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Serum level and single-nucleotide polymorphisms of toll-like receptor-7 among urinary bladder cancer Iraqi patients

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Abstract

Background: Toll-like receptor 7 (TLR7), a member of TLR family, plays a pivotal role in pathogenesis of different malignancies. Among these is urinary bladder cancer (UBC), which has not been extensively studied. Therefore, it was aimed to determine TLR7 serum level in UBC patients and evaluate its association with some demographic and clinicopathological characteristics. In addition, four *TLR7* single-nucleotide polymorphisms (SNPs: rs179018, rs179019, rs179020, and rs179021) were investigated to determine their susceptibility role in UBC and inspect SNP's impact on TLR7 level. Sixty-six UBC Iraqi patients were enrolled in this case-control study. Two control samples were also involved, 40 urinary tract infection (UTI) patients, and 48 healthy control subjects.

Results: Male gender, older age, and cigarette-smoking are risk factors for UBC. TLR7 level showed a significant decreased median in UBC patients compared to UTI patients or control (1.4 vs. 8.1 and 9.5 ng/ml, respectively; $p < 0.001$). The decrease was more pronounced in males, age group ≥ 48 years, cigarette-smokers, alcohol non-consumers, clinical stages I–II, and superficial tumor, as well as patients with family history of cancer and untreated patients. Mitomycin C and Bacillus Calmette–Guérin therapies tended to increase TLR7 level. Among the four investigated SNPs, only rs179019 *C* allele showed significantly uncorrected increased frequency in UBC males compared to control males ($p = 0.038$), while among UTI females, *C* allele frequency maintained a significantly corrected decreased frequency compared to control females ($p = 0.005$). Some SNPs influenced serum level of TLR7, but a significant impact was recorded for rs179019 in UTI females ($p = 0.006$).

Conclusions: Downregulation of TLR7 is suggested to have a role in etiology and pathogenesis of UBC, especially the male, elderly and smoker patients. Mitomycin C and Bacillus Calmette–Guérin may enhance TLR7 production in the blood of UBC patients. *TLR7* SNPs are suggested to influence susceptibility to develop UBC, and their potential in impacting TLR7 serum level is augmented.

Keywords: Urinary bladder cancer, Urinary tract infection, Toll-like receptor 7, Single-nucleotide polymorphism, Enzyme-linked immunosorbent assay, DNA-sequencing, Odds ratio

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Background

Urinary bladder cancer (UBC) is a global disease and ranked worldwide as the seventh most prevalent cancer in men with a male to female ratio of 4:1. In the Western world, UBC is the fourth most common cancer in men, while in women; it is the eighth most common cancer [1]. In Iraq, UBC is the sixth most common malignancy, and three times more frequently occurs in men than in women (Iraqi Cancer Board 2018) [2].

Environmental factors are involved in the etiology of UBC, and a list of risk factors has been established; for instance, occupational exposure to some chemical compounds, pelvic radiation, use of certain medications (cyclophosphamide), chronic urinary tract infection (UTI), and cigarette-smoking. Other risk factors include male gender, older age, race, family history of UBC and other cancers, obesity, diabetes mellitus, infection with human papillomavirus, and *Schistosoma haematobium* infestation [3]. In addition, genetic factors influence susceptibility to UBC, and its genetic basis has gained an increasing interest. Twin studies revealed that 31% of UBC risk can be explained by heritable factors; however, heritability assessment in sporadic cases was in favor of a small inherited component, while genetic polymorphism studies reported inconsistent associations between certain genetic markers and risk of UBC [4]. In a recent systemic analysis, gene-environment interactions have been introduced as the main etiological risk factors in UBC [5].

Among the genetic markers that have been investigated are toll-like receptors (TLRs). They are a family of transmembrane receptors involved in recognizing conserved molecular patterns of a microbial origin. In addition to their role in maintaining tissue hemostasis due to inflammation, TLRs recruit leukocytes for microbial-infected tissues, and consequently, the innate and adaptive immune responses are induced [6]. These receptors are expressed by peripheral immune cells and urinary bladder epithelium, and their role in activating anti-UBC immune response has been introduced; especially, TLR-2, TLR-4, TLR-7, and TLR-9 [7]. In vitro and in vivo evaluations revealed their agonist effects in activating anti-tumor immunity and to determine the immunotherapy potential. The results are valuable, and TLR-7 agonists against UBC have been recognized; therefore, it is suggested that targeting TLR-7 is a promising strategy for both antiviral and anti-tumor therapies [8].

