Sex hormone and receptor imbalance in women with bladder cancer: A molecular and physiological study

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Abstract. Bladder cancer is one of the most common malignancies of the urinary tract, characterized by high recurrence and morbidity rates. The majority of bladder cancer cases occur in older individuals, with the prevalence rising with age. The present study aimed to evaluate the levels of sex hormones (estrogen and testosterone) and their corresponding receptors [androgen receptor (AR) and estrogen receptor 1 (ESR1)] to determine whether they are risk factors for the pathogenesis and progression of bladder cancer in women. A total of 122 female participants were enrolled in the present study, including 50 patients diagnosed with bladder cancer and 72 age-matched participants who served as the control group. Blood samples and tissue biopsies were collected from the participants. Hormone levels were measured using the Cobase automated analyzer, while the gene expression levels of receptors were assessed using reverse transcription-quantitative PCR. The results showed a significant increase in the levels of the estrogen and testosterone hormones in the patient group. The results also demonstrated a significant increase in AR gene expression, but a decrease in ESR1 gene expression in the patients compared with the control group. These findings support a potential link between sex hormone imbalance and altered receptor expression in the pathophysiology of bladder cancer in women.

Introduction

Bladder cancer is the ninth most common cancer worldwide, with 614,298 new bladder cancer cases reported globally in 2022; it is the sixth most common cancer in men (523,674 cases; 5.4% of all newly diagnosed cancer cases) and the 17th in women (143,005 cases; age-standardized rate of 2.4 per 100,000), showing a marked male predominance (1). Bladder cancer accounts for ~2.7% of all cancer fatalities and 4.2% of all newly discovered cancer cases, with a 5-year relative overall survival rate of 77.9% (2). The prevalence of bladder cancer increases with age progression and the majority of cases (80%) occur in individuals aged >65 years (3). In total, >95% of bladder cancer originates from the urothelium, the inner lining of the urinary tract, and is referred to as urothelial carcinoma (4). As the urothelium spans both the upper and lower urinary tracts, tumors can develop in either region. Bladder cancer is classified by tumor invasiveness, as non-muscle invasive bladder cancer or muscle-invasive blader cancer, and by cellular grade (2). Hematuria is the most common initial symptom leading to diagnosis; thus, an assessment for bladder cancer is conducted only after presenting with symptoms such as hematuria, and notably, 20% of patients will have locally advanced or metastatic bladder cancer (5).

Cells in multicellular organisms communicate through signaling mechanisms that regulate growth, development and homeostasis (6). This occurs via direct contact or chemical messengers that bind to specific receptors on or inside the target cells. Cellular receptors can be either intracellular or cell surface proteins; intracellular receptors bind lipid-soluble messengers, while cell surface receptors interact with water-soluble molecules (7). Disruptions in these pathways can lead to cancer-related changes such as uncontrolled growth and resistance to cell death (8). Several tissues, including the testes, ovaries, endometrium, prostate, kidneys, liver, circulatory system, brain, neurological system, skeletal muscle, skin and bladder, have been shown to express and act upon androgens and estrogen binding to their receptors (9). Androgens, mainly testosterone and its effective derivative dihydrotestosterone (DHT), act through the androgen receptor (AR), a ligand-activated transcription factor of the nuclear receptor superfamily (10). The AR, a 110 kDa protein with 919 amino acids, serves a key role in regulating the expression of genes involved in numerous physiological and pathological processes (11). Conversely, estrogens, particularly estradiol (E2), act mainly via two estrogen receptors (ERs), Erα and Erβ [which are encoded by estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) genes, respectively]. Important physiological processes, including reproduction, metabolism, bone density maintenance and the development of estrogen-responsive malignancies, such as endometrial and breast cancer, are

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known to be mediated by these receptors (12). The ERs belong to a nuclear receptor superfamily that has the capacity to convert extracellular signals into transcriptional signals (13).

Understanding the interplay between androgen/estrogen hormones and their receptors may not only clarify the observed sex disparity in bladder cancer incidence but also provide new therapeutic targets. Hormone receptor modulators, such as AR antagonists or selective ER modulators, are being investigated for their potential to halt disease progression and improve treatment responses in patients with bladder cancer (11). The present study aimed to examine the levels of sex hormones, specifically estrogen and testosterone, in women diagnosed with bladder cancer, and to evaluate alterations in the expression of their corresponding receptors, including AR and ESR1, within bladder tissue. Furthermore, the present study aimed to investigate whether hormonal and receptor disorder may serve as risk factors in the pathogenesis and progression of bladder cancer in women.

Patients and methods

Study design and participants. In the present study, participants were prospectively recruited between July 2024 and January 2025, from various locations, including the Medicine City (Ghazi Al-Hariri Surgical Specialties Hospital and Al-Amal National Hospital in Baghdad, Iraq) as well as Al-Shifa Center for Oncology Treatment in Maysan, Iraq. Sample collection and data acquisition were conducted in accordance with standardized clinical procedures and predefined study protocols.

