IMMUNOHISTOCHEMICAL EXPRESSION OF ANDROGEN RECEPTOR IN TRIPLE NEGATIVE BREAST CARCINOMA AT ARMED FORCES INSTITUTE OF PATHOLOGY, RAWALPINDI

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ABSTRACT

Objective: To determine the frequency of immunohistochemical expression of androgen receptor in triple negative breast carcinoma.

Study Design: Cross sectional study.

Duration and Place of Study: This study included 30 cases confirmed as triple negative breast carcinoma at Armed Forces Institute of Pathology Rawalpindi, from Jan to Jul 2018.

Methodology: Anti-androgen receptor antibody was applied and assessed. Positive expression was defined as greater and equal to 10% nuclear immunostaining. SPSS-24 was used for analyzing data.

Results: Out of 30 cases of triple negative breast carcinoma (TNBC), all patients were female. Patients' ages ranged between 21-72 years with a mean age of 46.35 years and a standard deviation of ± 13.4. Androgen receptor expression was positive in 8 cases (27%) of all triple negative breast carcinomas. Out of these androgen receptor (AR) triple negative breast carcinomas; all 8 cases were of histological subtype invasive ductal (mammary) carcinoma, non special type, 7 cases (23%) were of histological grade 3 and 1 was of histological grade 2.

Conclusion: Androgen receptor expression is observed in 8 cases (27%) of triple negative breast carcinoma cases. Such patients can be selected as candidates for anti- androgen receptor targeted therapy.

Keywords: Androgen receptor, Breast carcinoma, Hormone receptors, Prognosis, Triple negative breast carcinoma.

INTRODUCTION

Breast cancer is a heterogeneous group of diseases which comprises a spectrum of natural history, clinical characteristics, histopathological features and molecular subtypes, with different prognostic and therapeutic implications[1,2]. Breast cancer is not only the most prevalent female malignancy worldwide but is also the commonest cause of female mortality in Pakistan. One in every nine women is diagnosed with breast cancer in Pakistan[3]. Histopathologic factors including biomarkers, oncogenes, hormones and their receptors are valuable to predict outcome and select management strategies, as they are involved in development and genesis of breast cancer. However, there are significant differences in outcome and treatment responses and long term survival among patients with the same histological subtype, grade and stage[4].

Risk factors for breast cancer include family history, menstrual and reproductive history, fibrocystic disease, exogenous estrogens, contraceptive agents, ionizing radiation and genetics such as BRCA-1, BRCA-2, p53, retinoblastoma and human epidermal growth factor receptor-2 (HER-2) receptor status. Poor prognostic factors include younger age, higher tumor size, high grade and stage, nodal involvement, lymph vascular invasion, hormone receptor negativity and HER-2 expression.

With the advancement of molecular techniques, molecular classification of breast cancer has been developed. These molecular subtypes have a predictive and prognostic value in the management of breast cancer. Gene expression profiling is the gold standard for molecular classification. Individualized treatment programs and more

importantly targeted therapies have become possible with the implementation of this classification[5]. However due to high cost and technical difficulties involved in gene profiling, immunohistochemistry is a suitable alternative[6].

There are five main intrinsic or molecular subtypes of breast cancer. These include luminal A, luminal B, HER 2 positive, Triple Negative Breast Cancers and normal breast like[7]. Luminal A breast cancer is hormone-receptor positive, HER-2 negative carcinoma. These are low-grade; having the best prognosis. Luminal B breast cancer is hormone-receptor positive and either HER-2 positive or HER-2 negative and their prognosis is slightly worse as compared to Luminal A.

Triple Negative Breast Carcinoma (TNBC), constitute 15% of all invasive ductal carcinomas, currently there is no specific targeted therapy. There is no standard treatment regimen for TNBC. These TNBC have aggressive clinical behavior, younger age at presentation, BRCA-1 gene mutations, p53 mutations, high tumor grade, high mitotic rate and increased risk of recurrence. They have low overall survival, unresponsiveness to hormonal and anti-Her2 therapy[8,9].

The treatment options of TNBC include, chemotherapeutic agents like, Taxanes and Anthracyclines as well as targeted therapies like Anti-VEGF, Anti-EGFR, Anti c-KIT, PARP1 inhibitor and anti Androgen Receptors therapy[8].

Androgen Receptor (AR) has emerged as a potential therapeutic target in TNBC. Recently, it has been observed that androgen and AR play an important role in the development and genesis of breast cancer. As AR belongs to nuclear steroid hormone receptor family, it has high functional, structural and topographical similarity to estrogen receptors (ER) and progesterone receptor (PR). Transcription of many androgen-regulated genes contributes to breast development and/or tumorigenesis. Existing evidence suggests an association between a higher level of AR expression and improved outcomes[10].

