Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

Original research article

Exploration of the correlation between clinic-pathological patterns and androgen receptor expression in triple negative breast cancer

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Abstract

Androgen receptor, also known as AR, has been shown to have a strong correlation with both the development and progression of breast cancer; however, the clinical relevance of AR in triple negative breast cancer, also known as TNBC, has been the subject of debate. There has only been a modest amount of study conducted on the topic of how neoadjuvant chemotherapy affects the expression of AR. The purpose of the current study is to investigate the clinical importance of AR and offer evidence for AR-directed treatment in TNBC. This will be accomplished through the examination of the expression of AR in patients who have TNBC. The results of further research demonstrated that AR expression did not have any link with the disease-free and overall survival of patients with general TNBC. Instead, it predicted a worse survival rate for patients with stage III TNBC in comparison to those who were diagnosed at earlier stages. AR expression happens more frequently in cases where there is no lymph node metastases or in cases of small TNBC tumours. It is linked to a dismal prognosis for patients whose malignancies have progressed to a more advanced stage.

Keywords: Breast cancer, triple negative breast cancer, androgen receptor prognosis, tumors

Introduction

Breast cancer is one of the most common types of cancer in women and a major public health problem around the world. In 2012, 1.7 million women had breast cancer, which made up about 23% of all cancers in women. Another 6.3 million women who had breast cancer in the last 5 years are still alive. The rates are highest in North America, Australia/New Zealand, and western and northern Europe. They are lowest in Asia and sub-Saharan Africa [2]. These differences between countries are probably caused by changes in society brought on by industrialization, such as changes in fat intake, body weight, age at menarche, and/or lactation, as well as changes in reproductive patterns, such as having fewer children and giving birth at a later age [3].

In India, the average age at which a woman gets breast cancer has changed a lot over the past few decades. Breast cancer is now the most common type of cancer in most cities in India. In rural areas, it is the second most common type of cancer. Between 25% and 32% of all female cancers in big cities are caused by breast cancer [4]. It's likely that the end of hormone replacement therapy (HRT) and the saturation or levelling off of mammography rates both had something to do with this. Out of these things, stopping HRT has probably had the most effect [5, 6]. This was shown in a report from the Women's Health Initiative, which showed that breast cancer rates dropped quickly among trial participants who stopped using HRT [7]. The use of mammograms did not change when HRT was stopped, which suggests that mammograms did not contribute to the drop in incidence rate [8].

Material and Methods

Study Area: Apollo Specialty Cancer Hospital and Tertiary Care Centre, Teynampet, Chennai (Tamil Nadu, India).

Study population: All Triple Negative Breast Cancer.

Type of the study: A Prospective, Observational, Cohort Study.

Study duration: May 2015 to Feb 2017.

Sample size: The required sample size is 77 cases.

Sample calculation: The sample size required for this study to be significant was determined by the statistician by reviewing the currently available literature. 77 patients diagnosed with triple negative breast cancer during the study period and who fulfilled the inclusion/exclusion criteria were allotted to the study.

Inclusion criteria: Newly diagnosed triple negative breast cancer patients for whom

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

immunohistochemistry was done either preoperatively or postoperatively for ER and PR receptor and Her2 Neu expression.

Exclusion criteria

1. Patients who underwent treatment in the past for breast cancer.
2. Patients presenting with insufficient data.
3. Patients with non-epithelial breast cancer i.e., sarcoma, phyllodes tumour.

Methodology

All newly diagnosed TNBC patients will join the study and be evaluated clinically and radiologically. After getting informed consent and a patient information sheet, immunohistochemistry was used to look at pathological features. The results of ER, PR and AR immunostaining were evaluated in a semi-quantitative way, and a tumour was considered positive if more than 10% of its cells had nuclear immunostaining. Ki-67 immunostaining was thought to be positive if the nuclei of more than 10% of the tumour cells were stained [9, 10].
The results need to be looked at using the right kind of statistical analysis.