The present case-control study involved 122 participants, all of whom were women of different ages ranging from 35-80 years, with a median age of 64 years. Among the participants, 50 patients who were diagnosed with bladder cancer (Table I) provided blood samples for hormonal analysis, of whom 22 also contributed tissue specimens [as formalin-fixed, paraffin-embedded (FFPE) blocks] for molecular studies. The control group included 72 age-matched individuals of whom 50 provided blood samples for hormonal comparison with the patient group, whereas the remaining 22 had benign tumors that were histologically diagnosed as benign urothelial papilloma and endometriosis of the urinary bladder. These diagnoses were confirmed by histopathological examination, with no evidence of malignancy or dysplasia. FFPE tissue specimens were collected from these cases and used as a comparison group for the bladder cancer tissue data (Fig. 1). For both the patient and control groups, venous blood samples were obtained and immediately processed by centrifugation at 3,000 x g for 10 min at room temperature (20-25˚C) to separate the serum, which was then aliquoted and stored in a deep freeze until further analysis. The present study was performed in compliance with the Declaration of Helsinki and approved by the Institutional Review Committee of College of Science, in cooperation with the Local Ethics Committee of the Biology Department, Mustansiriyah University (Baghdad, Iraq; January 2024; approval. no. BCSMU/1024/0062Z).

Inclusion criteria. The inclusion criteria were as follows: Patient group: i) Female patients of any age with a confirmed diagnosis of malignant bladder tumor based on histopathological examination; and ii) participants who provided informed consent for study participation. Disease stage or grade was not used as an eligibility criterion as all stages and grades of bladder cancer were included. The control group was: i) Healthy female volunteers age-matched to the patient group; and ii) female patients of any age with a confirmed diagnosis of benign bladder tumor based on histopathological examination.

Exclusion criteria. The exclusion criteria were as follows: i) Male participants; ii) individuals with any other diseases, including malignancies other than bladder cancer, autoimmune or chronic inflammatory conditions or infectious diseases; iii) FFPE tissue samples with inadequate quantity, poor preservation or RNA of insufficient quality for gene expression analysis; and iv) participants who had recently undergone chemotherapy, radiotherapy or immunotherapy.

Primer design and gene targets. Primers were designed using Primer 3 Plus (version 4; https://www.primer3plus.com) and their reference sequences were validated against the National Center for Biotechnology Information database using UCSC Genome Browser Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/blast.cgi). The primers were synthesized and lyophilized by Alpha DNA. Table II contains all of the primer sequences utilized in the present study.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). The TransZol Up Plus RNA Kit Reagent (Tissue) (TransGen Biotech Co., Ltd.; cat. no. ER501-01) was used to extract total RNA from each bladder tissue sample according to the manufacturer's instructions. RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.) and RNA purity was assessed by the A_{260}/A_{280} ratio, which was ~2.0. Total RNA was reverse-transcribed to complementary DNA (cDNA) using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech Co., Ltd.; cat. no. AE311-02) according to the manufacturer's instructions. The reaction was performed at 25˚C for 10 min in a final volume of 20 μl, with 4 μl of total RNA and a random primer.

Target gene expression was confirmed by qPCR, a sensitive technique for assessing steady-state mRNA levels. The Qiagen Rotor Gene Q Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to perform qPCR. The expression levels and fold changes of the target genes and the housekeeping gene, GAPDH, were quantified using the TransStart® Top Green qPCR SuperMix Kit (TransGen Biotech Co., Ltd.; cat. no. AQ131-01) in a 20 μl reaction mixture consisting of 10 μl 2X qPCR SuperMix, 4 μl nuclease-free water, 1 μl of each forward and reverse primer (10 μM) and 4 μl of cDNA. The thermocycler protocol included an initial enzyme activation step at 94˚C for 30 sec, followed by 40 cycles of denaturation at 94˚C for 5 sec, annealing at 58˚C for 15 sec (60˚C for GAPDH) and extension at 72˚C for 20 sec. A dissociation curve analysis was performed at 55-95˚C to verify product specificity.

The RT-qPCR efficiency of each primer set was determined by serial dilutions of cDNA template from the bladder tissue of women that diagnosed with malignant and benign tumor. standard curve was generated by preparing serial dilutions of a cDNA template, and each primer pair was tested across the dilution series. The slope of the linear equation was

Table I. Clinicopathological characteristics of patients (n=50) with bladder cancer.

Characteristic	No. of patients
Median age, years (range)	67 (37-80)
Sex	
Female	50 (100)
T stage	
Ta	2 (4)
T1	19 (38)
T2	16 (32)
T3	13 (26)
N stage	
N0	50 (100)
M stage	
M0	50 (100)
Tumor grade	
G1	11 (22)
G2	15 (30)
G3	24 (48)

Data presented as n (%). T, tumor; N, lymph node; M, metastasis; G, grade.

applied to calculate the efficiency according to the equation $E=(10^{(-1/slope)}-1)$ x100 where E=PCR efficiency (%) and slope=slope of standard curve. The PCR efficiency of studied genes ranged from 89.4 to 103.8%, slope from -3.61 to -3.23 and R^2 from 0.995 to 0.998.