Rationale of this study was to evaluate expression of androgen receptor in TNBC. So as to let the clinician select TNBC patients for targeted therapy against androgen receptor, this may improve overall survival.

METHODOLOGY

This cross sectional study was held at the department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi from Jan to Jul 2018. Method of sampling was non-probability, consecutive sampling. Sample size was calculated using WHO calculator, however sample size was limited due to number of resection samples received during these 6 months and inadequate fixation. Thirty histopathologically proven TNBC female patients were included in this study[11]. Tissue samples that were included in the study were with either incisional / excisional biopsies or mastectomy specimens that had been processed and diagnosed at department of Histopathology of Armed Forces Institute of Pathology. Autolyzed specimens or specimen showing extensive necrosis were excluded from in this study. Study was approved by Institutional Review Board held at AFIP (FC-HSP 17-24/READ-IRB/18/901). Baseline clinico-pathologic data including patients' particulars, histological type and histological grade was noted.

Streptavidin-biotin method for immune staining was used. Formalin-fixed, paraffin-embedded blocks were sectioned at 3μm thickness. They were de-paraffinized in xylene. Rehydration was done with decreasing concentration of ethanol. Heat induced epitope retrieval in Tris/EDTA buffer at pH 9.0 buffer was used for ready to use primary antibodies. Anti-Androgen Receptor (EP120) was used. An expression of ≥10% was defined as AR-positive expression while less than 10% AR expression was interpreted as loss of AR expression[12].

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 24. Frequency and percentage was calculated for catgorical variables such as gender, histological type, histological grade and androgen receptor expression. Continuous variables were expressed as mean and standard deviation such as age.

Percentage of categorical variables was compared using Pearson's chi-square test. The *p*-value ≤0.05 was considered to be statistically significant.

RESULTS

AR expression was positive in 8/30 (26.7%) patients, with cut off staining ≥10% of cells. Ages of patients ranged between 21 to 72 years with a mean age of 46.35 years and a standard deviation of ± 13.4 (fig-1). All the cases were diagnosed as invasive ductal carcinoma (IDC) of no special type (30/30). Grade 3 disease was seen in 14/30 (46.6%) of the cases. Whereas, 14/30 (46.6%) cases had grade 2 and 1/30 (3.3%) case had grade 1 disease. Of the 8 cases of positive androgen expression, 7 cases had histological grade 3 disease and 1 case had histological grade 2. No androgen expression was seen in cases having histological grade 1 (*p*-value=0.025) (fig-2).
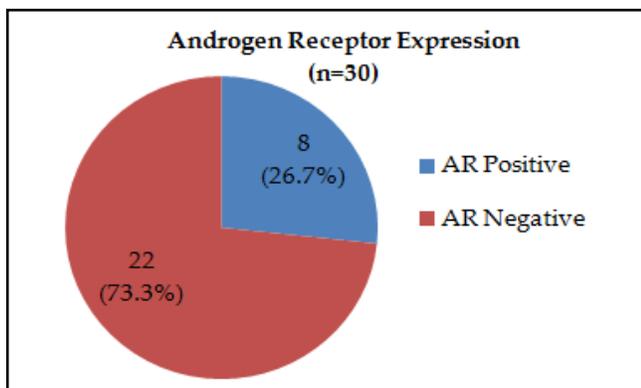

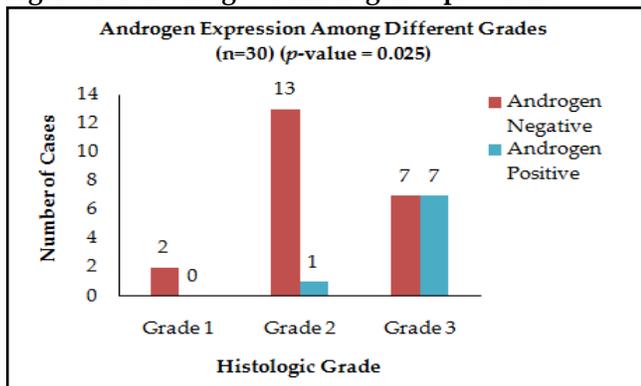**Figure-1: Percentage of Androgen expression.**

Figure 2: Androgen expression among different grades.

Lack of Androgen receptor was more in younger patients (<30 years) 8/9 (88.8%) patients. Among 9 patients with lymph node metastasis, 5

patients showed positive AR expression (*p*-value =0.019). Of the 27 cases that did not show presence of DCIS, 7 had positive androgen expression (*p*-value=0.783). In 23 cases lacking presence of lymphovascular invasion 4 cases showed androgen expression (*p*-value=0.96) (table-I & II).

