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Evaluation of Androgen Receptor Expression and Its Association with Clinicopathological Features in Iraqi Women with Breast Cancer

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Abstract

Breast cancer is the most prevalent carcinoma among women worldwide. Regarding the androgen receptor's existence as a likely predictive indicator, there has been interest in its relationship with various molecular markers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), as well as its association with clinicopathological features and therapeutic targets. Despite the global interest in evaluating androgen receptor (AR) expression in breast cancer, data from Iraq remain extremely limited, with only a few small studies addressing this topic. This scarcity of local evidence highlights the need for further investigation to better understand the biological and clinicopathological behavior of AR-positive breast cancers among Iraqi women. The objective of this research was to investigate the AR expression and its association with clinicopathological characteristics in Iraqi females recently diagnosed with breast cancer. We retrospectively analyzed AR expression by immunohistochemistry in 96 breast cancer tissue samples collected between February 2024 and January 2025. The association between AR and clinicopathological features, including age, tumor size, lymph node involvement, distant metastases, TNM stage, histological grade, molecular subtype, ER state, PR state, and HER2 state, was assessed. Tumors with at least 10% nuclear-stained cells were classified as AR-positive. The relationship between AR expression and other factors was determined using a chi-square test and logistic regression in SPSS. Approximately 63 (65.6%) of the 96 individuals were AR-positive. There was no significant connection between AR expression and clinicopathological parameters ($P > 0.05$). In conclusion, while AR is present in 65.6% of patients with breast cancer, there is no significant association with conventional clinicopathological characteristics. This indicates that AR has limited prognostic value in this population. However, its high expression necessitates additional research into its biological significance and possibility of a therapeutic target in some breast cancer subtypes.
Keywords: Androgen receptor, Breast cancer, Clinicopathological features, Immunohistochemistry, Iraqi women.

Introduction

Breast cancer is the most prevalent female carcinoma in all national cancer registries [1,2]. It ranks as the second leading cause of mortality among women [3,4]. Early identification and screening, particularly when accompanied by effective therapy, provide the most immediate hope for a decrease in breast cancer mortality among the approaches advocated by the World Health Organization [5]. According to the most recent Iraqi cancer registry, breast cancer is the most prevalent carcinoma among females, accounting for almost one-third of all reported cancer cases, and it is regarded as the second largest cause of death behind cardiovascular disease [6]. Studies conducted locally by Iraqi experts reveal that over 40% of cases found in Iraq are still in advanced stages [7,8].

It has been demonstrated that BC is not a single illness but encompasses certain biologic entities with diverse pathological traits and clinical consequences that differ according to their gene expression patterns, which contribute to their prognosis and prediction [9].

Breast cancer is classified into several histologic categories. The most frequent kind is invasive ductal carcinoma, which makes up between 50% and 70% of all cases of invasive breast cancer. Ten percent of breast cancers are invasive lobular carcinomas, making it the second most frequent type [10]. It is divided into molecular subtypes based on receptor expression statuses and unique phenotypes, which are identified by known biomarkers such as the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2

(HER2) [11]. Currently, assessing HR (hormone receptors, including ER and PR) and HER2 status is a key step in prognosis and predictive assessment [12]. Breast cancer is categorized into four subgroups depending on the markers as mentioned above: HR + /HER2-, HR + /HER2 +, HR-/HER2 +, and HR-/HER2- (triple-negative breast cancer, TNBC) [13].

Managing breast cancer involves determining the prognostic variables such as age, tumor size, histological grade, axillary lymph node status, and known biomarkers such as ER, PR, and HER2, which are well-documented prognostic factors for breast cancer. The expression of ER, PR, and HER2 in breast cancer has been well researched. Targeted therapy targeting these receptors is now a legitimate therapeutic option alongside surgery, radiation, and chemotherapy. In recent years, it has been demonstrated that androgens and the Androgen receptor (AR) play an essential role in the origin and progression of breast carcinoma [14].

