Prevalence of Androgen Receptors Expression in Triple Negative Breast Cancer Patients and its Correlation with Clinicopathological Criteria: Our Institutes Experience

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Abstract:

Background: Triple negative breast cancer (TNBC) is a term that has been applied to breast cancers which lack expression of three receptors: estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2). It represents about 20% of breast cancers diagnosed worldwide. TNBC is a challenging type by its presentation criteria and limited options of treatment. Continuous research for finding specific target is the aim of scientists. Androgen receptors (AR) expression take special attention in this type of breast cancer as its expression can help for finding special targeted treatment as anti-androgen therapy.

Purpose: To assess the AR expression in TNBC patients and to correlate its expression with clinicopathological parameters and disease outcome of patients in study populations.

Methods: This prospective study included 90 female patients confirmed as TNBC patients in medical oncology and clinical oncology departments, in Mansoura University and Zagazig University, Egypt, from December 2013 to May 2016. AR positive expression was defined as ≥10% nuclear immunostaining.

Results: AR expression was positive in twenty seven (27/90) patients (30%), and lack of its expression was significantly associated with younger age group ($p < 0.001$), higher grade ($p = 0.017$) & higher tumor stage ($p < 0.001$), presence of lymph node metastasis ($p < 0.001$) & distant metastases ($p = 0.032$), vascular ($p = 0.044$) & perineural invasion and high baseline CA 15-3 level ($p < 0.001$). Median follow up duration was 17.5 months (range 6-40), 32/90 died (35.6%). Mean overall survival (OS) was 28 months for AR negative TNBC patients versus 32 months for AR positive patients. Twenty four of died patients (24/32) were AR negative. Three years OS was 50.8% and 44.1% for AR positive and AR negative respectively, but with non-significant P-value.

Conclusions: Our study confirmed that AR positive expression in TNBC is a good prognostic feature and it can be sued as target for anti-androgen therapy in this group who are lacking any target treatment.

Keywords: Androgen receptor, Triple negative, Breast cancer

Background:

Breast cancer is a major health problem worldwide; it ranks as the first cancer type in female in Egypt [1]. Triple negative breast cancer (TNBC) which lacks the expression of: estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2), so it cannot benefit from endocrine or targeted treatment, and it was proved biologically to be a separate entity of breast cancer with different cell behavior and prognosis [2].

TNBC accounts for 9-21% of all breast cancer cases [3], and it is a challenging subtype that is associated with younger age at presentation. Because of absence of targeted therapy for treating TNBC a lot of researches have been done to find other treatment modalities to improve the patients' outcome. Androgen receptors (AR) are steroid hormone receptors which are expressed in 60%-70% of breast cancer patients, and has been involved in breast cancer (BC) pathogenesis [4], and it may serve as a therapeutic target for this subset of distinct breast cancer subtype [5].

The aim of our work is to assess the AR expression in triple negative breast cancer patients who presented to our hospitals, in addition to correlate such expression with clinico-pathological parameters and patient's survival.

Patients and Methods:

This prospective study was carried out in Zagazig University Hospital, Mansura University Hospital, and Mansura Oncology Center, in the period between December 2013 and May 2016, 90 pathologically proven TNBC female patients were included in the study. Tissue samples were taken either by excision biopsy or mastectomy samples, then they had been processed and diagnosed in pathology department, faculty of medicine Zagazig University [Informed consent was obtained from each patient, this study was approved by Zagazig University, Mansura University and Mansura Oncology Center Institutional review board (IRB)]. Baseline clinico-pathological data were collected for all patients. Follow up for a median of 17.5 months (range 6-40) for all patients was done by regular visit every three months, in which; clinical examination, chest x ray, pelvi-abdominal U/S, and any other investigations which were needed according to patients' complaints were done.

