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ORIGINAL ARTICLE

Androgen receptor expression as a prognostic and predictive marker in triple-negative breast cancer patients

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KEYWORDS

Triple-negative breast cancer;
Androgen receptor immuno-histochemistry;
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Abstract *Purpose:* It is clear that triple-negative breast cancer (TNBC) tumors are heterogeneous group, but clinically important sub-sets have begun to emerge. We investigate the immunohistochemical expression of androgen receptor (AR) among those hormonal insensitive groups which have only the option of chemotherapy. Exploiting this knowledge for therapy has been challenging.
Patients & methods: Seventy seven patients with TNBC subtype, treated from January 2009 until February 2011 were evaluated for AR expression where AR-positive expression group (⩾10% nuclear stained cells) was conducted to receive anti-androgen therapy post adjuvant chemotherapy (Bicalutamide "Casodex®") 50 mg, once daily with or without meals at the same time each day, to date. AR expression was correlated with other prognostic factors and survival (disease free survival (DFS) and overall survival (OS)). Cox proportional hazard model was used to assess variables in the multivariate analysis.
Results: The median age in the present study was 35.6 year **(19–63 years)**. The median follow-up period was 24 months **(3–60 months)**. AR-positive expression in the present study was **(21\77) 27.27%** correlated with clinical outcomes, for recurrent event **(*n* = 4, 19.05%), (*P* = 0.000, HR 12.750, CI 95% 3.668–44.318)** and for death event, no body died in AR positive expression group **(*P* = 0.000, HR 0.644, CI 95% 0.533–0.779)**. Improved survival with AR-positive expression group for 2-year and 3-year DFS was 85% and 78% respectively with **(*P* = <0.001, CI 95% 39.17–51.39)** and for OS at 2-year and 3-year was 100% **(*P* = 0.0005)**. In univariate and multivariate analysis, AR positive expression with anti-androgen therapy in TNBC patients in our present study had retained their independent prognostic value for DFS **(*P* = 0.0006, HR 4.659, CI 95% 1.553–13.977)**. Bicalutamide was well-tolerated therapy with no grade 3/4 treatment-related adverse events.
Conclusions: Bicalumide is well tolerated in AR positive TNBC subtype patients and could offer an alternative to cytotoxic chemotherapy in those patients with better OS and DFS.

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1. Introduction

Androgen receptor (AR) positivity has been detected in approximately half of all breast carcinoma cases.[1,2] High (AR) expression in breast cancer has been correlated with a low risk of recurrence and death.[1]

AR has been shown to have prognostic implications in breast carcinoma, and higher AR expression levels have been associated with older age at diagnosis, higher expression of ER or PR, lower nuclear grades, and smaller tumor size.[2,3]

Triple-negative breast cancers (TNBC) were recently divided into further subtypes including a subtype with high AR expression.[4]

Previous studies looking at AR expression in TNBC have demonstrated that AR negativity has been associated with a shorter disease-free interval and overall survival than AR-positive TN cancer. These studies suggest that AR expression could be a useful prognostic marker in TN tumors.[4]

AR is expressed in 60–70% of breast tumors independent of estrogen status, and in 20–32% of TNBC patients. Estrogen and Progesterone and the gene HER-2, these are the three big markers and/or targets in breast cancer. Evidence presented at the AACR Annual Meeting 2013 adds a fourth: androgen receptors.[5]

2. Patients and methods

This Retrospective study was conducted in Clinical Oncology Department and Histopathological Department, Tanta University 2009 until February 2011. The study included 77 consecutively treated patients with TNBC. The established clinical and histo-morphological factors of all patients were assessed. The steroid hormones status including (ER, PR, Her.2, AR and KI-67) was evaluated by immunohistochemistry (IHC).

The aim of this study focused on predictive and prognostic value of AR expression as a hormonal marker in TNBC patients.

