Original Article

Androgen receptor expression and its relationship with clinicopathological parameters in an Iranian population with invasive breast carcinoma

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Abstract

Background: Androgen receptor (AR) status and its association with prognosis in Iranian breast cancer population are uncertain. We examined AR expression and its relationship with clinicopathological parameters among Iranian patients with invasive breast carcinoma.

Materials and Methods: This study was performed on formalin fixed and paraffin embedded tissue specimens with a diagnosis of invasive breast carcinoma archived at two University Hospitals in Isfahan city, Iran. Antibodies were used for evaluation of AR, human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR). Other data were gathered from patients' documents.

Results: A total of 70 cases were evaluated including 55 (78.6%) ductal, 9 (12.9%) lobular, 2 (2.9%) medullary, and 4 (5.7%) mucinous carcinomas. Overall, 48.6%, 42.9%, 64.3%, and 57.1% of the samples were positive for ER, PR, AR, and HER2, respectively. Thirty three (47.1%) cases were ER^- PR^- and 17.1% were triple negative. AR + cases were younger and more frequently positive for ER and showed less frequently tumor size of > 2 cm. Although tumor grade and stage were relatively higher among AR^- cases compared to AR^+ ones, the difference between the two groups was not statistically significant.

Conclusions: AR expression was found to be frequently present in breast carcinoma in the studied population. Since half of the ER negative and half of the triple negative tumors were found to be AR positive, AR positive cases may benefit from alternative endocrine therapeutic strategies other than the conventional endocrine-targeted medications.

Key Words: Androgen receptor, breast cancer, estrogen receptor, progesterone receptor, targeted cancer therapy

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Received: 12.03.2013, **Accepted:** 04.05.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.133260

INTRODUCTION

Breast carcinoma is one of the most common malignant tumors and the leading cause of cancer mortality among women.[1] In Iran, breast cancer is the most common type of cancer among women comprising 24.4% of all malignancies.[2] Optimizing breast cancer therapies to increase cure rates in early

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How to cite this article: Mohammadizadeh F, Sajadieh S, Sajjadieh H, Kasaei Z. Androgen receptor expression and its relationship with clinicopathological parameters in an Iranian population with invasive breast carcinoma. Adv Biomed Res 2014;3:132.

stages is a critical need and crucial area of research in medical oncology. Currently, the studies on treatment strategies for different kinds of cancers are focusing on targeted therapies as specific annihilator for the tumoral cells. These treatment strategies do not target other tissues in the body and thus, in contrast to other anti-cancer treatments, other organs are not adversely affected. In this regard, immunotherapy is being used as one of the known targeted therapies for breast cancer, in which monoclonal antibodies against the hormonal receptors expressed by the breast cancer cells are being applied.[3]

Estrogen and progesterone receptors (ER and PR), which express in from 50% to 80% of breast malignancies are well-demonstrated as targets for monoclonal antibodies.[4] Androgens also play a role in normal breast physiology and therefore androgen receptor (AR) signaling is becoming increasingly recognized as an important contributor toward breast carcinogenesis.[5] Considering the high frequency of AR expression in breast cancer, targeted cancer therapies have been focused on AR as a target for cancer treatment.[6] On the other hand, common histopathological factors such as 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,[7,8] However, there is not enough evidence to demonstrate the prognostic value of AR especially in Iranian population. Consequently, more studies are required to validate AR as a new target in breast cancer and to determine the prognostic and therapeutic value of this receptor. The aim of the present study was to investigate the prevalence of AR expression in invasive breast carcinoma by immunohistochemistry and to determine its relationship with well-documented clinicopathologic prognostic determinants in an Iranian population.

MATERIALS AND METHODS

This observational study was conducted on archived formalin fixed and paraffin embedded tissue specimens in the laboratories of Alzahra and Beheshti University Hospitals in Isfahan (Iran) with a diagnosis of invasive breast carcinoma between 2006 and 2010. The cases of invasive ductal carcinoma were graded according to Bloom and Richardson grading system.[9] The paraffin embedded tissue blocks were sectioned in 4 μm cuts. The standard avidin biotin peroxidase complex method was used and heat-induced antigen retrieval using the microwave method was applied for all immunohistochemical staining to evaluate ER, PR, HER2, and AR expression in tissue samples.

