THE PROGNOSTIC VALUE OF ANDROGEN RECEPTOR EXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER PATIENTS

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Abstract

Background: Triple negative breast (TNBC) cancer is a distinct subtype of BC that is characterized by frequent recurrence and metastasis. It is suggested that androgen receptor (AR) may be a valuable prognostic marker in TNBC. **Aim of work:** To assess the relation between AR and clinicopathological features. **Methods:** This retrospective study included 35 patients with non-metastatic TNBC treated at Medical Oncology Department, Maadi Armed Forces Medical complex from January 2015 to June 2019. All patients were subjected to full documentation of their history. The general and local examination were the two main components of the clinical examination done for all subjects. Some laboratory investigations were performed. All patients underwent preoperative imaging tests. Immunohistochemistry was performed on Formalin fixed Paraffin Embedded tissue sections from tumor specimens using standard procedure to evaluate AR expression more than 1% of tumor cells nuclei stained were considered positive. **Results:** Low proliferative index was more frequent in patients with positive AR than those with negative AR (6/13; 46.2% vs 1/22; 4.5%, respectively) while High proliferative index (Ki 67 > 20 %) was more frequent in patients with negative AR than those with positive AR (19/22; 86.4% vs 7/13; 53%, respectively), a statistically significant difference (P = 0.009).**Conclusion:** Triple-negative breast cancer is an aggressive disease with mixed heterogenicity associated with poor prognostic outcome. Androgen receptor positivity was associated with lower risk of disease recurrence and mortality.

Key words: Breast; Cancer; Androgen; Triple.

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Introduction:

Worldwide Breast Cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 11.7% of the total cancer cases, it is the fifth leading cause of cancer mortality worldwide, 6.9% of the cancer deaths(1). Molecular diagnosis allows the stratification of BC into four major subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67.Triple negative breast (TNBC) cancer is a distinct subtype of BC that is characterized by frequent recurrence and metastasis(2).

Androgen receptor (AR) is a nuclear receptor, which upon the binding of androgen forms a hormone-receptor complex that acts on the androgen response elements of target genes to mediate gene transcription.Androgen receptor has drawn increasing attention in the management Of BC in recent years, as AR is expressed in 70-90% of primary BC and often at a higher level in comparison with ER(3).

This AR alteration explains the clinical benefit rate of 20-25% in patients with breast cancer treated by testosterone. Testosterone was later replaced with Tamoxifen and Aromatase inhibitors (AI), due to its masculinizing effects. These ER-modulating drugs have been widely used; however, their efficacy can be limited by patient intolerance. The observation that AI elevates androgen levels highlights the potential significance of AR-modulating agents(4).There are six subcategories of TNBC classified by gene expression profiles: Basal-like 1, Basal-like 2, Immunomodulatory, Mesenchymal and Mesenchymal stem-like, Luminal androgen receptor (LAR) and unstable. LAR-type tumors are usually abundant with AR up regulation. Unsurprisingly, a preclinical study demonstrated that LAR-type breast cancer cell lines are sensitive to AR antagonists. These findings suggest AR may be a valuable prognostic marker in TNBC(5).

Aim of work:
To assess the relation between AR and clinicopathological features.

Methods:
This is a clinicopathological and immunohistochemical retrospective study included 35 patients with non-metastatic TNBC treated at Medical Oncology Department, Maadi Armed Forces Medical complex from January 2015 to June 2019.The local ethics committees gave their approval for the study [IRB]and was performed in accordance with the Helsinki Declaration. All the participants were asked for their written informed permission prior to their actual involvement in the study.Females > 18 years old and pathologically proved to be triple negative breast cancer were only included in the study. On the other hand, we excluded patients with previous diagnosis of cancer, significant co morbidities or brain metastasis. All the participating patients were subjected tofulldocumentation of their history. The general and local examination were the two main components of the clinical examination done for all subjects. Some laboratory investigations were performed as complete blood picture, renal functions, liver functions and Tumor marker (CA15.3). All patients underwent preoperative imaging tests as breast ultrasound, diagnostic mammography and/or MRI breast, echocardiography, plain X-ray chest or CT chest if needed, pelvi-abdominal ultrasound and/or CT abdomen and pelvis if needed in addition to bone scan.Immunohistochemistry was performed on Formalin fixed Paraffin Embedded tissue sections from tumor specimens using standard procedure to evaluate AR expression more than 1% of tumor cells nuclei stained were considered positive.

