An Observational Study of AR Expression in Indian Women with TNBC and Their Treatment Response and Overall Survival

Tejesh Krishna Chatradi[1]

[1] Department of Medical Oncology, Yenepoya Medical College Hospital, Mangalore, Karnataka, India

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Address for correspondence Tejesh Krishna Chatradi, MBBS, DNB, DrNB, Department of Medical Oncology, Yenepoya Medical College Hospital, University Road, Mangalore 575022, Karnataka, India (e-mail: chtejeshkrishna@gmail.com).

Abstract

Introduction Triple-negative breast cancer (TNBC) is a heterogeneous subtype of breast cancer with the worst outcome, is not amenable for known target therapies, and has a poor response to chemotherapy. Androgen receptor (AR) was evaluated as a predictive, prognostic, and therapeutic factor in TNBC, but with contradicting results and the cutoff used for positive was also varied among studies. Thus, we studied 104 Indian women with TNBC for proportion showing AR expression and responses and survival outcomes compared over a period of 60 months with standard of care.

Objectives Primary: To observe the proportion of AR expression in TNBC. Secondary: To compare (1) pathological response to neoadjuvant chemotherapy (NACT) and (2) overall survival (OS) of both groups.

Materials and Methods This is a prospective descriptive study of 104 female patients with TNBC who visited the medical oncology department at a tertiary hospital in South India over a period of 2 years (June 2018–June 2020). They were diagnosed with TNBC by Immunohistochemistry (IHC); hence, they tested negative for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) receptors. Patients with HER2 2+ IHC were confirmed negative by fluorescence in situ hybridization testing. They were analyzed with IHC for AR expression using EP140 rabbit monoclonal primary antibody interpreted by a single breast pathologist, and proportion of TNBC expressing AR were identified with a cutoff $>10\%$ nuclear expression. They were managed as per the standard of care necessary for a particular stage. Results were analyzed comparing the baseline characteristic, treatment response, recurrence rate, and survival outcomes over a follow-up period of 60 months.

Results Percentage of AR expression in TNBC in this study was 35%. Twenty-five percent patients (28/104) were treated with NACT and complete pathological response was seen in 1/13 (7.69%) versus 7/22 (31.8%) in AR-positive compared with AR negative group (p 0.003). The AR-positive group showed significant survival advantage compared with the AR-negative group. Median OS of AR-negative group was reached at 29 months whereas for the AR-positive group it was 49 months ($p=0.58$). The AR-positive group showed late recurrences after 24 months in follow-up.

Keywords
- TNBC
- AR
- 5-year-follow-up
- IHC
- prognosis
- response
- pCR

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Conclusion This study showed that the proportion of TNBCs showing AR expression was much higher. Assessment of AR status in patients with TNBC provides additional information on prognosis and also predicts response to chemotherapy. AR-positive TNBC represents a breast cancer subtype with unique features with potential targeted therapies.

Introduction

Breast cancer is the most frequently occurring cancer in women and the second leading cause of cancer deaths worldwide. Breast cancer incidence increases as socioeconomic status increases.[1] Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by the lack of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2) receptors. This designation masks the heterogeneity of this patient population and the challenge of stratifying them for optimal treatment selection.[2]

Due to the paucity of treatment targets, cytotoxic chemotherapy is still the standard of care for TNBC. There is a need to develop new and more effective targeted treatments for these patients. Approximately 20% patients with TNBC are cured by standard therapy (tumor resection, radiation, and cytotoxic chemotherapy), but the remaining 80% patients progress to the metastatic stage of the disease.

One of the several therapeutic targets currently under study for the management of TNBC is the androgen receptor (AR).[3] It is a nuclear steroid hormone receptor that is expressed in 10 to 43% of TNBCs.[4,5] In the absence of ERα, AR drives "luminal-like" gene expression patterns. One of the TNBC molecular subtypes consistently identified via gene expression profiling is the luminal AR subtype.[3]

Studies have explored the prognostic role of AR in TNBC to better understand androgen action in TNBC, identify actionable factors that drive outcomes, and determine if testing for AR status should be included in routine clinical practice for diagnosing TNBC. The prevalence of AR positivity in female patients with TNBC is highly varying across the world (range: 11–56%) but data on the Indian population is lacking. The prevalence of TNBC worldwide is 11 to 15%.[6–14]

