Studying the Correlation between Androgen Receptor (AR) Expression and Prognostic Factors in Invasive Breast Carcinoma

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Abstract

Background: Breast cancer is the most common malignancy among women throughout the world. Androgen receptor is a receptor that belongs to the family of nuclear hormone receptors. This receptor functions in the cytosol of target cells as copying factors. These receptors are generally expressed on the neoplastic tissue of breast. However, the role of AR marker in breast cancer has not been defined clearly yet. The present research seeks to study the role of androgens in pre-awareness of breast cancer among women.

Materials and Method: This is a cross sectional-analytical research conducted on 110 Mastectomy and Lumpectomy samples of breast cancer referred to the pathology unit of Shahid Beheshti Hospital of Kashan and Alzahra University of Isfahan from 2010 to 2014. Androgen expression in 110 tissue samples was measured using anti-AR antibody in accordance with immunohistochemistry method principles. Furthermore, the correlation between AR expression and receptor of estrogen, progesterone, HER2neu, metastasis to lymph nodes, tumor grade, age and tumor size in patients' paraffin blocks was also studied.

Results: The following frequencies were reported for each tumor grade: 30.9% for grade one, 50.9% for grade two, and 18.2% for grade three. Totally, 61.8% of all cases had AR expression. Positive cases of ER were reported among 69.1% of the participants, while positive cases of PR and HER2 were reported among 64.5% and 29.1% of cases respectively. Considering the correlation between AR expression and tumor grade, the following frequencies were reported for positive AR in each grade: 39.7% in grade one, 54.7% in grade two, and 2.9% in grade three. However, the negative cases of AR had the following frequencies: 16.7% in grade one, 40.5% in grade two, and 42.9% in grade three and the difference was statistically significant (P-value < 0.001). However, no correlation was observed between AR expression and other indicators such as patient's age, tumor size, lymph node status, and ER, PR, and HER2neu hormone receptors.

Conclusion: Further expression of ER in tumors with lower grades is predictable. As a result, it can be used as an indicator to predict better prognosis among patients with invasive breast cancer.

Key words: Breast Cancer, Metastasis, Androgen Receptor, Tumor Grade, Lymph Nodes.

Introduction

Breast cancer is the most common malignant tumor and constitutes the most important cause of death as a result of cancer among women throughout the world. As many as 1 million cases of this disease are recorded every year around the world (1). Breast cancer cases are on the rise in Asian countries like Iran (2).

Invasive breast carcinoma constitutes a large and variable spectrum of complications with different radiographic, pathological, and biological properties in their clinical expression. The most widely used method of classification has been suggested by World Health Organization (WHO) (2nd edition) where this carcinoma is divided into the lobular and ductal types based upon growth structure and cytological properties of tumoral cells (3).

Old histochemical cases including tumor size, metastasis to axillary lymph nodes, histological grade and biomarkers such as ER, PR, and HER2neu steroid hormone receptors (Estrogen Receptor, Progesterone receptor, Neoadjuvant Human Epidermal Growth Factor Receptor 2) are valuable prognostic and therapeutic factors that are very useful in breast carcinoma (4).

The progress and treatment of breast cancers has shown that presence of hormone receptors such as estrogen and progesterone and biomarkers such as HER2neu in tumoral cells is associated with response to hormone treatments and chemotherapy and helps improve pre-awareness among patients. However, as many as 10 to 24 percent of patients exhibited none of these markers and did not benefit from these treatments. As a result, the need to discover newer exclusive tumoral molecules is obviously required(5).

Androgen receptor (AR) is a biomarker which has recently attracted a lot of attention in the field of breast cancer. As AR belongs to the family of hormone-steroid receptors with nuclear expression, it has a great deal of similarity with estrogen and progesterone receptors in terms of the functioning structure and topography (6).

AR is generally observed in 70% of all cases of breast cancer. The effect of this receptor on breast cancer is yet to be identified but androgen seems to cause proliferative changes in breast tissue (7). The risk of breast cancer among women in the post Menopause period increases as the androgen levels go up (8).

The high rate of breast cancer highlights the necessity of studying variable risk factors (such as clinical parameters and biological markers) in order to assess prognosis and obtain preemptive and therapeutic strategies. The role hormone receptors such as ER, PR, and HER2neu play in pre-awareness of breast cancer is proved (9). In spite of research conducted on androgen receptor, its role as a prognostic factor and its therapeutic importance in breast carcinoma is yet to be identified.

