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Effect of androgen receptors as prognostic factors in patients with Bladder Cancer

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Abstract: Bladder cancer (BC) is one of the most common cancers in the world. These cases occur mainly in developed countries, but in developing countries, the incidence is also increasing due to changes in lifestyle. Bladder cancer is the 7th most common cancer worldwide among men and 17th among women. Subjects included in this case-controlled study constituted 100 cases presenting patients of bladder cancer which were selected from outpatient's clinic of urology and nephrology center, Mansoura University, Mansoura, Egypt, and 100 healthy as controls.

DNA extracted from whole blood DNA was genotyped androgen receptors (AR). The genes polymorphisms were determined using ARMS-PCR and detection of the results of genotyping by gel electrophoresis system and preparing 2.5% agarose gel with ethidium bromide for gel staining. Gels were electrophoresed for 30 minutes at 100 volts. Then the gels were photographed under UV light (320 nm) and scored for the resulting genotypes. The aim of this work aimed to investigate the effect of AR G1733A[rs6152] gene polymorphism on the genetic susceptibility for bladder cancer among Egyptian subjects.

Results

The present study showed no significance difference between BC and control group regarding age and sex respectively. Analysis of AR (rs6152 G>A) gene variant showed no significant differences between the BC and control group regarding to the genotypes AA and GA vs. GG and A allele vs. G frequencies. The testing of genetic models Codominant, Dominant, Recessive and of AR (rs6152 G>A) variant showed no significant association with the risk of BC. No significant association of the AR (rs6152 G>A) variant was found using the dominant and recessive models regarding demographic data and histopathological investigation (Tumor grade and stage). No significant association of the AR (rs6152 G>A) variant was found using the dominant and recessive models regarding the biochemical and hematological lab results.

Conclusion

Our results show that AR gene polymorphism (rs6152 G>A) is not associated with bladder cancer. However, future research with a larger sample size is needed to confirm these findings.

Key word: Bladder Cancer - Androgen receptor

1.Introduction

Bladder cancer (BC) is one of the most common cancers in the world. These cases occur mainly in developed countries, but in developing countries, the incidence is also increasing due to changes in lifestyle. Bladder cancer is the 7th most common cancer worldwide among men and 17th among women. At the same time, there are no biomarkers

available that provide accurate diagnostic information. Specifically, the Surveillance. The main risk factors for bladder cancer are age, smoking, alcohol abuse, obesity, and excessive consumption of red meat. It has been shown that chemicals such as aromatic amines and aniline dyes may also contribute to the development of metastatic bladder cancer (). Androgen binding begins with a conformational change in the androgen receptor and then transport of the receptor dimer to the nucleus where it binds to specific androgen response elements (AREs) in the regulatory regions of its target genes. In this way, the androgen receptor transmits the androgen message directly to the level of genetic programs **(1 and 2).** Absence of a ligand, AR is found primarily in the cytosol in a multiprotein complex with heat shock proteins, such as heat shock protein, immunophilins, p23, FKBP51, FKBP52, Cyp40, and serine/threonine phosphatases ()**.**

This study aimed to investigate the effect of Androgen receptors (AR) on the genetic susceptibility for bladder cancer among Egyptian patients.

Subjects & Methods

Subjects:

This study included 100 cases suffering from bladder cancer. The patients were collected randomly from the Outpatients' Clinic of Neurology and urology Center, Mansoura University, Egypt.

For comparison, a control sample was taken in the form of 100 healthy subjects with a negative family history of inflammatory disorders. Their approval was obtained from the local ethical and scientific committees in addition to an informed agreement that was obtained from all participants in the study.

Blood sampling

Venous blood samples were collected subsequently from each patient as well as from all the individuals in the control group. Each blood sample was divided into two aliquots: 2 ml was collected in sterilized vacutainers containing ethylene diamine tetra acetic acid (EDTA), Another sample of 3 milliliter blood was taken for assessment biochemical analysis was allowed to clot, centrifuged at 4000 rpm for 10 min, and then serum was separated and stored at -20° until used.

