Prevalence of androgen receptors in invasive breast carcinoma and its relation with estrogen receptor, progesterone receptor and Her2/neu expression

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Abstract  Background and aims: Although Breast carcinoma had many targeted biomarkers for its treatment, however, it is a heterogeneous disease with different outcomes and need new markers especially for the triple negative group when estrogen receptor, progesterone receptors and Her2/neu are negative. Androgen receptor is a new target with unclear role. The aim of this study was to examine the prevalence of androgen receptors in invasive breast cancer and tries to elucidate its relation to some well recognized clinicopathological and immunohistochemical markers.

Materials and methods: One hundred and fifty cases of invasive breast carcinoma were evaluated for type, grade and stage and studied immunohistochemically for estrogen receptor, progesterone receptor, Her2/neu and androgen expression. Androgen receptor expression was correlated with histopathological factors and the three studied markers separately then the studied cases were classified into three groups according to estrogen, progesterone receptor and Her2/neu expression and correlated with androgen receptor expression.

Results: Androgen receptor was expressed in 71% of breast cancer cases. Its expression is associated significantly with both the stage and the grade. Also it was significantly associated with estrogen receptor and Her2/neu expression. It was expressed in a significant number of triple negative breast carcinoma, in Her2/neu positive cases and in estrogen negative cases which indicate that androgen receptor could be a new target for the treatment of these groups.

Conclusions: Although the impact of androgen receptor on breast cancer outcomes had not been clearly established, this result may provide evidence that androgen receptor is a good prognostic and predictive marker.

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Introduction

Breast cancer is the most common malignancy in females. Traditional histopathologic factors including tumor size, axillary lymph node metastasis and histological grade, as well as many biomarkers including oncogenes, tumor suppressor genes, sex steroid hormones and their receptors are involved in the genesis and development of breast cancer and are valuable to predict outcomes and select management strategies [1]. However, breast cancers have heterogeneous features that are diverse in behavior, outcome and response to therapy. It is difficult to predict outcomes in all breast cancer patients using traditional histopathologic factors and the same biomarkers [2].

Perou et al. [3] and Sotiriou et al. [4] in separate studies suggested a categorization of invasive breast cancers based on genetic profiles into five subtypes with clinical implications: luminal A, luminal B, HER-2 overexpressing, normal breast-like and triple negative phenotype. In other words it can be classified into estrogen receptor (ER)-positive (luminal A and B) and ER-negative (nonluminal) subtypes with a further subdivision of the ER-negative types into Her2-positive and triple negative phenotype [negative for (ER), progesterone receptor (PR) and Her-2/neu]. Luminal A tumors differ from luminal B tumours by a higher expression of ER-related genes and lower expression of proliferation-associated genes [5–7].

Conventional histopathological and molecular analyses of breast cancers have shown that triple negative breast cancer (TNBC) account for about 15% of all invasive ductal carcinomas of no specific type and are often high grade, have areas of necrosis, may have a typical and atypical medullary phenotype and have a distinct pattern of genetic alteration, including frequent P53 mutation [6].

Currently, invasive breast cancer is treated by multi-modality therapy. Approximately 75% of breast cancers are positive for ER and/or PR. This group of tumors is generally responsive to selective estrogen receptor modulators. The remaining 20–25% of breast cancers is ER and PR negative and is not amenable to selective ER modulators. This hormone receptor-negative group includes triple-negative tumors and a subset of tumors, which are positive for Her-2/neu [8].

Her-2/neu-positive tumors are now often treated with humanized monoclonal antibody to Her-2/neu protein called trastuzumab. Although, the therapy with trastuzumab is effective, it is not without serious cardiotoxicity in some patients and is very expensive. However, TNBC lack any specific targeted therapy at the current time. Standard treatment regimen for TNBC has not been established. The present treatment modality for TNBC is combined chemotherapy [9]. So the search for more predictive biomarkers for TNBC is a primary aim and goal of breast cancer research.

The androgen receptor (AR) is one such newly emerging biomarker [10]. AR has biological and therapeutic utilization in prostate cancer, but its use in breast cancer treatment is limited because of the widespread and effective use of anti-estrogen hormonal therapies [11,12].

