of breast cancer received as lumpectomy or mastectomy specimens in the Department of Pathology, of our institute. Ethical clearance was obtained for the commencement of the study. History was obtained from the proforma attached. The tissue was formalin

fixed and paraffin embedded. All the cases were subjected to immunohistochemistry for ER and PR expression. The antigen retrieval was done by using pressure cooker method with 10 mmol citrate buffer at pH 6.0. Tris buffer was used as the wash buffer and Diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. Hydrogen peroxide was used to block the endogenous activity. After protein blocking, the slides were incubated overnight with the available ER and PR primary antibodies and they were conjugated with streptavidin Horse Radish Peroxidase (HRP). Target antigen retrieval was done by heat induced epitope retrieval technique. Primary antibody used for ER was Monoclonal rabbit Estrogen Receptor antibody, RMAB001(Diagonostic Biosystem). Primary antibodies for PR was Monoclonal rabbit Progestrone Receptor antibody, RMAB002 (Diagonostic Biosystem). Antigen retrieval was followed by avidin biotin method of immunostaining. 3–5 µm sections were cut and mounted on poly–l–lysine coated slides. Slides were dried overnight at 37°C and dewaxed in xylene and hydrated. The slides were counterstained with hematoxylin and were examined by light microscopy. Positive and negative controls were run with every batch of the IHC. Only membrane staining of tumor cells was considered as positive. Figure 1 shows photomicrograph of stained section. IHC scoring for estrogen receptor is given in table 1 and for progesterone receptor in table 2. Brown nuclei were taken as positive for estrogen receptor and cytoplasmic brownish colouration was ignored. Brown nuclei were taken as positive for Progesterone Receptor. Cytoplasmic brownish colouration was ignored. Quick score method of assessment was used to assess the range of immunostaining.

Total Score (TS) = PS + IS (Range 0-7)

Negative–quick score 0

Low - quick score 2-3

Medium - quick score 4-5

High - quick score 6-7

Statistical Analysis

Chi square test was used for statistical analysis. p value <0.05 was considered as significant value.

RESULTS

The data revealed that all the patients were in the fifth to sixth decade of age. Estrogen receptor positivity was seen in 16 cases comprising 32% of the total cases. Percentage of positive cells vary from 12 to 92% with moderate and strong intensity (table 3).

Progesterone receptor positivity was seen in 19 cases comprising 38% of the total cases. Percentage of positive cells vary from 1 to 84% with mild, moderate and strong intensity (table 4).

Combination of ER and PR

On basis of ER PR positivity tumors were separated into four categories: ER+PR+, ER+PR-, ER-PR+ and ER-PR-. Maximum number of cases were combined ER and PR negative constituting 56% followed by ER and PR positive cases constituting 26% cases (table 5).

Correlation of ER and PR with lymph node

ER PR positivity although showed no definite correlation with nodal staging with p value=0.86 (table 6).

Correlation of ER and PR with tumour size

In the present study majority of cases (38) were having size varying from 2 to 5 cm and no definite correlation (p=0.09) of ER, PR was observed with increase in size of tumor (table 7).

DISCUSSION

Although women in India are less likely than women in west-

Percentage positivity	Score	Staining intensity	Score
<10 %	1	Low	1
10–50 %	2	Moderate	2
50–75 %	3	Strong	3
>75 %	4		

Table-1: Scoring criteria for Estrogen Receptor

Percentage positivity	Score (PS)	Staining Intensity	Score (IS)
No staining	0	Negative	0
1-25%	1	Weak	1
26-50%	2	Medium	2
51-75%	3	High	3
76-100%	4		

Table-2: Scoring criteria for Progestrone Receptor

Estrogen receptor	No of cases	%age
Positive	16	32%
Negative	34	68%
Total	50	100%

Table-3: Cases with ER positivity

Progesterone receptor	No of cases	%age
Positive	19	38%
Negative	31	62%
Total	50	100%

Table-4: Cases with PR positivity

Combination of ER and PR	Number of cases (n=50)	Percentage
ER+PR+	13	26%
ER+PR-	3	6%
ER-PR+	6	12%
ER-PR-	28	56%

