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ANDROGEN RECEPTOR EXPRESSION IN BREAST CANCER

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ABSTRACT

Background: *Breast cancer is a heterogeneous disease. Accumulating evidence suggests a role for androgen signaling in breast cancer. Androgen receptor (AR) is often expressed in breast cancer and several studies suggest that its role depends on the tumor microenvironment as well as the relative levels of circulating estrogens and androgens. Therefore, the aim is to evaluate role of AR in four molecular subtypes of breast cancer.*

Methods: *AR expression was studied on tumor tissues by immunohistochemistry in 125 breast cancer patients and correlated with established clinicopathological parameters and disease status. The SPSS version 20 for statistical analysis of the data.*

Results: *In this study, nuclear AR expression was seen in 34% of the breast cancer patients. AR expression was seen significantly higher in stage III patients (P=0.05) and low BR tumors (P=0.003). With regards to disease status, patients who undergo disease remission had significant higher AR expression (38%) than patients who developed disease metastases (14%, P=0.03). In Kaplan and Meier survival analysis, patients with AR expression had better disease free- (P=0.02) and overall survival (P=0.01). In relation to molecular subtypes, AR expression was seen higher in Luminal A subtype (64%) and Luminal B subtype (52%) as compared to Her-2 subtype (16%) and TNBC subtype (18%, P=0.0001).*

Conclusion: *AR expressing breast cancer can be benefited with AR inhibitors.*

Key Message: *AR is an emerging new therapeutic target in breast cancer. AR expressing breast cancer have improved disease free- and overall survival and can be benefited with AR inhibitors such as bicalutamide, enzalutamide, and apalutamide.*

Key words: *androgen receptor, immunohistochemistry, molecular subtypes, breast cancer*

INTRODUCTION

Breast cancer a heterogeneous disease featuring distinct four main molecular subtypes identified based on ER, PR and Her-2 expression are Luminal A, Luminal B, Her-2 enriched and basal like. These molecular subtypes could provide information on prognosis and influence treatment planning. Gene expression microarray studies have shown evidence of androgen signaling in breast cancers (1–3). Circulating androgens are detected at physiological conditions in females, and their levels differ during different phases of life. Androgens are converting in to estradiol or binding to a subset of estrogen-responsive element or direct binding to androgen receptor (AR). AR is thought to play a central role in its initiation, progression of breast cancer, and its response to therapy. However, regarding genetic alterations of AR in breast cancer are not well known. AR appears to have diverse functions in molecular subtypes of breast cancer (4). In ER positive subtype, there is a dynamic relationship between ER and AR, where the two receptors can transcriptionally regulate each other through heterodimerization and binding to the same DNA sequence (5). In ER-α-negative/HER 2-positive breast cancer, activation of Wnt/β-catenin pathway facilitates the transcriptional activity of AR promoting tumor growth. In TNBC, androgens seem to initiate second-messenger signaling cascades, which often results in a feedback loop, leading to the progression of the tumor.

AR is now considered as a prognostic marker and therapeutic target for breast cancer. Like

prostatecancer, AR expressing breast cancers are potential candidates for AR inhibitors such as Bicalutamide or Enzalutamide. In view of this, the present study evaluated incidence of androgenreceptor expression by immunohistochemistry method in molecular subtypes of breast cancer.

MATERIALS AND METHODS PATIENTS

In this retrospective study, 100 breast cancer patients who had been diagnosed and treated at GCRIduring 2014 to 2019 were included in the study. The detailed clinical history such as patient's age,menopausal status, disease stage, histopathological findings, treatment offered and disease status was recorded from the case files maintained at the Institutional Medical Record Department.

Formalin fixed paraffin embedded tumor tissue (FFPE) blocks were collected from Histopathologydepartment of the institute. Disease staging was done according to UICC TNM classification. Disease status was assessed by clinical examination, radiological investigations and biochemical investigations. The study was approved by Institutional Scientific Review Board and Ethics Committee. Patients other than TNBC subtype and subjected to neo-adjuvant therapy (either Radiotherapy or Chemotherapy before surgery) and patient with stage IV TNBC were excluded from the study.