GAPDH was selected as the internal reference gene for normalization of the qPCR data; it encodes a key glycolytic enzyme that is constitutively and ubiquitously expressed across mammalian tissues, demonstrating high amplification efficiency and stable expression in both normal and pathological conditions, including malignant and benign tissues, ensuring reliable and consistent detection across all samples (14). Fold changes in the quantified expression levels of mRNA were determined using the relative cycle threshold ($2^{-\Delta\Delta Cq}$) method as originally described by Livak and Schmittgen (15). Each sample was run in duplicate and the mean Cq values for both GAPDH and the target genes were recorded for the patients and controls.

Statistical analysis. Statistical analyses were performed using GraphPad Prism (version 9.2; Dotmatics). Additionally, *a priori* power analysis was performed using G*Power (version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany). The present study included 50 patients and 72 control participants. Based on *a priori* power analysis assuming a medium effect size (Cohen's d=0.5), a significance level of α=0.05 and a two-sided test, the statistical power of the present sample was 80%. Normality of the continuous variables was evaluated using the Kolmogorov-Smirnov (KS) and Shapiro-Wilk (SW) tests. Based on the normality evaluation, for variables that satisfied the assumption of normality, comparisons between two independent groups were performed using an unpaired two-tailed t-test. For variables that did not satisfy the assumption of normality, non-parametric statistical methods were applied: Comparisons between two independent groups were performed using the Mann-Whitney U test. Monotonic associations between variables were examined using Spearman's rank correlation coefficient (ρ). P<0.05 was considered to indicate a statistically significant difference.

Results

Age of the patient and control groups. The age distribution of participants was divided into five categories. Among the 50 patients with bladder cancer, the age groups were 30-39 years (n=3; 6%), 40-49 years (n=6; 12%), 50-59 years (n=7; 14%), 60-69 years (n=13; 26%) and ≥70 years (n=21; 42%) (Fig. 2). In the control group (n=72), the age distributions were similar, with 30-39 years (n=6; 8%), 40-49 years (n=10; 14%), 50-59 years (n=12; 17%), 60-69 years (n=17; 24%) and ≥70 years (n=27; 37%) (Fig. 2). Unpaired t-test analysis revealed no statistically significant differences in the age distribution between the patients and controls (P>0.05), indicating appropriate age-matching across the groups.

Normality testing. Prior to conducting the statistical analysis, the distribution of the data was assessed using the KS and SW tests for normality (Table III). Regarding the serum hormone levels, testosterone demonstrated normal distribution in both the control (KS test: P=0.199; SW test: P=0.140) and patient (KS test: P=0.200; SW test: P=0.181) groups; however, the estrogen levels in the control group showed a normal distribution (KS test: P=0.200; SW test: P=0.955), while the patient group exhibited significant deviation from normality (KS test: P=0.006; SW test: P=0.001).

For receptor gene expression in bladder tissues, ESR1 expression showed mixed results, with the control group demonstrating normal distribution (KS test: P=0.186; SW test: P=0.045), while the patient group showed significant deviation from normality (KS test: P=0.000; SW test: P=0.003). AR expression in the control group showed normal distribution (KS test: P=0.200; SW test: P=0.557), whereas the patient group demonstrated significant deviation from normality (KS test: P=0.085; SW test: P=0.029). These results are summarized in Table IV.

Serum reproductive hormone levels. The serum levels of estrogen and testosterone in the patient (n=50) and healthy control (n=50) groups were evaluated and compared using the Mann-Whitney U test (Table V). The serum testosterone levels were significantly higher in the patient group (mean rank=54.40) compared with the control group (mean rank=26.60), with a Mann-Whitney U value of 244.000 (Z=-5.350; P<0.001). Similarly, serum estrogen levels showed a significant increase in the patient group (mean rank=55.52) relative to the control group (mean rank=35.48), with a Mann-Whitney U value of 561.500 (Z=-3.640; P<0.001). These findings indicate marked dysregulation of the circulating reproductive hormones in female patients with bladder cancer, characterized by elevated levels of both androgens and estrogens.