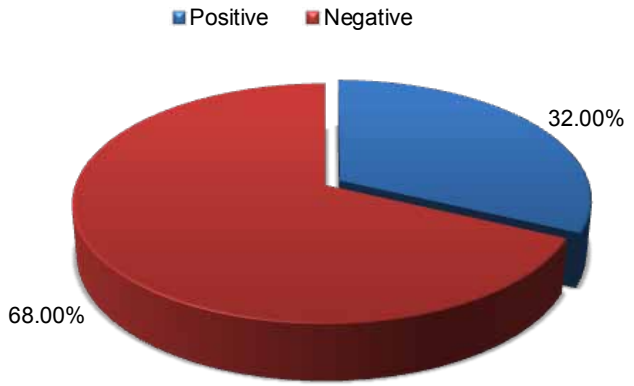
Table-5: Combination of ER and PR cases

Lymph Nodes	ER+	PR+	ER+PR+	ER+PR-	ER-PR+
N0	4 (30.7%)	7 (43.8%)	3 (30%)	-	2 (33.3%)
N1	3 (23.1%)	3 (18.8%)	2 (20%)	-	2 (33.3%)
N2	2 (15.5%)	1 (6.3%)	1 (10%)	2 (100%)	-
N3	4 (30.7%)	5 (31.1%)	4 (40%)	-	2 (33.3%)

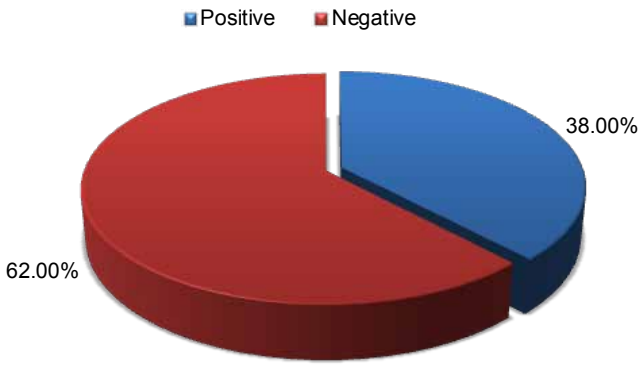
Table 6: Correlation of ER and PR with lymph node

Size	ER+	PR+	ER+PR+	ER+PR-	ER-PR+
<2	3 (18.8%)	4 (21%)	3 (23.1%)	-	1 (16.7%)
2 – 5cm	11 (68.8%)	13 (68.5%)	8 (61.5%)	3 (100%)	5 (83.3%)
>5 cm	2 (12.4%)	2 (10.5%)	2 (15.4%)	-	-

Table-7: Correlation of ER and PR with tumour size



Graph I: Cases with ER positivity



Graph II: Cases with PR positivity

ern countries to develop breast cancer they are more likely to die of it due to paucity of screening, early diagnosis and treatment options.[7] Eighty percent of carcinomas that are ER and PR positive respond to hormonal manipulation whereas only about 40% of those with either ER or PR alone respond. ER-positive cancers are less likely to respond to chemotherapy. Conversely cancers that fail to express either ER or PR have a less than 10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy.[8]

In normal human mammary tissue estrogen is the major epithelial cell mitogen and is known to induce progesterone receptor (PR) expression which is maximally induced at low estrogen concentrations while a higher amount of estrogen is required to induce proliferation. The cells expressing the estrogen receptor alpha (ERα) invariably contained the PR. Estrogen is necessary to induce progesterone receptors.[9] Thus, it is important to screen the ER and PR status of the patient before commencement of the treatment. Henceforth, the present study was conducted.

In the present study, estrogen receptor positivity was seen in 16 cases comprising 32% of the total cases. This percentage is less than the number of ER positive cases reported in the western literature (50–70%) however in Indian literature the positivity is 30-50%.[4] Kaul R et al[10] and Tiwari et al[11] reported the incidence as 34.5% and 42.6% respectively. In Mumbai, Desai et al[12] found 32.6% breast tumors as ER positive. A study from UK by Barnes et al[13] observed 73% ER positivity. In France, M Caly et al reported 74% ER expression in breast tumors.[14] Thus, the present study also found a marked difference between the incidence of India and other countries which may be due to racial difference, low prevalence of oral contraceptives and hormone replacement therapy. However a recent study from Chennai by Ambroise M et al[15] in 2011 found ER over expression in 59% of cases.

Estrogen receptor (ER) positive breast cancers generally have a better prognosis and are responsive to anti-estrogen therapy which targets on this specific protein. Unfortunately ER-negative breast cancers are more aggressive and unresponsive to anti-estrogens. A Hankison SE et al[16] reported that estrogen receptor assays predict clinical response correctly in about 2/3rd of patients with estrogen receptor positive tumors i.e. two third of ER positive tumors respond to hormonal manipulators whereas virtually all (96%) of the ER negative tumors fail to respond to endocrinal therapy. Hence estrogen receptor positive tumors have a longer disease free survival as compared to estrogen receptor negative tumors. Thus Estrogen Receptor has become one of the most important prognostic and predictive markers for breast cancer.