Androgen receptor (AR), a nuclear steroid hormone receptor, is one of the newly emerging biomarkers that are present in 60–80% of women with breast cancer [15]. The expression of AR differs among breast cancer subtypes and is mainly responsible for ER-positive tumors [16]. According to reports, AR can speed up the cell proliferation of triple-negative breast cancer (TNBC) and ER-negative breast cancer [17]. The primary cause is that AR and ER compete with one another to bind to the androgen-responsive component, which promotes the growth of tumor cells [18]. A positive feedback loop mechanism strengthens the synergy between HER2 and AR by encouraging HER2 transcriptional upregulation, which in turn triggers related downstream pathways, speeding up the development of AR-positive cancers [19].

The aim of this investigation was to evaluate the androgen receptor expression in Iraqi breast cancer women and to investigate its relationship with clinicopathological characteristic.

Materials and Methods

This study was conducted retrospectively between February 2024 and January 2025 at the Oncology Teaching Hospital-Medical City (Baghdad, Iraq). The study included 96 female patients who had just received a BC diagnosis with an age range of 33-75 years. Among the patient data obtained were: the patient's age, tumor size, lymph nodes involvement, distant metastases, TNM stage, histological grade, molecular subtype, ER state, PR state, and HER2 state. Disease staging was done using the UICC TNM classification [20]. Clinical examination, radiographic analysis, biochemical analysis, and advanced imaging techniques (MRI and/or PET-CT) were used to assess the disease state.

The study received approval from the Ethics Committee of the College of Pharmacy, University of Baghdad (Approval number: REC042462A; Date: 25/10/2025). Approval of the Ministry of Health was obtained. Each patient received an explanation of the study's purpose.

Inclusion criteria included females who had just received an invasive breast cancer diagnosis. Exclusion criteria included male patients, Individuals with cancer disease other than breast cancer, patients with Androgen abnormality disorders (such as polycystic ovary syndrome (PCOS), congenital adrenal hyperplasia (CAH), metabolic syndrome, persistent acne, hirsutism, androgenic alopecia, Cushing syndrome and insulin resistance), as these conditions can alter systemic androgen levels and potentially influence AR expression in breast tissue, leading to confounding results. Patients who had received anti-cancer or anti-androgenic drugs prior to sample collection were also excluded. Verification of these conditions was performed through medical record review and patient self-reporting to ensure accurate exclusion.

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks served as a primary source for histopathological and immunohistochemical evaluation. These archived samples were provided by the pathology department of the oncology teaching hospital, where they had been kept after initial diagnostic tests. For each patient, a piece of 4 microns in thickness was extracted from the paraffin block of the tumor previously diagnosed with invasive breast cancer. For AR immunohistochemical investigation, these tissue sections were prepared on positively charged glass slides. A commercially available polyclonal rabbit anti-human androgen receptor (Fine Test, China) was used. Immunoreactivity was visualized using the liquid 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate chromogen system, and counterstaining was performed with hematoxylin. AR-related tumors≥10% of nuclear-stained cells were determined to be AR-positive [21]. Two experienced pathologists who were blind to patient outcomes assessed the immunohistochemistry expression of AR.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 28, was used to conduct the statistical analysis. We used the Chi-square test to analyze the correlation between AR expression and clinicopathological variables. A multivariate logistic regression model was employed to assess essential risk factors for AR expression, with a p-value threshold of less than 0.05 deemed significant.

Results and Discussion

This research was performed on 96 paraffin-embedded tissue specimens from females diagnosed

with invasive breast carcinoma. All the patients were females. The patients' mean age was 53.59±9.55 years (33-75 years range). In this study, 84 patients were diagnosed with invasive ductal carcinoma, nine patients with invasive lobular carcinoma, and three patients with mixed ductal and lobular carcinoma. Most tumors were T2 (44.8%) in size, and lymph node involvement was present in (58.4%) of cases. Distant metastasis was documented in (25%) of patients. The majority of cases were diagnosed at TNM stage II (27.1%). Histologically, grade II tumors were the most common (69.8%). Regarding molecular subtype, HR(+)/HER2(–) was the predominant subtype (59.4%). AR positivity was detected in (65.6%) of cases. HR was positive in (79.2%) of cases, whereas HER2 was negative in (72.9%) of cases, as demonstrated in (Table 1).