We have used streptavidin-biotin method for immune-staining, where we have cut paraffin-embedded samples into four micron sections then we have baked them at sixty five °C for half an hour. We have deparaffinized sections from all samples with xylene and then rehydrated them. We have submerged sections into EDTA buffer for antigenic retrieval; then we have put them into antigenic retrieval microwaved. After that we have treated sections with hydrogen peroxide 3% in methyl-alcohol to antagonize endogenous peroxidase activity, and then we have incubated sections with bovine serum albumin (BSA) 1 % to antagonize nonspecific stain binding. We have incubated sections of all samples with primary mouse monoclonal anti-AR (abcam, clone [AR 441] (ab9474) dilution 1:100) antibody overnight at 4°C. After that we have washed sections and incubated them all with a biotinylated secondary anti-rabbit antibody (Abcam). The tissue sections were counterstained with ten percent Mayer's hematoxylin and dehydrated. The degree of AR positivity was reviewed, evaluated and analyzed by two independent pathologists [6]. AR-positive expressions were defined as ≥10% nuclear staining while less than 10% considered loss of AR expression [7, 8].

Statistical analysis:

Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Shapiro-Wilk test. Mann Whitney U test was used to compare between two groups of non-normally distributed variables. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when was appropriate. Trend of change in distribution of relative frequencies between ordinal data were compared using Chi-square test for trend. Overall Survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of OS was done according AR. These time-to-event distributions were estimated using the method of Kaplan-Meier plot, and compared using two-sided exact log-rank test. All tests were two sided. A p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

Results:

Our patients' criteria are detailed in table (1), all patients were female, with the age ranged from 25- 77 (median=46.5 y), 32 patients were ≤ 35y (35.6%). 57.8% of patients were premenopausal at presentation. The majority of patients were diagnosed with invasive duct carcinoma (IDC) of no special type (74/90), 40% of patients had GIII disease. Stage I was detected in eight patients, stage II &III presented in most of studied patients 72.2% (65/90), and 17 patients had stage IV disease (18.9%). AR expression was positive in twenty seven (27/90) patients (30%), with cutoff of staining >10% of cells (Figure 1).

Lack of AR expression was seen more with younger patients (≤35years) (30 /32) patients (93.8%) (Figure 2).

Among 78 patients who had positive lymph nodes metastasis, only twenty of them showed positive AR expression. Also, between 17 patients who presented with metastatic disease; only one patient expressed AR positivity.

Twenty three patients (23/27) who were positive for AR expression were with normal baseline CA 15-3 levels ($P < 0.001$) (Table 2).

The expression of AR was significantly correlated with age, tumor grade, vascular invasion, perineural invasion, stage, and baseline CA 15-3 level. But no

correlation was found between AR expression and menopausal state, positive family history, performance status (ECOG PS), or disease laterality.

Survival and follow-up data analysis

- In our study the median follow-up duration was 17.5 months (range 6-40).
- The mean OS for AR positive group was 32 months comparing to 28 months for AR negative group (p=0.185) (Figure 3)
- 1 year OS was 92.2 %, 2years OS was 81.3% and 3years OS was 50.8% for AR positive group while 1year OS was 85.5 %, 2years OS was 59.6 % and 3years OS was 44.1 % for AR negative group.
- We found that 32 patients were died; 24 (75%) patients of them had AR negative expression (Table 3)

So better overall survival was seen in patients with AR positive expression in comparison to AR negative patients but it was non-significant (p= 0.185) (Table 3).

Table (1): Clinopathological parameters, Androgen Receptor (AR) expression and survival of our patients

Characteristics	No.	Percent
Age (year)		
Mean ± SD	46.59	± 13.86
Median (range)	46.50	(25 – 77)
≤ 35 years	32	35.6%
> 35 years	58	64.4%
Menopause		
Premenopausal	52	57.8%
Postmenopausal	38	72.2%
Family history		
Negative	79	87.8%
Positive	11	12.2%
ECOG PS		
ECOG 0	15	16.7%
ECOG 1	59	65.6%
ECOG 2	16	17.8%
Side		
Right breast	37	41.1%
Left breast	53	58.9%
Histopathology		
IDC	74	82.2%
ILC	9	10%
Others	7	7.8%
Grade		
Grade I	10	11.1%
Grade II	44	48.9%
Grade III	36	40%
Vascular invasion		
Negative	21	23.3%
Positive	69	76.7%
Perineural invasion		
Negative	42	46.7%
Positive	48	53.3%