EDTA blood samples were used for the evaluation of the presence of single nucleotide polymorphisms (SNPs) for Androgen variants of genes, by ARMS-PCR. The samples also were used to make a complete blood count (CBC). The collected sera were used for performing some routine biochemical tests, including; liver enzymes [(alanine aminotransferase (ALT), aspartate aminotransferase (AST)], albumin, T. bilirubin, creatinine, Na and K.

Inclusion criteria

Patients are diagnosed with bladder cancer using all clinical and laboratory examinations. Healthy individuals were of matched age and sex, with normal CBC. Individuals in control groups appeared to be healthy and had no family history of cancer.

Exclusion criteria

Typical patients with co-morbidities such as renal insufficiency, hepatitis and heart diseases. Patients with inflammatory diseases and autoimmune disorders. Patients who received any interfering medication (inducers/inhibitors) for any chronic disease.

Detection of AR G1733A (rs6152) gene polymorphis according to **(3).** The DNA was amplified using specific oligonucleotide primers:

Forward inner (FI): 5-CAG CAG CGG GAG AGC GAG GTA G-3

Reverse inner (RI) : 5-AAG TGG GAG CCC CCG AGT CT-3

Table (1): Demographic data of the groups studied.

Variable	BC (n=120)	Control (n=100)	p-value
Age (years) mean ± SD	58.02 ± 11.68	58.0 ±10.0	p=0.98 t=0.01
Sex, n(%)			
Males	97 (81%)	80 (80%)	p=0.87 x2=0.02
Females	23 (19%)	20 (20%)	

Forward outer (FO) : 5-CAAGCCCATCGTAGAGGCCC-3

Reverse outer (RO): 5-GCCAATGGGGCACAAGGAGT-3

Measuring the concentration of Potassium and sodium using Enzymatic method Fixed Time (Coromatest, Linear Chemicals. S.L):

PCR amplification: Denature DNA for separation of the 2 strands. Step 2: Primers Anneal for binding of the primers with their complementary sequences on the single strands of DNA. Step 3: DNA polymerase extends the DNA by adding nucleotides to the 3' ends of the primers.

Gel Electrophoresis: The Agarose gel electrophoresis process depend on the charge of DNA; DNA is negatively charged, to separate it by size it is put in a solution which pulls the negatively charged DNA to the opposite end, DNA fragments migrate according to their weight while smaller parts go faster than larger ones. When the electrical current runs, we add a dye to see the bands of DNA. The size of the fragment can therefore be determined by calibrating the gel, by the use of known size standards and comparing the distance of unknown complex DNA**.**

Data interpretation

Data analysis was performed by Statistical Package for the Social Sciences (SPSS) software version 26 (SPSS Inc., PASW Statistics for Windows version 26. Chicago: SPSS Inc.).

Table (2): The histopathological investigations of the tumor grade and TNM stages of BC group.

Variable	n (%)
Tumor grade	
GI	58 (48.3%)
GII	23 (19.1%)
GIII	39 (32.6%)
T stage	
T0	15 (12.5%)
T1	25 (20.8%)
T2	37 (30.8%)
T3	39 (32.5%)
T4	4 (3.4%)
N stage	
N0	23 (19%)
N1	25 (20.8%)
N2	25 (20.8%)
N3	15 (12.7%)
Nx	32 (26.7%)
M stage	
Mo	97 (81%)
M1	23 (19%)
TURBT	
Performed	45 (37.5%)
Not-performed	75 (62.5%)

Results

Demographic data of studied groups

The current study was conducted on 100 patients with bladder cancer (BC), having a mean age (58.02 ± 11.68 y), ranged from 26 y to 78 y; and divided to (81%) males and (19%) females. In addition, 100 healthy individuals were included as a control group, Figures (1, 2). Table (1) showed no significant difference between BC and control groups regarding age and sex (p=0.98 and 0.87, respectively).