Table 1. The clinicopathological characteristics of patients

Clinicopathological characteristic		**N (%)**
Age	30-40	8 (8.4%)
	41-50	29 (30.2%)
	51-60	34 (35.4%)
	>60	25 (26%)
Tumor size	T1	28 (29.1%)
	T2	43 (44.8%)
	T3	18 (18.8%)
	T4	7 (7.3%)
Lymph node involvement	N0	40 (41.6%)
	N1	26 (27.1%)
	N2	22 (22.9%)
	N3	8 (8.4%)
Distant Metastases	Metastases	24 (25%)
	Non	72 (75%)
TNM Stage	I	21 (21.9%)
	II	26 (27.1%)
	III	25 (26%)
	IV	24 (25%)
Histological Grade	I	8 (8.3%)
	II	67 (69.8%)
	III	21 (21.9%)
Molecular Subtype	HR (-), HER2 (+)	7 (7.3%)
	HR (+), HER2 (-)	57 (59.4%)
	HR (+), HER2 (+)	19 (19.8%)
	TNBC	13 (13.5%)
AR	Positive	63 (65.6%)
	Negative	33 (34.4%)
HR	Positive	76 (79.2%)
	Negative	20 (20.8%)
HER2	Positive	26 (27.1%)
	Negative	70 (72.9%)

HR: hormone receptors (Estrogen and Progesterone receptors), HER2: human epidermal growth factor receptor 2, TNBC: Triple-negative breast cancer, AR: Androgen receptor, N: number of patients.

The comparison of patients' ages across different tumor stages demonstrated no statistically significant variations ($p>0.05$). This suggests that tumor stage in the studied population was not influenced by patient age, as demonstrated in (Table 2).

Table 2. Relationship between patient age and tumor stage

Variables	**Stage I (N=21)**	**Stage II (N=26)**	**Stage III (N=25)**	**Stage IV (N=24)**	**p-value**
Age (year)	54.14 ± 9.77	53.62 ± 9.12	53.38 ± 10.48	53.32 ± 9.42	0.992

N: number of patients

Immunohistochemical analysis demonstrated that 63 (65.6%) patients exhibited AR positivity, while 33 (34.4%) patients were AR-negative. According to the Chi-square test, no significant correlation was observed between the level of AR expression and age, tumor size, lymph node involvement, distant metastases, TNM stage, histological grade, molecular subtype, HR, and HER2 (P >0.05). The relationship between AR expression and clinicopathological variables is demonstrated in (Table 3).

Table 3. The relationship between androgen receptor expression and clinicopathological variables

Clinicopathological variables		AR expression		$\chi 2$	p-value
		Positive (N=63)	Negative (N=33)		
Age	30-40	6 (75%)	2 (25%)	2.397	0.494
	41-50	21 (72.4%)	8 (27.6%)		
	51-60	19 (55.9%)	15 (44.1%)		
	>60	17 (68%)	8 (32%)		
Tumor size	T1	18 (64.3%)	10 (35.7%)	0.598	0.905
	T2	28 (65.1%)	15 (34.9%)		
	T3	13 (72.2%)	5 (27.8%)		
	T4	4 (57.1%)	3 (42.9%)		
Lymph node involvement	N0	22 (55%)	18 (45%)	4.402	0.228
	N1	19 (73.1%)	7 (26.9%)		
	N2	15 (68.2%)	7 (31.8%)		
	N3	7 (87.5%)	1 (12.5%)		
Distant metastases	Metastases	15 (62.5%)	9 (37.5%)	0.139	0.710
	Non	48 (66.7%)	24 (33.3%)		
TNM stage	I	12 (57.1%)	9 (42.9%)	3.256	0.354
	II	16 (61.5%)	10 (38.5%)		
	III	20 (80%)	5 (20%)		
	IV	15 (62.5%)	9 (37.5%)		
Histological grade	I	3 (37.5%)	5 (62.5%)	3.189	0.203
	II	45 (67.1%)	22 (32.9%)		
	III	15 (71.4%)	6 (28.6%)		
Molecular Subtype	HR (−), HER2 (+)	6 (85.7%)	1 (14.3%)	5.756	0.124
	HR (+), HER2 (-)	32 (56.1%)	25 (43.9%)		
	HR (+), HER2 (+)	15 (78.9%)	4 (21.1%)		
	TNBC	10 (76.9%)	3 (23.1%)		
HR	Positive	47 (61.8%)	29 (38.2%)	2.314	0.128
	Negative	16 (80%)	4 (20%)		
HER2	Positive	21 (80.8%)	5 (19.2%)	3.625	0.057
	Negative	42 (60%)	28 (40%)		