In the recent years, it has been shown that androgen and AR also play an important role in the genesis and development of breast cancer [13,14]. Since AR belongs to the nuclear steroid hormone receptor family, it shows high structural, functional and topographic similarity to ER and PR [15]. However, AR has not been well characterized in terms of its role as a predictive or a prognostic factor and the clinical significance of its expression in breast cancer patients remains unknown and its effects in different subtypes of breast cancer are still unclear [16].

The aim of our study was to examine the prevalence of AR positivity in invasive breast cancer and to try elucidate its correlation with the clinicopathologic features and prognosis of both TNBC and non TNBC.

Materials and methods

Patient selection and clinicopathologic analysis

We reviewed retrospectively the data of 150 invasive breast carcinoma female patients who were brought to the Department of Pathology, Tanta University, Faculty of Medicine from November 2009 to March 2011. We excluded all the patients who had received preoperative chemotherapy or radiotherapy and those who were diagnosed as recurrent breast cancers at the time of operation. Data regarding patient demographics and histopathology of primary tumor were obtained by reviewing medical records including patient age at initial diagnosis and lymph node status and any distant metastasis. The mean age of the patients at time of diagnosis was $58.3 \pm 10.1$ years.

Routine histopathological study was done and tumor stage was classified on the basis of criteria of the Sixth American Joint Committee on Cancer. Histological type and grading followed the World Health Organization classification [17].

Immunohistochemical staining of AR, ER, PR and Her-2/neu

We evaluated AR, ER, PR and Her-2/neu expression in the specimens from formalin-fixed, paraffin-embedded whole sections of surgically resected breast cancer specimens using immunohistochemistry (IHC).

Primary antibodies for AR (monoclonal, clone AR441; Dako, Glostrup, Denmark, diluted1:50), ER (monoclonal, clone SP1; NeoMarkers for Lab Vision, Fremont, CA, diluted 1:50), PR (monoclonal, clone PR 636; Dako, diluted 1:100) and Her-2/neu (polyclonal; Dako, diluted 1:100) were used.

Briefly, 4-μm-thick sections of formalin-fixed, paraffin embedded tissues were deparaffinized and rehydrated. After treatment with 3% hydrogen peroxide solution for 10 min to block endogenous peroxidases, the sections were pretreated in citrate buffer (pH 6.0) for antigen retrieval in a microwave oven for 20 min. The aforementioned primary antibodies were incubated, and then, the sections were processed with EnVision Detection Systems (Dako) according to the manufacturer’s instructions and 3, 3’-diaminobenzidine tetrahydrochloride was used as a chromogen. The sections were counterstained with hematoxylin. Breast and prostatic cancer tissues were used as positive controls. Negative controls were done by doing the same steps without putting the primary antibody.

Interpretation of AR, ER, PR and Her-2/neu

The cells in five high power fields were counted. Tumors with $\geq 10\%$ nuclear-stained cells were considered positive for ER and PR expression.
Prevalence of androgen receptors in invasive breast carcinoma and its relation

Her-2/neu immunohistochemical staining was scored from 0 to 3+ according to the guideline indicated for Hercep Test (Dako) [18] using the following categories: 0, no immunostaining; 1, weak incomplete membranous staining in any proportion of tumor cells; 2, complete membranous staining, either non-uniform or weak in at least 10% of tumor cells; and 3 uniform intense membranous staining in >30% of tumor cells. Cases only with score 3 were regarded as positive.

The expression of AR in breast cancer tissues were assessed according to the percentage of positive cells and staining intensity: a percentage of positive cells of ≤10% scored 0, >10% ≤25% scored 1, >25% ≤50% scored 2, >50% ≤75% scored 3, >75% scored 4; weak expression (+) with slight yellow staining or only individual cells were stained in yellow to Brown yellow scored 1, strong expression (+++) with staining from brown yellow to brown scored 3, moderate expression (+ +) with staining intensity between weakly positive and strongly positive scored 2. Scores of staining intensity x score of percentage of positive cells was the integrated score: a score of ≥2 represented positive result, and ≤1 represented negative result [9].

Based on the previously described semiquantitative methods, the studied cases were classified into 3 groups included TNBC = group1, non TNBC with ER-positive and/or PR-positive and HER2-negative = group2, and the last group was HER2-positive breast cancers = group3. TNBC was defined as ER, PR and Her-2/neu negative by IHC.

Statistical analysis

The differences between the discrete variables were evaluated by chi-square test. Fisher’s exact test was used when appropriate. For the comparison p-test was used. A P value ≤0.05 was considered statistically significant. SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL) was used for all statistical analyses.