The high rate of breast cancer highlights the necessity of conducting further research in order to identify the prognostic causes of this disease and find useful therapeutic strategies. As a result, the present research was designed to measure the expression of AR marker in malignant breast cells and study the possible correlation of these prognostic factors including ER, PR, and HER2neu metastasis to lymph nodes, histological grades, tumor size, and patient's age.

Materials and Method

This is a cross sectional-analytical research conducted on mastectomy and lumpectomy samples of breast cancer sent to the pathology unit of Shahid Beheshti Hospital of Kashan and Alzahra Hospital of Isfahan. The list of the patients was first explored and samples of breast cancer were randomly selected and studied. The background information of cases was extracted from patients' clinical files. H&E lams underwent morphological assessment so that the best paraffin blocks could be selected for Immunohistochemistry staining. Paraffin blocks and tissue pieces as thick as 5 microns were extracted from samples and they were stained using anti-AR antibody in accordance with Immunohistochemistry method principles.

All these steps were repeated once again to stain ER, PR, HER2neu markers. The stained lams were studied separately for each marker by two pathologists. If more than 10% of cells were positive regardless of staining level, the lams stained for AR marker were considered to be positive. [Figures 1, 2, and 3] present the tumoral tissue and staining level in terms of AR marker as no staining, weak staining and strong staining [Figures 1, 2, and 3].

Considering the frequency of ER marker occurrence among positive AR patients, with respective rates of 84 and 58 percent and statistical power of 80%, the sample size was 108 (54 positive AR patients and 54 negative AR patients) and finally 110 patients took part in the research. The following definitions were given for each prognostic factor:

ER: if it exists in more than 10% of the cells, it will be nuclear positive.

PR: if it exists in more than 10% of the cells, it will be nuclear positive.

HER2neu based upon rating from 0 to 3 and membranous coloring. Grades 0 and 1 are negative, grade 2 is the intermediate level and grade 3 is considered to be positive. Considering the results of FISH experiment, those in the intermediate level were finally categorized as positive or negative.

Lymph nodes engagement: no engagement, engagement in less than 4, engagement in 4 to 9 and engagement in more than 9 lymph nodes.

Tumor size: equal to or less than 2 cm, 2 to 5 cm, and more than 5 cm.

Tumor grade: based on Nottingham classification which considers the following three factors: tubular differentiation in malignant cells, polymorphism of nucleus, and mitosis level (each ranging from 1 to 3). It is finally classified into 3 grades: I: 3-5, II: 6-7, and III: 8-9.

Inclusion criteria:
- all samples of breast cancer who have undergone operation and had referred to the pathology center of Shahid Beheshti Hospital of Kashan and Alzahra University of Isfahan from 2010 to 2014.
- existence of sufficient tissue to study immunohistochemistry

Exclusion criteria:
- not sufficient tissue to study.
- incomplete clinical file or when it was not possible to trace particulars in hospital file.

All information of each sample including tumor grade, number of lymph nodes engaged, the positive or negative value of the recorded immunohistochemistry along with patient's age, tumor size, and the answer of FISH underwent statistical analysis.

Using the proper software (SPSS) and after removing the problems and errors, proper statistical methods were utilized to analyze the variables. Chi-square or Fischer's exact tests were used for qualitative variables mentioned in the assumptions of proposal, while t-student test was utilized for quantitative variables.

Methodology

As many as 100 patients with invasive breast cancer were studied. 12.7% of the participants were younger than 35, 23.6% aged 36 to 45 years old, 26.4% aged 46 to 55, 20.9% aged 56 to 65, and 16.4% were older than 65 years. The average age of the patients was 51.71 ± 12.68 years ranging from 28 to 83 years old.

14.5% of tumors were smaller than 2 cm, 57.3% were between 2 to 5 cm, and the remaining 28.2% had a size of 5 cm or more. The average tumor size was 4.41 ± 2.38 cm with a range of 1 to 12.5 cm.

No lymph node metastasis was observed in 31.8% of cases, 28.2% had 1 to 3 engaged nodes, 25.5% had 4 to 9 engaged lymph nodes, and 14.5% had metastasis to 9 lymph nodes or more.

30.9% of tumors were grade I, 50.9% were grade II, and the remaining 18.2% were grade III.

Positive cases of ER were observed among 69.1% of participants, while positive cases of PR were observed among 64.5% of the patients. HER2neu marker coloring was positive for 29 patients, but it was negative for 71 patients and 10 patients were in the intermediate level. Considering intermediate patients' responses to FISH, 3 were positive and the remaining 7 were diagnosed negative. Positive cases of HER2neu marker were reported among 29.1% of participants and, totally, 61.8% had AR expression [Table 1, 2].