AR: androgen receptor, HR: hormone receptors (estrogen and progesterone receptors), HER2: human epidermal growth factor receptor 2, TNBC: triple negative breast cancer, N: number of patients.

Multivariate logistic regression analysis demonstrated that age, tumor size, lymph node involvement, distant metastasis, TNM stage, histological grade, HR, and HER-2 were not considered significant risk factors for AR ($p > 0.05$) as shown in (Table 4).

Despite the variables considered non-significant risk factors, several patterns were noted. For stage III patients, the odds ratio (OR) was 3.00 (95% CI: 0.81–11.08), indicating that they had a 3.00 times higher chance of being AR-positive than stage I patients. Similarly, in grade III patients, the odds ratio (OR) was 4.17 (95% CI: 0.75–23.18), indicating that they had a 4.17 times higher chance of being AR-positive than grade I patients. In addition, for patients with N3 lymph node involvement, the odds ratio (OR) was 5.73 (95% CI: 0.64–50.97), indicating that they had a 5.73 times higher chance of being AR-positive than other N patients. HER2-positive patients also indicated that they had a 2.80 times higher chance of being AR-positive than HER2-negative patients; the odds ratio (OR) was 2.80 (95% CI: 0.95–8.30), as shown in (Table 4).

Table 4. Multivariate logistic regression for AR

Clinicopathological feature		OR (95% CI)	p-value
Age group	30–40	Reference	---
	41–50	0.88 (0.15–5.27)	0.884
	51–60	0.42 (0.07–2.40)	0.331
	>60	0.71 (0.12–4.32)	0.708
Tumor size	T1	Reference	---
	T2	1.04 (0.38–2.81)	0.943
	T3	1.44 (0.40–5.24)	0.576
	T4	0.74 (0.14–3.99)	0.727
Lymph Node involvement	N0	Reference	---
	N1	2.22 (0.76–6.46)	0.143
	N2	1.75 (0.59–5.23)	0.314
	N3	5.73 (0.64–50.97)	0.118
Distant Metastases	Non	Reference	---
	Metastases	0.83 (0.32–2.18)	0.71
TNM Stage	I	Reference	---
	II	1.20 (0.37–3.87)	0.76
	III	3.00 (0.81–11.08)	0.099
	IV	1.25 (0.38–4.13)	0.715
Histological Grade	I	Reference	---
	II	3.41 (0.75–15.58)	0.114
	III	4.17 (0.75–23.18)	0.103
Molecular Subtype	HR (-), HER2 (+)	Reference	---
	HR (+), HER2 (-)	0.21 (0.02–1.89)	0.165
	HR (+), HER2 (+)	0.63 (0.06–6.80)	0.7
	TNBC	0.56 (0.05–6.63)	0.642
HR	Positive	0.41 (0.12–1.33)	0.137
	Negative	Reference	---
HER2	Positive	2.80 (0.95–8.30)	0.063
	Negative	Reference	---

AR: androgen receptor, HR: hormone receptors (estrogen and progesterone receptors), HER2: human epidermal growth factor receptor 2, TNBC: triple negative breast cancer.