However, there are conflicting reports about the value of AR in treating TNBC.[13,15] AR-positive cells can be targeted with antiandrogen therapy.[1–5] There is no standard cutoff for AR expression in TNBC and various studies used different cutoffs.[6–14]

In this study, the AR expression was evaluated in 104 female patients with TNBC attending to the medical oncology department at a tertiary hospital in South India. All of the tumor samples were processed identically. Any differences in treatment protocols were noted to observe the innate differences between AR-positive and AR-negative subgroups of TNBC to standard treatment and survival outcome. These protocols were performed to solve our hypothesis on whether tailored management based on AR expression would be needed to treat TNBC.

In this study, our principal objective was to observe the proportion of AR expression in Indian women with TNBC and its implication on their response and survival outcomes. The primary objective was to identify the percentage of female patients with TNBC showing AR expression. The secondary objectives were to compare the pathological response and overall survival (OS) rate to neoadjuvant chemotherapy (NACT) between AR-positive and AR-negative groups.

Material and Methods

Study Design

A prospective descriptive study of 104 adult female patients diagnosed with TNBC (both nonmetastatic and metastatic) who presented to the medical oncology department and met the inclusion criteria.

They were evaluated for AR expression by immunohistochemistry (IHC) in the same specimen used for testing ER, PR, and HER2 receptors. The period of study was 2 years (June 2018–June 2020) and 5 years of follow-up period (June 2018–June 2024).

Sample Size

One hundred and four female patients diagnosed with TNBC (both nonmetastatic and metastatic) were included in the study.

Inclusion Criteria

(1) Women with breast cancer aged $\geq$ 18 years
(2) Patients with microscopic evidence of breast cancer
(3) Patients negative for ER, PR expression (by IHC), and HER2 amplification (by IHC; if equivocal (2 +) they are confirmed to be negative by a fluorescence in situ hybridization [FISH] test)
(4) All patients who received standard treatment as per stage

Exclusion Criteria

(1) Patients who are $\leq$ 18 years old
(2) Male patients with breast cancer
(3) Patients without microscopic confirmation of cancer
(4) Patients who were not treated at our hospital

Intervention

The same tissue sample used for ER, PR, and HER2 testing were used to evaluate AR status.

Inpatient and outpatient female patients with TNBC attending the department of medical oncology were thoroughly assessed for staging and pathological variants. Patient recruitment to study was completed in 2 years.

Specimens analyzed for the study were surgical samples collected from patients treated with primary surgery, patients with stage IV disease at presentation who had a diagnostic core biopsy, and patients treated with NACT followed by surgery. They were evaluated for AR expression by IHC by EP120 rabbit monoclonal antibody on the same specimen used for ER, PR, and HER2 testing (by IHC, if HER2neu was equivocal 2+ confirmed to be negative by FISH test). All slides were interpreted by a single breast oncopathologist. As majority of the prior studies have used a cutoff of $>10\%$, positive AR expression was defined as $>10\%$ nuclear staining.

All patients were treated with combined modality treatment according to the stage. All patients received standard chemotherapy with anthracycline and taxane (for two ineligible patients, alternative regimens like Cyclophosphamide, Methotrexate, Flurouracil Regimen (CMF) were provided).

Breast conservation surgery (BCS) was performed in 11 female patients with TNBC who presented at an early stage and all of them were given radiation treatment at the breast region subsequently. Modified radical mastectomy (MRM) with axillary nodal dissection was performed in 87 female patients with TNBC. Upfront surgery followed by adjuvant chemotherapy was performed in 70 female patients who were found to be operable at presentation. Adequate nodal yield and resection margins were achieved as per pathology report in all operated cases. MRM patients underwent radiation as per indication.

Complete NACT was provided presurgery to 35 patients who presented with locally advanced TNBC or had inoperable tumors. MRM was performed irrespective of response. Surgery was performed immediately if there was progression on NACT. During this study, the standard of treatment did not include adjuvant capecitabine, if complete pathological response (pCR) was not achieved after total NACT.