**Table 1: Tumor properties in pathological examinations**

| Pathological properties		Finding
Average tumor size (cm)	Frequency ≤ 2%	16 (14.5%)
	Frequency 2 – 5%	63 (57.3%)
	Frequency more than 5%	31 (28.2%)
Average lymph nodes engaged	Frequency (%) no engagement	35 (31.8%)
	Frequency (%) 1-3 nodes	31 (28.2%)
	Frequency (%) 4-9 nodes	28 (25.5%)
	Frequency (%) more than 9	16 (14.5%)
Tumor grade	I	34 (30.9%)
	II	56 (50.9%)
	III	20 (18.2%)

Table 2: Statistical findings of Immunohistochemical analysis of prognostic factors

| Immunohistochemical marker		Finding
Average percent of ER staining	Frequency (%) positive ER	76 (69.1%)
	Frequency (%) negative ER	34 (30.9%)
Average percent of PR staining	Frequency (%) positive PR	71 (64.5%)
	Frequency (%) negative PR	39 (35.5%)
HER2neu	Frequency (%) positive HER2neu	32 (29.1%)
	Frequency (%) negative HER2neu	78 (70.9%)
Average percent of AR staining	Frequency (%) positive AR	68 (61.8%)
	Frequency (%) negative AR	42 (38.2%)