Characteristics	No.	Percent
T		
T0	5	5.6%
T1	17	18.9%
T2	30	33.3%
T3	28	31.1%
T4	10	11.1%
N		
N0	12	13.3%
N1	29	32.2%
N2	27	30%
N3	22	24.4%
M		
M0	73	81.1%
M1	17	18.9%
AJCC Stage		
Stage I	8	8.9%
Stage II	25	27.8%
Stage III	40	44.4%
Stage IV	17	18.9%
CA15-3		
Normal	42	46.7%
High	48	53.3%
AR		
Negative	63	70%
Positive	27	30%
Follow-up (month)		
Mean ± SD	19.86	± 10.08
Median (range)	17.50	(6 – 40)
Mortality		
Alive	58	64.4%
Died	32	35.6%

Continuous variables were expressed as mean ± SD & median (range); categorical variables were expressed as number (percentage). IDC Invasive Ductal Carcinoma, ILC Invasive Lobular Carcinoma .AR Androgen Receptors.

Table (2): association between clinopathological parameters and Androgen Receptor (AR) expression in our patients

Characteristics	All patients (N=90)		AR				p-value
			Negative (N=63)		Positive (N=27)		
	No.	(%)	No.	(%)	No.	(%)	
Age (year)							
Mean ± SD	46.59	± 13.86	45.06	± 14.88	50.15	± 10.52	0.066
Median (range)	46.50	(25 – 77)	44	(25 – 77)	49	(29 – 70)	
≤ 35 years	32	(35.6%)	30	(93.8%)	2	(6.3%)	**<0.001**
> 35 years	58	(64.4%)	33	(56.9%)	25	(43.1%)	
Menopause							
Premenopausal	52	(57.8%)	37	(71.2%)	15	(28.8%)	0.780
Postmenopausal	38	(72.2%)	26	(68.4%)	12	(31.6%)	
Family history							
Negative	79	(87.8%)	54	(68.4%)	25	(31.6%)	0.494
Positive	11	(12.2%)	9	(81.8%)	2	(18.2%)	
ECOG PS							
ECOG 0	15	(16.7%)	10	(66.7%)	5	(33.3%)	0.242
ECOG 1	59	(65.6%)	39	(66.1%)	20	(33.9%)	
ECOG 2	16	(17.8%)	14	(87.5%)	2	(12.5%)	
Side							
Right breast	37	(41.1%)	27	(73%)	10	(27%)	0.607
Left breast	53	(58.9%)	36	(67.9%)	17	(32.1%)	
Histopathology							
IDC	74	(82.2%)	48	(64.9%)	26	(35.1%)	0.061
ILC	9	(10%)	9	(100%)	0	(0%)	
others	7	(7.8%)	6	(85.7%)	1	(14.3%)	
Grade							
Grade I	10	(11.1%)	6	(60%)	4	(40%)	**0.017**
Grade II	44	(48.9%)	26	(59.1%)	18	(40.9%)	
Grade III	36	(40%)	31	(86.1%)	5	(13.9%)	
Vascular invasion							
Negative	21	(23.3%)	11	(52.4%)	10	(47.6%)	**0.044**
Positive	69	(76.7%)	52	(75.4%)	17	(24.6%)	
Perineural invasion							
Negative	42	(46.7%)	22	(52.4%)	20	(47.6%)	**0.001**
Positive	48	(53.3%)	41	(85.4%)	7	(14.6%)	
T							
T0	5	(5.6%)	3	(60%)	2	**(40%)**	**0.014**
T1	17	(18.9%)	8	(47.1%)	9	(52.9%)	
T2	30	(33.3%)	21	(70%)	9	(30%)	
T3	28	(31.1%)	22	(78.6%)	6	(21.4%)	
T4	10	(11.1%)	9	(90%)	1	(10%)	
N							
N0	12	(13.3%)	5	(41.7%)	7	(58.3%)	**<0.001**
N1	29	(32.2%)	15	(51.7%)	14	(48.3%)	
N2	27	(30%)	24	(88.9%)	3	(11.1%)	
N3	22	(24.4%)	19	(86.4%)	3	(13.6%)	
M							
M0	74	(82.2%)	47	(64.4%)	26	(35.6%)	**0.032**
M1	16	(17.8%)	16	(94.1%)	1	(5.9%)	
AJCC Stage							
Stage I	8	(8.9%)	3	(37.5%)	5	(62.5%)	**<0.001**
Stage II	25	(27.8%)	12	(48%)	13	(52%)	
Stage III	40	(44.4%)	32	(80%)	8	(20%)	
Stage IV	17	(18.9%)	16	(94.1%)	1	(5.9%)	
CA15-3							
Normal	42	(46.7%)	19	(45.2%)	23	(54.8%)	**<0.001**
High	48	(53.3%)	44	(91.7%)	4	(8.3%)	

