

# Correlation of expression of Androgen Receptors and Estrogen Receptors in female Breast Cancer cases.

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**Abstract:** Estrogen receptors and progesterone receptors have been extensively studied and assayed in breast cancer management as independent prognostic markers for hormonal therapy. Androgen receptors are known to induce proliferative changes in breast cancer and this has led to investigations regarding expression of androgen receptors in breast cancer. Androgen receptor expression has been found to be associated with poor breast cancer subtypes and could be an independent predictor in invasive breast cancer.

**Keywords:** *Breast cancer, androgen receptors.*

## Introduction

Breast cancer is the second most common malignancy of the women in India, next only to cervix cancer. In the year 2001, 800,000 new cancer cases were reported, out of which about 80,000 cases were of breast cancer. The average incidence rate of breast cancer India is 16/100,000, varying from 22-28/100,000 females in urban settings to 6/100,000 in rural areas. In cities like Mumbai, Trivandrum and Delhi, it forms the most frequent female cancer. The age of presentation among Indian females is a decade later than the western population i.e. 45-54 years. About 80-90% cases of breast cancer present at a locally advanced stage. Recent advances in molecular pathology enable identification of molecular mechanisms underlying development and progression of breast cancer. Several prognostic markers have been identified for breast cancer viz. estrogen receptor (ER), PR, p53, CerbB-2 and others. Of these, estrogen receptor has long been studied and assayed for breast cancer management. Anti-estrogens (Tamoxifen) and ER blockers (SERMS) have, of late, become powerful therapeutic agents to treat breast cancers that express ER's. In addition, aromatase inhibitors are now used in postmenopausal women to block the *in-situ* conversion of adrenal androgens to estrogens.

Androgens have been found to be carcinogenic in prostate cancer. Lately, they have also been thought to play an important role in breast cancer. The risk of breast cancer is increased in postmenopausal women with high estrogen levels as also high androgen levels. Androgens are known to induce proliferative changes in breast cancer by a direct activation of ER by DHEA, 5-androstene-3bba ER-negative tumors. The expression of androgen receptors (AR) in breast cancer cases, particularly those that are ER negative, has long been a matter of debate, including whether AR expression has prognostic significance in ER negative tumors. Present study was undertaken with the following *aims and objectives* : 1. To study status of Androgen receptor (AR) expression in 25 cases of breast cancer. (2) To correlate Androgen receptor (AR) expression with estrogen receptor (ER) expression. (3) To correlate Androgen receptor expression with various clinical and histopathological parameters.

## Review of literature

Breast cancer is the most common malignancy in females all over the world. In India it is the second most common malignancy next only to cervix cancer. In the year 2003, about 240000 cases of breast cancer were diagnosed worldwide, out of which 40000 patients died of it. In India, 80000 cases of breast cancer were reported in the year 2001. The average age of reporting in Indians is 45-54 years, which is a decade later than that in western countries. Thus the need is felt for better prognosis using better molecular markers, as every cancer, including breast carcinoma has a molecular basis for its genesis.

The genesis of breast cancers is largely hormonal and genetic. Sex hormones like estrogen and progesterone have been studied and their effects on the breast tissue used as prognostic tools in breast cancer management. Genetic factors include genetic mutations that are autosomal, defective DNA repair and some familial cases due to germ line mutations. The expression of a large number of carcinogenic genes is now detectable via assay of selected proteins using immunohistochemistry techniques (ER, PR, HER/neu, e-cadherin, p53, ki-67 etc.). This technique assays actual protein content. Immunohistochemistry uses antibodies to detect proteins on tissue sections and has the advantage of being able to identify the cell type expressing the protein and also its accurate location.

This major sex steroid hormones viz estrogens and androgens have been known to cause proliferation in tumor cells. In case of breast cancer, estrogen receptor expression is particularly thought to be of great importance, as ER + cases are known to respond better and more frequently to hormonal therapy (50-75% response rate) while ER-negative tumors has less than 10% chance of response. In addition ER positivity also is prognostic of delayed recurrence in primary breast cancer. Also, estrogen is thought to play a major role in the development and progression of breast cancer.

Estrogen receptor expression has been studied and assayed in breast cancer management as a prognostic indicator in breast cancer hormonal therapy. Antiestrogen (Tarduxitic) ER blockers (SERMs) and aromatase inhibitors (that block *in situ* conversion of androgens to estrogens) have gained widespread acceptance in cancer therapy. Other markers that are routinely assayed in breast carcinomas are PR, HER2/neu, e-cadherin, p53 and Ki-67). The role of adrenal androgens in carcinogenesis has also been studied. They are known to cause prostate cancer. The expression of androgen receptors, in breast cancer cases, particular house that

or ER has long been a matter of debate including whether AR expression has prognostic significance in ER-negative tumors.