In the present study, progesterone receptor positivity was seen in 19 cases comprising 38% of the total cases which was in concordance with other studies conducted in India. In the Indian study by Shet et al[4] found PR expression of 42%. PR expression was found to range from 33.6% to 46% in other Indian studies.[4,5] whereas in western studies expression varies from 57 to 61.3% respectively as found in studies conducted by Di Stefano et al[17] and Stierer et al. [18] PR assessment is a valuable tool in breast cancer patients as it indicates the disease free survival in these patients.[13] There has been controversy about importance of PR receptor in breast cancer but recent

studies have shown that patients with PR positive tumors benefit with endocrine therapy although PR is a weak predictor of response to endocrine therapy.

Aaltoma et al[19] studied prognostic variables of 281 women with breast cancer that were followed up for 8 years. Clinical and histological features, nuclear characteristics and mitotic studies were analyzed separately in PR-negative and PR-positive tumors. In PR-negative tumors axillary lymph node status and tumor size with histologic subtypes were predictors of survival. In PR positive tumors, size of tumor and lymph node status was used to predict survival independently. It was observed that mitotic indices and nuclear- morphometric variables are of limited value in predicting patient survival in hormone negative breast cancers and the study concluded that a separate analysis is advocated for hormone negative and hormone positive tumors for predicting survival. Gown et al[20] stated that patients with ER or PR-positive tumors had a better prognosis than patients with ER-positive or PR-negative tumors, who had a better prognosis than patients with ER-negative/PR-negative tumors.

Some workers have hypothesized that the low incidence of hormone receptor negative breast tumors in Indian setup may be because of the low frequency of oral contraceptive pills (OCPs) and hormone replacement therapy (HRT) in Indian population. Tewari M et al[11] carried out an analysis of 150 breast cancer patients who had a history of intake of OCP or hormone replacement therapy for a minimum of 1 year was carried out and compared with an equal number of age, stage and histopathology matched non-users. The study concluded that the proportion of hormone receptor positive tumors were found to be more in the patients who were on oral contraceptive pills and hormone replacement therapy.

The present study found that maximum number of cases were combined ER and PR negative constituting 56% followed by ER and PR positive cases (26%) and this is in concordance when compared to other studies carried out by Desai et al and Mehta et al (table 8).

Survival and response to hormonal therapy are most favourable among women diagnosed with tumors positive for both ER and PR, intermediate for tumors discordant on receptor status and least favourable for tumors negative for both receptors.[22] Fisher et al[23] studied estrogen receptor status of 178 invasive breast cancer casesand found that positive ER was associated with high nuclear and low histological grades, absence of tumor necrosis, presence of marked tumor elastosis and older patients. It was also observed that well differentiated tumors were more frequently ER+ in older women.

In the present study majority of cases (38) were having tumor size varying from 2 to 5 cm but no definite correlation of ER and PR was observed with increase in size of tumor. However, Anderson et al[24] supported the fact that ER positive phenotype was associated with smaller tumor size, better grade and cancer specific survival than ER negative tumors.

Author	Combination of ER and PR			
	ER+PR+	ER+PR-	ER-PR+	ER-PR-
Desai et al[11]	25%	7.4%	21.1%	46.5%
Mehta et al[21]	30.3%	20.9%	7.7%	41.2%
Present study	26%	6%	12%.	56%

Table-8: Comparative analysis of combination of ER and PR with previous literature

In the present study, ER and PR positivity showed no definite correlation with nodal staging. Various studies have also failed to find any direct association of ER positivity with lymph node metastasis. Similar to present study, Chariyalertsak et al[25] also found no statistically significant difference in lymph node status and the positivity of ER.[71] However, Allegra JC et al[22] reported that Survival and response to hormonal therapy are favourable among women diagnosed with tumors positive for both ER and PR, intermediate for tumors discordant on receptor status (ER+PR-, ER-PR+) and least favourable for tumors negative for both receptors .

CONCLUSION

Knowledge of prognostic factors is of utmost significance in planning therapeutic approach to the disease. Hormonal receptor status is a factor to determine the hormonal therapy and prognosis of the disease. Studies suggest survival and response to hormonal therapy is favourable among women diagnosed with tumors positive for both ER and PR. Clinical trials have shown that the survival advantage for women with hormone receptor-positive tumors is enhanced by treatment with adjuvant hormonal and/or chemotherapeutic regimens. ER PR positive tumors have superior survival as compared to ER PR negative tumors. Consequently, the present study concludes that all the carcinomas of breast should be subjected to immunohistochemistry to find out the status of hormone receptors i.e ER, PR and other biomarkers for recommending required hormonal therapy.

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