Statistics/data analysis:
The collected data were analyzed by computer using Statistical Package of Social Services version 23 (SPSS), Data were represented in tables and graphs, Continuous Quantitative variables were expressed as the mean ± SD & median (range), and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Suitable statistical tests of significance were used after checked for normality. The results were considered statistically significant when the significant probability was less than 0.05 ($P < 0.05$). P-value < 0.001 was considered highly statistically significant, and P-value ≥ 0.05 was considered statistically insignificant.

Results:
Table (1) shows that Androgen Receptor expression was demonstrated in (13/35; 37.1%) of patients, AR positive tumor was more frequent in patients older than 60 years at diagnosis (8/13; 61.5%) but without statistically significant difference ($P = 0.1$). Regarding menstrual status, AR positive tumor was more frequent in post-menopausal women (10/13; 76.9%) than premenopausal patients (3/13; 23.1%) but without statistically significant difference ($P = 0.7$). Androgen Receptor positive tumor was predominant in obese patients (7/13; 53.8%) rather than those with normal BMI (2/13; 15.4%) and overweight patients (4/13; 30.8%) without statistically significant difference ($P = 0.4$). Finally, in patients with positive family history, there was no statistically significant difference between distribution of androgen receptor expression as shown (30.8% vs 27.3%) for positive and negative AR, respectively ($P = 0.8$).As shown in table (2), there was no statistically significant difference between patients with IDC and ILC as regard AR expression where, IDC (12/13) represented (92.3% vs 7.7%) for ILC (1/13) in positive AR patients ($P = 0.8$). Positive AR expression was statistically higher in Grade II tumors than in Grade III (8/13; 61.5% vs 5/13; 38.5%, respectively; $P = 0.04$). Regarding pathological tumor size, AR expression was higher in T2 tumors (61.5%) vs (0.0% & 38.5%) for T1 and T3, respectively with no statistically significant difference ($P = 0.3$). Low proliferative index was more frequent in patients with positive AR than those with negative AR (6/13; 46.2% vs 1/22; 4.5%, respectively) while High proliferative index (Ki 67 > 20 %) was more frequent in patients with negative AR than those with positive AR (19/22; 86.4% vs 7/13; 53%, respectively), a statistically significant difference ($P = 0.009$).As shown in table (3), AR expression was more frequent in tumors with lymph node infiltration than tumors without lymph node infiltration (9/13; 69.2% vs 4/13; 30.8%, respectively) with no statistically significant difference ($P = 0.9$). There was no statistically significant difference between patients with positive and negative Lymphovascular invasion as regard AR expression where, AR expression was less frequent in tumors with Lymphovascular invasion than tumors without Lymphovascular invasion (3/13; 23.1% vs 5/13; 38.5%, respectively; $P = 0.1$). Also, AR expression was more frequent in patients without Extra capsular infiltration than in patients with Extra capsular infiltration (23.1% vs 7.7%, respectively) but with no statistically significant difference ($P = 0.9$).As shown in table (4), distant metastasis was more frequent with AR negative patients than AR positive (86.4% vs 76.9%, respectively) while local recurrence was more frequent in AR positive than in AR negative (23.1% vs 13.6%, respectively) with no statistically significant difference ($P = 0.4$). In AR-positive patients, the progression rate was 84.6% (11/13), while in AR-negative patients was 100% (22/22). During this period, the mortality in AR-positive TNBC was 53.8% (7/13) and the mortality in AR-negative TNBC was 90.9% (20/22). With statistically significant difference between AR-positive and AR-negative in relation to mortality rate ($P = 0.012$, respectively) as shown in figure (1) and table (5).

Table (1): Relation between AR expression and (age, menstrual status, body mass index and family history).