In summary, the patients were treated and followed-up through the study period on standard of care by various modalities as indicated. Additionally, they were followed-up postcompletion of treatment and assessed regularly. Patient follow-up period was for a median of 5 years (60 months).

Outcome was noted in the form of response to chemotherapy in neoadjuvant setting as pCR, partial response, stable disease, or progression on NACT. OS outcome for all was assessed in the follow-up period of 60 months/5 years after initiation of treatment.

Outcome

Patients were grouped into two groups based on their AR expression as group A, AR-positive TNBC, and group B, AR-negative TNBC.

Statistical Analysis

Patient data was collected in the study format (►**Supplementary material**) Available in online version, and entered in Microsoft Excel sheet for statistical analysis. Descriptive statistics were performed for patient demographics and clinical characteristics. For continuous variables, median and quartiles were computed. The chi-square test or the Mann–Whitney nonparametric test was used to study association between variables, according to their nature (categorical or continuous).

OS was defined as the time from initiation of treatment to death from any cause. The Kaplan–Meier method was used to estimate survival curves, and the log-rank test was used to test difference between groups. All reported p-values are two-sided, and a p-value of <0.05 is considered to be statistically significant. All analyses were done using SPSS (software version 27.0, IBM).

Ethical Approval

This study was approved by Krishna Institute of Medical Sciences (KIMS) Ethics Committee for Thesis (KIMS/EC/2018/23–13) on July 18, 2018. Written informed consent was obtained prior to study, and all procedures performed in studies involving human patients were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration, and its later amendments or comparable ethical standards.

Results

A total of 158 patients were diagnosed with TNBC from June 2018 to June 2020. One hundred and four of them met the inclusion and exclusion criteria and were analyzed irrespective of stage for AR expression by IHC.

Descriptive Characteristics

The median age at presentation was 53 years and 25% of patients were of premenopausal age (18–45 years). Twenty-five percent patients had radiological tumor size >5 cm, 65% patients had stage 3 disease, 92% patients had grade 3 tumors, and 50% patients had high K_i-67 ($>50\%$). Ten percent patients had rare histological subtypes like apocrine, metaplastic, medullary, papillary, lobular, and sebaceous of TNBC similar to previous studies.[12–15]

Percentage of AR Expression in TNBC

Among the 104 patients analyzed, 36 were found to express AR by IHC at a cutoff $>10\%$. Thus, the percentage of AR expression in TNBC in this study was 35%.

Comparison of Patient Characteristics between Two Groups

Based on AR presence, two subgroups were defined as "AR positive" and "AR negative" and were compared.

Median age, radiological tumor size, nodal status, and K_i-67 were comparable in both groups. However, the AR-negative group (57%) had higher proportion of patients with

grade 3 tumors than the AR-positive group (36%) (p 0.25). All apocrine subtypes were deemed AR positive, whereas lobular and sebaceous subtypes were deemed AR negative. Proportion of patients receiving NACT and adjuvant treatment was similar in both groups, 22/68 (32%) in the AR-negative group and 13/36 (36%) in the AR-positive group.

Surgical and Multimodality Treatment Outcomes

All patients had uneventfully completed the multimodality treatments like surgery and radiation on this study without any major adverse effects.

Patients were followed after the completion of treatment and were regularly assessed for any recurrence. Patients who were lost to follow-up were censored. Among the patients who received NACT ($n = 35$), their response was assessed in MRM specimens acquired postsurgery and was categorized based on response to chemotherapy into four groups.

Three patients had local tumor progression while on NACT (1/22), 4.5% in the AR-negative group, and 2/13 (15.4%) in the AR-positive group (p 0.03). All three patients had only local radiological tumor progression and immediately underwent MRM and remaining taxane chemotherapy was administered as adjuvant chemotherapy. One in each group had stable disease and partial responses, 13/22 (59%) in the AR-negative and 9/13 (69%) in AR-positive group. pCR was noted in 7/22 (31.81%) patients in the AR-negative group and 1/13 (7.69%) patients in the AR-positive group (p 0.03) (►**Table 1**) (►**Fig. 1**).