One out of every eight women will develop breast cancer, making it the most prevalent cancer in the world [22]. However, breast cancer mortality rates had reduced in recent decades due to the development of various novel therapeutic approaches and diagnostic techniques to identify tumors in their early stages [23]. It has been suggested that BC is a prevalent and heterogeneous illness. In this context, certain clinical characteristics, including age, lymph node involvement, tumor size, tumor type, grade, and neural invasion, are considered significant in determining prognosis and even therapy [24]. There is also considerable variability in the proliferation and progression of breast cancer cells, which influences the clinical course of the disease and constitutes a crucial factor in determining prognosis [25].

It is widely recognized that estrogen stimulates the development of breast cancer and androgen stimulates the development of prostate cancer. The role of AR in breast cancer has drawn a lot of interest. Previous investigations indicated that AR plays a permissive function in the onset and spread of breast cancers [26]. According to our analysis, 65.6% of breast cancer patients expressed AR, which is in line with other previous research that indicated AR positivity in approximately 60–80% of breast cancer patients [27,28,29,30].

Regarding molecular typing in patients with breast cancer, no significant correlation was observed between the AR receptor and molecular subtype; however, AR positivity was found in all subtypes. The frequencies of AR positive in various subtypes, HR-/HER2 +, HR + /HER2-, HR + /HER2 +, and TNBC, were found to be 85.7%, 56.1%, 78.9%, and 76.9%, respectively. In breast tumors that are ER-positive, the AR positive rate is 61.8%, which is in line with the 60%–90% reported rate [31]. However, some evidences suggest AR may behave as a tumor suppressor within this subtype [32,33]. In the meantime, ER and AR expression are connected. Among the most probable ways is that AR can block the transcriptionally active components of ER by competitively binding to estrogen-responsive elements [32]. Furthermore, p300, a coactivator that competes with ER, can be directly bound by AR, which subsequently suppresses ER function and downstream signaling

pathways [32], thus luminal breast cancer tumor growth is suppressed.

In the current study, AR expression in patients with HER-2-positive breast cancer accounts for 80%. Additionally, AR expression in breast cancer with HER-2 positive individuals suggests a poorer outcome and might be a mediator of HER-2 signaling pathways, which can override the potentially protective effects of AR signaling and contribute to more aggressive tumor biology [34]. TNBC is highly heterogeneous and lacks specific therapies, such as HER-2 and HR, which indicate an early metastasis and a miserable prognosis. There are presently no legally available targeted treatments for TNBC; thus, new treatment methods are urgently needed. However, in our study, AR is expressed in 76.9% of TNBC patients, and further evidences suggest that AR is a potential target for therapy [35].

Notably, our analysis revealed no statistically significant associations between AR expression and clinicopathological variables, including age, tumor size, lymph node status, distant metastasis, TNM stage, histological grade, molecular subtype, HR, or HER2 expression (all p-values > 0.05). These nonsignificant results were similar to those of other research that reported no significant association between clinicopathological factors and androgen [14,29]. In contrast, some studies have indicated significant positive relationship between AR expression and molecular subtypes, as well as ER, PR, and HER-2 [36,37]. In similar lines, recent studies had shown that AR expression is strongly linked to age and tumor size [38]. Numerous investigations indicate a correlation between AR expression and histological grade [30, 38, 39,40] and TNM stage [40]. The differences between our results and those of previous studies may be due to sample size limitation, patient population, variability in breast cancer subtypes, and methodological variations that includes the cutoff used to define AR positivity. The borderline association with HER2 (P=0.057) suggests a possible trend that may have reached statistical significance with a larger sample size or in more homogeneous patient population. Further large-scale studies are needed to clarify this relationship.

Conclusion

While AR is expressed in 65.6% of breast cancer patients, there is no significant association with conventional clinicopathological characteristics. This indicates that AR has limited prognostic value in this population. However, its high expression necessitates additional research into its biological significance and potential as a target for therapy in some subtypes of breast cancer.

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Conflicts of Interest

There is no conflict of interest.

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Ethics Statements

The study was approved by the Ethics Committee of the College of Pharmacy, University of Baghdad with the number (REC042462A). Approval of Ministry of health was obtained.