Immunohistochemical localization

4µm thin sections were cut on microtome (Leica, Germany) and taken on 3-aminopropyl triethoxysilane (APES) coated slides. Immunohistochemical localization of ER, PR, Her-2 and AR was performed on FFPE tissue blocks containing primary tumor and evaluated by Haematoxylene and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer usingVentana reagents (Ventana, USA). Briefly, the protocol includes following steps of deparaffinization using EZ solution, antigen retrieval using cell conditioning (CC1), incubation with ultra-view DAB inhibitor for 4 minutes, 100µl of primary antibody, ultra view HRP multimerfor 8 minutes, ultra view DAB detection kit for 8 minutes, counterstain with haematoxylin for 8 minutes, bluing reagent for 4 minutes and mounted with DPX. The primary antibody clone, company, dilution, antigen retrieval time and antibody dilution used are as follows:

Primary antibody	Clone	Company	Dilution	Cell Conditioning	Primary antibody incubation time (mins)
ER	SP1	Roche Ventana	RTU	Standard	16
PR	1E2	Roche Ventana	RTU	Standard	16
Her-2	4B5	Roche Ventana	RTU	Mild	32
AR	SP107	Cell Marque	1:100	Mild	32

Scoring

Two independent observers familiar with Immunohistochemistry and unaware of the clinical outcome scored all the sections. The sections were scored with semi-quantitative scoring ranging from negative (no staining) to 3+ (1+: staining in <10% of cells, 2+: staining in 10% to 50% of cells, and 3+: staining in >50% of cells) For statistical evaluation, scores 1+, 2+ and 3+ were takentogether as positive group.

Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlationand significance between the two parameters. In case of patient number less than 5 in the cells of 2 x 2 tables, Yates' Continuity Correction value along with its significance was taken into consideration. Univariate survival analysis was carried out by Kaplan and Meier method and LogRank statistics was used to assess the prognostic significance of disease free survival (DFS) and overall survival (OS). Multivariate survival analysis was performed using Cox regression model with forward stepwise (likelihood ratio) method. The Wald statistics and relative risk [Exp(B)] with 95% confidence interval (CI) for Exp(B) were used to evaluate the prognostic significance. Pvalues ≤ 0.05 were considered significant.

Results

AR expression

AR is known as nuclear receptor activated by binding of any androgenic hormones which gives nuclear staining by immunohistochemistry on formalin fixed paraffin embedded tissue blocks. ARexpression was found negative in 66% (83/125)and positive in 34% (42/125) of breast cancer withan intensity of 1+, 2+ and 3+ in13% (16/125), 15% (18/125) and 6% (8/125), respectively.

Correlation of AR expression with clinical parameters

In relation with clinical parameters a similar expression of AR was observed between patients with
≤50 years (31%, 24/77) and age>50 years (37%, 18/48; p=0.46) as well as between patients with pre-menopausal (38%, 17/44) as compared to patients with post-menopausal status (30%, 25/81;p=0.38).
Correlation of AR expression with pathological parameters

In relation to pathological parameters, a trend of higher AR expression was noted with in patientswith T3 and T4 tumour size (42%, 8/19), than T1 and T2 tumor size(34%, 34/106);in patients withpositive lymph node status (38%, 26/69) than lymph node negative (28%,16/56); and patients withstage III disease (46%,17/37)than stage I and stage II disease (28%,25/88). With histological subtype, patients having IDC with DCIS had significantly higher expression than IDC. None of the patients with medullary carcinoma exhibited AR expression. Further, a significant decreasingincidence of AR expression was seen in from low BR score tumors (63%, 5/8) to intermediate BRscore tumors (42%, 30/72) to high BR score tumors (10%, 3/29, P=0.003). A similar trend was seen from Grade I (37%, 3/8) and II tumors (38%, 38/84) to grade III tumors (21%, 7/33).
Correlation of AR expression with disease status

Of 125 patients, 22 patients developed disease metastases. One patient developed local recurrence and 21 patients developed visceral metastases. Patients who undergo disease remission had higherAR expression (38%, 39/103) than patients who developed disease metastases (14%, 3/22).
Kaplan & Meier Survival AnalysisDisease Free Survival
InAR negative group, 19 (33%) patients developed disease relapse whereas in AR positive group,3 (07%) patients developed disease relapse. So, AR negative patients had worse disease free survival (33% patients relapsed; mean +months 58.50 +2.69) than AR positive patients (7% patients relapsed; mean +months 69.17 +2.12; Log Rank=5.16, df=1, P=0.02; Figure).