Survival Outcome Analysis

All the analyzed patients (104) after completion of standard of care treatment as per stage were on follow-up over the next 60 months. The following consort diagram shows the numerical flow of the study at intervals (►**Fig. 2**). An unplanned interim analysis was done at 24 months as the study suffered severe follow-up loss due to pandemic and lockdown restrictions. After confirming adequacy of sample size in both groups, the final OS analysis was done at 50 months without any modifications in the study, once median OS was reached in both the groups.

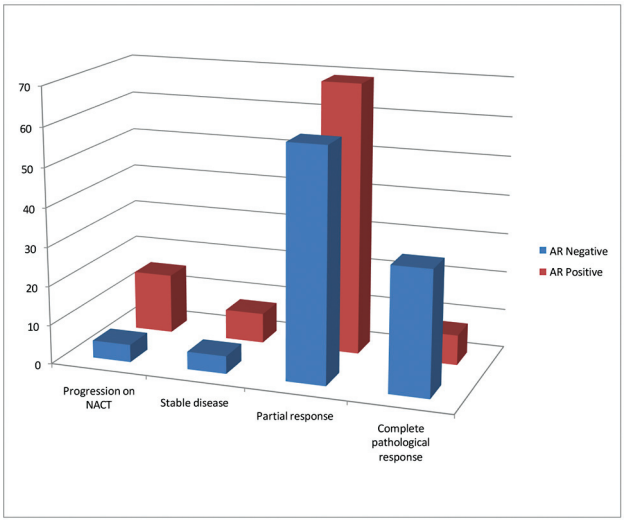

Fig. 1 Three-dimensional (3D) bar chart showing response to neoadjuvant chemotherapy (NACT) in each group.

Survival outcomes were assessed among both groups: AR positive and AR negative. OS was calculated as the period between initiation of treatment and death due to any cause (►**Fig. 2**). Median OS of the AR-negative group was calculated as 29 months whereas for the AR-positive group it was evaluated as 49 months. However, these calculations were not statistically significant ($p = 0.58$) (►**Fig. 3**).

Among upfront stage 4 patients who received palliative chemotherapy, the AR-positive group showed a median OS of 20 months, whereas in the AR-negative group it was 11 months, but not statistically significant ($p = 0.377$).

Discussion

TNBC is a heterogeneous disease. The identification of the subtypes is necessary for a better characterization and for

Table 1 Distribution and proportion of patients in both groups as per their response to NACT

Response to NACT, $n = 35$				
AR				
		Negative	**Positive**	**Total**
Progression on NACT	Count	1	2	3
	% within AR	**4.5%**	**15.4%**	8.57%
Stable disease	Count	1	1	2
	% within AR	4.54%	7.69%	5.71%
Partial response	Count	13	9	15
	% within AR	59.09%	69.23%	42.85%
Complete pathological response	Count	7	1	8
	% within AR	**31.81%**	**7.69%**	22.85%
Total	Count	22	13	35

Abbreviations: AR, androgen receptor; NACT, neoadjuvant chemotherapy.
Note: Table showing number and percentage of patients in either group in their respective response to NACT. The differences in proportions of progression on NACT and PCR in both the roups are highlighted.

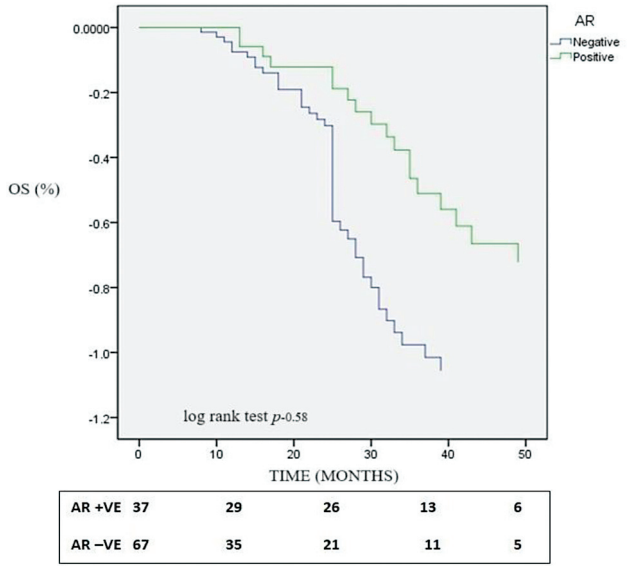

Fig. 2 Consort diagram of the study.