With respect to genetic-association studies, four *TLR* single-nucleotide polymorphisms (SNPs), *TLR2* (-196 to-174del), *TLR3* (C1377T), *TLR4* (Thr399Ile), and *TLR9* (G2848A) genes, were investigated in North Indian UBC patients. It was demonstrated that *TLR2* SNP is involved in UBC susceptibility, while the other SNPs were not associated with risk of disease [9]. In a further study, two *TLR4* SNPs (-729G/C and -260G/C) were genotyped in Chinese patients, and the data were in favor of a positive association with UBC [10]. The $TLR4_{-729}$ G/C SNP has also been confirmed to be associated with risk of developing UBC in Chinese [11].

In the present work, it was aimed to determine TLR7 level in a sera of UBC patients and evaluate its association with some demographic and clinicopathological characteristics. A focus on four *TLR7* gene SNPs (rs179018, rs179019, rs179020, and rs179021) was also implemented to determine their susceptibility role in UBC. Equally important, SNP's impact on TLR7 serum level was inspected in UBC patients. To the best of our knowledge, it is the first detailed evaluation of TLR7 in UBC.

Methods

Populations studied

During May 2017–February 2018, 66 diagnosed UBC Iraqi patients were recruited at the outpatient Urology Clinic of Baghdad Teaching Hospital (Baghdad, Iraq). Their age median (range) was 62 (24–90) years. Clinical characteristics of patients were defined by the consultant surgeons at the hospital. For each patient, the following information was obtained: gender, age, status of cigarette-smoking and alcohol-consuming, family history of UBC or other cancers in their blood relatives and up to the third degree, clinical stage and invasiveness of tumor, and current types of therapy (Table 1). Two control samples were also enrolled. The first included 40 chronic UTI patients. They were also recruited at the outpatient urology clinic, in which their diagnosis was made by the urologists. Urine profile (microscopical examination and culture) was suggestive of chronic UTI. Their age median (range) was 41.5 (22–70) years. The second control involved 48 potential blood donors who were healthy and their blood-testing profile at the Blood Bank (Baghdad, Iraq) was negative. Their age median (range) was 40.5 (18–68) years. All participants were randomly selected, and they were genetically unrelated. The participants provided their written informed consent to be included in the study. The study protocol was approved by the Ethics Committee at the Iraqi Ministry of Health.

Blood collection

Five milliliters of venous blood were collected from each participant. The blood was divided into two aliquots; the first was dispensed in a plain tube to collect serum, while the second was drawn in EDTA tube and stored at − 20 °C until DNA isolation.

Table 1 Baseline characteristics of urinary bladder cancer and chronic urinary tract infection patients and control

Characteristic	UBC (*N* = 66)		UTI (*N* = 40)		Control (*N* = 48)		*p*
	N	%	*N*	%	*N*	%	
Gender							< 0.001
Male	59	89.4	8	20.0	24	50.0	
Female	7	10.6	32	80.0	24	50.0	
Age group							< 0.001
< 48 years	18	27.3	28	70.0	40	83.3	
≥ 48 year	48	72.7	12	30.0	8	16.7	
Cigarette-smoking							< 0.001
Yes	51	77.3	6	15.0	20	41.7	
No	15	22.7	34	85.0	28	58.3	
Alcohol-consuming							< 0.010
Yes	20	30.3	2	5.0	4	8.3	
No	46	69.7	38	95.0	44	91.7	
Family history							
Yes	13	19.7					
No	53	80.3					
Clinical stage							
I–II	41	62.1					
III–IV	25	37.9					
Tumor invasiveness							
Superficial	29	43.9					
Invasive	37	56.1					
Type of therapy							
Untreated	19	28.8					
Methotrexate	33	50.0					
BCG	14	21.2					

BCG Bacillus Calmette–Guérin, *N* absolute number, *p* Pearson's chi-square test probability, *UBC* urinary bladder cancer, *UTI* urinary tract infection

Serum level of TLR7

Enzyme-linked immunosorbent assay kit (MyBioSource, Canada) was used to determine serum level of TLR7, and instructions of the manufacturer were followed.