Overall Survival

InAR negative group, 19 (33%) patients whereas in AR positive group, 3 (07%) patients died dueto disease. So, AR negative patients (33% patients died; mean +months 64.60 +2.79) had worse overall survival than AR positive patients (7% patients died; mean +months 71.28 +1.05; Log Rank=5.88, df=1, P=0.01; Figure).
Correlation of AR expression with molecular subtypes

In relation with molecular subtype, higher expression of AR was observed in Luminal A subtype (64%, 16/25) and Luminal B subtype (52%, 13/25) as comparedto Her-2 subtype (16%, 4/25) andTNBC subtype (18%, 9/50, P=0.0001).

DISCUSSION

AR is emerging as an important factor in the pathogenesis of breast cancer and a potential therapeutic target. In the current study, AR expression was evaluated in four molecular subtypes of breast cancer such as Luminal A, Luminal B, Her-2 positive and triple negative. The AR expression was noted in 34% of breast cancer. With respect to molecular subtypes, 64% of LuminalA, 52% of Luminal B, 16% of Her-2 positive and 18% of triple negative exhibited AR expression.It was observed that Luminal A and Luminal B exhibited significant higher AR expression than Her-2 positive and triple negative. Various other studies on breast cancer have observed AR positivity is the range of 60% to 80% (6-8).

AR has been investigated extensively in hormone-dependent cancers such as prostate and breast cancers. Increasing evidence support the role of AR signaling in other hormone-independent tumortypes, including bladder, kidney, pancreatic, liver, endometrial, mantle cell lymphoma, andsalivary gland cancers, etc. However, the critical role of AR signaling in other hormone- independent human malignancies is poorly understood (9).

With respect to correlation of AR expression with clinical parameters such as age and menopausalstatus did not show any significant findings. A study by Gujam et al (10) observed similar findings with age and a study of Hu et al (11) showed significant higher AR expression in postmenopausal patients. Regarding correlation of AR expression with pathological parameters, a trend of higher expression of AR was seen in patients with larger tumor size, positive lymph node status and stageIII disease. Similar to our findings, Aleskandarany et al (12) observed significant association of AR with smaller tumour size (≤ 2 cm) and Astvatsaturyan et al (13) show significant association with positive lymph node status and early stage disease. In relation to histopathological subtype, higher expression of AR observed with in IDC with DCIS subtype. A study of Meijnen et al also showed a correlation between AR expression and DCIS histology of breast cancer (14). While thestudy of Wang et al did show no correlation between AR level with the ductal or other histopathogical type of breast cancer (3). This study observed a significant higher AR expression in low BR score tumors and a similar trend with histologic grade of the tumor. Contrary to our studies, few studies have shown significant association of AR with advanced histological grade (7-9, 15). A study of Astvatsaturyan et al (10) showed no association between AR expression and BRscore.

In this study, AR expression was significantly associated with disease status. The data depicted that high number of patients with AR expression were in remission period (38%). Further, patientswith AR expression had significant better disease free and overall survival than patients with no expression of AR. Two other studies showed no association between AR expression with the disease free survival as well as overall survival.

Additionally, AR expression was correlated with molecular subtypes of breast cancer. Luminal Aand Luminal B subtypes exhibited significant higher AR expression as compared to HER-2 positive and TNBC subtypes which was in concordance with two studies (7,13). Some studies alsoobserved higher AR expression in Her-2 positive breast cancer (18-19). A study by He et al indicated that AR plays an important role in promoting the growth of HER2 positive breast cancerby cross-talking with the HER2 signaling. AR drug may be used as an alternative second line therapy for treating HER2 positive breast cancer (20).

TNBC is an aggressive subtype characterized by the lack of ER, PR, and Her2 receptors. This designation masks the heterogeneity of this patient population and the challenge of stratifying them for optimal treatment selection. Due to the paucity of treatment targets, cytotoxic chemotherapy isstill the standard of care for TNBC, and there is a need to develop new and more effective targeted treatments for these patients (21). Studies on TNBC noted AR expression in the range of 13%- 37%. In a meta-analysis study, AR positivity is associated with lower risk of disease recurrence inTNBC was described by Wang et al (3). Tiffany et al in phase II study have shown TNBC that express AR may benefit from an AR inhibitor enzalutamide (22).

CONCLUSION

In conclusion, variable expression of AR is seen in diverse molecular subtypes. Luminal A and Luminal B exhibited higher expression whereas Her2+ and TNBC exhibited lower expression. Further AR expression emerged as a prognostic marker in breast cancer predicting favourable disease free- and overall survival. Thus, AR is an emerging new target and AR positive advancedbreast cancer can be benefited with as anti-AR drugs such as Bicalutamide or Enzalutamide.

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