the construction of appropriate therapeutic strategies. AR expression varied widely as per countries' cohorts—8.3% in the Nigerian women with TNBC cohort, 55% women with TNBC in the United Kingdom cohort, etc.[8]

Baseline Characteristics

This study observed that the median age (53 years), tumor size (4.2 cm), and stage at presentation of Indian women with TNBC were similar to world data.[1]

Histologically, in majority of patients with TNBC, that is, 87.5% (91/104), invasive ductal carcinomas were diagnosed and other histological types consisted of $< 10\%$ of patients with TNBC. More than 90% patients had a high-grade tumor, whereas K_i-67 was $> 50\%$ in approximately 50% patients. All the clinical and pathological parameters were comparable with previous study data of TNBCs.[6,7,10,13]

Proportion of AR Expression

In this study, AR expression was observed in 35% patients at a cutoff of 10%. Compared with various studies worldwide and to the present Indian data (►**Table 2**), our study's value was found to be higher.[6–14] Thus, the role of AR expression on TNBC's tumor biology and clinical response in our population might be substantial.

Approximately 10% (11/104) patients with TNBC underwent BCS upfront. Approximately 7/104 (6.7%) patients with TNBC were diagnosed with stage 4 at presentation, 28/104 (25%) patients were treated with NACT, and the rest 70/104 (70%) patients were operated upfront.

Baseline characteristics of our study group (median age, menstrual status, grade, histological types, K_i-67, stage, and

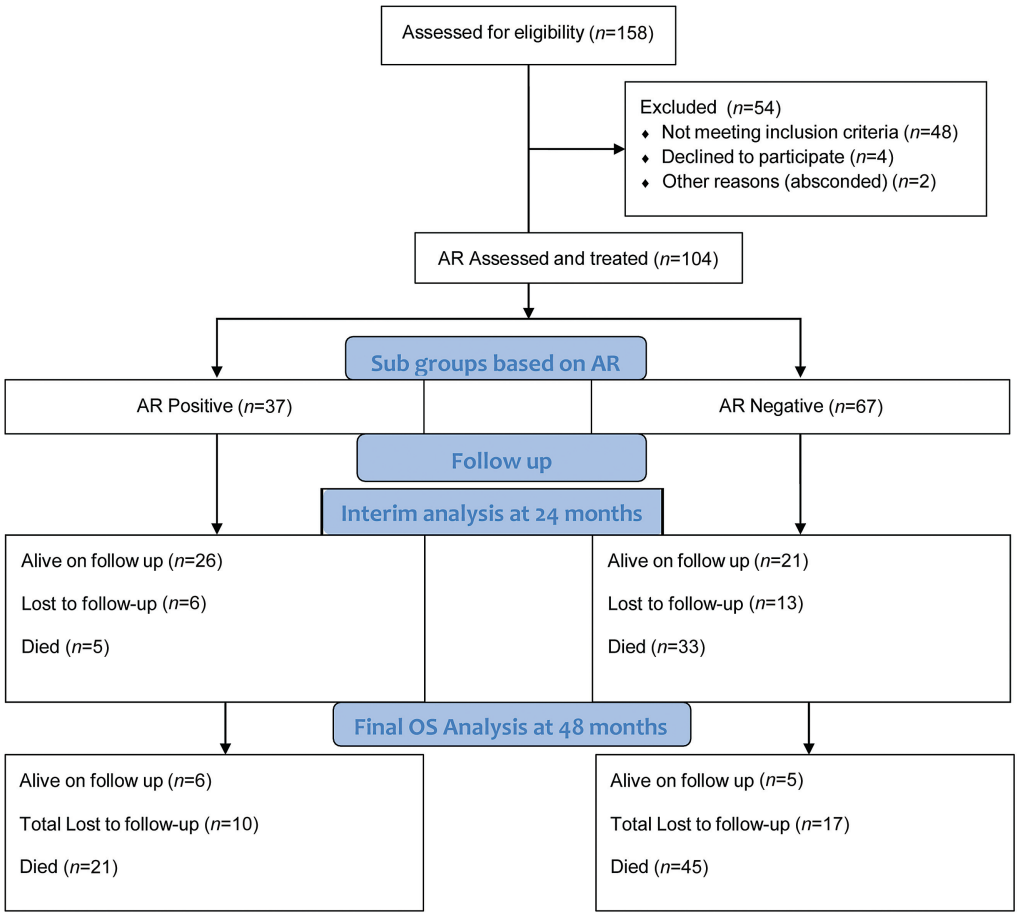

Fig. 3 Overall survival among both groups over the 5-year follow-up period.