TLR7 gene SNPs

Genomic DNA was isolated from EDTA blood by the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). The isolated DNA was subjected to PCR amplification using designed forward (5′-GGTGAGAAAC CCTGCTATAAA-3′) and reverse (5′-CAAACATCTG CTCCTCCTC-3′) primers, which were employed to amplify intron 2 region of human chromosome X containing rs179018, rs179019, rs179020, and rs179021 SNPs (chrX:12866832+12867830). The PCR protocol and conditions were as previously described [12]. The amplified PCR fragments were subjected to Sanger's sequencing using ABI3730XL automated DNA sequencer (Macrogen Corporation—Korea). SNP genotypes were revealed by the Geneious software version 10.2.6 after alignment with reference gene sequences in the Gene Bank (Fig. 1).

Statistical analysis

Data of TLR7 serum level were first tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test), and accordingly, the level was given as median and range. Significant differences between medians were assessed by Mann–Whitney *U* or Kruskal–Wallis test. The statistical package SPSS (version 19.0) was computed to carry out these analyses. As *TLR7* is an X-linked gene, allele and genotype frequencies of SNPs were analyzed independently for males and females. Deviation from Hardy–Weinberg equilibrium (HWE) was assessed by Pearson chi-square test. Odds ratio (OR) and its 95% confidence interval (CI) were used to define the association between SNP and disease. Significance of association was assessed by two-tailed Fisher's exact probability (*p*). These estimations were carried out using the WINPEPI package (version 11.36). A probability (*p*) value ≤ 0.05 was considered significant after applying false

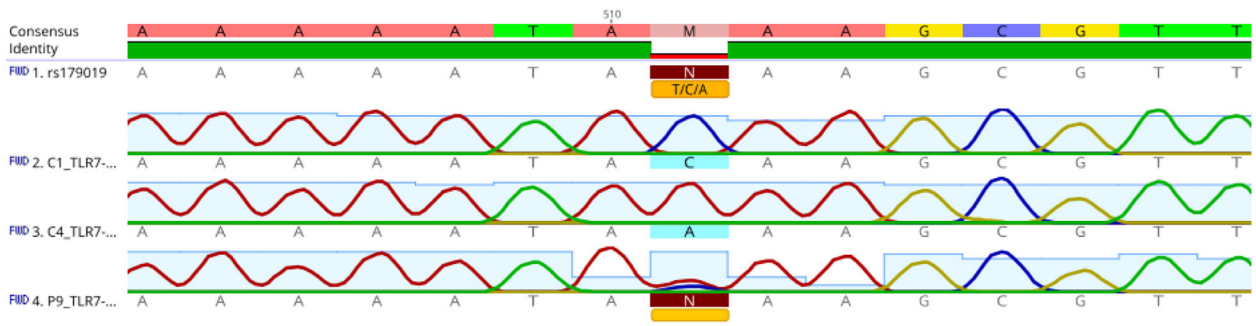

Fig. 1 DNA sequence chromatogram of *TLR7* gene SNP (T/C/A: rs179019) showing three genotypes: CC (sequence 2), CA (sequence 4) and AA (sequence 3), in addition to the reference sequence (rs179019), as revealed by Geneious software (version 10.2.6) analysis

discovery rate (FDR) to correct the p value due to multiple comparisons.

Results

Baseline characteristics of investigated samples

UBC patients had the highest median of age (62 years) compared to UTI patients (41.5 years) or control (40.5 years), and the difference was significant ($p < 0.001$). The age group ≥ 48 years was the most frequent among UBC patients (72.7%), while most of UTI patients were at the age group < 48 years (70.0%). With respect to gender, 89.4% of UBC patients were males, while 80.0% of UTI patients were females. Most UBC patients were cigarette-smokers (77.3%), while smoker UTI patients showed a lower frequency (15.0%). Alcohol-consuming was observed in 30.3% of UBC patients compared to 5.0% in UTI patients. Family history of UBC or other cancers was recorded in 19.7% of UBC blood relatives. More than 50% of UBC patients were at the clinical stages I–II (62.1%) and 56.1% had an invasive tumor. With respect to therapy, 21.2% of UBC patients received Bacillus Calmette–Guérin (BCG) vaccine, while 50% was administrated with mitomycin C (MMC) (Table 1).