Variable		Androgen Receptor						P
		Negative		Positive		Total		
		N=22		N=13		N=35		
		N	%	N	%	N	%	
Age	<60y	14	63.6%	5	38.5%	19	54.3%	0.149
	≥60y	8	36.4%	8	61.5%	16	45.7%	
Menstrual Status	Postmenopausal	18	81.8%	10	76.9%	28	80.0%	0.726
	Premenopausal	4	18.2%	3	23.1%	7	20.0%	
Body mass index	Normal	1	4.5%	2	15.4%	3	8.6%	0.413
	Obese	16	72.7%	7	53.8%	23	65.7%	
	Overweight	5	22.7%	4	30.8%	9	25.7%	
Family history	-VE	12	54.5%	6	46.2%	18	51.4%	0.885
	+VE	6	27.3%	4	30.8%	10	28.6%	
	NA	4	18.2%	3	23.1%	7	20.0%	

Table (2): Relation between AR expression and (Histologic subtypes, Tumor grade, Tumor size and Ki-67).

Variable		Androgen Receptor						P
		Negative		Positive		Total		
		N=22		N=13		N=35		
		N	%	N	%	N	%	
Histologic subtype	IDC	20	90.9%	12	92.3%	32	91.4%	0.886
	ILC	2	9.1%	1	7.7%	3	8.6%	
Tumor grade	G2	6	27.3%	8	61.5%	14	40.0%	0.046
	G3	16	72.7%	5	38.5%	21	60.0%	
Tumor size	T1	3	13.6%	0	0.0%	3	8.6%	0.349
	T2	13	59.1%	8	61.5%	21	60.0%	
	T3	6	27.3%	5	38.5%	11	31.4%	
Ki-67	High	19	86.4%	7	53.8%	26	74.3%	0.009
	Low	1	4.5%	6	46.2%	7	20.0%	
	NA	2	9.1%	0	0.0%	2	5.7%	

Table (3): Relation between AR expression and (Axillary LNs, Lymphovascular invasion and Extra capsular infiltration).

Variable		Androgen Receptor						P
		Negative		Positive		Total		
		N=22		N=13		N=35		
		N	%	N	%	N	%	
Axillary LNs	-Ve	7	31.8%	4	30.8%	11	31.4%	0.948
	+VE	15	68.2%	9	69.2%	24	68.6%	
Lymphovascular invasion	-Ve	6	27.3%	5	38.5%	11	31.4%	0.172
	+VE	12	54.5%	3	23.1%	15	42.9%	
	NA	4	18.2%	5	38.5%	9	25.7%	
Extra capsular infiltration	-Ve	4	18.2%	3	23.1%	7	20.0%	0.937
	+Ve	2	9.1%	1	7.7%	3	8.6%	
	NA	16	72.7%	9	69.2%	25	71.4%	

Table (4): Relation between AR expression and site of progression.

Variable		Androgen Receptor						P
		Negative		Positive		Total		
		N=22		N=13		N=35		
		N	%	N	%	N	%	
Site of Progression	Distant	19	86.4%	10	76.9%	29	82.9%	0.474
	Local	3	13.6%	3	23.1%	6	17.1%	

Table (5): Patients' clinical outcome as regard Androgen Receptor expression.

Clinical outcome		Androgen Receptor						P
		Negative		Positive		Total		
		N=22		N=13		N=35		
		N	%	N	%	N	%	
Recurrence	No	0	0.0%	2	15.4%	2	5.7%	0.058
	Yes	22	100.0%	11	84.6%	33	94.3%	
Mortality	No	2	9.1%	6	46.2%	8	22.9%	0.012
	Yes	20	90.9%	7	53.8%	27	77.1%	