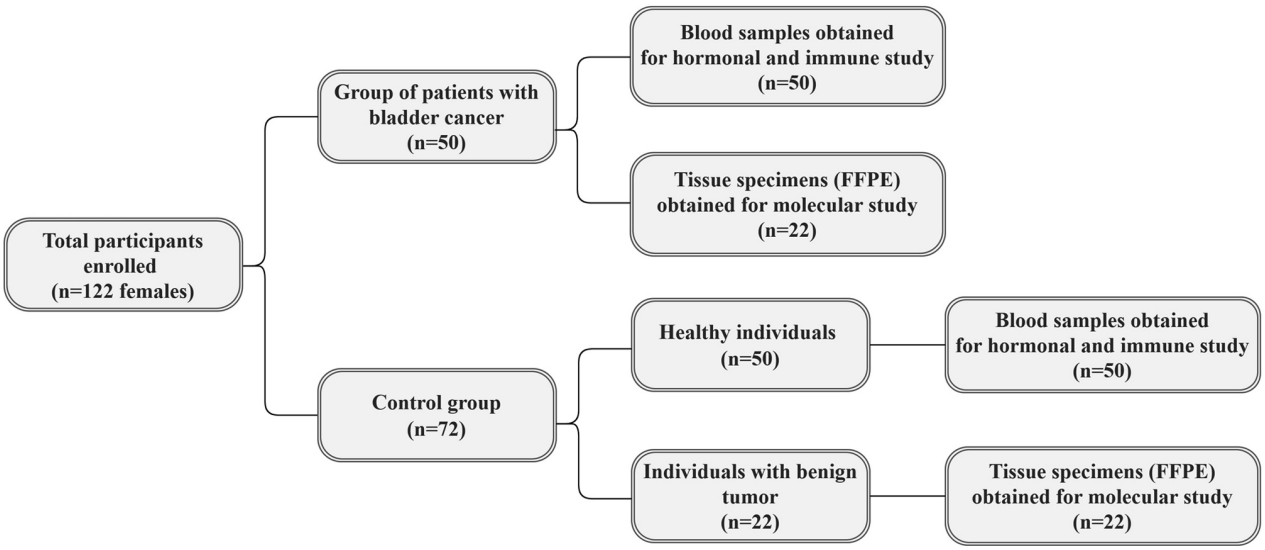

Figure 1. Flowchart illustrating the distribution and number of participants, as well as the type and number of blood and tissue samples collected in the present study. FFPE, formalin-fixed paraffin-embedded.

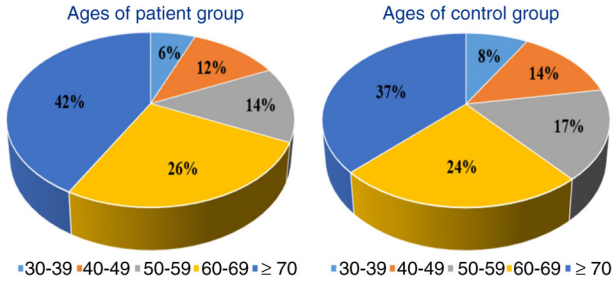

Figure 2. Age distribution of the patient and control groups, categorized into intervals beginning with 30-39 years and extending to >70 years.

Analysis of gene expression. The expression levels of the target genes, AR and ESR1, in the bladder cancer tissue samples were assessed using RT-qPCR amplification curves, which were compared with the housekeeping gene internal control, GAPDH (Figs. S1-S3). The stable and consistent expression of GAPDH was confirmed by the amplification plots, which showed early Cq values with little change between samples, demonstrating its appropriateness for the normalization of the target genes. However, the AR and ESR1 amplification curves showed inconsistency in the Cq values, suggesting that their transcript abundances were different to GAPDH. The specificity and effectiveness of the reactions were validated by the sigmoidal amplification patterns shown for every gene, which were distinguished by discrete exponential phases and notable separation from the negative controls. The assumption of identical amplification efficiencies was further supported by the comparable slopes of the target and housekeeping gene curves, which guaranteed the accuracy of the relative quantification.

Statistical analysis of gene expression in the bladder tissues revealed contrasting patterns for AR and ESR1 expression (Table VI). AR gene expression was significantly upregulated in the bladder cancer tissues (mean rank=28.05) compared with the benign control tissues (mean rank=14.30), with a Mann-Whitney U value of 76.000 (Z=-3.629; P=0.002). This marked increase in AR expression suggests enhanced androgenic signaling capacity within the bladder cancer tumor microenvironment. By contrast, ESR1 gene expression was significantly downregulated in the bladder cancer tissues (mean rank=17.59) compared with the control tissues (mean rank=25.80), with a Mann-Whitney U value of 134.000 (Z=-2.169; P=0.030). This reduction in ESR1 expression, occurring despite elevated circulating estrogen levels, indicates a disruption in the estrogen-mediated signaling pathways.

Collectively, these findings demonstrate a distinct pattern of hormone receptor dysregulation in female patients with bladder cancer, characterized by enhanced AR expression coupled with diminished ESR1 expression, which may contribute to an imbalance favoring pro-oncogenic androgen signaling while reducing the estrogen-mediated protective effects.

Correlation between hormone levels and bladder cancer stage. To further explore the clinical significance of hormonal dysregulation, Spearman's correlation analysis was performed to examine the correlation between serum hormone levels and bladder cancer stage in female patients (n=50; Table VII). The analysis revealed a weak positive correlation between serum testosterone levels and cancer stage (ρ=0.263; P=0.05), suggesting a pattern where higher testosterone levels tended to be correlated with more advanced disease stages. More notably, the serum estrogen levels demonstrated a moderate positive correlation with cancer stage (ρ=0.512; P=0.0015), indicating a significant correlation between elevated estrogen levels and disease progression. This finding suggests that estrogen levels may serve as a potential indicator of tumor advancement in female patients with bladder cancer.

Discussion

Age represents a key demographic parameter consistently included in cancer epidemiological studies; it is recognized as one of the influential and thoroughly investigated determinants of cancer risk. Since the occurrence of most malignancies

Table II. Sequences and specifications of the primers used in the present study.