Author Contribution

The authors contributed equally to this work.

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تقييم مستقبلات الأندروجين وعلاقتها بالخصائص السريرية والمرضية لدى النساء العراقيات المصابات بسرطان الثدي

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الخلاصة

يُعَدُّ سرطان الثدي أكثر أنواع السرطان شيوعًا بين النساء في جميع أنحاء العالم. وبالنسبة لوجود مستقبل الأندروجين كمؤشر انذاري محتمل، فقد ازداد الاهتمام بدراسة علاقته بمختلف المؤشرات الجزيئية، مثل مستقبل الإستروجين، ومستقبل البروجستيرون، ومستقبل عامل نمو البشرة البشري الثاني، إضافةً إلى ارتباطه بالخصائص السريرية والمرضية والأهداف العلاجية. وعلى الرغم من الاهتمام العالمي بتقييم تعبير مستقبل الأندروجين في سرطان الثدي، فإن البيانات المتوفرة في العراق ما تزال محدودة للغاية، إذ لا توجد سوى دراسات قليلة صغيرة الحجم تناولت هذا الموضوع. ويبرز هذا النقص في الأدلة المحلية الحاجة إلى مزيد من الدراسات لفهم السلوك البيولوجي والسريري والمرضي لسرطانات الثدي الايجابية لمستقبل الأندروجين لدى النساء العراقيات بصورة أفضل. هدفت هذه الدراسة إلى تقصي تعبير مستقبل الأندروجين وعلاقته بالخصائص السريرية والمرضية لدى النساء العراقيات اللواتي شُخِّصن حديثًا بسرطان الثدي. وقد أُجري تحليل تعبير مستقبل الأندروجين بأثر رجعي باستخدام تقنية التلوين المناعي النسيجي في ٩٦ عينة نسيجية من سرطان الثدي جُمعت خلال الفترة الممتدة من شباط ٢٠٢٤ إلى كانون الثاني ٢٠٢٥. كما جرى تقييم العلاقة بين مستقبل الأندروجين والخصائص السريرية والمرضية، بما في ذلك العمر، وحجم الورم، وإصابة العقد اللمفاوية، والنقائل البعيدة، ومرحلة الورم، والدرجة النسيجية، والنمط الجزيئي، وحالة مستقبل الإستروجين، وحالة مستقبل البروجستيرون، وحالة مستقبل عامل نمو البشرة البشري الثاني. وصُنِّفت الأورام التي أظهرت تلوينًا نوويًا في ما لا يقل عن ١٠٪ من الخلايا على أنها موجبة لمستقبل الأندروجين. وتم تحديد العلاقة بين تعبير مستقبل الأندروجين والعوامل الأخرى باستخدام اختبار مربع كاي والانحدار اللوجستي. وأظهرت النتائج أن ٦٣ مريضة بنسبة (٦٥,٦٪) من أصل ٩٦ مريضة كانت ايجابيه لمستقبل الأندروجين. ولم تُسجَّل علاقة ذات دلالة إحصائية بين تعبير مستقبل الأندروجين والخصائص السريرية والمرضية المدروسة (قيمة الاحتمال أكبر من ٠,٠٥). وفي الختام، على الرغم من ان مستقبل الأندروجين كان إيجابياً بنسبة ٦٥,٦٪ لدى مريضات سرطان الثدي، إلا إنه لا توجد علاقة ذات دلالة إحصائية مع الخصائص السريرية والمرضية التقليدية. وتشير هذه النتيجة إلى أن قيمته التنبؤية لمسار المرض محدودة في هذه الفئة السكانية. ومع ذلك، فإن ارتفاع نسبة التعبير عنه يستدعي إجراء مزيد من البحوث لاستكشاف أهميته البيولوجية وإمكان الاستفادة منه كهدفًا علاجيًا محتملا في بعض الأنماط الفرعية من سرطان الثدي.

الكلمات المفتاحية: مستقبلات الاندروجين، سرطان الثدي، الخصائص السريرية والمرضية، الاختبار المناعي النسيجي، النساء العراقيات.