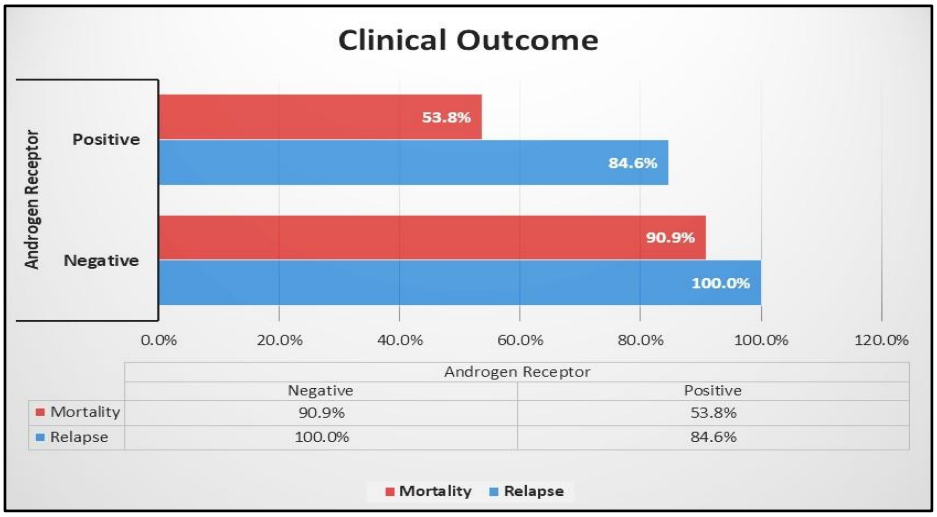

Figure (1): Patients' clinical outcome as regard Androgen Receptor expression.

Discussion:

Breast cancer can be classified according to molecular cytology into luminal A, luminal B, basal-like, human epidermal growth factor receptor 2 (HER2) over expressing. Different subtypes require different therapeutic strategies. To date, ER, PR and HER2 have been proved to be important prognostic indicators for breast cancer. They are also essential in determining the use of hormone therapy, chemotherapy, and targeted therapy in different subtypes [(6)]. The AR is a nuclear receptor that binds testosterone and DHT in the cytoplasm, translocating to the nucleus to regulate gene transcription. Female androgens are derived from the adrenal glands and ovaries; after menopause, the ovaries become mainly androgen-secreting organs. Both testosterone and its potent metabolite (DHT) have been measured in normal breast tissue. Higher concentrations have been measured in both invasive and in situ breast carcinomas [(7)].

This is a clinicopathological and immunohistochemical retrospective study included 35 patients with non-metastatic TNBC treated at Medical Oncology Department, Maadi Armed Forces Medical complex from January 2015 to June 2019. The AR is expressed in approximately 70–90 % of overall breast cancer cases. AR is also expressed in DCIS with 60–90 % co-expression with ER. In TNBCs, the AR is expressed in 10–50 % of cases [(8)]. Consistent with these data, we found that AR was positive in (13/35; 37%) of the patients which is comparable to results reported by Hu et al., Mcghan et al., and He et al., who reported AR expression in 42%, 23% and 26% respectively [(9-11)]. A systematic review and meta-analysis done by Vera-Badillo et al. reviewed 19 studies including 7693 patients and concluded that ER positive tumors were more likely to express AR than ER-negative tumors (74.8% vs 31.8%; $P < 0.001$) [(12)]. This wide range in reported incidence of AR expression in TNBC can be attributed to variations in number of involved patients in each study or the cutoff value of AR positivity (≥1% or ≥10%), also the primary antibody source. Currently, there are no standard or consensus guidelines for scoring AR immunoreactivity in tissue sections. We used 1% as the cut point to define AR positivity and evaluate AR immunoreactivity in whole sections of TNBC. Also, the cut point that should be used to evaluate ER and PR positivity in breast cancers according to the ASCO/CAP guidelines is 1% [(13)].

Regarding the relation between AR expression and clinicopathological parameters, there was no significant relation between the age of the patients, menopausal status, and BMI with AR expression ($P = 0.1$; $P = 0.7$; $P = 0.4$, respectively) in agreement with He et al. [(11)]. McGhan et al. reported that AR expression was significantly associated with older age ($P = 0.05$) but with no significant relation with menopausal status ($P = 0.1$) [(10)].

On the other hand, AR positivity was significantly higher in older patients (P = 0.002), as reported by Dieci et al. [14]. Although, we found in our study that AR expression was more frequent with old age results were not statistically significant, this difference in significance may be due to different sample size between studies, race and follow up time.

In our study there was no significant relation between AR expression and family history (P = 0.8) which was comparable with YA-XUAN et al. [15]. Also, there was no significant relation between AR expression and pathologic subtypes (P= 0.8) which was similar to Asano et al. [16]. On the other side, Dieci et al. reported that there was a significant relation between AR and pathological subtypes (P<0.001) and this difference in significance may be attributed to large sample size as the study included 263 TNBC patients [14]. In our study there was no significant relation between AR expression and tumor size (P= 0.3) in agreement with Hu et al. and mcGhan et al. [9, 11].