Figure 1: Immunohistochemical staining of AR, negative (×10)
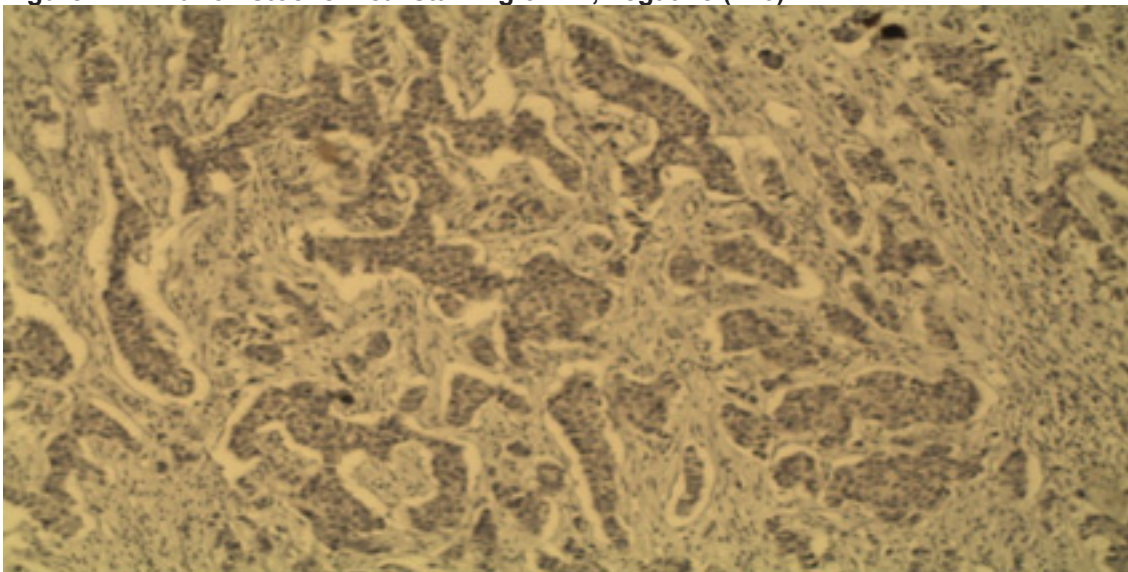
Figure 2: Immunohistochemical staining of AR, weak positive (×10)
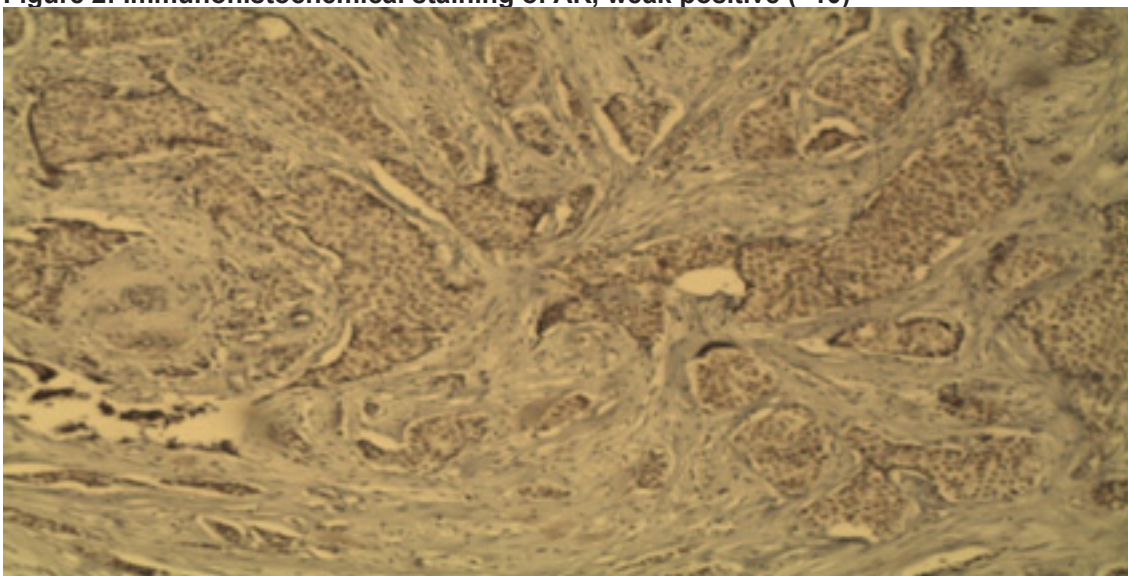
Figure 3: Immunohistochemical staining of AR, strong positive (×10)
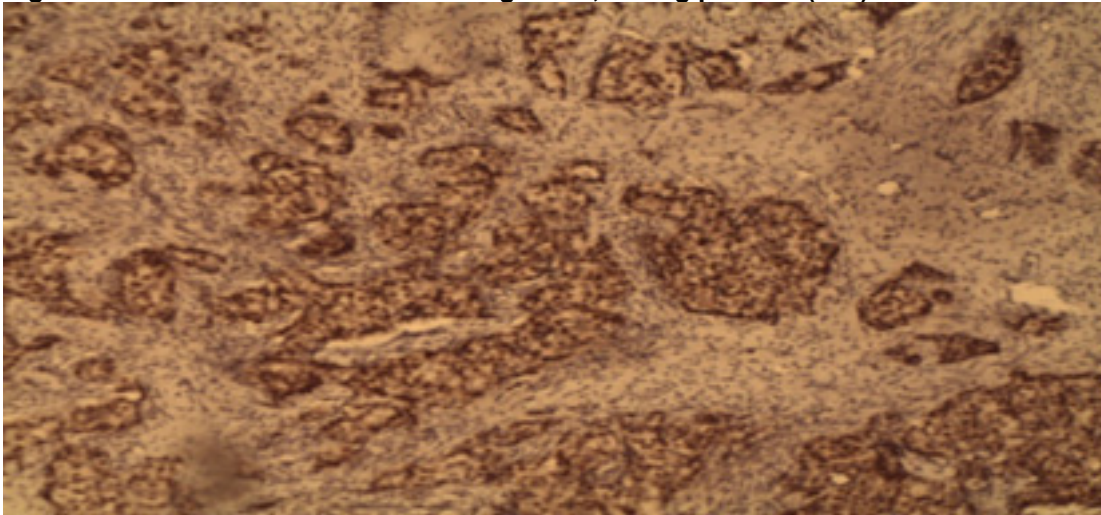
Considering the correlation between AR marker and patients' age, the average age in the group with AR expression was 51.44 ± 12.43 years old, while this mean in the group without AR expression was 52.14 ± 13.23 years with no significant difference reported between the two groups (P-value = 0.779) [Figure 4].

