IMMUNOHISTOCHEMICAL STUDY OF ESTROGEN AND PROGESTRONE RECEPTORS IN BREAST CARCINOMA.

Medicine

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ABSTRACT

Background-Worldwide, more than a million women are diagnosed with breast cancer every year accounting for a tenth of all new cancers and 23% of all female cancer cases. Incidence rates vary considerably, with the highest rates in the developed world and the lowest rates in Africa and Asia[1].To know the ER,PR receptor positivity is very important for the treatment and prognosis of patient.
Methods-This is a prospective study which includes 50 diagnosed cases of Breast Carcinoma. The representative histopathology formalin fixed paraffin embedded tissue blocks of the patients are studied by IHC for ER, PR positivity.
Results-Grade I tumors showed ER and PR Positivity in 55.55% and While Grade III showed ER &PR positivity of 27.27%
Conclusion- Hence, ER & PR positivity indicates better prognosis than the ER & PR negativity. From the point of view of prognosis and adjuvant chemotherapy, the knowledge of receptor status and other markers is very important.

KEYWORDS

ER, PR, IHC, Breast Carcinoma

INTRODUCTION

Worldwide, more than a million women are diagnosed with breast cancer (BC) every year accounting for a tenth of all new cancers and 23% of all female cancer cases. Incidence rates vary considerably, with the highest rates in the developed world and the lowest rates in Africa and Asia.[1] The role of estrogen receptor (ER) & progesterone receptors (PR) in BC is very important as it determines the modality of treatment and thus helpful in prognosis.

These are steroid receptors expressed in nuclei of normal mammary epithelial cells. ER regulates the growth proliferation and differentiation of breast epithelium. It also plays an important role in development and progression of BC. In addition ER is a prognostic as well as a strong predictive marker of hormonal response in BC. The degree of hormonal response increases with increase in expression of ER. The physiological effect of progesterone completely depends upon the presence of PR. Estrogen is necessary for inducing the PR. In normal mammary glands, progesterone promotes epithelial cell proliferation and is essential for lobulo-alveolar outgrowth.[2]

The present study was conducted to detect ER and PR by IHC (IHC) in diagnosed cases of BC and its correlation with age, menopausal status, stage and lymph node metastasis.

MATERIAL AND METHODS

This study of IHC ER and PR in BC was undertaken in Department of Pathology at a Tertiary Care Hospital. This was a prospective study of two years on fifty cases of BC. The clinical details of women were noted with special reference to local examination, lymph node metastasis and stage of tumour. An informed consent was taken from every patient. Cases of recurrence, necrotic tumour due to prior therapy were excluded.

Immunostaining was performed with mouse monoclonal anti human ER and PR provided as ready to use liquid tissue culture supernatant, presented in 5% horse serum phosphate buffered saline containing 12 mm sodium azide as preservative. This anti ER & anti PR belonged to IgG1 class & stored at 2-8°c. The technique used was based on the labeled streptavidin biotin (LSAB) method. Endogenous peroxidase was blocked by first inactivating the section in 0.6 H_2O_2. The specimen was then incubated with biotinylated linked antibody and peroxidase labeled streptavidin staining was completed after incubation with substrate chromogen solution. The substrate chromogen (DAB) resulted in a brown coloured precipitate at antigen site. Biogenex EZ-Retriever system V.2 was been used in our study for antigen retrieval of specimen. The technology is based on the application of heat for varying length of time to formalin fixed, paraffin embedded (FFPE) tissue sections immersed in "Antigen Retrieval solution" with unique feature of temperature monitoring.

RESULTS

In the present study, 50 cases were classified as ER+PR+, ER-PR-, ER+PR- and ER-PR+ based on presence or absence of receptor binding activity. The results of ER/ PR were correlated with clinical parameters like age, menopausal status, stage of the tumour and histopathological parameters like grade, lymph node status and size of tumour.

Incidence of ER+PR+ group was maximum (i.e. 46%) followed by ER-PR- (i.e.40%), ER+PR- (12%) and ER-PR+ (2%).

Table 1: Showing correlation of different age groups with hormone receptor status in study group

Receptor status	Age of patient (yrs)							
	≤45 (T.N=12)		46-45 (T.N=16)		56-65 (T.N=13)		>65 (T.N=9)	
	N	%	N	%	N	%	N	%
ER+PR+	4	33.33	8	50	6	46.15	5	55.55
ER-PR-	7	58.33	7	43.75	5	38.47	1	11.11
ER+PR-	-	-	1	6.25	2	15.38	3	33.33
ER-PR+	1	8.33	-	-	-	-	-	-

(T.N = Total no. of cases)

ERPR status showed higher incidence of reactivity with advancing age. Patients below or equal to age of 45 years were found more ER & PR negative (i.e. 58.33%) and less ER & PR positive (i.e. 33.33%). Patients more than 65 years were found more ER & PR positive (i.e. 55.55%) and less ER & PR negative (i.e. 11.11%).