In our study the statistical analysis of the AR showed no evidence of significant relation between AR expression and some prognostic factors like lymph node status, lymph-vascular invasion, and extra capsular Infiltration (P= 0.9; P= 0.1; P= 0.9, respectively) which was comparable with Hu et al. [9]. On the other hand, it was reported that there was a significant relation between expression of AR and lymph node status (P= 0.03) [15]. In our study there was a statistically significant relation between AR and tumor grade where AR expression was more common with low grade tumors (P= 0.04) which is comparable with Veli Sunar et al. [17]. In our study AR expression was higher in tumors with low Ki-67 less than 20% (P = 0.009) which is similar to Hu et al., who reported that AR expression was higher in low Ki-67 (P= 0.007) [9]. On the other side, YA-XUAN et al. and Asano et al. reported that there was no statistically significant relation between AR expression and Ki-67 (P = 0.7; P = 0.9, respectively)[15, 16].

Conclusion:

Triple-negative breast cancer is an aggressive disease with mixed heterogenicity associated with poor prognostic outcome. 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 can be a predictive factor for new targeted treatment in this distinct dismal type of breast cancer.Androgen receptor positivity was associated with lower risk of disease recurrence and mortality.

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REFERENCES:

1. Sung H, Ferlay J, Siegel RL, et al. GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 0:1–41.
2. Marra A, Trapani D, Viale G, et al. Practical classification of triple- negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies. npj Breast Cancer. 2020; 6:54.
3. Giovannelli P, Di Donato M, Galasso G, et al. The Androgen Receptor in Breast Cancer. Front Endocrinol (Lausanne). 2018; 9:492.
4. Mizushima T, Miyamoto H. The Role of Androgen Receptor Signaling in Ovarian Cancer. Cells. 2019;8(2):176.
5. Ana C. Garrido-Castro, Nancy U, et al. Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. Cancer Discov 2019; 9(2) 176-198.
6. Eroles P, Bosch A, Pérez-Fidalgo JA, et al. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev. 2012 Oct;38(6):698-707.
7. Hickey T, Robinson J, Carroll J, et al. Minireview: The Androgen Receptor in Breast Tissues: Growth Inhibitor, Tumor Suppressor, Oncogene?" Molecular endocrinology 2012; 26(8): 1252-1267.
8. Gucalp A, Traina TA. Targeting the androgen receptor in triple-negative breast cancer. CurrProbl Cancer 2016; 40:141–50.
9. Hu R, Dawood S, Holmes MD, et al. Androgen Receptor Expression and Breast Cancer Survival in Post-menopausal Women." Clinical Cancer Research 2011; 17(7):1867-1874
10. He J, Peng R, Yuan Z, et al. Prognostic Value of Androgen Receptor Expression in Operable Triple-Negative Breast Cancer: A Retrospective Analysis Based on a Tissue Microarray. Medical oncology 2012; 29(2): 406-410.
11. McGhan LJ, McCullough AE, Protheroe CA, et al. Androgen Receptor-Positive Triple Negative Breast Cancer: A Unique Breast Cancer Subtype. Annals of surgical oncology 2014; 21(2): 361-367.
12. Vera-Badillo FE, Templeton AJ, de Gouveia P, et al. Androgen Receptor Expression and Outcomes in Early Breast Cancer: A Systematic Review and Meta-Analysis. Journal of the National Cancer Institute2013; 319.
13. Hammond M, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med. 2010;134(6):907–22.
14. Dieci MV, Tsvetkova V, Griguolo G, et al., Androgen Receptor Expression and Association with Distant Disease-Free Survival in Triple Negative Breast Cancer. Front. Oncol.2019; 9:452.
15. YA-XUAN LIU, KE-JING ZHANG, LI-LI TANG. Clinical significance of androgen receptor expression in triple negative breast cancer-an immunohistochemistry study. ONCOLOGY LETTERS 2018; 15: 10008-10016.
16. Asano Y, Kashiwagi S, Goto W, et al. Expression and clinical significance of androgen receptor in triple-negative breast cancer. Cancers 2017; 9:4.
17. Veli Sunar, Hayriye T. Dogan, Furkan Sarici, et al. Association between androgen receptor status and prognosis in triple negative breast cancer. JBUON.2018; 23(5): 1325-1330.