Protocol for immunohistochemistry: Immunostaining for the ER, PR, HER2 and AR receptors, ki 67. After optimised epitope retrieval, immunohistochemistry was done with mouse monoclonal antibodies ER (1D5) (1:50) and PR (PgR 636) (1:400) and a polymer-based detection system (DAKO) (DAKO reagents). The antigen retrieval process for IHC staining of ER and PR was as follows: Five micron-thick sections were taken out of the paraffin and put back into deionized water. They were heated in citrate buffer (pH 6.0) for three minutes at 12 to 15 pounds per square inch (PSI) and 120 degrees Celsius in an electric pressure cooker. Then, they are left to cool for about ten minutes before being stained with immunostain [11].
The slides were then put into an automated system (DAKO Autostainer plus) where they were exposed to 3% hydrogen peroxide for five minutes, incubated with the primary antibody for thirty minutes, labelled polymer for thirty minutes, 3, 3'-diaminobenzidine (DAB) as a chromogen for five minutes, and then hematoxylin as a counterstain for five minutes [12].
Between incubations, pieces were washed with Tris-buffered saline. The incubations were done at room temperature (TBS). The Tissue-Tek SCA (Sakura Finetek) Cover slipper was used to make the cover slip. Positive controls were made from known positive tissues (like the endometrium and breast), and negative controls were made by replacing the primary antibody with TBS and running them alongside the patient slides [13].
Using the ASCO/CAP ER and PgR Guideline Recommendations to interpret the results:
Positive for ER or PgR if immunoreactivity is found in more than or equal to 1% of tumour cell nuclei.
Negative for ER or PgR if finding of <1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls are seen).

1. The slides were uninterpretable for ER or PgR, if finding that no tumour nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack a nuclear staining.
2. H Score, Allred score or Quick score may be provided.

The IHC score must be 0 or 1+, Fluorescent in situ hybridization (FISH)HER2/neu/CEP17 ration must be <1.8 or the gene amplification copy should be<4 [14].

HER2 Neu staining: The Her cep Test was used to find the HER2 antigen. For the procedure, the slides were put in a calibrated water bath with 10 mmol/L of citrate buffer at a temperature of 95-99 °C. The slides are then kept at 95-99 °C for 40 minutes. After pouring out the epitope retrieval solution, the sections are rinsed in the wash buffer and then soaked in the buffer for 5-20 minutes before they are stained. The Hercep Test programme is used to load the slides onto the auto-stainer, as explained in the manufacturer's insert. The slides are rinsed in the auto-stainer, then put in 200uL Peroxidase-Blocking Reagent for five minutes, rinsed again, put in 200uL primary Anti-HER2 Protein (or Negative Control Reagent) for thirty minutes, rinsed twice and then put in 200uL substrate-chromogen solution (DAB) for ten minutes. After the slides were taken out of the auto stainer, they were counterstained with hematoxylin and then given a cover slip [15].
A pathologist looked at the stained slides through the light microscope. At our hospital, the negative cutoff for ER and PR is when less than 10% of the tumour is stained. HER2 results are based on the maximum intensity and distribution of staining, as shown below:
0 = no staining; 1+ = weak and incomplete membrane staining in invasive tumour cells;
2+: circumferential membrane staining is moderate in at least 10% of invasive tumour cells; 3+: strong membrane staining is seen in more than 30% of invasive carcinoma cells. For HER2 IHC, tumours with a

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

score of 0 or 1+ are considered negative, 2+ is equivocal, and a score of 3+ is positive. IHC 2+ cases should be checked with FISH for HER2 to confirm the positive result [16].