The process of immunohistochemistry was applied as follows: (1) placing the sections in 37°C oven for 48 h, (2) rinsing in 100% xylol, graded ethanol (100%, 85%, and 75%), and distilled water, (3) rinsing in 10% phosphate buffered saline (PBS) solution, (4) exposure to 10% H_2O_2 and methanol at a ratio of 1:9 for 30 min, (5) rinsing in PBS, (6) placing in citrate buffered solution (pH = 6.1) for 14 min at a microwave with power 800, (7) rinsing in 10% PBS, (8) adding blocking serum to the slides for 30 min and then drying, (9) separately adding the specific antibodies including HER2 (Dako, clone PN2A, 1/200 dilution), AR antibody (Dako, clone R441,1/100 dilution), ER antibody (Dako, clone ID-5,1/50 dilution) and PR antibody (Dako, clone PgR; 1/300 dilution) for 30 min at room temperature, (10) rinsing in PBS, (11) adding broad spectrum antibody for 30 min, (12) adding HRP-streptavidin for 30 min, (13) addition of diaminobenzidine for 10 min, (14) rinsing in 10% PBS, (15) dehydration in distilled water, graded alcohols (75%, 85%, 100%), and xylol, (16) counterstaining with hematoxylin, five dips, and (17) mounting. The slides were then evaluated by light microscopy.

Negative controls omitting the primary antibodies were included with each run of staining. Normal breast tissue in the samples served as an internal control for ER and PR. Normal prostate tissue was used as the control for AR. Nuclear staining in 1% or more of the tumor cell nuclei were considered as positive for ER and PR. For HER2, 2 + and 3 + membrane staining of the tumor cells were considered as positive.[10] The quick score method[10] was used for semi-quantitation of AR status as follow:

- Intensity of staining; slides were assessed for the average degree of nuclear staining at low power (×10) and the following scores allocated: Negative (0) weak (1), moderate (2), or strong (3)
- The percentage of cells with positive nuclei was counted at high power (×40) and the following scores were allocated: <25% = 1, 25-50% = 2, 50-75% = 3, and >75% = 4. The scores from 1 to 2 were added together to give a final score ranging from 0 to 7. The final score was designated as negative or positive as follow: score of 0-3 as negative and score of 4-7 as positive.[10]

Other data including age at the time of diagnosis, tumor size (greatest tumor dimension), and the status of axillary lymph node were obtained from the records available in the patient's documents.

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. A $P < 0.05$ was considered significant in all analyses.

RESULTS

In this study, 70 paraffin blocks of breast carcinoma were evaluated. Cancer characteristics are presented in Table 1. Overall, 48.6%, 42.9%, 64.3%, and 57.1% of the samples were positive for ER, PR, AR, and HER2, respectively. Thirty three (47.1%) cases were ER^- PR^- and 17.1% were triple negative (ER^-, PR^-, and $HER2^-$). Among triple negative cases, half (6/12) were AR^+.

Comparison between AR^+ and AR^- cases are demonstrated in Table 2. AR^+ cases were about 7 years younger and were more frequently positive for ER. The difference between the expression of either markers of PR and HER2 was not statistically significant between AR^+ and AR^- groups. Mean of tumor size was not significantly different between AR^+ and AR^- cases, but tumor size of >2 cm was more frequent among AR^- cases. Although tumor grade was relatively higher among AR^- cases compared to AR + cases, the difference between the two groups was not statistically significant. A statistically significant difference was not observed in nodal status between the AR^+ and AR^- cases.

There was a significant inverse relationship between age and AR score ($r = -260$, $P = 0.03$), the score decreases with increase of the age. Furthermore, there was a significant inverse relationship between tumor size and AR score ($r = -0.41$, $P < 0.001$), the score decreases with increase of the tumor size. There was no statistically significant relationship between the AR score and the number of involved lymph nodes ($r = 0.01$, $P = 0.92$), but a strong direct relationship between the tumor size and number of involved lymph nodes was found ($r = 0.41$, $P < 0.001$).

DISCUSSION

In cancer research, development of targeted therapies has always been an ideal goal. The specific targets in breast cancer, which have been successfully used include HER2 and ER.[6] Previous evidence have shown that androgens can directly affect the growth of breast cancer cells,[5,11] In this regard, some studies were conducted to establish the levels of different kinds of androgens in breast cancer patients and found that high level of androgen can be associated with different types of breast carcinoma,[12,13] In this study, we demonstrated that AR expression is present in about 65% of all the invasive breast carcinoma variants. Our data showed that AR negativity is associated with older age at the time of diagnosis. We found that half of the ER negative tumors are AR positive, which can suggest an endocrine therapeutic strategy for patients who do not benefit from anti-estrogen based medications. As the reports have indicated before,