Results

The study included 150 cases; 102 of infiltrating duct carcinoma, 7 cases of tubular carcinoma, 7 cases of cribriform carcinoma, 7 cases of mucinous carcinoma, 15 cases of medullary carcinoma, 4 cases of papillary carcinoma and the remaining 4 cases were other rare types (2 cases of neuroendocrine carcinoma, 1 case juvenile secretory and one case metastatic sarcomatoid carcinoma) (Table 1).

Prevalence of AR expression and its relation with the studied clinicopathological factors

AR was expressed in the nuclei of 71% of breast cancer cases. AR was expressed in younger cases as 66% of AR positive cases were less than 50 years with statistical significance (p = 0.032). According to the histological type, 72% of AR positive cases were IDC-NOS (Figs. 1 and 3), but there was no statistical significance between the different types (p = 0.054). On the other hand, there was a strong statistical significance between AR positivity and the TNM staging as the expression was higher in stage I and II (p = 0.029). Also there was a significant difference between the histological grade as most of AR positive cases were either grade I or II (28%, 59%, respectively, p = 0.001). These data were summarized in Table 1.

Association between AR expression and the studied immunohistochemical markers

ER, PR and Her2/neu were evaluated in all the studied cases and the results were shown in Table 2. AR expression was studied in association with ER, PR and Her2/neu separately and revealed that AR positivity is correlated significantly with ER positivity (Figs. 2 and 3), (p = 0.002) but still there is 44 cases out of 52 ER negative cases expressing AR (Figs. 1 and 4). AR expression was not significantly correlated with the PR expression (p = 0.072), while there was a strong statistical significance between AR positivity and Her2/neu negativity (Figs. 1 and 2), (p = 0.0006). On the other hand there were 34 AR positive cases out of the 62 Her2/neu positive cases (Figs. 3 and 4). These data were summarized in Table 2.

Association between AR expression and the three studied groups

The studied cases were classified into 3 groups: TNBC group = group1 (number of cases = 48) (Fig. 1), Non TNBC (ER positive & Her2/neu negative) group = group2 (number of cases = 40) (Fig. 2) and Non TNBC (Her2/neu positive) = group3 (number of cases = 62) (Figs. 3 and 4). These three groups were correlated with AR expression and revealed that there was statistical difference between the groups as AR positivity was the highest in the group2 (ER positive, Her2/neu negative) then in the TNBC group (group1) and lastly in group3 (Her2/neu positive). Also 65% the AR negative cases were Her2/neu positive. These data were summarized in Table 3.

Discussion

Although ER, PR and Her2/neu promote the genesis and development of breast cancer, and are associated with its prognosis, it is important to identify and validate new biomarkers for better prediction and prognostication [19]. This study was carried out to analyze AR expression in breast carcinoma with respect to IHC-defined molecular classes and also its relationship to other well recognized clinical–pathological factors.

AR is a member of the steroid receptor subfamily. However, the role of AR in breast cancer is still uncertain. There is some evidence supporting a role for AR in the pathogenesis and outcome of breast cancer. Nahleh [20] and Ana et al. [11] studied the frequency of AR expression and found that AR is expressed in >70% of breast carcinomas and positive rates of AR are comparable with or higher than those of ER or PR. Moinfar et al. [10] found 60% of all invasive carcinomas and 46% of ER-negative invasive carcinomas to be AR positive. Both Gonzalez et al. [14] and Leo et al. [12] had been observed AR expression in more than 75% cases of breast carcinoma. All these figures are concordant with this study, where AR was expressed in 71% of invasive breast carcinoma.

It has been shown that AR is frequently expressed in some types of breast carcinoma, including IDC-NOS, and less expressed in other types such as mucinous carcinoma [16,21]. Our study showed that AR positivity was of higher in IDC-NOS. Also high positive rates of AR were shown in cribriform, tubular, and papillary types and negative rates in mucinous and medullary carcinoma. These results were somewhat limited by our small sample size.
Our results show that AR expression is associated with lower histological grade and early TNM stage. In many previous studies it has been documented that AR expression is related to good prognostic factors, including lower histological grade, smaller tumour size and negative nodal metastasis [8,9,15]. Even in Meijnen et al. [7] study on ductal carcinoma insitu, AR expression was most frequently seen in intermediately and well-differentiated grades.