Primer	Sequence (5'→3')	Primer size, bp	Ta, ˚C
AR			58
Forward	GAGATGATACCCTCCCAGCA	20	
Reverse	CATTTGCAGGGTTTCTGGTT	20	
ESR1			58
Forward	AGCACCCTGAAGTCTCTGGA	20	
Reverse	GATGTGGGAGAGGATGAGGA	20	
GAPDH			60
Forward	GGCCTCCAAGGAGTAAGACC	20	
Reverse	AGGGGTCTACATGGCAACTG	20	

AR, androgen receptor; ESR1, estrogen receptor 1; Ta, annealing temperature.

Table III. Normal distribution assessment of serum estrogen and testosterone concentrations in patients (n=50) with bladder cancer and healthy controls (n=50).

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group	Statistic	df	P-value	Statistic	df	P-value
Testosterone						
Control	0.217	50	0.199	0.883	50	0.140
Patient	0.202	50	0.200	0.899	50	0.181
Estrogen						
Control	0.134	50	0.200	0.978	50	0.955
Patient	0.302	50	0.006	0.723	50	0.001

[a]Non-parametric test. df, degrees of freedom.

Table IV. Normal distribution assessment of ESR1 and AR gene expression in bladder tissues from the patient (n=22) and control groups (n=22).

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group	Statistic	df	P-value	Statistic	df	P-value
ESR1						
Control	0.220	22	0.186	0.841	22	0.045
Patient	0.290	22	0.000	0.709	22	0.003
AR						
Control	0.192	22	0.200	0.940	22	0.557
Patient	0.237	22	0.085	0.837	22	0.029

[a]Non-parametric test. ESR1, estrogen receptor 1; AR, androgen receptor; df, degrees of freedom.

notably rises with advancing years, cancer is widely considered an age-related condition (16,17). Beyond its role as a demographic factor, advancing age is biologically linked to changes that may contribute to bladder cancer development. For example, aging is associated with cumulative genetic and epigenetic alterations, impaired DNA repair mechanisms and increased oxidative stress, all of which can promote carcinogenesis (18). Additionally, age-related hormonal alterations, particularly fluctuations in androgen and estrogen levels, can influence urothelial cell function and proliferation, thereby increasing vulnerability to bladder cancer (19). This age-hormone relationship provides important context for

Table V. Statistical analysis of the serum testosterone and estrogen levels in the patient (n=50) and control (n=50) groups using the Mann-Whitney U test.

Statisitical term	Testosterone	Estrogen
Mean rank		
Control	26.60	35.48
Patient	54.40	55.52
Mann-Whitney U	244.000	561.500
Wilcoxon W	1,064.000	1,596.500
Z-value	-5.350	-3.640
P-value	<0.001	<0.001

Z, standardized test statistic.

Table VI. Statistical comparison of the androgen and estrogen receptor gene expression levels in the bladder tissues from the patient (n=22) and control (n=22) groups using the Mann-Whitney U test.

Statistical term	Androgen receptor	Estrogen receptor-1
Mean rank		
Control	14.30	25.80
Patient	28.05	17.59
Mann-Whitney U	76.000	134.000
Wilcoxon W	286.000	387.000
Z-value	-3.629	-2.169
P-value	0.002	0.030

Z, standardized test statistic.

Table VII. Correlation of the serum testosterone and estrogen levels with bladder cancer stage using Spearman's rank correlation coefficient.

Statistical term	Correlation with stage of bladder cancer
Testosterone	
Spearman's correlation	0.263
P-value (two-tailed)	0.05
No. of samples	50
Estrogen	
Spearman's correlation	0.512
P-value (two-tailed)	0.0015
No. of samples	50

understanding the hormonal dysregulation observed in the present study.

Hormonal signals serve a critical role in regulating the growth, differentiation and survival of cells throughout the body. These same biological signals can also contribute to cancer when they become dysregulated. Hormones act through highly specific communication systems, including endocrine, paracrine and autocrine signaling, that influence gene expression and cellular behavior (20). The findings of the present study demonstrated a clear hormonal imbalance in women with bladder cancer, characterized by significantly elevated levels of both estrogen and testosterone in serum compared with the control group. Increased testosterone levels in women with bladder cancer suggests dysregulation of androgen metabolism or adrenal androgen overproduction. Testosterone and its more potent derivative, DHT, signal through the AR, which is also expressed in bladder tissues (21). Additionally, the increased circulating estrogen levels in female patients with bladder cancer may result from altered systemic hormone metabolism or local estrogen synthesis within the tumor microenvironment, facilitated by elevated aromatase enzyme activity that converts androgens to estrogens (22).

