Immunohistochemical Expression of Androgen Receptor (AR) and its Relationship with Clinicpathological Parameters in Breast Cancer

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Abstract

Breast carcinoma is one of the most common malignant and the leading cause of cancer related death among women all around the world. Gene expression profiling characterized 4 major groups of BC, which classified patients into Luminal A, Luminal B, HER-2/neu enriched, and Triple Negative BC (TNBC) . Among the members of the steroid receptor superfamily the role of estrogen and progesterone receptors (ER and PR) and HER2 are play a role in breast cancer as both predictive and prognostic markers and management of therapy. Androgen Receptor (AR) is a steroid nuclear receptor involved in complex signaling pathways that are thought to play a role in cell proliferation. Greater than70% of human breast cancers expresses the androgen receptor (AR) and varies significantly among molecular subtypes of breast cancer and its contribution to the progression of disease may differ depending on the stage.

In this study, we aimed to examine the expression pattern of AR and its association with clinicopathological parameters and IHC markers.

Methods: Immunohistochemistry (IHC) was performed on breast cancers using antibodies against androgen receptor (AR), esterogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type-2 (HER2). The levels of expression were scored semiquantitatively.

Results: AR positivity was noted in Thirty-four of the cases, whereas the other thirteen cases were negative. AR was significantly related to tumors histological type (p= 0.002), tumor grade (p= 0.002) and positive PR/HER2 status (p= 0.04). No statistical difference was demonstrated in AR expression in relation to tumor size (p= 0.12), lymph node status (p= 0.68) and expression of ER (p= 0.23).

Conclusion: breast carcinomas express AR positivity more than ER, PR and HER2 status. In addition, the expression of AR correlated to lower tumor grade which could serve as a good prognostic factor and potential therapeutic target. However, this finding will need to be confirmed by large cohort studies.

Keywords: Breast cancer, Androgen receptor, Estrogen receptor, Progesterone receptor, Immunohistochemistry.

تعبير المناعة النسيجية الكيميائية لمستقبلات الاندروجين وعلاقته بالمعلمات الاكلينيكية في سرطان الثدي

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الخلاصة

يعد سرطان الثدي السرطان الاكثر شيوعا الذي يصيب النساء في مختلف انحاء العالم. وقد أجريت الدراسة على47 عينة من نساء مصابات بسرطان الثدي وكانت عبارة عن أنسجة مثبتة بالفورمالين ومطمورة بالبرافين على هيئة قوالب . استخدم طريقة كيمياء النسيج المناعية لتقييم تعبير المستقبلات لكل من HER-2 و الاستروجين و البروجيستيرون وكذلك Androgenلإيجاد العلاقة بين نتائج كيمياء المناعة النسيجية ومؤشرات التقدم لسرطان الثدي المتمثلة بعمر المريض ، حجم الورم ،النوع النسيجي وكذلك درجتة. حيث اظهرت النتائج بان 43 حالة من 47 اعطت التعبير الهرموني الموجب للمستقبل Androgen. كم أظهرت النتائج وجود فروقات معنوية مرتبطة بين مستوى التعبير للمستقبل Androgen مع نوع ودرجة الورم وكذلك كلا من البروجستيرون و HER-2 وبالمقابل فان تعبير Androgen الموجب يعد بمثابة نذير جيد وكهدف علاجي محتمل.

الكلمات المفتاحية: سرطان الثدي, مستقبل الاندروجين, مستقبل الاستروجين, مستقبل البروجستيرون, المناعة النسيجية الكيميائية.