Continuous variables were expressed as mean ± SD & median (range); categorical variables were expressed as number (percentage); *Independent samples Student's test; • Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

Table (3): The effect of Androgen Receptor (AR) expression on TNBC patients' survival

Outcome	All patients (N=90)		AR				p-value
			Negative (N=63)		Positive (N=27)		
	No.	(%)	No.	(%)	No.	(%)	
Mortality							
Alive	58	(64.4%)	39	(67.2%)	19	(32.8%)	0.442
Died	32	(35.6%)	24	(75%)	8	(25%)	
OS							
Mean (month) (95%CI)	29 months (26 – 32)		28 months (25 – 32)		32 months (27 – 36)		0.185
6 month OS (%)	98.9%		98.4%		100%		
12 month OS (%)	87.6%		85.5%		92.2%		
24 month OS (%)	61.7%		59.6%		81.3%		
36 month OS (%)	45.6%		44.1%		50.8%		

Continuous variables were expressed as mean (95%CI); Categorical variables were expressed as number (percentage); 95%CI: 95% Confidence Interval; ‡ Chi-square test; † Log rank test; $p<0.05$ is significant.

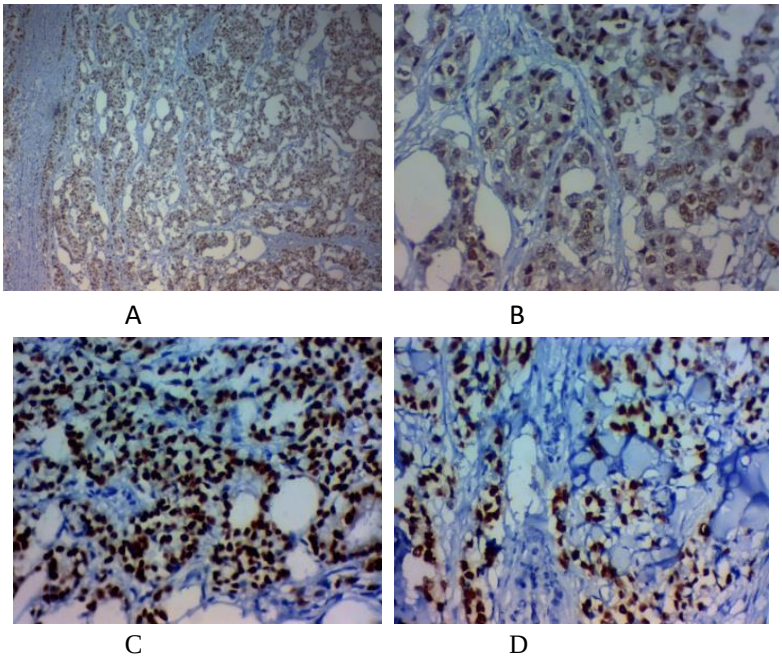

Figure1: Immunohistochemical expression of androgen receptors (AR) in breast carcinoma of various types :(A) positive AR expression in the nucleus of high grade invasive duct carcinoma of no special type cellsx100 (B) High power view of the previous image showed positive AR expression in the nucleus of high grade invasive duct carcinoma of no special type cells 400. (C) & (D) positive AR expression in the nucleus of high grade invasive lobular carcinoma x400. The original magnification was ×100 B, C& D the original magnification was ×400