Table-I: Correlation between Androgen expression and tumor stage.

Stage	Androgen		n (%)	*p*–value
	Negative	Positive		
Stage 1	2	-	16%	0.223
Stage 2	2	3	41%	
Stage 3	4	1	41%	

Table-II: Correlation between lymph node metastasis and androgen expression.

Stage	Androgen		n (%)	*p*–value
	Negative	Positive		
Not Seen	18	3	70%	0.019
Seen	4	5	30%	

DISCUSSION

The study aimed to assess Androgen receptor expression prevalence in 30 female patients. These patients were diagnosed as triple negative breast carcinoma at Armed Forces Institute of Pathology, Rawalpindi. It was observed that AR was expressed positively in 8/30 (26.7%) patients. This positive expression of AR was significantly correlated with tumor grade and lymph node metastasis with *p*-value <0.05. However, no significant correlation was established between AR expression and age, presence of DCIS or lymphovascular invasion.

In comparison to other studies, a large systematic review was done that included 7693 breast cancer patients in 19 studies, it was observed that out of all estrogen positive cases 74.8% showed Androgen expression. This percentage was 31.8% in estrogen negative tumors[13]. Androgen expression was seen in 74 out of 287 patients with TNBC (25.8%) according to He *et al*[13]. Similarly, 23% of a total 94 patients with TNBC showed AR expression in a study by Mc Ghan *et al*[14]. Ten percent of patients having TNBC were reported to have androgen expression by Neimeier *et al*[16]. Other similar studies observed that 10-43% of TNBCs reported expression of AR[17,18]. In a meta-

analysis including 13 studies of 2826 patients, 24.4% patients shows positive AR expression[19]. This study results are in concordance with these studies.

TNBC is a heterogeneous disease and numerous studies have shown further classification of TNBC based on its genetic profile, AR positive TNBC is one such subtype[10,15]. It also shows that preserved androgenic signaling could be a potential therapeutic molecular target similar to estrogen receptor positive carcinoma of breast[18]. In addition to this, 70-90% of breast carcinoma cases

TNBC prognosis is poor when compared to patients of other subtypes and this difference in mortality rates and recurrences can be attributed partly to their different genetic subtype in this entity of breast cancer. Adverse effects in proliferation of breast cancer cells as a result of signals generated by AR expression has also been observed in few cancer cell lines given 5-alpha-dihydrotestosterone treatment. This mechanism could lead to relapse of breast cancer[24]. Exact characteristics of AR-positive TNBCs can only be determined through additional studies (table-III).

Table-III: Comparison with other studies.

	Present Study (AFIP) (n=30)	Farag *et al* (Egypt) (n=90)	Asano *et al.* (Japan) (n=190)	Zakaria *et al.* (Egypt) (n=77)
Age (years)	21-72 Mean = 46.35	27-77 Mean = 46.5	Mean = 55	19-63 Mean = 35.6
Histologic type (IDC)	100%	82%	84%	92%
Tumour grade III	37%	40%	56.3%	32%
Lympovascular Invasion, Present	21.1%	-	28.9%	-
Ductal carcinoma in situ, Present	22/30	-	-	-
Androgen receptor expression	8/30 (27%)	27/90 (30%)	56/190(29.5%)	21/77 (27%)

give AR expression which is comparable to the rate of estrogen expression. Previous studies suggest that androgens are involved in the reduction of breast cancer evolution however, it is still uncertain what exact mechanisms and clinical significance of androgen receptor in breast cancer is involved in this process[20-22]. Role of Androgen signaling in breast cancer development remains controversial, however, androgen is involved in influencing breast cancer risk throughout various methods: either though binding of AR in turn stimulating production of malignant cells or through ER binding leading to competitive inhibition of 17 bestradiol stimulatory effect on tumor cells, or through conversion to estradiol[23]. AR negativity was significantly associated with distant metastasis, recurrences and histologic grade according to a study by Lou *et al*[17]. While in a study conducted by Wang *et al*, AR positive cases had lower tumor grade ($p<0.001$), but had lymph node metastases ($p<0.0.1$)[19].

CONCLUSION

Androgen receptor expression was observed in 8/30 (26.7%) patients of triple negative breast carcinoma. Such patients can be selected as candidates for outcome of disease as well as a predictive marker of novel targeted therapy in this distinct subtype of breast cancer. More such studies with greater number of patients and longer follow up is required to reduce controversial results.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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