Data were expressed as mean ± SD, or frequency (percentage). Student t-test and Chi-square test were applied, BC: Bladder cancer.

Data were expressed as frequency (percentage). . BC: Bladder cancer, TURBT: Trans urethral removal of bladder tumor.

Our data shows that no significant difference between the BC and control group considering the lab results (p>0.05).

Table (3): Comparison of the lab measurements between the BC and control groups.

Parameter	BC (n=120)	Control (n=100)	*p-value*
WBCs ($\times10^9$/L)	7.7 ± 1.50	8.0 ± 2.0	*t=1.26 p=0.20*
Lymphocytes ($\times10^9$/L)	1.81 ± 0.66	1.98 ± 0.80	*t=1.72 p=0.08*
Neutrophils ($\times10^9$/L)	4.7 ± 1.42	5.0 ± 0.92	*t=1.81 p=0.07*
Platelets ($\times10^9$/L)	*251 ± 60*	*250 ± 50*	*t=0.13 p=0.89*
RBCs ($\times10^{12}$/L)	*4.51 ± 0.76*	*4.4 ± 0.68*	t=1.12 *P*=0.26
Hemoglobin (g/dl)	12.97 ± 1.21	13.01 ± 0.88	*t=0.27 p=0.78*
ALT (U/L)	30.26 ± 4.14	29.46 ± 4.04	*t=1.44 p=0.15*
AST (U/L)	31.18 ± 5.34	29.9 ± 4.7	*t=1.86 p=0.06*
Bilirubin (mg/dl)	0.81 ± 0.19	0.76 ± 0.77	*t=0.64 p=0.50*
Albumin (g/dl)	4.2 ± 0.36	4.27 ± 0.30	*t=1.9 p=0.06*
ALP (U/L)	180 ± 71.0	202 ± 56.0	*t=1.94 p=0.05*
Creatinine (mg/dl)	1.1 ± 0.13	1.07 ± 0.18	*t=1.43 p=0.15*
RBS (mg/dl)	124.9 ± 8.05	127 ± 9.0	*t=1.82 p=0.07*
Sodium (mmol/L)	138.8 ± 6.0	140 ± 7.0	*t=1.36 p=0.17*
Potassium (mmol/L)	4.2 ± 0.8	4.0 ± 0.79	*t=1.85 p=0.06*

Parameters were expressed as mean ± SD, Student-t test was applied. BC: Bladder cancer,

WBCs: White blood cells, RBCs: Red blood cells, ALT: Alanine aminotransferase, AST: Aspartate, ALP: Alkaline phosphatase, RBS: Random blood sugar

Analysis of Androgen Receptor (AR) (rs6152 G>A) gene variant

Analysis of AR (rs6152 G>A) gene variant showed no significant difference between the BC and control group regarding the genotypes and allele frequencies, Table 4

Association of the AR (rs6152 G>A) variant with clinical data& lab results

No significant association of the AR (rs6152 G>A) variant was found using the dominant and recessive models regarding the demographic data and histopathological investigation, (Table 5).

Table (4): The genotypic and allelic frequencies of the AR (rs6152 G>A)variant among the study participants

Genetic polymorphisms	**BC (n=120)**	**Controls (n=100)**	**OR (95% CI)**	***p-value***
AR (rs6152 G>A)				
Genotypic frequencies	n (%)	n (%)		
GG	55 (45.8%)	56 (60%)	1.0	
GA	45 (37.5%)	35 (36%)	1.3 (0.73 – 2.3)	0.30
AA	20 (16.7%)	9 (60%)	2.26 (0.94 – 5.4)	0.06
HWE	χ^2=0.22 *p*=0.59	χ^2=0.23, *p*=0.63		
Allelic frequencies				
G	155 (64.5%)	147 (73.5%)	1.0	
A	85 (35.4%)	53 (26.5%)	1.52 (1.00 – 2.3)	0.05

Data are presented as frequency (percentage); Chi-square test was applied. **BC:** Bladder cancer; **OR:** Odds Ratio; **CI:** Confidence Intervals; **HWE:** Hardy-Weinberg equilibrium.