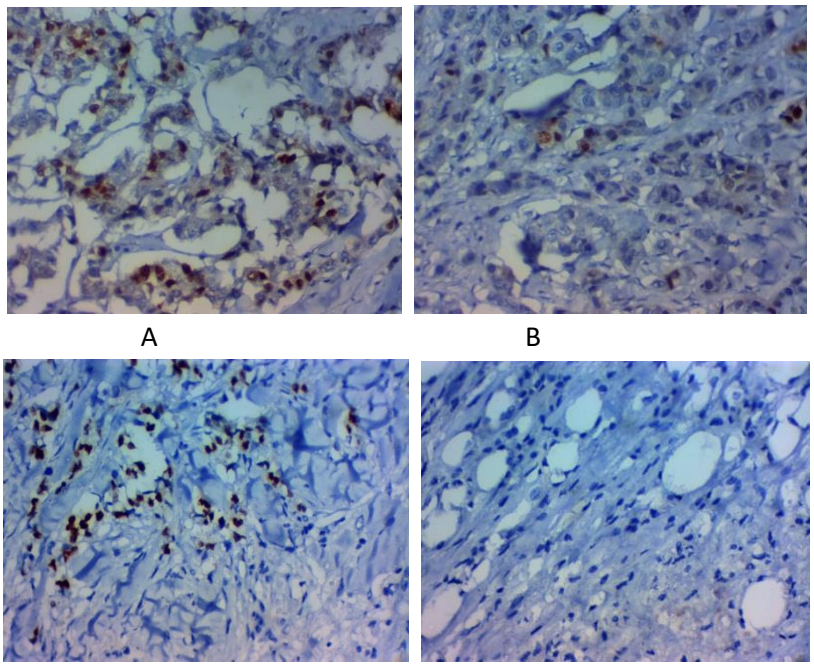

Figure 2: Immunohistochemical expression of androgen receptors (AR) in breast carcinoma of various types: (A) & (B) loss of AR expression in the nucleus of low grade invasive duct carcinoma of no special type cellsx400. (C) & (D) loss of AR expression in the nucleus of invasive lobular carcinoma x400. A, B, C& D. The original magnification was ×400

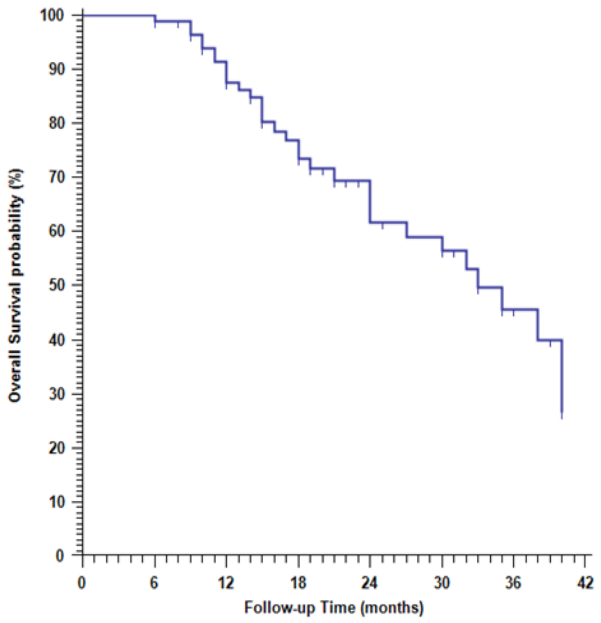

Figure 3A: OS in the entire study group, Mean= 29 (26-32)