Table 1: Cancer characteristics

Demographic and disease data	Mean±SD or number (%)
Age, year	50.94±1.27
Carcinoma type	
Ductal	55 (78.6)
Lobular	9 (12.9)
Medullary	2 (2.9)
Mucinous	4 (5.7)
Tumor size, cm	3.95±2.01
Tumor grade in invasive ductal carcinomas	
I	6 (10.9)
II	24 (43.6)
III	25 (45.5)
Nodal involvement	
None	30 (42.9)
1-3	15 (21.4)
4-9	16 (22.9)
≥10	9 (12.9)
ER^+	34 (48.6)
PR^+	30 (42.9)
AR^+	45 (64.3)
$HER2^+$	40 (57.1)

ER: Estrogen receptor, PR: Progesterone receptor, AR: Androgen receptor, HER2: Human epidermal growth factor receptor 2

Table 2: Comparison between AR^- and AR^+ samples

Characteristic	AR^- *n*=25 (%)	AR^+ *n*=45 (%)	*P* value
Age, years	55.4±13.7	48.4±11.5	0.026*
Carcinoma type			
Ductal, *n*=55	22 (40)	33 (60)	0.026**
Lobular, *n*=9	0	9 (100)	
Medullary, *n*=2	2 (100)	0	
Mucinous, *n*=4	1 (25)	3 (75)	
ER±	8 (32)/17 (68)	26 (57.7)/19 (42.2)	0.034**
PR±	8 (32)/17 (68)	22 (48.8)/23 (51.1)	0.132**
HER2±	13 (52)/12 (48)	27 (60)/18 (40)	0.345**
Nodal involvement			
None	10 (40)	20 (44.4)	0.708***
1-3	8 (32)	7 (15.5)	
4-9	5 (20)	11 (24.4)	
≥10	2 (8)	7 (15.5)	
Tumor size, cm	4.3±1.8	3.7±2.0	0.253*
Tumor size, ≤2/>2 cm	2 (8)/23 (92)	13 (28.8)/32 (71.1)	0.050**
Histological grade			
I	1 (4.5)	5 (15.1)	0.192**
II	8 (36.3)	16 (48.4)	0.073***
III	13 (59)	12 (36.3)	

Data are presented as mean±SD or number (%); ER: Estrogen receptor, PR: Progesterone receptor, AR: Androgen receptor, HER2: Human epidermal growth factor receptor 2, *Independent *t* test, **Chi-square test, ***Mann-Whitney test

treatment of ER negative tumors is more complicated, hence target therapies against AR seems to be effective in these patients.[6] Furthermore, more than a quarter of our samples were triple negative (ER^-, PR^-, and $HER2^-$). Triple negative variants of breast carcinoma generally have more aggressive clinical course and validated targeted therapies for them are currently unavailable.[14] We found that half of these tumors were AR positive. Previous studies have also shown that up to 53% of triple negative tumors are AR positive with variable results among different populations.[15] These results provide evidence that AR is an important target for cancer therapy.[16]

In the study by Gonzalez *et al*. on 250 blocks of invasive breast carcinoma consisting of 212 ductal and 38 lobular neoplasms, AR expression was observed in about 60% of the cases. The same as our result, AR expression was associated with ER expression. Their results also showed lower proliferative index and low or intermediate histological grade in AR positive tumors. Moreover, patients with AR positive breast carcinoma have been reported to have a significant longer overall survival compared to those with AR negative tumors.[17] Furthermore, a study on the prognostic value of AR expression in ER negative carcinomas showed that AR expression was associated with increased age, tumor grade, tumor size, and HER-2/ν overexpression. In addition, patients with AR positive tumors had better disease free survival.[18] Among a cohort on 215 invasive ductal breast carcinomas, AR and ER were expressed together in the majority (80-90%) of ductal breast carcinomas. Their analysis showed that AR is an independent prognostic factor in ER positive group and increases the risk of cancer related death in ductal subtype.[19] In a study among 1467 breast cancer patients, 78.7% were AR positive and AR positivity significantly improved the prognosis of the patients and decreased overall mortality. However, there was a non-significant association between AR status and breast cancer death among women with ER negative tumors, which indicates that the association of AR status and breast cancer survival may depend on ER status.[20] The pathophysiological studies on the role of androgen in breast cancer have suggested that androgens can prevent the tumoral cell growth.[6] According to a recent *in vivo* study, AR can prevent the activation of target genes that mediate the stimulatory effects of 17 beta estradiol on breast cancer cells. Thus, AR expression seems to be a positive prognostic factor in breast carcinoma.[19]

Our study suffers from some limitations including the rather small sample size and lack of availability of data concerning the survival of patients. Studies with larger sample size and adequate follow-up are required to validate the significance of prognostic role of AR expression in breast carcinoma.