Table 2 Comparison of AR cutoff values and percentage AR positives in various studies to this study

Author of the study (year of publication)	Cutoff percentage on IHC	Percentage of AR+
McGhan et al (2014)[7]	> 10	23
Ricciardi et al (2015)[9]	> 10	26.6
Choi et al (2015)[10]	> 10	17.7
Grogg et al (2015)[11]	≥ 1	11.4
Rampurwala et al (2016)[12]	> 10	15
Narayanan and Dalton (2016)[13]	> 10	15–70
Collignon et al (2016)[8]	≥ 1	12
Anand et al (2017)[15]	≥ 1	30
Patnayak et al (2018)[14]	> 10	20
Bhattarai et al (2019)[6]	≥ 1	22.7
This study	> **10**	**35**

Abbreviations: +, positive; AR, androgen receptor; IHC, immunohistochemistry.
Note: Comparison of our study AR cutoff and percentage to various Indian and other publications in tabular form shows higher AR expression even with higher cutoff. Study Result and AR Cutoff are highlighted.

nodal involvement at presentation) were comparable to previous studies.[6,12–15]

Comparison of Baseline Characteristics of Both Groups

Patients aged < 35 years were observed to be more in the AR-negative group (9/68, 13%) compared with the AR-positive group (1/36, 3%). The AR-negative group had more patients (43/68, 63%) aged < 55 years than the AR-positive group (18/36, 50%). But the mean age in both the groups were identical at 52 years in AR negative and 55 years in AR positive.

Similar proportion of pre- (26% vs. 22%) and postmenopausal patients (73% vs. 77%) were comparable between the AR-negative and AR-positive groups, respectively. Mean tumor size at presentation was smaller in the AR-positive group than the AR-negative group (4.2 vs. 5.2 cm). Proportion of patients with higher stage was more in the AR-negative group compared with the AR-positive group. The AR-positive group had significantly higher proportion of patients of stage 1 than the AR-negative group (11% vs. 3%) ($p = 0.077$). K_i-67 correlated with higher grade but was not significantly different between the two groups ($p = 0.79$).

Histological subtypes showed comparable proportion of Invasive ductal carcinoma (IDC) type, which was majority in both groups (88% vs. 87%). For the patients with apocrine type, all had AR expression of 100% (3/3), whereas among papillary, lobular, sebaceous, and signet cell type, none had AR expression. Medullary and metaplastic type occurrences were equal in both the groups. Both groups were comparable in the treatment pattern as there was no significant difference in rate of BCS (11% vs. 10%) or patients undergoing upfront surgery or NACT (27% vs. 26%).

Response to NACT

Only one patient in the AR-negative group had progressed, compared with two AR-positive patients on NACT. Thus, indicating the AR-positive group is chemoresistant than the AR-negative group. Even pCRs were significantly different in both groups—31.8% patients in the AR-negative group achieved pCR whereas only 7.69% patients in the AR-positive group achieved pCR ($p = 0.003$). This result was comparable to previous studies, which have suggested lower pCR rates in the AR-positive group than Quadraple negative breast cancer (QNBC).[13–15] Distant recurrence rate within 1 year of completion of treatment was higher in the AR-negative group (34%) compared with the AR-positive group (24%).

Survival Analysis

Mortality rate within 1 year was also higher in the AR-negative group than the AR-positive group (18/68, 26.5% vs. 7/36, 19.4%). Though the response to chemotherapy was more in the AR-negative group compared with the AR-positive group, the recurrence and mortality occurred earlier in the AR-negative group than in the AR-positive group.