Immunohistochemistry for androgen receptor

For the IHC staining for AR, the antigen was retrieved by deparaffinizing and rehydrating five-micron sections in deionized water. They are heated in citrate buffer (pH6.0) for three minutes at 12 to 15 pounds per square inch (PSI) or about 120 degrees Celsius in an electric pressure cooker. Then, they are cooled for ten minutes before being stained with immunostain [17]. All of the slides were put into an automated system (DAKO Autostainer plus) and exposed to 3% hydrogen peroxide for 5 minutes. They were then incubated with primary antibody for 30 minutes, labelled polymer for 30 minutes, 3, 3' diaminobenzidine (DAB) as a chromogen for 5 minutes and hematoxylin as a counterstain for 5 minutes [18]. These incubations are done at room temperature and sections are washed with Tris-buffered saline between each one (TBS). The Tissue-Tek SCA (Sakura Finetek) Cover slipper was used to do cover slipping. With the patient slides, positive controls were made from known positive tissues (like prostate cancer) and negative controls were made by replacing the primary antibody with TBS [19]. When at least 10% of the nuclei of tumour cells were immunoreactive, the sample was given a positive score for AR. Further, AR positivity was categorised as low (less than 10% of tumour cell nuclei were immunoreactive), medium (about 10% of tumour cell nuclei were immunoreactive), or high (more than 10% of tumour cell nuclei were immunoreactive) [20].

Observations and Results

Part A: A total of 77 patients with TNBC were enrolled in this study and analyzed.

Part B: The TNBC patients were further analyzed based on the Androgen receptor expression, clinical and pathological factors were cross tabulated.

Table 1: Region wise distribution

States	Frequency	Percent (%)
Tamil Nadu	52	67.5
Andhra Pradesh	10	13
Northeast	10	13
Kerala	2	2.6
Karnataka	3	3.9
Total	77	100

In our study 67.5% (n=52) female from Tamil Nadu, 13% (n=10) from Andhra Pradesh and North East, 2.6(n=2) from Kerala, 3.9% (n=3) from Karnataka.

Table 2: Religion wise distribution

Religion	Frequency	Percent (%)
Hindu	55	71.4
Christian	6	7.8
Muslim	5	6.5
Others	11	14.3
Total	77	100

In our study, the religion wise distribution among hindus 71.4%, Christian 7.8%, Muslim 6.5%, others 14.3%.

Table 3: Mode of detection

Mode of detection	TNBC Group	
	Symptomatic	Screened
Frequency	61	16
Percentage (%)	79.2	20.8

Majority of the patients were symptomatic. Symptomatic patients often presented with a lump in the breast. In our study 20.8% of the patients are detected by screening test.

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Table 4: Age wise distribution

Age	Frequency	Percent (%)
<41	17	22.1
41-60	44	57.1
>61	16	20.8
Total	77	100

In our study, the mean age of presentation in TNBC group was 50.3 years. Majority of the patients (n=44, 57.1%) were in the age group of 41to 60 years. In the present study the incidence of TNBC was low among the patients over the age of 60 years (n=16, 20.8%).

Table 5: Personal Habits

Habits	Frequency	Valid Percentage
Nil	74	96.1
Alcoholism	1	1.3
Smoking	2	2.6
Total	77	100

Table 6: Menopausal Status and Androgen Receptor Expression

Menopausal Status vs. and rogen receptor expression	AR expression negative		AR expression positive	
	Number of patients	Percentage	Number of patients	Percentage
Premenopausal	21	38.9	11	47.8
Perimenopausal	9	16.7	4	17.4
Postmenopausal	24	44	8	34.8
Total	54	100	23	100

Both in the Androgen receptor positive and negative, group, the distribution of the cases where predominantly among the pre and perimenopausal women.