Correspondingly, the results of the present study demonstrated a significant increase in AR gene expression in bladder cancer tissues compared with benign control tissues. This elevation was consistently observed across the analyzed samples, indicating a clear difference in expression levels between malignant and non-malignant bladder tissues. The alignment between elevated testosterone levels and increased AR expression suggests that enhanced androgenic signaling may contribute to tumor development or progression (21). Notably, even in women, androgen/AR signaling can influence bladder cancer growth, indicating that androgens are not exclusively male hormones in the context of cancer pathogenesis (11). These findings align with numerous investigations examining AR expression variations in human bladder tumors and the association with different disease characteristics (23,24). Early research has shown that bladder cancer tissue has higher levels of AR than control tissue, indicating that AR is upregulated in malignancy (25). Furthermore, AR expression has been strongly associated with tumor stage and grade, with patients harboring AR-positive tumors demonstrating a worse prognosis than those with AR-negative tumors (26). Analysis of both non-metastatic and metastatic malignancies has revealed that a higher percentage of metastatic lesions express AR compared with primary tumors, supporting the concept that AR-positivity is associated with metastatic potential (27). However, other research has shown no statistically significant difference in AR expression between male and female patients, with AR expressed at comparable levels in both sexes (28).

The interplay between elevated androgen levels and AR expression contributes to bladder cancer proliferation and differentiation through multiple interconnected mechanisms that promote cellular proliferation and malignant transformation (29). Upon androgen binding, the AR complex translocates to the nucleus and functions as a transcription factor, upregulating cell cycle regulators, including cyclins and cyclin-dependent kinases, that drive G_1/S phase transition while simultaneously suppressing apoptotic pathways through increased Bcl-2 expression and decreased pro-apoptotic factor activity (30). AR signaling also exhibits cross-talks with critical growth factor pathways, enhancing EGFR activity and activating the PI3K/AKT/mTOR cascade, both of which are essential for cell survival and proliferation, while stimulating VEGF expression to promote tumor angiogenesis (31). Given these findings, previous hypotheses have emphasized androgenic activity, rather than AR expression

alone, as a key driver of bladder cancer biology, since systemic testosterone levels strongly influence AR activation (32).

In contrast to the upregulated androgen pathway, the findings of the present study demonstrated elevated estrogen levels accompanied by downregulation of ESR1 expression in bladder cancer tissue, indicating a disruption in estrogen/ER signaling. This disruption in the estrogen/ER axis has notable implications for bladder cancer pathogenesis, as estrogen signaling has been demonstrated to regulate critical cellular mechanisms including cell cycle control, apoptosis and differentiation, thereby exerting protective effects against tumorigenesis in various tissues (33). The protective role of estrogen in bladder carcinogenesis is supported by epidemiological evidence showing that postmenopausal women have a higher risk of bladder cancer than premenopausal women (34). Furthermore, women who reach menopause at a younger age have a markedly increased risk of bladder cancer, supporting the concept that estrogens may inhibit bladder cancer incidence (35). Additional clinical studies have demonstrated that higher frequencies of estrogen exposure lead to lower bladder cancer incidence. For example, parous women who have experienced elevated E2 during pregnancy and those who used estrogen and progestin for hormonal therapy have a lower risk of bladder cancer formation, again suggesting that high estrogen exposure decreases bladder cancer risk (36). Molecularly, ESR1 expression has been found to be significantly downregulated in high-grade or muscle-invasive bladder tumors (37). Consistently, Kontos *et al* (38) reported a clear and significant downregulation of ESR1 expression in bladder cancer tissue, further highlighting the homogeneity in ESR1 expression profiles across different bladder cancer subtypes and stages.

Several mechanisms have been proposed to explain how altered ER expression and impaired ER signaling contribute to bladder cancer progression. Gucalp *et al* (27) proposed that the observed reduction in ER expression may result from epigenetic silencing through promoter hypermethylation of the ESR1 gene, a well-characterized phenomenon documented across various malignancies. A mechanistic study has suggested that ESR1 controls the expression of inositol polyphosphate-4-phosphatase type IIB to reduce AKT activity and consequently suppress bladder cancer cell proliferation (39). Beyond transcriptional regulation, ER signaling has been implicated in modulating non-coding RNA networks, including microRNAs (miRs), circular RNAs (circRNAs) and enhancer RNAs (eRNAs), all of which serve crucial roles in bladder cancer progression. ESR1 has been demonstrated to induce miR-4324 expression through direct promoter binding in bladder cancer cells, thereby suppressing cellular proliferation and metastatic potential (40). Furthermore, ESR1 downregulates circ_0023642 expression by modulating its host gene, UVRAG, which subsequently upregulates miR-490-5p and leads to decreased EGFR expression, ultimately inhibiting bladder cancer cell invasion (41). Conversely, knockdown experiments targeting estrogen-responsive eRNAs, specifically eGREB1 and P2RY2e, in bladder cancer cell lines resulted in notable inhibition of proliferation, migration and invasion, coupled with enhanced apoptosis, suggesting that these eRNAs may exert oncogenic functions (42). The simultaneous elevation of AR signaling and suppression of ER signaling observed in the present study suggests that a critical hormonal imbalance may drive bladder cancer progression in female patients by tipping cellular signaling towards growth and survival rather than differentiation or suppression.

The present study has certain limitations that should be considered. Specifically, only AR and ESR1 were evaluated, while other sex hormone receptors, including ESR2 and progesterone receptor, which may also have role in the pathogenesis of bladder cancer. Furthermore, receptor expression was assessed only at the mRNA level through gene expression analysis, therefore, the functional relevance of these findings needs to be further confirmed at the protein level in future studies.