The purpose of the present study was to correlate the expression of androgen receptors with their ER status in breast carcinoma, along with age tumor grade and size and lymph node status in Indian patients.

## Material and Methods

The present study included 25 cases diagnosed as breast cancer at the Institute of Pathology, referred from Surgery Department, Safdarjung Hospital, New Delhi. The histopathologic diagnosis was established on conventional haematoxyline and eosin (H&E) staining. Various clinical parameters like age of the patients, tumor stage (TNM), histopathological parameter like tumor type, lymph node (LN) status, and histopathological grade, were noted. AR expression was identified in all cases and correlated with ER expression, using immunoperoxidase ABC (avidin-biotin complex) method.

**Materials included :** (1) blocking antibody (2) primary antibodies (AR: Monoclonal antibody Clone AR441, Neomarker), (ER: Rabbit monoclonal antibody Clone SP1, Labvision, CA USA) (3) secondary antibody (4) ABC kit (Vector labs) (5) diaminobenzidine (DAB) chromogen agent.

**Methods :** Paraffin embedded, methanol fixed slides were preincubated 37.3°C for 30 minutes. Xylene treatment in two changes, each of 30 minutes was used to remove the wax. Methanol treatment in two changes each of 20 minutes was used for dehydration. Peroxide treatment in mixture of 47ml methanol and 3 ml H<sub>2</sub>O<sub>2</sub> for 45 minutes was given. Slides were then washed in citrate buffer (pH 6.9). Heat induced epitopes retrieval was done in microwave. Slides were placed in citrate buffer (pH 6.9) and heated for 90 seconds at 800X followed by 5-minute treatment at 80X. The slides were cooled and the heating and cooling process was repeated four times. Similar process was repeated in citrate buffer (pH 6.0) another four times. After cooling blocking antibody was applied and slides were left for incubation for 1 hour at 37.3 °C. Then, anti-AR antibody was applied on to the slides (without TBS washing), and left for overnight incubation in a refrigerator. Secondary antibody was added after washing with TBS buffer pH 7.6 and left for incubation at 37 °C for 2 hours. Next, ABC antibody was added after washing with TBS buffer pH 7.6 and left for incubation at 37 °C for 2 hour. Slides were then washed again with TBS buffer. Diaminobenzidine (DAB) solution was added on to the slides with a micropipette slides were left for 3 minutes. Counterstaining was done after washing the DAB-stained slides with distilled water and applying hematoxyline with micropipette. The slides were dried and mounted with DPX mountant.

Interpretation: Tumor cells showing more than 10% expression for ER, AR were included as positive.

## Results

A total of 25 cases of breast carcinoma were analyzed for present study. The age of the patients varied from 26 to 70 years, with maximum number of patients in the age group 40-49 years i.e. 10 patients (40%), followed by 9 patients in the age-group >50 years and 6 patients with age <39 years. Among the cases with varying tumor stage, maximum number of cases were in stage 2B (5 cases), followed by, (in descending order) 3 B (4 cases), 1

and 3a stage (3 cases each), 2 cases with stage 2A and 1 case of tumor stage 4.

Cases were categorized into different histomorphological types, with maximum number of cases of infiltrating ductal carcinoma (IDC) NOS (14 cases), followed by infiltrating lobular carcinoma (ILC) (5 cases), 2 cases each of ductal carcinoma-in-situ and mixed IDC and ILC type and 1 case each of medullary and comedocarcinoma.

Fourteen cases showed lymph node positivity while remaining 11 cases had negative lymph node status. Among 12 cases, with details of family history of breast and/ or other cancers, 7 cases revealed a positive family history of the same.

Androgen receptor (AR) expression was analyzed in all 25 cases and further correlated with the various clinical, histopathological markers. Further AR expression was correlated with ER expression. Out of 25 cases, 12 (48%) cases showed estrogen positivity and 13(52%) cases revealed negative ER expression. Expression, with various parameters is as follows:

**Table No 1(Total cases=9)**

AGE	AR	%
<39	2	22
40-49	3	33
>50	4	44
<b>TOTAL</b>	<b>9</b>	<b>100</b>

**AR expression and age (Table-1):**

There were nine cases that tested AR positive. Out of these four case (44%) were reported in women over 50 year of age. Least frequency of AR expression was reported in woman with age < 40 years. Thus, AR positivity has been observed directly correlating with increasing age. (Fig: 1)

STAGE	AR	%
1	1	12.5
2A	0	0
2B	2	25
3A	2	25
3B	3	37.5
4	0	0
<b>TOTAL</b>	<b>8</b>	<b>100</b>

AR expression and stage (Table-2): Out of eight AR positive cases for which status of tumor stage was known, most cases (3/8 or 37.5%) were reported at an advanced stage 3B. 25% cases were reported each at 2B and 3A stages. Very few (only 1/8 or 12.5%) cases were reported at primary stage-1.