Table 7: Cross Tabulation (Age at Menarche is Androgen Receptor Expression)

Age at menarche is androgen receptor expression	AR expression negative		AR expression positive	
	Number of patients	Percentage	Number of patients	Percentage
Early	1	1.9	1	4.3
Late	4	7.4	2	8.7
Normal	49	90.7	20	87.0
Total	54	100	23	100

Table 8: Cross Tabulation (Age at Menopause vs. Androgen receptor expression)

Age at menopause vs. androgen receptor expression	AR expression negative		AR expression positive	
	Number of patients	Percentage	Number of patients	Percentage
Early	6	11.1	4	18.2
Late	1	1.9	1	4.5
Not Attained	47	87.0	18	77.3
Total	54	100	23	100

There was statistically no difference the line AR expression distribution between the groups.

Table 9: Cross tabulation (Stage Group vs. Androgen Receptor Expression)

Group	AR expression negative		AR expression negative	
	Number of patients	Percentage	Number of patients	Percentage
IA	7	13	3	13
IB	0	0	0	0
IIA	21	38.9	7	30.4
IIB	13	24.1	8	34.8
IIIA	6	11.1	2	8.7
IIIB	1	1.9	0	0
IIIC	4	7.4	2	8.7
IV	2	3.7	1	4.3

The Stage wise distribution among the androgen receptor positive and negative patient was not different.

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Table 10: Cross tabulation (Histology type vs. androgen receptor expression)

Group	AR expression negative		AR expression positive	
	Number of patients	Percentage	Number of patients	Percentage
IDC	39	72.2	20	87
IDCNOS	9	16.7	3	13
Mixed	2	3.7		
ILC	3	5.6		
Medullary	1	1.9		

On comparing the histological subtypes, it was found that Infiltrating Ductal Carcinoma (IDC) was the commonest occurrence in both groups (72.2% and 80%).

Table 11: Cross tabulation (Extra nodal spread vs. Androgen receptor expression)

Group	Extra nodal Spread			
	AR negative		AR positive	
	Present	Absent	Present	Absent
Number of patients	48	6	2	21
Percentage	88.9	11.1	8.7	91.3%

In AR negative tumours the extra nodal spread rate was slightly higher (1.1%) than AR positive group (8.7%) but was not statistically significant (*P*=0.72).

Table 12: Cross Tabulation (Lymphovascular Invasion vs. Androgen Receptor Expression)

Group	Lymphovascular Invasion			
	AR negative		AR positive	
	Present	Absent	Present	Absent
Number of patients	12	42	6	17
Percentage	22.2	77.8	26.1	73.9

In AR negative tumours the lymphovascular invasion rate was slightly lower (22.2%) than AR positive group (26.1%) but was not statistically significant (*P*=0.71).

Table 13: Cross Tabulation (Grade of tumor vs. Androgen Receptor Expression)

Grade	AR negative		AR positive	
	Number of patients	Percentage	Number of patients	Percentage
I	2	3.7	1	4.3
II	17	31.5	2	8.7
III	35	64.8	20	87

On grade wise comparison between the groups, there were no statistically significant differences. Most of the women had Grade III disease at the time of their presentation in both the groups.

Table 14: Cross tabulation (T stage vs. Androgen receptor expression)

T Stage	AR negative		AR positive	
	Number of patients	Percentage	Number of patients	Percentage
T1	10	18.5	5	21.7
T2	36	66.7	14	60.9
T3	8	14.8	4	17.3
T4	0	0	0	0
Total	54	100	23	100

Most of the patients had T2 disease during their presentation (66.7% vs. 60.9%). There was a slightly higher proportion of women presenting with T1 disease in negative group (18.5% vs. 21.7%), although statistically not significant (*P*=0.88).

Table 15: Cross tabulation (N stage vs. Androgen receptor expression)

N Stage	AR negative		AR positive	
	Number of patients	Percentage	Number of patients	Percentage
N0	29	53.7	13	56.5
N1	15	27.7	6	26.08
N2	5	9.25	2	8.69
N3	5	9.25	2	8.69
Total	54	100	23	100

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The overall node negative rate was 59% in AB positive group and 45% in AR Negative group, but not statistically not significant (P=0.99).