Introduction

Breast cancer is a highly heterogeneous disease characterized by various predisposing risk factors, clinicopathologic features and molecular characteristics [1]; it is the most frequent malignancy among women worldwide. Its rate rising rapidly in Asian women and the developing world and it is recognized to be the second most common cause of death in women [2]. According to the Ministry of Health / Iraqi cancer registry 2014, breast cancer was the most common malignancy affecting women and leading cause of death after cardiovascular diseases [3,4]. The etiology of breast cancer involves a combination of genetic, environmental and hormonal factors that collectively alter normal breast cancer tissue. Breast cancer is a heterogeneous disease and shows various subtypes such as Luminal A (ER and/or PR +, Her-2/neu−), Luminal B (ER and/or PR+, HER2+), basal-like (ER, PR−, Her2−, CK5/6+, and/or Her2+), normal-like, and unclassified negative for all the markers [5]. On gene expression profiling and immunohistochemistry, breast cancer is divided into five subtypes: luminal A (ER and/or PR+, HER2-), luminal B (ER and/or PR+, HER2+), basal cell-like (BCL) or 'triple-negative' phenotype (ER, PR -, HER2- with expression of basal/myoepithelial markers), HER-2 positive tumors (ER-, PR-, HER2+), and normal breast like [6]. This helped stratification of BC patients for prognostic and therapeutic purposes [7]. Management of breast carcinoma involves assessment of prognostic factors such as age, tumor size, histological grade, axillary lymph node status, and identified biomarkers such as ER, PR and human epidermal growth factor receptor 2 (HER2) are well-documented prognostic factors for breast cancer. The expression of estrogen

receptor (ER), progesterone receptor (PR), and the epidermal growth factor receptor 2 (HER-2) in breast cancer has been extensively studied targeted therapy against these receptors is now validated modality of treatment in conjunction with surgery, radiotherapy, and chemotherapy [8]. In the recent years, it has been showed that androgen and AR also play an important role in the genesis and development of breast cancer [9]. It's more widely expressed in breast cancer than ER or PR, with more than70% invasive cells expressing AR [10]. Another receptor of the nuclear steroid hormone family is the androgen receptor (AR). It belongs to steroid nuclear receptor family involved in complex signaling network that plays role in cell proliferation. It was found to be associated with good prognosis and to be related to ER and PR expression in BC [11,12]. Recently, the androgen receptor (AR) has been suggested to play a key role in breast cancer biology in certain disease subgroups [13,14]. AR gene is found on chromosome Xq11–12. The AR is bound to heat shock proteins that stabilize it to expose the C-terminal ligand-binding domain. Circulating androgens bind to this C-terminal ligand binding domain then leads to AR dimerization and the requisite phosphorylation of its tyrosine kinase, which in turn causes translocation of the AR complex to the nucleus. The DNA-binding domain binds to androgen response elements in both the promoter and enhancer regions of target genes and creates an active transcription complex upon recruitment of coregulatory proteins [15]. Androgen receptor (AR) is expressed in 70-80% of BC cells and is more frequently expressed in ER-positive than in ER-negative tumors and metastatic breast tumors, respectively [16,17].

The effect of AR expression on prognosis also varies according to the breast cancer subtype. Previous studies have suggested that AR may be both a prognostic factor for survival and a predictive factor for response to endocrine treatment in patients with breast cancer [18]. Therefore, the aim of this study was to examine the relationship between androgen receptor and clinicopathological characteristics.

Material and methods

This observational study was conducted on 47 archival cases of breast cancer patients in the Medical city, Baghdad Teaching Hospital and Al-Kadhimya Teaching Hospital between November 2017 to June 2018. All patients underwent lumpectomy/mastectomy with or without axillary dissection.

The clinical parameters in this study included age, tumor histological type were classified according WHO, the histological grade was determined for each case according to the Bloom and Richardson Grading System, tumor size (<2, 2-4, ≥5 cm), lymph node status (positive or negative), ER, PR, HER2 and AR expression status.