Table 1  Correlation between AR expression and studied clinicopathological factors.

<table>
<thead>
<tr>
<th>Clinicopathological factor</th>
<th>AR positive n = 107 (%)</th>
<th>AR negative n = 43 (%)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50 (n = 78)</td>
<td>66 (62%)</td>
<td>12 (28%)</td>
<td>150</td>
<td>0.032</td>
</tr>
<tr>
<td>50 (n = 72)</td>
<td>41 (38%)</td>
<td>31 (72%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDC-NOS (n = 102)</td>
<td>78 (72%)</td>
<td>24 (56%)</td>
<td>150</td>
<td>0.054</td>
</tr>
<tr>
<td>Tubular (n = 9)</td>
<td>7 (7%)</td>
<td>2 (5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cribriform (n = 7)</td>
<td>6 (6%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous (n = 7)</td>
<td>3 (3%)</td>
<td>4 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary (n = 15)</td>
<td>8 (7%)</td>
<td>7 (16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary (n = 6)</td>
<td>4 (4%)</td>
<td>2 (5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others (n = 4)</td>
<td>1 (1%)</td>
<td>3 (7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (n = 57)</td>
<td>40 (37%)</td>
<td>17 (39%)</td>
<td>150</td>
<td>0.029</td>
</tr>
<tr>
<td>Stage II (n = 66)</td>
<td>58 (54%)</td>
<td>8 (19%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III (n = 20)</td>
<td>8 (8%)</td>
<td>12 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV (n = 7)</td>
<td>1 (1%)</td>
<td>6 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (n = 33)</td>
<td>30 (28%)</td>
<td>3 (7%)</td>
<td>150</td>
<td>0.001</td>
</tr>
<tr>
<td>Grade 2 (n = 87)</td>
<td>63 (59%)</td>
<td>24 (56%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3 (n = 30)</td>
<td>14 (13%)</td>
<td>16 (37%)</td>
<td></td>
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</tr>
</tbody>
</table>

AR: androgen receptor, IDC-NOS: infiltrating duct carcinoma-not otherwise specified.

Figure 1  A case of IDC-NOS, TNBC, showing AR positive staining (streptavidin–biotin-DAB, 200x). AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; IDC-NOS, infiltrating duct carcinoma non otherwise specified; TNBC, triple negative breast cancer.

Table 2  Correlation between AR expression and studied immunohistochemical markers.

<table>
<thead>
<tr>
<th>Immunohistochemical marker</th>
<th>AR positive n = 107</th>
<th>AR negative n = 43</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n = 98)</td>
<td>63 (59%)</td>
<td>35 (81%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Negative (n = 52)</td>
<td>44 (41%)</td>
<td>8 (19%)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n = 57)</td>
<td>34 (32%)</td>
<td>23 (53%)</td>
<td>0.072</td>
</tr>
<tr>
<td>Negative (93)</td>
<td>73 (68%)</td>
<td>20 (47%)</td>
<td></td>
</tr>
<tr>
<td>Her2/neu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (62)</td>
<td>34 (32%)</td>
<td>28 (65%)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Negative (88)</td>
<td>73 (68%)</td>
<td>15 (35%)</td>
<td></td>
</tr>
</tbody>
</table>

AR: androgen receptor, ER: estrogen receptor, PR: progesterone receptor.

In the current study AR expression was also associated with high ER, PR and low Her2/neu expression (good prognostic factors) and this was in accordance with Ana et al. [11] and Park et al. [16]. Also Leo et al. [12] demonstrated that androgen signaling may also activate estrogen-responsive genes.

However, in this study, a significant percentage of tumors with poor prognostic histological factors, were positive for AR and negative for ER and/or PR. This finding which was
also found by Seung et al. [8] and Ana et al. [11] who found that among poorly differentiated invasive carcinomas, 39% were ER and PR negative but AR positive. These data may represent the independent expression of AR in human breast cancer.

Table 3  Correlation between AR expression and the three studied groups.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>AR positive</th>
<th>AR negative</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: TNBC</td>
<td>36 (34%)</td>
<td>12 (28%)</td>
<td>48</td>
<td>0.043</td>
</tr>
<tr>
<td>Group 2: Non TNBC (ER+ve &amp; Her2/neu−ve)</td>
<td>37 (35%)</td>
<td>3 (7%)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Group 3: Non TNBC (Her2/neu+ve)</td>
<td>34 (31%)</td>
<td>28 (65%)</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>43</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

TNBC: triple negative breast cancer, AR: androgen receptor, ER: estrogen receptor, PR: progesterone receptor.