Table (5**):** Association of AR *(rs6152 G>A)* with demographic data and histopathological investigation among the BC patients

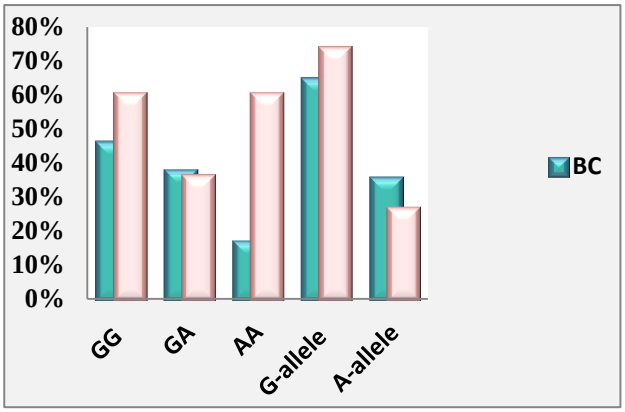
Parameters	**ANR (rs6152 G>A)**					
	BC (n = 120)		***p-value***	**BC (n = 120)**		***p-value***
	DominantGA+AA (n = 65)	**GG (n = 55)**		**Recessive AA (n = 20)**	**GG + GA (n = 100)**	
Demographic data						
1. Age (Years), mean ± SD	59.04 ± 11.31	56.79 ± 12.1	0.29	60.1 ± 11.5	57.6 ± 11.73	0.38
2. Sex, n (%)MalesFemales	54 (83%)11 (17%)	43 (78.2%) 12 (21.8%)	0.49	18 (90%) 2 (10%)	79 (79%) 21 (21%)	0.25
Pathological investigations						
1. Tumor grade, n(%) GI+GIIGIII	41 (34.1%) 24 65.9%)	40 (72.7%) 15 (27.3%)	0.26	10 (50%) 10 (50%)	71 (71%) 29 (29%)	0.06
2. T stage, n(%)T0+T1 T2+T3+T4	19 (29.2%) 46(70.8%)	21 (38.2%) 34 (61.8%)	0.30	5 (25%) 15 (75%)	35 (35%) 65 (65%)	0.36
3. N stage, n(%)N0+N1 N2+N3+Nx	33 (50.7%) 32(49.3%)	15 (27.3%) 40 (72.7%)	0.70	6 (30%) 14 (60%)	42 (42%) 58 (58%)	0.31
4. M stage, n(%)M0 M1	53 (81.5%) 12(18.5%)	46 (83.6%) 9 (16.4%)	0.06	18 (90%) 2 (10%)	79 (79%) 21 (21%)	0.25

Table (6): The genotypic and allelic frequencies of the *AR (rs6152 G>A)*variant among the study participants

Genetic polymorphisms	**BC(n=120)**	**Controls(n=100)**	**OR (95% CI)**	***p-value***
AR (rs6152 G>A)				
Genotypic frequencies	**n (%)**	**n (%)**		
GG	55 (45.8%)	56 (60%)	1.0	
GA	45 (37.5%)	35 (36%)	1.3 (0.73 – 2.3)	**0.30**
AA	20 (16.7%)	9 (60%)	2.26 (0.94 – 5.4)	**0.06**
HWE	χ^2=0.22 *p*=0.59	χ^2=0.23, *p*=0.63		
Allelic frequencies				
G	155 (64.5%)	147 (73.5%)	1.0	
A	85 (35.4%)	53 (26.5%)	1.52 (1.00 – 2.3)	**0.05**

Data are presented as frequency (percentage); Chi-square test was applied. **BC:** Bladder cancer; **OR:** Odds Ratio; **CI:** Confidence Intervals; **HWE:** Hardy-Weinberg equilibrium.