Through semi quantitative immunohistochemistry (IHC) staining depending on the staining intensity and the percentages of the positively stained tumor cells of ER, PR, HER2 and AR expressions in formalin-fixed and paraffin-embedded breast cancer tissues. Immunostaining was performed to study the correlations between AR, ER, PR, HER2/neu expressions in human breast cancer. Formalin-fixed paraffin-embedded tissue sections (5 lm) were deparaffinized in xylene and rehydrated in a graded series of ethanol. The slides were treated with methanol containing 0.3% hydrogen peroxide to block any endogenous peroxidase activity. Heat-mediated antigen retrieval with the pressure cooker method was used for all staining. Specific antibodies were used for immunohistochemical studies on serial tissue sections. Primary antibodies used in this study included ER (Dako, clone ID-5,1/50 dilution), PR (Dako, clone PgR; 1/300 dilution), HER2 (clone PN2A, 1/200 dilution), AR (Dako, clone R441,1/100 dilution), all utilizing Dako kits ™ (Dako, Denmark). The final product of the reaction was visualized with 3,3'-diaminobenzidine (DAB) and the nuclei were stained with hematoxylin. Staining results were assessed independently by two pathologists. AR staining was classified using the H-score10, which ranges from 0 to 300 and is calculated according to the following formula: (1 × percentage of cells staining weakly positive) + (2 × percentage of cells staining moderately positive) + (3 × percentage of cells staining strongly positive). H-score ≤150 was considered weak AR expression and H-score >150 were considered strong AR expression. ER and PR were considered positive if ≥1% of tumor cells stained positive, HER2 staining was assessed using a semiquantitative score ranging from 0 to 3+, where 0 or 1+ indicate a negative, 2+ indicates an indeterminate, and 3+ indicates a positive HER2 test result. The data were analyzed using the SPSS software (version 16.0) for windows. Quantitative and qualitative variables are presented as mean±SD and number (%), respectively. Independent sample *t*-test and Chi- square test were used for comparisons. P <0.05 was considered significant in all analyses.

Statistical analysis

The data were analyzed using the SPSS software (version 18.0). Quantitative and qualitative variables are presented as mean ± SD and number (%), respectively. Independent sample t-test and Chi-square test were used for comparisons. P < 0.05 was considered significant in all analyses.

Results

The clinicopathological characteristics of the patients are shown in Table (I). All patients were women. This study was performed on 47 paraffin tissue blocks of patients with breast cancer. The mean age of patients was 50.47±11.92 with range of 23 to 80 years. The majority of tumors were ductal carcinomas 41 (87.2%), with a few mucinous carcinomas 1 (2.1%). Twenty-three percent of tumors (48.9%) were high stage (III). Twenty-four (51.1%) tumor size less and equal 2 cm, 23 (48.9%) were of higher grade III and 26 (55.27%) cases showed positive lymph node status. According to the histological type, 61.7% of AR positive cases were IDC, but there was no statistical significance between the different types (p =0.741). On the other hand, there was a strong statistical significance between AR positivity and the TNM staging as the expression was higher in stage III (p =0.002). Also there was a significant difference between the histological grade as most of AR positive cases were grade III (40.4% p =0.002), these data were summarized in Table I1.

Table 1: Clinicopathology and immunohistochemistry of breast cancers.

Characteristics	**Mean±SD or n (%)**
Age (Year)	50.47±11.93
Histological type	
Ductal	41 (87.2%)
Lobular	3 (6.4%)
Medullary	2 (4.3%)
Mucinous	1 (2.1%)
Lymph node involvement	
Positive	26 (55.27%)
Negative	21 (44.73%)
Tumor size cm	
≤ 2	24 (51.1%)
> 2	23 (48.9%)
TNM stage	
I	6 (12.8%)
II	9 (19.1%)
III	23 (48.9%)
IV	9 (19.1%)
Histological grade	
Grade I	6 (12.8%)
Grade II	18 (38.3%)
Grade III	23 (48.9%)

ER status	
Positive	21 (44.7%)
Negative	26 (55.3%)
PR status	
Positive	18 (38.3%)
Negative	29 (61.7%)
Her2 status	
Positive	14 (29.8%)
Negative	33 (70.2%)
AR status	
Positive	34 (72.3%)
Negative	13 (27.7%)

Table 2: Relationship between androgen receptor (AR) expression in breast cancer and clinicopathological variables.

Characteristics	AR (-ve) n=13 (%)	AR (+ve) n=34 (%)	P value
Age (Year)	47.62±9.014	51.56±12.816	0.316
Histological type			
Ductal	12 (25.5%)	29 (61.7%)	0.741
Lobular	1 (2.1%)	2 (4.3%)	
Medullary	0 (0%)	2 (4.3%)	
Mucinous	0 (0%)	1 (2.1%)	
Lymph node involvement			
Positive	9 (19.2%)	17 (36.2%)	0.683
Negative	4 (8.5%)	17 (36.2%)	
Tumor size cm			
≤ 2	6 (12.8%)	8 (17 %)	0.127
> 2	7 (14.9%)	26 (55.4%)	
TNM stage			
I	0 (0%)	6 (12.8%)	0.002
II	2 (4.3%)	7 (14.9%)	