Table 16: Cross Tabulation (AR expression positive vs. KI-67)

AR expression positive	Frequency	Percentage
LowKI67	15	65.2
HighKI67	8	34.8

Among the AR positive patients, 65.2% (n=15), had lower ki-67 and 34.8(n=8) had higher KI 67

Table 17: Cross Tabulation (AR expression negative vs. KI-67)

AR expression negative	Frequency	Percentage
Low KI67	4	7.4
High KI67	50	92.6

In our present study in negative group 7.4% (n=4), had lowerki-67 and 92.65 (n=50) had higher KI 67.

Discussion

Triple-negative breast cancer is a group of cancers that are very different from each other. In his study, Chenag M, C, U *et al*. showed that some types of triple-negative breast cancer are known to be more aggressive and have a poor prognosis, while other types have a prognosis that is the same as or better than hormone receptor positive breast cancers. Small sample sizes have made it hard to do studies on TNBC patients so far. The goal of this study was to look at 77 TNBC patients' clinical features, histological features, androgen receptor expression, as well as any links between those things [21].

TNBC-Age wise distribution

In our study, the TNBC group had a mean age of presentation of 50,3 years. Most of the patients (n = 44, or 57.1%) were between 41 and 60 years old. The number of people with TNBC who were over 60 years old (n=16) was low. Our patients were a little bit younger than those in the Western Data. There was no statistically significant difference in the number of cases of TNBC by age (p=0.12) [22].

TNBC-Hormonal risk factors

Premenopausal state has been linked to TNBC30. In this study, premenopausal and postmenopausal individuals were evenly distributed. Pre-and perimenopausal women were more likely to have TNBC than post-menopausal women (58.4% vs. 41.6%). A study by Kabat *et al*. found that smoking and alcohol intake were not linked to TNBC, but may be linked to ER-positive breast cancer. In this study, the connection between TNBC and personal habits such as smoking, drinking, and tobacco could not be investigated due to their low prevalence (3%). The survey included 68 (88.3%) housewives. This study couldn't compare marital status because just 1% of patients were unmarried. Age at menarche, menopause and hormone replacement treatment were not statistically significant in our study [23].

Analysis of the histopathological characteristics

In our study, the most common presentation was T2 (64.9%), and the least common was T4 (0%). N1 pat ients (27.3%), N2 patients (9.1%), and N3 patients (9.1%) made up the largest group (54.5%). This proba bly shows the bias in the way our private tertiary care cancer is presented and screened [24].

TNBC-Histology

Infiltrating Ductal Carcinoma (IDC) was found to be the most common histological subtype in our study (76.6%, N=59). Infiltrating Lobular Carcinoma Not Otherwise Specified (15.6%), Medullary Carcinoma (1.3%), Mixed Histology Carcinoma (2.6%), and ILC (3.9%) were the other pathological types in the TNBC group. In the TNBC group, we didn't find any mucinous or papillary tumours [25].

TNBC-Type of surgery & margin positivity

In our study, of the 77 people who had surgery, 65 (84.4%) had modified radical mastectomy (MRM), which was what the patients wanted, and 12 (15.6%) had breast-conserving surgery (BCS). Since the type of surgery was up to the patient, it was not possible to use statistics to compare how many women in each group were able to keep their breasts. MRM was done on all NACT patients [26].

TNBC-Stage and Grade

Stage II was the most common stage of disease in our study (63.7%), followed by stage III and stage I. Most of the cancers in TNBC (71.4%) were high grade. In a subgroup analysis within the TNBC group, it was found that Grade 3 tumours are most common at early stages I and II [27].

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

TNBC-Extra nodal spread and lymphovascular invasion
In our study, Extra nodal spread was present in 8 of 77 patients (10.4%). Lymphovascular Invasion Is Present in 18 of 77 Patients (23.4%).