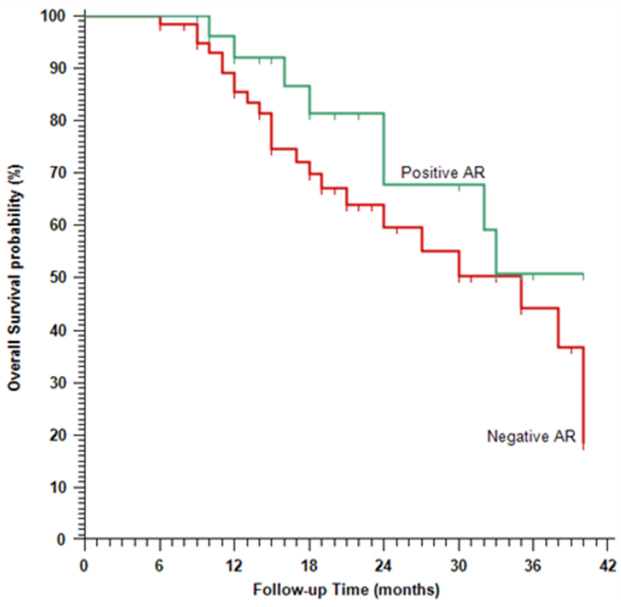

Figure 3B: OS in both study groups according to AR-expression (AR +ve and AR-ve).

Discussion:

In our study we assessed the prevalence of AR expression in 90 female patients who were newly diagnosed as triple negative breast cancer in our institutes, we found that it was positively expressed in 30% (27/90) of our patients. In Egypt, the prevalence of AR expression among TNBC Egyptian patients was investigated in other centers; AR positive expression was demonstrated in 27.2% of patients in Tanta University [9]. Reviewing other studies, demonstrates wide range of AR expression among patients with TNBC, from 6.6% to 75% [8, 10-13], this may be explained by the variations in number of involved patients in each study, or the cutoff value of AR positivity (≥1% or ≥10%), also the primary antibody source, the methodology of testing and patient selection criteria in prospective studies could be other reasons for this variability among different studies. In large systematic review included 7693 breast cancers in 19 studies, AR positive expression was 74.8% in ER-positive tumors and 31.8% in ER-negative tumors [14].

He et al., 2012 found AR expression in 74 patients of 287 patients with TNBC (25.8%) [15]. Mc Ghan et al., 2014 showed that AR expression was 23% of 94 TNBC patients [11]. Niemeier et al., 2010 reported AR expression was in 10 % TNBCs [16]. Other studies, reported that the AR is expressed in 10-43 % of TNBCs [17, 18]. And in a meta-analysis of thirteen studies including 2826 TNBC patients; AR positive expression rate was 24.4 %. [19].

TNBC is a heterogeneous disease, and numerous studies showed that TNBC can be more classified according to its genetic profile; AR +ve TNBC is one of these subtypes [11] [13], and it shows preserved androgenic signaling that could be a potential therapeutic molecular target like ER +ve BC [18, 20]. In addition, AR expression has been recognized in up to 70%–90% of BC, comparable to the rate of ER expression in breast cancer [21]. While prior reports reported that androgens can reduce the evolution of BC, the exact mechanisms and clinical significance of AR in BC still uncertain [22-24].

The role of Androgen signaling in breast cancer development still controversial, however, androgen influences the risk of BC throughout different contradictory methods: either by AR binding which stimulates malignant cell production or via binding to ER with consequent competitive inhibition of 17 b-estradiol stimulatory effect on tumor cells, or through conversion to estradiol [25].

As the AR expression has a wide range of expression, also the prognostic significance of its expression is a matter of controversy. We detected significant positive correlations between AR positivity in TNBC with older age, lower stage, and lower histological grade at presentation. Similarly, in TNBC tumors, many studies have shown that positivity of AR immunostaining is accompanied with the same presentations [26, 8, 27]. Luo et al., 2010 reported that AR negativity was significantly associated with higher histological grade, development of recurrences, and distant metastasis [17]. While Wang C et al., 2016 reported AR+ patients tended to have lower tumor grade ($p< 0.001$), but more lymph node metastases ($p < 0.01$) [19].

The prognosis of TNBC patients is significantly poor in comparison to patients with other subtypes of BC, and the underlying differences in recurrence and mortality rates may be clarified in part by different genetic subtype in this special entity of BC. Also signals generated by AR expression have been confirmed to display adverse effects on cellular proliferation in some breast cancer cell lines treated with 5-alpha-dihydrotestosterone [28], this molecular mechanism could be involved in delaying disease relapse.