Nonetheless, among stage 3 patients who were treated in an adjuvant setting, they showed significant survival advantage in the AR-negative group compared with the AR-positive group. At 18 months, 21/25 (84%) patients were alive in the AR-negative group and 8/11 (76%) patients in the AR-positive group ($p = 0.023$). Over the next 3 years, this advantage was lost as 63% of the AR-negative group versus only 27% in the AR-positive group who were on follow-up, showed recurrence and mortality ($p = 0.023$). Most of the recurrences were at metastatic sites and were treated with later lines of palliative chemotherapy and OS was noted at mortality.

In NACT setting, stage 3 patients showed significant survival advantage in the AR-positive group irrespective of the pathological response to chemotherapy (22 vs. 15 months) ($p = 0.003$). Thus, this occurrence suggested that the AR-positive group retains their survival advantage despite failing on chemotherapy. This also indicates that different biological drivers work in pathophysiology of both the groups and specific molecular targets need to be identified to treat either of them more effectively than usual.

Even in palliative setting for those with stage 4 disease at presentation, the AR-positive group showed survival advantage with a median OS difference of approximately 9 months (20 vs. 11 months) ($p = 0.3$).

Survival outcomes were assessed among both groups. The AR-positive group showed survival advantage irrespective of the pathological response to chemotherapy or in adjuvant setting compared with the AR-negative group (►**Fig. 3**).

Overall Survival

Median OS of the AR-negative group was reached at 29 months whereas for the AR-positive group it was 49 months, which is not statistically significant ($p = 0.58$).

In the AR-positive subgroup, most of those patients who crossed the 2-year follow-up period without recurrence had their follow-up period relaxed to a 6-month interval. After approximately 3 years, these individuals began to exhibit recurrence, reaching median OS at 4 years. Therefore, in this subgroup, a prolonged close follow-up period (beyond 24 months) and a long-term maintenance treatment, such as AR-targeted therapy, may be helpful.

Strengths

A well-executed, real-world, objective, prospective observational study with a sufficient sample size was performed in a single institution under the supervision of a skilled team of surgeons, pathologists, and mentors. All patients completed the course of therapy and there was more than 5 years of follow-up data.

Limitations

Patients on this study had a notable loss to follow-up during the pandemic period especially those who had lower risk of recurrence and had to be censored from final analysis. Majority of patients who had a recurrence had received subsequent lines of treatment, hence were on follow-up.

Generalizability

TNBC was managed as per standard of care during the study period. Though standard of care has changed to total NACT and addition of adjuvant capecitabine in later years, outcomes are still poor. Newer insights on the tumor behavior and subtyping especially with inexpensive and widely available tests like IHC is feasible and would have potential not only to predict but also to tailor the treatment based on AR expression.

Future Research and Directions

This study shows that the AR-positive subgroup of TNBC is innately more resistant to chemotherapy and behaves indolent like luminal A and shows delayed recurrences than the AR-negative subgroup. Hence, further studies are needed to compare with the addition of AR-targeted therapies for maintenance after standard treatment, which might benefit the AR-positive group in Indian women with TNBC as the proportion of AR expression is found to be higher than usual.

Conclusion

Percentage of AR expression in TNBC in this study was 35% at a cutoff of 10% for positivity, when compared with various studies worldwide and to Indian data this was much higher.

The AR-positive group had lower grade and lesser response rates to NACT. The AR-negative group despite showing better response to NACT had early recurrence and higher mortality reaching median OS within 3 years of completion of treatment.

The AR-positive group, despite having better survival compared with the AR-negative subgroup in the first 3 years, had significant late recurrence and mortality and even reached the median OS at approximately 4 years.

TNBC has poor prognosis and limited management options. Understanding subsets like the AR-positive subgroup and their natural course of disease will help tailoring the treatment strategy by avoiding unnecessary toxicity due to chemotherapy due to TNBC's_less chemosensitivity. It will also help in developing better follow-up strategies to detect late recurrence.

Further studies to target available treatment options like androgen blockers in the AR- positive subgroup for long-term maintenance might be beneficial for preventing the recurrence and mortality caused by TNBC.

Patient Consent

Informed patient consent was obtained for this study.

Conflict of Interest

None declared.

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