Figure (4): The genotypic and allelic frequencies of the AR (rs6152 G>A) variant among patients with BC and controls**.**



The genetic models of AR (rs6152 G>A) variant showed no significant association with the risk of bladder cancer, Table (5).

No significant association of the AR (rs6152 G>A) variant was found using the dominant and recessive models regarding the biochemical and hematological lab results, (Table 7).

Discussion

Bladder Cancer (BC) is a complex, multifactorial disease and a very common malignancy of the genitourinary tract (Parkin, 2004). Bladder cancer develops through a series of genetic changes such as chromosomal alterations and loss of cell cycle regulation that leads to tumor development **(4).** Therefore, it is necessary to explore different gene polymorphisms such as VDR polymorphism in the search for candidate genes that may be useful for screening and assessing bladder cancer risk.

The androgen receptor is a ubiquitous receptor responsible for responses to androgen stimuli. Androgens, a hormone related to testosterone, are essential for normal male sexual development. However, one polymorphism in the AR gene, G1733A (rs6152), has been associated with several clinical risks such as cardiovascular disease (CD), androgenetic alopecia, high prostate-specific antigen (PSA) levels, male infertility, recurrent spontaneous abortion and prostate cancer **(3).** Androgen receptors (AR) widespread expression in urothelial carcinoma, its putative role in UC progression, and the availability of several novel androgen receptor-targeted therapies make it an attractive therapeutic target in the clinic. Androgen receptor signaling plays an important role in bladder cancer formation and progression and mediates resistance to cisplatin-based chemotherapy, providing a basis for targeting androgen receptors in bladder cancer **(5).**

Signaling of androgen receptor is very important in the development of bladder cancer. Previous clinical studies shown that AR activates several potential tumor-promoting signaling pathways and appears to significantly alter antitumor immunity. Thus, suppression of androgen signaling in multiple clinical populations has been associated with a reduced risk of bladder cancer development and recurrence **(6).**

The role of androgen receptors in the development of bladder cancer remains unclear, with scant information in the literature **(7).** Few studies, with conflicting results, have examined the role of androgen receptors in bladder cancer, probably due to different investigational methods. Lore and colleagues reported that androgen receptor expression is higher in bladder cancer than in normal bladder lining **(8).** Nam et al., **(9)** reported that expression of androgen receptors was associated with a lower risk of disease recurrence in patients with non-muscle invasive bladder cancer (NMIBC). This report contradicts our results that showed no association between the SNP AR gene (rs6152 G>A) variant and bladder cancer patients while our results similar to another study found no significant association between AR expression and outcomes **(10).**

The current study, which contrasts with **(11),** explored that AR is involved in bladder carcinogenesis and thus represents a potential target for bladder cancer therapy. In previous years, several studies have sought to characterize AR expression in bladder cancers and determine whether differences in expression are associated with different aspects of the disease. Early studies showed that AR was present in bladder cancer tissue and that AR was present at higher levels in tumor tissue compared to control tissue suggesting upregulation of AR in malignant tumors (8). These studies support the our results **(12 - 17).**

The present study showed no significant association between the AR variant (rs6152 G>A) using dominant and recessive models

with demographic data (age and sex) (p=0.98 and 0.87, respectively) and histological investigation (tumor grade and stage) p>0.05. No significant association was found between the AR variant (rs6152 G>A) using dominant and recessive models with respect to biochemical and hematological laboratory findings (p>0.05).

The current study shows that the analysis of ulcerative colitis patients found no association between AR receptor expression and gender (p = 0.23). Our results supported by the study **(18),** there were no associations between androgen receptor expression and tumor stage at diagnosis or subsequent treatment. On the other hand, our result consistent with **(19),** explored the expression patterns of AR tested in relation to tumor stage and grade, and were not associated with tumor stage and grade. In contrast to **(20),** who found that the AR gene was significantly associated with an increased risk of grade 4-5 prostate cancer and pT3b/4 disease (all p < 0.05).

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