**Table no 3: Total cases = 9**

LN Status	AR	%
LN +	4	44
LN -	5	55
<b>TOTAL</b>	<b>9</b>	<b>100</b>

AR expression and lymph node status (Table-3): LN status was available for all the nine AR positive cases. Of these 44% (4/9) cases showed LN metastasis while the rest 55% (5/9) cases were LN negative. Thus there was no significant correlation between AR and

**Table 4 (Total cases = 5)**

GRADE	AR	%
Low	1	20
Intermediate	2	40
High	2	40
<b>TOTAL</b>	<b>5</b>	<b>100</b>

**AR expression and grade (Table-4):**

Data for tumor grade was available for 5/9 cases. Of these 40% cases or 2/5 were each of intermediate and high grade. Only 20% cases (1/5) were of low grade. (Tumor grade was noted in IDC cases), ILC cases were excluded.

**Table 5 (Total cases = 3)**

GRADE	AR	%
Low	1	20
Intermediate	2	40
High	2	40
<b>TOTAL</b>	<b>5</b>	<b>100</b>

**AR expression and family history (Table-5):**

Family history in AR positive tumours were available only in three cases. Of these 66% or 2/3 cases showed no family history of breast cancer. Only one patient had a relative with breast cancer. Thus, family history is not significant with AR expression.

**Table 6 (Total cases = 9)**

TYPE	AR	%
1	3	33
2	3	33
3	2	22
4	1	11
5	0	0
6	0	0
7	0	0
8	0	0
<b>TOTAL</b>	<b>9</b>	<b>100</b>

**AR expression and tumour type**

(Table-6): Out of the nine AR positive cases, maximum cases were IDC NOS type and ILC types (33% or 3/9 cases each, respectively). Two cases (22%) were of mixed type where as only one case was of medullary carcinoma, showing positive AR expression. The maximum frequency of AR positivity in IDC and ILC tumors shows that AR positivity is marker of bad prognostic cancer types.

**Table 7 (Total no. of cases = 25)**

	ER+	%	ER-	%
AR+	7	58.33	2	18
AR-	5	41.66	11	82
<b>TOTAL</b>	<b>12</b>	<b>48</b>	<b>13</b>	<b>52</b>

**AR expression and ER expression**

(Table-7): Out of total 25 cases included in the study, twelve (48%) cases were ER positive and rest 13 (52%) cases were ER negative. Of the ER positive cases seven (58.33%) cases were AR positive and the rest five (41.66%) cases were AR negative. Of the ER negative cases, two (18%) cases were AR positive and the rest 11 (82%) cases were AR negative. (Fig. 2, 3, 4, 5, 6.)

## Discussion

Utility of ER expression in breast cancer, as a prognostic marker, is well documented. Lately, there has been a need to identify more prognostic factors. Among these, AR expression is less documented. Earlier studies have focused on ER positive tumors and have shown some association between AR status and disease free survival, as well as response to endocrine therapy. Our study focused on AR expression in breast carcinoma cases and correlation with various established prognostic markers, including ER expression, more so in cases having negative ER expression. Positive AR expression was observed in 9 (36%) cases. Among the various prognostic parameters analyzed, AR expression showed positivity with increasing age of the patients. Agoff et al and Bryan et al in concordance with a similar study conducted this. The incidence of breast cancer is high in elderly females, when the androgenic levels are high, and the risk of breast cancer is high in women with high estrogen levels than those with high androgen levels. It has been shown in animal models that

testosterone in combination with estrogen can induce high incidence of mammary carcinoma. Increasing tumor grade and relatively poor prognostic types like IDC NOS, also showed increase in AR positivity. This was in concordance with study performed by Isola and Agoff et al. The expression was also found more in cases with higher tumor stage i.e. 37.5% cases in stage 3B. Lymph node positivity and family history, however, failed to show any significant associations.

Our study focused on identifying association of AR expression in cases of ER negative tumors. Only 18% cases with ER negativity showed positive AR expression. On the other hand 58.33% cases showed ER and AR positivity. This higher percentage was also noted by Agoff et al. However, lesser number of ER negative cases showing AR positivity was contrasting with observations by Agoff et al. This might have been due to inclusion of lesser number of ER negative cases. On the whole, however AR can be included as an independent prognostic marker for breast carcinoma. The results of Agoff et al and Bryan et al, revealing significance of survival of patients with AR expression and lacking ER expression, further substantiates the utility of studies like the present one in identifying more prognostic factors for breast carcinoma.

## Conclusion:

1. Androgen receptor (AR) expression is associated with increasing age, tumor grade, stage and poor prognostic breast cancer types.
2. AR expression was seen more in ER positive cases, than ER negative cases.
3. AR expression could be an independent prognostic factor in invasive duct breast carcinomas.

## Recommend Reading

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