TNBC-T Stage & N Stage
In our study, the average tumour size after surgery was 3.2 cm (0-9 cm). There was no clear link between the size of the tumour and the number of lymph nodes that were affected. T4 disease had not been seen in any patient (0%). LABC was found in 18 patients, or 23.3%. Three people who had surgery outside the hospital and were sent back for more care were found to have oligometastatic disease [28].

TNBC-KI-67
>20% Ki67 was regarded high. There is no consensus on a conventional cut-off figure for clinical practise, however 10%-20% is the most typical range to dichotomize groups. High ki-67 expression was linked to greater histological grade, large tumour size, and positive lymph nodes in breast cancer. Several studies found a positive association between ki-67 and Androgen receptor expression. In our study, 75.3% (n=58) had elevated ki-67, indicating TNBC's aggressiveness. We studied 77 TNBC individuals. AR's prognostic relevance varies. Some research has contradictory results, causing controversy. Although data supports the significance of androgens and AR in breast cancer, the AR pathway's relevance in TNBC is unknown. Literature is variable. This heterogeneity is caused by differences in study size and AR positive criteria (1% or >10%). Variability across investigations is also caused by the primary antibody source and testing procedure. AR expression was 74.8% in ER-positive tumours and 31.8% in ER-negative tumours in one of the largest systematic evaluations.
In this study, 29.9% (n=23) patients expressed androgen receptor. These studies are similar. Our study analysed TNBC by AR status [29].

AR-Age and Hormonal risk factors
In our study, 50% (n=8) of women over 60 had AR. Bryan discovered no correlation between AR and menorrhoea. In our study, pre and perimenopausal women (n=15) expressed AR higher. In our study, the AR negative expression group, 1.9% of the patients had early menarche, whereas 4.3% of the patients in AR positive expression group had early menarche. Both groups experienced late menarche. Age at menarche, menopause and hormone replacement therapy indicated no statistical difference between the two groups [30].

AR-Stage
AR expression was associated with small tumour size, well-differentiated tumours, lower proliferative index, lower grade, lack of lymph node metastasis, and ductal type. AR immunostaining was inversely correlated with higher clinical stage. 13% of AR-positive patients were in stage I, 65.2% in stage II and 17.4% in stage III. Stages III through IV have few patients. Androgen receptor versus stage showed no statistical difference [31].

AR-Types of histology
On comparing the histological subtypes, it was found that Infiltrating Ductal Carcinoma (IDC) was the commonest occurrence in both groups (72.7% and 87%). This is similar to published data [32].

AR-T Stage
AR expression is linked to modest tumour size, according to Luo X *et al*. Most patients in our study had T2 disease (66.7% vs. 60.9%). In the AR positive expression group, 21.7% of women presented with T1 disease, however this was not statistically significant (P=0.88). This resembles Luo *et al*. [33].

Conclusions
The ways that Triple Negative Breast Cancer looks and acts in the body are. TNBC often showed up in women before or just before menopause. At presentation, the average age was 50,3 years. TNBC was rare in women over 65. There was a strange link between TNBC and second-degree family history, but not with first-degree family history. No matter what stage they were in when they were found, most TNBC tumours were of a higher grade. TNBC was usually less aggressive in people over the age of 65. It usually showed up as early breast cancer with few metastases in the lymph nodes, most of the time N1. Even low-grade TNBC tumours spread to more lymph nodes. Even though TNBC tumours were worse, they did not spread to more lymph nodes as often. This showed that there was no link between the grade of the tumour and its spread to other lymph nodes in TNBC. In this study, androgen receptor expression was positive in 29.9% (n=23) of the patients, but it was not positive in 70.1% (n=54) of the TNBC group. Androgen receptor expression is highest in women before menopause and lowest in women going through menopause. Androgen receptor expression is only seen in IDS, IDS NOS histology. AR expression-positive TNBC had ki-67 that was lower. Our data suggest that the combination of AR

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

expression and Ki-67 status could be used to sub-classify the risk of TNBC patients.

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