Figure 4: Average age of patients in +AR and –AR groups
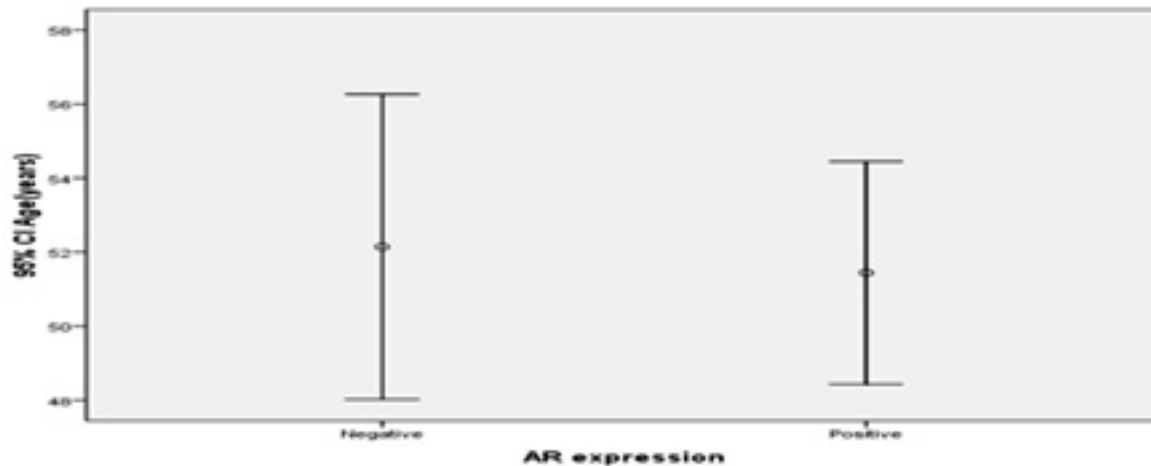
Considering the correlation between AR expression and tumor size, the average tumor size in the group with AR was 4.33 ± 2.32 cm, while this average in the group without AR expression was 4.54 ± 2.49 cm with no significant difference observed between them (P-value = 0.64) [Figure 5].

Figure 5: Average tumor size of the patients in +AR and –AR groups
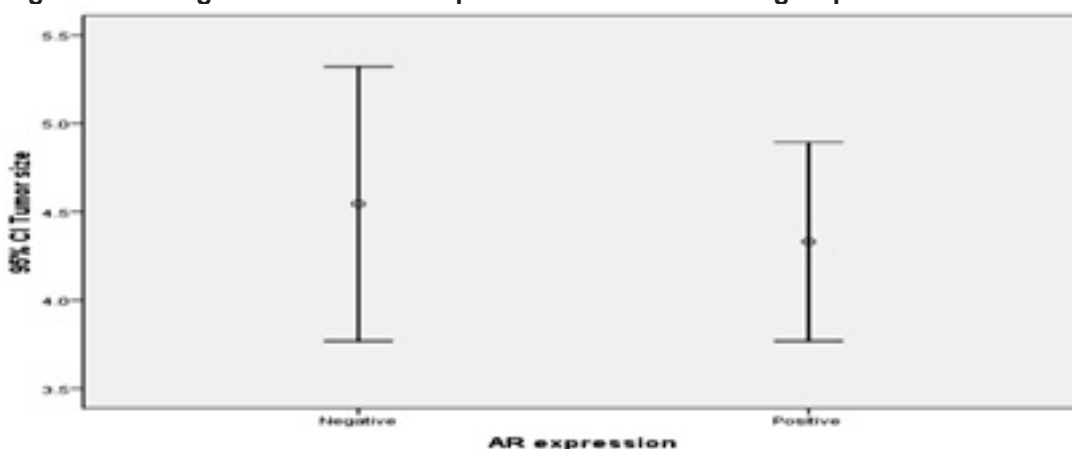
Cases of no metastasis to lymph node in groups with and without positive AR were 32.4% and 31% respectively. Cases of metastasis to one to three lymph nodes in +AR and –AR groups were 29.4% and 26.2% respectively, while cases of metastasis to four to nine lymph nodes in +AR and –AR groups were 23.5% and 28.6%. Cases of metastasis to more than nine lymph nodes in +AR and –AR groups were 14.7% and 14.3% respectively and this difference was not statistically significant (P-value=0.946).

In terms of the correlation between AR expression and tumor grade, the following frequencies were reported for each grade with positive AR: 39.7% for grade I, 54.7% for grade II, and 2.9% for grade III. The following frequencies were observed for each grade with negative AR: 16.7% for grade I, 40.5% for grade II, and 42.9% for grade III. This difference was not statistically significant (P-value < 0.001) [Table 3].

The frequency of AR expression in positive ER group was 75%, while this frequency in negative ER group was 25% which constitutes no statistically significant difference (P-value < 0.088). The frequency of AR expression in positive PR group was 69.1%, while this frequency in negative PR group was 30.9% which constitutes no statistically significant difference (P-value < 0.202).

The frequency of AR expression in the positive HER2neu group was 32.4%, while this frequency in negative HER2neu group was 67.6% which constitutes no statistically significant difference (P-value = 0.338) [Table 4].