III	4 (8.5%)	19 (40.4%)	
IV	7 (14.9%)	2 (4.3%)	
Histological grade			
Grade I	0 (0%)	6 (12.8%)	0.002
Grade II	9 (18.9%)	9 (19.2%)	
Grade III	4 (8.5%)	19 (40.4%)	
ER status			
Positive	4 (8.5%)	17 (36.2%)	0.236
Negative	9 (19.1%)	17 (36.2%)	
PR status			
Positive	2 (4.3%)	16 (34.0%)	0.046
Negative	11 (23.4%)	18 (38.3%)	
Her2 status			
Positive	1 (2.1%)	13 (27.7%)	0.041
Negative	12 (25.5%)	21 (44.7%)	

Estrogen receptor (ER), Progesterone receptor (PR), and Human epidermal growth factor receptor type-2 (HER2) were expressed in 21 (44.7%), 18(38.3%), and 14 (29.8%) of cases, respectively. AR was expressed in 34 (72.3%) of cases. The relationship between AR expression and clinicopathological features is presented in Table II. Significant relationship were observed between PR, Hre2 expression and AR expression (P=0.04). Furthermore, there was a significant relationship between histological stage, grade and AR expression (P=0.002). There was no significant relationship between AR expression and histological type, tumor size, lymph node status, ER status ($P > 0.05$).

Discussion

Breast cancer is the most common malignancy worldwide among women in developing countries [19]. It is subclassified into distinct molecular subtypes as into luminal A, luminal B, Her2 (human epidermal growth factor receptor 2) like and basal-like or triple negative breast cancer [20]. The androgen receptor (AR) is a member of the steroid hormone nuclear receptor, which is frequently expressed in breast cancer [21]. Several studies have reported that androgen receptor positivity was identified in breast cancer patients who were estrogen receptor and progesterone-negative, and is expressed in more than 70% of primary breast cancer. Therefore, it is thought that the androgen receptor has an important role in breast cancer carcinogenesis [22,23].

In the current study AR expression in more than 70% cases of breast carcinoma, and this was in accordance with [22,24-26] studied found that AR is expressed in >70% of breast carcinomas and positive rates of AR are comparable higher than those of ER or PR expression. The AR is frequently expressed in many types of breast carcinoma, including both invasive and in situ ductal carcinomas. Its expression is correlated with a better prognosis for breast carcinoma. Our study included different types of breast carcinomas and the invasive ductal carcinoma (87.2%) represented the most frequent histological type, therefore, we showed that AR positivity was higher in invasive ductal carcinoma (61.7%). This result was agreement with several studies by [27-30]. Estrogen receptors and progesterone receptors are the most commonly studied hormone receptors in breast cancer and these hormonal receptors are important to determine the best medical treatment. We found no significant association between AR and ER expression in invasive breast carcinoma; nevertheless, there is a positive association between AR and PR expression. These finding in agreement with [31] found no significant association between AR and ER expression in invasive breast carcinoma; however, a strong positive association was found in their study between AR and PR expression. In contrast to other studies by [32-34] they find that AR expression was significantly associated with estrogen receptor and progesterone receptor expression. Other study by [35-37] found no significant differences between AR expression and the clinicopathological characteristics of the tumors, such as ER or PR status. On the other hand, there was a significant interaction between AR expression and HER2 in our study. This finding disagreement with the results of [38-41], they did not find any significant correlation between AR expression and HER2 overexpression. The difference in the number and nature of cases studied, in addition to technical differences may explain the disagreement between our study and those of others.

We found no significant relationship between AR expression and the clinicopathological characteristics of the tumors such as axillary lymph node involvement, tumor size and tumor histological type. These findings are supported by [9,39,42,43]. Nevertheless, we found a positive and significant relationship between AR expression and histological grade and stage of tumors. Many studies have found that AR expression correlates with tumor grade [28,44-46]. Lower grade tumors tend to have better prognosis, and hence, the expression of AR can serve as a good prognostic indicator.

Our study suffers from some limitations including the rather small sample

Conclusion

In conclusion, AR expression is significant relationship with PR and Her2 status. In addition, we found that AR expression is associated with tumor lower grade and stage. More studies among Iraqi women with breast cancer patients are needed to assess the actual rate of AR expression as aprognostic factor.

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