2.1. Immunohistochemistry for ER, PR, HER-2, AR and KI-67

For immune-staining, 3–5 mm sections were deparaffinized with 40 min incubation at 60_C and subsequent immersion in xylene, and were rehydrated in solutions of decreasing ethanol. Then specimens were incubated in 0.3% H_2O_2 for 30 min to inhibit activation of endogenous peroxidases. Slides were then washed with phosphate-buffered saline (PBS) and heated in an 830-W microwave oven for at least 15 min in 10 mmol/l sodium citrate buffer (pH 6.0) for antigen retrieval. Sections were incubated with primary antibodies against [mouse monoclonal, androgen receptors (ab9474 1:500 dilution), rabbit monoclonal Estrogen receptor (ab37438 1:25 dilution), rabbit monoclonal Progesterone receptor (ab2765 1:25 dilution), rabbit monoclonal HER-2 receptor (ab134182 1:100 dilution) and Rabbit polyclonal KI-67 (ab15580 1:100 dilution) overnight at 4_C. For the negative control, the primary antibody was replaced with phosphate buffered saline (PBS). Rabbit anti-mouse horseradish peroxidase-conjugated secondary antibody was added followed by incubation for 40 min at room temperature. The color was developed using diaminobenzidine (DAB) as a chromogen. Slides were extensively washed with PBS after each step. Finally they were counter-stained with Mayer's hematoxylin.

The immunostaining results for ER, PR & AR were assessed semiquantitatively and reported as positive if more than 10% of cells have nuclear immunostaining in a tumor. Tumor cells were considered positive for HER2 protein over-expression when more than 10% of the cells showed complete moderate or strong membrane staining. Ki 67 immunostaining was considered positive if there were nuclear staining in more than 10% of the tumor cells.[6]

2.1.1. Study design

Seventy seven TNBC patients were classified into two sub-groups according to AR-expression profile, where AR-positive expression group 21/77 (27.27%) designated to receive anti-androgen therapy post adjuvant traditional chemotherapy, Bicalutamid 50 mg once daily with or without meals at the same time each day still received to date.

At the time of primary treatment, none of the patients had any evidence of distant metastases. After the completion of the primary treatment, our TNBC patients underwent regular follow-up examinations at our department for DFS and OS as regards AR expression profile. All the procedures were in accordance with the ethical standards of our faculty's Ethical committee.

2.1.2. Statistical analysis

Statistical presentation and analysis of the present study were conducted, using Number and percentage for qualitative and tested by chi-square test. We used Kaplan–Meier and Cox regression for survival analysis by SPSS for windows version 18.0 software package (SPSS Inc., Chicago, 11) and P value = $<0.05\%$. Overall survival (OS) defined the length of time from the date of diagnosis and the patients still alive either free or not. Disease-free survival (DFS) expresses the period after curative treatment (disease eliminated) when no disease can be detected.

3. Results

At the time of the primary treatment, none of the patients had any evidence of distant metastases.

The tumor's, patient's and treatment's characteristics in 77 TNBC patients are presented in Table 1, the median age of the patients was 35.6 years (range, 19–63). Age >35 years was 50.65%, premenopausal at the presentation was 52.63%, patients had grade III tumors 32.47%, tumor size larger than 2 cm was 27.27%, and patients had invasive ducal carcinoma (IDC) (92.21%). At least one axillary lymph node was positive in 57.14% of patients, and positive mitotic index was 67.53%. In twenty one patients (21/77) 27.27% were positive for AR expression in TNBC patients in the present study. Positive AR immunostaining was inversely correlated with large tumor size ($P = 0.001$), nodal status ($P = 0.007$), high grade ($p = 0.000$) and higher Ki67 ($p = 0.08$). Positive AR expression was associated with age less than 35 years (42.86%), female patients (100%), premenopausal status (42.86%) and no patients had stage III at presentation. For AR negatively expressed tumors in TNBC patients in the present study 51.79% was associated with age <35, 3 male patients (5.36%), premenopausal status (56.36%). Stage III at