Postmenopausal women were found more ER & PR positive (i.e. 50%) as compared to premenopausal women (i.e. 35.7%). Premenopausal women were found more ER & PR negative (i.e. 50%) as compared of postmenopausal women (i.e.36.12%).

Table 2: Showing correlation of stage of the tumour with hormone receptor status in study group

Receptor status	Stage of the tumour					
	Stage – I (T.N = 7)		Stage – II (T.N = 22)		Stage – III (T.N = 21)	
	N	%	N	%	N	%
ER+PR+	5	71.44	11	50	7	33.33
ER-PR-	1	14.28	10	45.5	9	42.85
ER+PR-	1	14.28	1	4.5	4	19.04
ER-PR+	-	-	-	-	1	4.78

With increase in stage of tumour, ER & PR positivity decreased and ER & PR negativity increased.

It was found that ER & PR positivity was inversely proportional to tumour size. Tumour size from 0 to 1.9 cm showed 78.57% ER & PR positivity and 14.28% ER & PR negativity, while negative. Lymph node with no metastasis showed more ER & PR positivity, while lymph node with metastasis showed more ER & PR negativity. Patient with no lymph node involvement showed 66.66% ER & PR positivity and 27.27% ER & PR negativity, while patient with more than 9 lymph node involvement showed 30% ER & PR positivity and 50% ER & PR negativity.

Table 3: Showing correlation of grade of the tumour with hormone receptor status in study group

Receptor status	Grade of the tumour					
	Grade – I (N=9)		Grade – II (N=30)		Grade – III (N=11)	
	N	%	N	%	N	%
ER+PR+	5	55.55	15	50	3	27.27
ER-PR-	1	11.11	13	43.33	6	54.54
ER+PR-	3	33.33	2	6.66	1	9.09
ER-PR+	-	-	-	-	1	9.09

It was found that ER & PR positivity was inversely proportional to grade of tumour.

DISCUSSION

IHC is an established modality for studying the hormone receptor status in BC and for planning the most appropriate therapy. Estrogen remains the primary marker for hormone receptor status in BC with PR being a surrogate marker.

Present study correlated with the studies of Dunnwald LK et al[3] and Barnes DM et al[4].

Incidence Of Cases In Various Er/pr Groups In Previous Study And Present Study					
Authors	Year	ER+/PR+ (%)	ER - /PR- (%)	ER+ /PR- (%)	ER-/PR+ (%)
Redkar AA et al[5]	1992	23.8	53.4	20	2.8
Barnes DM et al[4]	1995	50	25	20	5
Desai SB et al[6]	2000	25	46.5	7.4	21.1
Dunnwald LK et al[3]	2007	63	21	13	3
Present study	2008-2010	46	40	12	2

Fisher ER et al carried out a study on correlation of ER with pathological and clinical characteristics of 178 BC.[7] Positive ER was found higher in older patients. Joslyn SA et al carried out a study on ER and PR in primary BC. [8] All women diagnosed with primary BC in Lowa from 1990 through 1992 were included in study (N = 6178). In this study they found older women were significantly more likely to be ER + PR +, while younger women were significantly more likely to be ER-PR-. Desai SB et al studied 789 cases of BC.[6] The objective of the study was to document the ER and PR status of BC in the Indian population and in their study ER and PR status showed higher incidence of reactivity with advancing age. Grazia A et al studied ERPR negative BC.[9] In that they found women more than or equal to 50 years of age were found more ER+PR- followed by ER+PR+. In BC research article, Dunnwald LK et al studied hormone receptor status of BC patient in 2007.[3] They found that older women more likely to be diagnosed with ER+PR+ tumours (68%), whereas more than one third of women 30-39 years old presented with ER-PR- tumours.

Present study also showed higher incidence of ER/PR reactivity with advancing age. One possibility to explain the increased ER levels in BC of older patients is the availability of unoccupied ER binding sites due to lower levels of circulating estrogens in the postmenopausal patients. Alternatively, ER expression is upregulated in older patients due to lower circulating endocrine hormones.[10] The reduced ER levels in younger patients may be attributed in part to ER occupancy by endogenous estrogens and/or downregulation of ER expression by the progesterone and other endocrine hormones. .[10]

In the study by Fisher ER et al ER status was correlated with

pathological and clinical characteristics of 178 BC cases[7]. They found the frequency of ER+ tumours were more in postmenopausal women than premenopausal women. Silfversward C et al carried out study on ER concentration in 269 cases of histologically classified human BC.[11] The ER concentration was significantly found lower in premenopausal women than in postmenopausal women. Thorpe SM studied ER and PR determination in the BC.[12] . He found that ER+PR- tumour occurs predominantly among postmenopausal women. Pujol P et al carried out the study on changing ER and PR patterns in BC during the menstrual cycle and menopause.[13] . They found the percentage of ER+ tumours were higher among the postmenopausal women than premenopausal women. Conversely, the percentage of PR+ tumours was higher among the postmenopausal women than premenopausal women.