CONCLUSIONS

Our study shows that AR expression is frequent in breast carcinoma. In addition, we found that AR expression is associated with smaller tumor size and younger age at the time of diagnosis. Moreover, half of the ER negative and half of the triple negative tumors were AR positive, which can suggest an endocrine therapeutic strategy for patients who do not benefit from other conventional endocrine-targeted medications.

ACKNOWLEDGMENT

This study was supported by the Isfahan University of Medical Sciences (Isfahan, Iran). Authors are thankful to Mr. Nasr and Mrs. Zeidi, the IHC technologists of the pathology laboratory of Alzahra Hospital (Isfahan, Iran) who did the IHC staining of the studied samples. We appreciate Dr. Ali Gholamrezaei who analyzed the data and edited the report.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Sadjadi A, Nouraie M, Ghorbani A, Alimohammadian M, Malekzadeh R. Epidemiology of breast cancer in the Islamic Republic of Iran: First results from a population-based cancer registry. East Mediterr Health J 2009;15:1426-31.
3. Bernard-Marty C, Lebrun F, Awada A, Piccart MJ. Monoclonal antibody-based targeted therapy in breast cancer: Current status and future directions. Drugs 2006;66:1577-91.
4. Perez EA. Breast cancer management: Opportunities and barriers to an individualized approach. Oncologist 2011;16 Suppl 1:20-2.
5. Birrell SN, Hall RE, Tilley WD. Role of the androgen receptor in human breast cancer. J Mammary Gland Biol Neoplasia 1998;3:95-103.
6. Garay JP, Park BH. Androgen receptor as a targeted therapy for breast cancer. Am J Cancer Res 2012;2:434-45.
7. Wang SY, Shamliyan T, Virnig BA, Kane R. Tumor characteristics as predictors of local recurrence after treatment of ductal carcinoma in situ: A meta-analysis. Breast Cancer Res Treat 2011;127:1-14.
8. Dowsett M, Dunbier AK. Emerging biomarkers and new understanding of traditional markers in personalized therapy for breast cancer. Clin Cancer Res 2008;14:8019-26.
9. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology 1991;19:403-10.
10. Detre S, Saclani Jotti G, Dowsett M. A "quickscore" method for immunohistochemical semiquantitation: Validation for oestrogen receptor in breast carcinomas. J Clin Pathol 1995;48:876-8.
11. Lippman M, Bolan G, Huff K. The effects of androgens and antiandrogens on hormone-responsive human breast cancer in long-term tissue culture. Cancer Res 1976;36:4610-8.
12. Micheli A, Muti P, Secreto G, Krogh V, Meneghini E, Venturelli E, et al. Endogenous sex hormones and subsequent breast cancer in premenopausal women. Int J Cancer 2004;112:312-8.
13. Zumoff B, Levin J, Rosenfeld RS, Markham M, Strain GW, Fukushima DK. Abnormal 24-hr mean plasma concentrations of dehydroisoandrosterone

and dehydroisoandrosterone sulfate in women with primary operable breast cancer. Cancer Res 1981;41:3360-3.
14. Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N. Triple-negative breast cancer: Current status and future directions. Ann Oncol 2009;20:1913-27.
15. McNamara KM, Yoda T, Takagi K, Miki Y, Suzuki T, Sasano H. Androgen receptor in triple negative breast cancer. J Steroid Biochem Mol Biol 2013;133:66-76.
16. Hudis CA, Gianni L. Triple-negative breast cancer: An unmet medical need. Oncologist 2011;16 Suppl 1:1-11.
17. Gonzalez LO, Corte MD, Vazquez J, Junquera S, Sanchez R, Alvarez AC, *et al.* Androgen receptor expresion in breast cancer: Relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. BMC Cancer 2008;8:149.
18. Agoff SN, Swanson PE, Linden H, Hawes SE, Lawton TJ. Androgen receptor expression in estrogen receptor-negative breast cancer. Immunohistochemical, clinical, and prognostic associations. Am J Clin Pathol 2003;120:725-31.
19. Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, *et al.* Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. Cancer Res 2009;69:6131-40.
20. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, *et al.* Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res 2011;17:1867-74.

Source of Support: Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.