In conclusion, the findings of the present study demonstrate a distinct pattern of hormonal dysregulation in female patients with bladder cancer, characterized by elevated serum levels of both testosterone and estrogen and significant changes in receptor expression. Despite an observed increase in circulating estrogen, ESR1 gene expression was downregulated in bladder cancer tissues, indicating disrupted estrogen-mediated protective signaling. Conversely, AR expression was significantly upregulated, which together with elevated testosterone levels, suggests enhanced pro-oncogenic androgenic activity within the tumor microenvironment. Collectively, these findings revealed a critical hormonal imbalance favoring pro-oncogenic androgen signaling while diminishing estrogen-related protection, which may represent a notable factor in bladder cancer pathogenesis and progression in women. These results highlighted the potential importance of the AR/ER axis as a therapeutic target and suggest that modulating AR activity or restoring ER function may offer novel treatment strategies for female patients with bladder cancer.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

NML and IEA contributed to design and planning of study. FGL collected and analyzed data and wrote the manuscript. NML reviewed and edited the manuscript. IEA participated in data processing. NML and IEA supervised the study. FGL and NML confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the relevant institutional review board of Mustansiriyah University (approval no. B CSMU/1024/0062Z). All data were prospectively obtained from medical records in compliance with the principles to 1964 Helsinki Declaration and its later amendments. Written