In the present study, ER+PR+ and ER+PR- tumours were found more in postmenopausal women while ER-PR- and ER-PR+ tumours were found more common in premenopausal women. So the present study correlated with most of the above studies.

The possibility of increased estrogen synthesis in adipose stores and greater bioavailability has been reflected in hormone receptor positive tumour. It is probably due to this reason that postmenopausal obesity has been found to be more constantly associated with increased risk of hormone receptor positive tumours, than hormone receptor negative tumours. .[14] . Fifty to eighty five percent of carcinomas express estrogen receptors and such tumours are more common in postmenopausal women. [15] Dunnwald LK et al showed following results for stage of tumour. [3] They found that ER+PR+ tumours decreased with stage, while ER-PR- tumours increased with stage. Results of present study correlated with the above study. Redhar AA et al carried out study on ER and PR measurement in BC. [5]

No correlation was observed between ER and PR status with tumour size, suggesting that ER and PR status was not depended on tumour size. The study carried out by Desai SB et al also showed no correlation of ER & PR status with size of tumour.[6] In the study carried out by Grazia A et al they found that ER+PR+ tumour cases were decreased with increasing tumour size.[9] Dunnwald LK et al showed inverse relation with tumour size.[3] Results of present study correlated with the studies of Grazia A et al[9] and Dunnwald LK et al[3].

Study carried out by Redkar AA et al did not show any correlation between axillary nodal involvement and ER/PR status.[5] Desai SB et al showed an average 58.3% patients with primary BC representing with axillary nodal metastasis. [6] Only 41.7% were node negative. The presence or absence of nodal metastasis did not correlated significantly with ER/PR status. Grazia A et al found decrease in ER+PR+ and ER + PR - status with increase in number of nodes involved.[9] Dunwald LK et al also showed decrease in ER + PR+ status with lymph node involvement while increase in ER - PR- status with lymph node involvement[3]. Results of present study correlated with the studies of Grazia A et al [9] and Dunnwald LK et al[3].Silfversward C at al provided a histological review of 269 female primary BC analysed for estrogen receptor.[11] The ER levels were higher in ductal carcinomas of lower grade than in higher histological malignancy grade. Desai SB et al reported that most of the patients in their study had high grade (grade 3) tumour.[6]

ER and PR reactivity decreased with the increasing tumour grade. Dunnwald LK et al carried out a study on hormone receptor status, tumour characteristic and prognosis.[3]

They found inverse relation of ER + PR + tumour with the grade of tumour. Mudduwa LK et al The majority of patients included in their study had high grade tumour and showed lower hormone receptor expression. [16] S. Mirunalini et al studied correlation of estrogen and progesterone receptor status with grade of the tumour. [17] Incidence of receptor expression were significantly more among the cases with grade 2 malignancy (53.3%) than compared with grade 1 (6.6%) and grade 3 (40%). Results of Present study correlated with the studies of Silfversward C et al[11], Desai SB et al[17], Dunnwald LK et al[3] and Mudduwa LK et al[19]

CONCLUSION

Presence or absence of estrogen and progesterone receptors were assessed by immunohistochemical study in 50 cases of BC. The incidence of ER + PR + cases was maximum (i.e. 46%) followed by the

incidence of ER - PR - cases (i.e. 40%). ERPR status showed higher incidence of reactivity with the advancing age. Postmenopausal women were found more ER & PR positive (i.e. 50%) as compared to premenopausal (i.e. 35.7%). It was observed that with increase in stage of tumour, ER PR positivity was decreased, while ER & PR negativity was increased. Lymph node with no metastasis showed more ER&PR positivity, while lymph node with metastasis showed more ER PR negative. Hence, ER & PR positivity indicates better prognosis than the ER & PR negativity. From the point of view of prognosis and adjuvant chemotherapy, the knowledge of receptor status and other markers is very important. It was found that ER PR positivity was inversely proportional to grade of tumour.
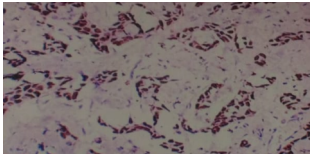
LEGENDS

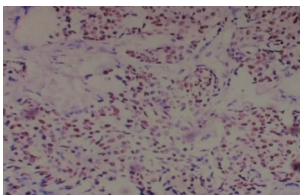**Figure 1: 60 % ER positivity in IDC (400X)**

Figure 2: 40 % PR positivity in IDC (400X)

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