Table 3: Correlation between AR expression and clinicopathological factors

Factor		Positive AR	Negative AR	P-value
Tumor size	≤ 2 cm	11 (16.2%)	5 (11.9%)	0.59
	2 to 5 cm	40 (58.8%)	23 (54.8%)	
	> 5 cm	17 (25.0%)	14 (33.3%)	
Number of lymph nodes engaged	No engagement	22 (32.4%)	13 (31%)	0.94
	1-3 nodes	20 (29.4%)	11 (26.2%)	
	4-9 nodes	16 (23.5%)	12 (28.6%)	
	> 9 nodes	10 (14.7%)	6 (14.3%)	
Tumor grade	I	27 (39.7%)	7 (16.7%)	< 0.001
	II	39 (57.4%)	17 (40.5%)	
	III	2 (2.9%)	18 (42.9%)	

Table 4: Correlation between AR expression and hormone receptors

Hormone receptor		Positive AR	Negative AR	P-value
ER	Positive	51 (75.0%)	25 (59.5%)	0.08
	Negative	17 (25%)	17 (40.5%)	
PR	Positive	47 (69.1%)	24 (57.1%)	0.20
	Negative	21 (30.9%)	18 (42.9%)	
HER2neu	Positive	22 (32.4%)	10 (23.8%)	0.33
	Negative	46 (67.6%)	32 (76.2%)	

Discussion

Although the role of ER, PR, and HER2neu markers as prognostic factors in breast cancer has been defined (10, 11), it is necessary to conduct further research in order to identify new factors and markers in this field. The present research has been designed to study AR marker expression on breast cancer tissue using IHC technique in order to determine its correlation with other prognostic factors such as hormone markers and clinicopathological findings.

A large number (61.8%) of the participants in our research expressed androgen marker which is in line with previous research in this field. However, various values have been reported regarding positive androgen in different studies. In researches by Sadighi et al., Monifar, and Alshenawy, as many as 49.1%, 60%, and 74.8% of patients expressed androgen receptor (12, 13, 14). This difference may be partially attributed to the extraction technique. Furthermore, difference in execution of Immunohistochemistry method including the type of antibody used to diagnose AR may

also contribute to this difference. These issues highlight the importance of conducting further research in this field. The results of our research failed to show a correlation between patients' age and expression of AR marker. In our research, expression of AR was associated with lower grades of disease (P-value < 0.001). As a matter of fact, expression of AR marker was observed in 39.7% of grade I and 57.4% of grade II tumors. Only 2.9% of tumors with grade III were positive in terms of AR. In a research by Ensani et al, the expression of AR marker in grade I and II tumors was 73.9% and 66.7% respectively but AR marker was never expressed by anyone of grade III tumors which is in line with the results reported in our research (15). Many studies point to the fact that expression of AR marker is usually associated with good prognostic factors such as smaller tumor size and no metastatic engagement of lymph nodes, although no such thing was observed in our research (16).

The results of our research show no correlation between other markers of breast tumor including estrogen, progesterone, and Her-3 receptors with AR occurrence.

These results highlight the fact that it is impossible to draw a link between these tumoral markers and expression and intensity of AR. However, the positive state of androgen receptor has been accompanied by ER expression in most research (17, 18, 19).

Maybe this difference can be attributed to the genetic differences of patients living in various regions of the world (Iranian patients vis-à-vis patients from other parts of the world).

Although our research failed to show a significant correlation between AR expression and ER, PR, and Her-2 markers, a large percent of patients with positive ER or positive PR or negative Her-2 (75%, 69%, and 67.6% respectively) express androgen receptor. As observation of ER and PR markers and absence of Her-2 in breast cancer indicate a better prognosis of disease, we may conclude that AR is a good predictor of pre-awareness of breast cancer.

It turned out in our research that as many as 40% of patients with TNBC (Triple-negative breast cancer) express AR marker. As many as one third of the participants in Park's study exhibited AR, while 36% of patients in Safarpour's research were positive in terms of AR marker (5, 18). As these patients had the worst prognosis and benefited from no antiestrogen medical treatment of Her-2 targeted therapy, the need for new and more effective medicines in order to improve these patients' prognosis is felt. Considering the relatively high expression of AR marker in this group of patients, the possibility of this marker's prognostic role in this group is proposed.

Conclusion

The results of our research showed that AR is expressed in a considerable group of breast cancers and is usually accompanied with lower grades of tumor. As AR is expressed in a significant number of TNBC patients, it may be a possible therapeutic target in this group of patients. This is a proposition that requires further investigation and research.

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