informed consent was obtained from all participants included in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. GLOBOCAN 2022: Bladder cancer 9th most common worldwide-World Bladder Cancer Patient Coalition.
2. Leslie SW, Soon-Sutton TL and Aeddula NR: Bladder cancer. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2025.
3. Mushtaq J, Thurairaja R and Nair R: Bladder cancer. Surgery (Oxford) 37: 529-537, 2019.
4. van Kessel K: Personalized bladder cancer management. Erasmus Universiteit Rotterdam (EUR), 2018.
5. Kamat AM and Black PC: Bladder cancer: A practical guide. Springer International Publishing, Cham, 2021.
6. Heldin CH, Lu B, Evans R and Gutkind JS: Signals and receptors. Cold Spring Harb Perspect Biol 8: a005900, 2016.
7. Khalil B, Miller EJ and Lappin SL: Physiology, cellular receptors. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2025.
8. Iqbal MJ, Kabeer A, Abbas Z, Siddiqui HA, Calina D, Sharifi-Rad J and Cho WC: Interplay of oxidative stress, cellular communication and signaling pathways in cancer. Cell Commun Signal 22: 7, 2024.
9. Hu H, Zhou H and Xu D: A review of the effects and molecular mechanisms of dimethylcurcumin (ASC-J9) on androgen receptor-related diseases. Chem Biol Drug Des 97: 821-835, 2021.
10. Estébanez-Perpiñá E, Bevan CL and McEwan IJ: Eighty years of targeting androgen receptor activity in prostate cancer: The fight goes on. Cancers (Basel) 13: 509, 2021.
11. Li P, Chen J and Miyamoto H: Androgen receptor signaling in bladder cancer. Cancers (Basel) 9: 20, 2017.
12. Chen P, Li B and Ou-Yang L: Role of estrogen receptors in health and disease. Front Endocrinol (Lausanne) 13: 839005, 2022.
13. Eyster KM: The estrogen receptors: An overview from different perspectives. Methods Mol Biol 1366: 1-10, 2016.
14. Joshi CJ, Ke W, Drangowska-Way A, O'Rourke EJ and Lewis NE: What are housekeeping genes? PLoS Comput Biol 18: e1010295, 2022.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. White MC, Holman DM, Boehm JE, Peipins LA, Grossman M and Henley SJ: Age and cancer risk: A potentially modifiable relationship. Am J Prev Med 46: S7-S15, 2014.
17. Al-Adilee MN and Al-Abassi HM: The Role of Caspase-8, MLKL and RIPK1 in Iraqi Patients' women with breast cancer. Al-Mustansiriyah J Sci 36: 22-33, 2025.
18. Rossi DJ, Jamieson CHM and Weissman IL: Stems cells and the pathways to aging and cancer. Cell 132: 681-696, 2008.
19. Biagetti B and Puig-Domingo M: Age-related hormones changes and its impact on health status and lifespan. Aging Dis 14: 605-620, 2023.
20. Ganguly S, Naik D, Muskara A and Mian OY: The nexus of endocrine signaling and cancer: How steroid hormones influence genomic stability. Endocrinology 162: bqaa177, 2021.
21. Miyamoto H, Yang Z, Chen YT, Ishiguro H, Uemura H, Kubota Y, Nagashima Y, Chang YJ, Hu YC, Tsai MY, *et al*: Promotion of bladder cancer development and progression by androgen receptor signals. J Natl Cancer Inst 99: 558-568, 2007.
22. Cao J, Yang X, Li J, Wu H, Li P, Yao Z, Dong Z and Tian J: Screening and identifying immune-related cells and genes in the tumor microenvironment of bladder urothelial carcinoma: Based on TCGA database and bioinformatics. Front Oncol 9: 1533, 2020.
23. Chen J, Huang CP, Quan C, Zu X, Ou Z, Tsai YC, Messing E, Yeh S and Chang C: The androgen receptor in bladder cancer. Nat Rev Urol 20: 560-574, 2023.
24. Sottnik JL, Vanderlinden L, Joshi M, Chauca-Diaz A, Owens C, Hansel DE, Sempeck C, Ghosh D and Theodorescu D: Androgen receptor regulates CD44 expression in bladder cancer. Cancer Res 81: 2833-2846, 2021.
25. Lombard AP and Mudryj M: The emerging role of the androgen receptor in bladder cancer. Endocrine-related cancer 22: R265-R277, 2015.
26. Rangel N, Rondon-Lagos M, Annaratone L, Osella-Abate S, Metovic J, Mano MP, Bertero L, Cassoni P, Sapino A and Castellano I: The role of the AR/ER ratio in ER-positive breast cancer patients. Endocr Relat Cancer 25: 163-172, 2018.
27. Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, Blackwell K, Rugo H, Nabell L, Forero A, *et al*: Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. Clin Cancer Res 19: 5505-5512, 2013.
28. Mashhadi R, Pourmand G, Kosari F, Mehrsai A, Salem S, Pourmand MR, Alatab S, Khonsari M, Heydari F, Beladi L and Alizadeh F: Role of steroid hormone receptors in formation and progression of bladder carcinoma: A case-control study. Urol J 11: 1968-1973, 2014.
29. Godoy G, Gakis G, Smith CL and Fahmy O: Effects of androgen and estrogen receptor signaling pathways on bladder cancer initiation and progression. Bladder Cancer 2: 127-137.
30. de Brot S and Mongan NP: The cell cycle and androgen signaling interactions in prostate cancer. In: Precision Molecular Pathology of Prostate Cancer. Robinson BD, Mosquera JM, Ro JY and Divatia M (eds). Springer International Publishing, Cham, pp381-404, 2018.
31. Izumi K, Zheng Y, Li Y, Zaengle J and Miyamoto H: Epidermal growth factor induces bladder cancer cell proliferation through activation of the androgen receptor. Int J Oncol 41: 1587-1592, 2012.
32. Izumi K, Mizokami A, Lin WJ, Lai KP and Chang C: Androgen receptor roles in the development of benign prostate hyperplasia. Am J Pathol 182: 1942-1949, 2013.
33. Goto T and Miyamoto H: The role of estrogen receptors in urothelial cancer. Front Endocrinol (Lausanne) 12: 643870, 2021.
34. McGrath M, Michaud DS and De Vivo I: Hormonal and reproductive factors and the risk of bladder cancer in women. Am J Epidemiol 163: 236-244, 2006.
35. Wolpert BJ, Amr S, Ezzat S, Saleh D, Gouda I, Loay I, Hifnawy T, Mikhail NN, Abdel-Hamid M, Zhan M, *et al*: Estrogen exposure and bladder cancer risk in Egyptian women. Maturitas 67: 353-357, 2010.
36. Davis-Dao CA, Henderson KD, Sullivan-Halley J, Ma H, West D, Xiang YB, Gago-Dominguez M, Stern MC, Castelao JE, Conti DV, *et al*: Lower risk in parous women suggests that hormonal factors are important in bladder cancer etiology. Cancer Epidemiol Biomarkers Prev 20: 1156-1170, 2011.
37. Miyamoto H, Yao JL, Chaux A, Zheng Y, Hsu I, Izumi K, Chang C, Messing EM, Netto GJ and Yeh S: Expression of androgen and oestrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. BJU Int 109: 1716-1726, 2012.
38. Kontos S, Kominea A, Melachrinou M, Balampani E and Sotiropoulou-Bonikou G: Inverse expression of estrogen receptor-β and nuclear factor-κB in urinary bladder carcinogenesis. Int J Urol 17: 801-809, 2010.
39. Flores-Estrada JJ, Jiménez A, Victoria-Acosta G, Cortés-Malagón EM, Ortiz-López MG, Alvarez-Sánchez ME, Nuñez-Olvera SI, Pérez-Navarro YF, Morales-Reyna M and Puente-Rivera J: Nuclear receptors in bladder cancer: Insights into miRNA-mediated regulation and potential therapeutic implications. Int J Mol Sci 26: 7340, 2025.
40. Ge Q, Lu M, Ju L, Qian K, Wang G, Wu CL, Liu X, Xiao Y and Wang X: miR-4324-RACGAP1-STAT3-ESR1 feedback loop inhibits proliferation and metastasis of bladder cancer. Int J Cancer 144: 3043-3055, 2019.
41. Wu L, Zhang M, Qi L, Zu X, Li Y, Liu L, Chen M, Li Y, He W, Hu X, *et al*: ERα-mediated alterations in circ_0023642 and miR-490-5p signaling suppress bladder cancer invasion. Cell Death Dis 10: 635, 2019.
42. Ding M, Liu Y, Li J, Yao L, Liao X, Xie H, Yang K, Zhou Q, Liu Y, Huang W and Cai Z: Oestrogen promotes tumorigenesis of bladder cancer by inducing the enhancer RNA-eGREB1. J Cell Mol Med 22: 5919-5927, 2018.