Figure 2  A case of cribriform carcinoma, non TNBC with ER+, PR+ and Her2/neu− (streptavidin–biotin-DAB, 200×) showing AR positive staining (streptavidin–biotin-DAB, 400×). AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; IDC-NOS, infiltrating duct carcinoma non otherwise specified; TNBC, triple negative breast cancer.

Figure 3  A case of IDC-NOS with ER+, PR+ and Her2/neu+ (streptavidin–biotin-DAB, 200×) showing AR positive staining (streptavidin–biotin-DAB, 400×). AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; IDC-NOS, infiltrating duct carcinoma non otherwise specified; TNBC, triple negative breast cancer.

Figure 4  A case of medullary carcinoma, ER−, PR+, Her2/neu+ showing AR positive staining (streptavidin–biotin-DAB, 200×). AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; IDC-NOS, infiltrating duct carcinoma non otherwise specified; TNBC, triple negative breast cancer.
cancer and also indicate that the presence of AR expression in ER-negative tumors has a particular prognostic significance.

Our study classified the breast cancer cases into three groups to focus on TNBC because TNBC has poor prognosis as compared with non TNBC, and TNBC cannot benefit from antiestrogen therapy and HER2-targeted therapy. In those cases, chemotherapy is the main adjuvant treatment. New biomarkers and additional effective treatment guidelines are necessary to improve prognosis in these groups.

Carey et al. [22] found that 26% of their studied cases were TNBC. These TNBC were mainly of high histological grade, showed high mitotic index, and were found more frequently in premenopausal women. TNBC was found in 16.3% of Emad et al. [23] cases. The most common histological types were ductal carcinomas of no specific type, metaplastic and salivary gland-like carcinomas; the majority of these tumors were grade 3. There were positive associations with larger size, pushing margins, poorer Nottingham Prognostic Index, development of recurrence and distant metastasis, and poorer outcome in terms of overall survival and disease-free interval. The current study showed the same results in the 48 TNBC (32%) cases, as these cases were of high grade, and mainly stage 3 and 4.

It has been indicated that AR is expressed in large proportion of TNBC and that it might have a role as a prognostic marker and a therapeutic target in this subgroup [24]. In our study, 36 cases out of 48 cases of TNBC showed AR positivity. Seung et al. [8] and Xiang et al. [9] found that in ER-negative tumors, AR-positive patients exhibited significantly better disease-free survival than AR-negative tumors especially in TNBC patients. Also Rakha et al. [1] demonstrated that in TNBC, especially lymph node-positive, as expression of AR was lost, there was an increased incidence of high nuclear grade, development of recurrence and distant metastasis. Therefore, AR could play a role as a prognostic factor in this poor group. Xiang et al. [9] study showed that TNBC could be divided into good prognosis subtype and poor prognosis subtype according to AR status.

AR positivity was significantly correlated with loss of Her2/neu expression, in these cases when ER was positive and Her2/neu is negative, AR positivity was very high (37 out of 40 cases) while in cases with positive Her2/neu which therapy is mainly though herceptin, which is very expensive, AR was also expressed in 34 out of 62 cases and this may be used as a good additional target therapy for these group of patients.

Most ER negative tumors (i.e., TNBC and non TNBC) are considered as aggressive tumors, but it appears that AR reactivity can be used to prognosticate these tumors [12]. One important implication of this study is the use of AR-related targeted therapy for breast cancer, especially for ER negative/AR positive tumors. Preclinical studies have shown inhibitory roles of androgens like Dehydroepiandrosterone and its sulfate on ER negative/AR positive cells lines. Similar approaches can be used for ER negative/AR positive human breast cancers as an adjunctive therapy [12].

In conclusion, AR is expressed in a significant number of most types of breast cancers. AR is also associated with lower tumor burdens and favorable differentiation. In addition, AR is expressed in a significant number of TNBC, and also in Her2/neu positive cancers which indicates that AR could be a new target for the treatment of these groups. Also AR expression in ER-negative tumors can be exploited for an additional targeted therapy.

**Conflict of interest**

The author declares that they have no conflict of interest.

**References**