In our study the OS for AR positive patients was better than for those with AR negative but it was non-significant which may be explained by small number of patients and short duration of follow up. AR expression had a good effect on survival in our study (unless it was not significant), and patients with AR +ve TNBCs survived longer than those with AR-ve TNBCs. This may suggest a difference in malignant potential between AR +ve and -ve TNBC. However, we could not determine any exact factor responsible for this increase in survival. Even though this was a prospective study, we didn't alter the management strategies for any patient as the result of AR expression. Therefore, the difference in survival may be due to the variations in sensitivity to conventional therapies or by the native character of the AR-positive TNBC phenotype. Additional studies are necessary to recognize the exact characters of AR-positive TNBCs.

In another study which was done for assessment of AR expression in all types of breast cancer, the subgroup analysis showed that androgen receptors expression in TNBC tumors has a trend toward an increase in OS and RFS [29].

In a meta-analysis of 13 studies including 2826 TNBC patients; AR positivity showed no effect on OS, but lowered recurrence risk in TNBC [19].

McGhan and colleagues had found AR expression to be associated with lymph node metastasis in TNBC [11]. However, McGhan's study [11], and Mehrdad et al., 2016[30] showed that OS was similar between AR-positive and AR-negative patients.

In a retrospective study done by He et al., 2012; the positive expression of AR was demonstrated as a favorable prognostic factor in terms of both the DFS and the OS compared to those negative AR (87.0% vs. 74.2% and 94.2% vs 82.3%, respectively) [15]. Also, Luo X et al., 2010 reported AR expression was correlated with the 5year

Disease-free survival (DFS) and overall survival (OS) of TNBC patients [17].

On the other hand, Hu et al., 2011 reported that women with AR-positive TNBCs had an 83 % increase in overall mortality compared to women with AR-negative tumors [17]. Also, Park et al., 2011 have shown a trend toward poorer outcomes in AR-positive, ER-negative breast cancers [31]. Choi and colleagues have reported AR positive expression as a significant

predictor of worse DFS and OS in TNBC without lymph node involvement. However, they could not identify AR as a prognostic marker in patients with TNBC and lymph node metastasis [32]. On the other side, among 81 Egyptian BC cases, AR was expressed in 37.04% (19% of cases were TNBC and 13.3% of them were expressed AR), AR expression was significantly correlated with older age (p=0.03), post-menopausal status (p=0.001), lower grade (p=0.003), and early stage of presentation (p=0.03), but AR expression didn't correlate with the OS in the studied cases[33], enrollment of all molecular subtypes of BC patients not only triple –ve in their study may be the cause of these different results from ours. Also, in another Egyptian study containing 150 cases of BC, AR was expressed in 71% of the cases. AR is also associated with lower tumor burdens and favorable differentiation. In addition, AR is expressed in a significant number of TNBC (36 cases out of 48 cases of TNBC showed AR positivity) [34].

Conclusion:

Our study results support the value of AR expression in TNBC as we can rely on AR expression as a prognostic factor for disease outcome and as a predictive factor for new targeted treatment in this distinct dismal type of breast cancer. And to solve the issue of controversy more studies with higher patients' numbers and longer follow up period are needed.

Conflict of Interest

There was no conflict of interest.

List of Abbreviations:

TNBC	Triple Negative Breast Cancer
ER	Estrogen Receptor
PR	Progesterone Receptor
HER2	Human Epidermal Growth Factor Receptor 2
AR	Androgen Receptors
OS	Overall Survival
BC	Breast Cancer
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma

Authors' Contributions:

All the authors helped in collecting the cases. KF, OF, SE, SB, RA and RS delivered chemotherapy and underwent follow-up. LG performed excision biopsy or mastectomy samples to all patients. OH and OM performed all histopathological and immune-histochemical assessment. All the authors helped in designing the subject and drafted the manuscript. All the authors read and approved the final manuscript.

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None

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