Serum level of TLR7

Serum level of TLR7 showed a significant decreased median in UBC patients (total and characteristic subgroups) compared to UTI patients or control ($p < 0.001$). Some the characteristic subgroups tended to have a more decreased level (males, age group ≥ 48 years, smokers, alcohol non-consumers, clinical stages I–II, and superficial tumor, as well as patients with family history of UBC or other cancers), but without significant difference compared to the corresponding subgroups. Untreated UBC patients also tended to have the lowest serum level of TLR7, while the level was increased in association with MMC or BCG therapy, but without significant differences. Among UTI patients and control, the TLR7 serum levels were approximated, and moreover, there were no significant variations between its medians in their characteristic subgroups. Two exceptions were encountered in the control sample; males and alcohol-consumers showed a significant decreased median of TLR7 compared to females and alcohol non-consumers, respectively (Table 2).

TLR7 gene SNPs

TLR7 gene SNPs were independently analyzed for UBC males and UTI females because the gene is X-linked. In addition, UBC females and UTI males were excluded from SNP analyses due to small sample sizes. Allele frequencies of the four investigated SNPs (rs179018, rs179019, rs179020, and rs179021) showed no significant variation between UBC males and control males. The rs179019 SNP was an exception; *C* allele showed a significant increased frequency in UBC males compared to control males ($p = 0.038$), but the significance was lost when the p value was corrected (Table 3). An opposite observation was made in UTI females, and the *C* allele frequency maintained a significantly corrected decreased frequency compared to control females ($p = 0.005$) (Table 4).

TLR7 SNP-impact on TLR7 serum level

Although some SNP's alleles and genotypes showed variation in serum level of TLR7 among the four investigated groups (UBC males, control males, UTI females, and control females), no statistically significant level was attended. The SNP rs179019 was an exception, and CA genotyped showed a significant increased median of TLR7 compared to CC and AA genotypes in UTI females ($p = 0.006$) (Tables 5 and 6).

Discussion

The results presented suggest a risk effect for age, male gender, and cigarette-smoking in etiology of UBC. With respect to age, UBC is regarded as a disease that mostly afflicts middle-aged or elderly people. The recorded age range at diagnosis of UBC is 60–69 years [13]. The present UBC age median was within this range, and the

Table 2 Serum level of TLR-7 in urinary bladder cancer and urinary tract infection patients and control distributed according to some characteristics

Characteristic	Median (range); ng/ml			*p* (*pc*)		
	UBC (*N* = 66)	UTI (*N* = 40)	C (*N* = 48)	UBC vs. C	UTI vs. C	UBC vs. UTI
Total	1.4 (0.1–22.2)	8.1 (0.7–14.6)	9.5 (1.9–25.1)	< 0.001 (S)	0.089 (NS)	< 0.001 (S)
Gender						
Male	1.3 (0.1–22.1)	7.1 (4.1–14.6)	6.5 (1.9–25.1)	< 0.001 (S)	0.564 (NS)	< 0.001 (S)
Female	1.6 (0.6–3.4)	8.5 (0.7–14.5)	11.7 (5.6–17.0)	< 0.001 (S)	0.003 (S)	0.001 (S)
p (*pc*)	0.827 (NS)	0.654 (NS)	0.002 (S)			
Age group						
< 48 years	2.0 (0.1–22.2)	8.3 (0.9–14.6)	9.1 (1.9–25.1)	< 0.001 (S)	0.199 (NS)	< 0.001 (S)
≥ 48 years	1.1 (0.1–9.2)	7.2 (0.7–14.5)	10.4 (6.9–13.9)	< 0.001 (S)	0.343 (NS)	< 0.001 (S)
p (*pc*)	0.152 (NS)	0.919 (NS)	0.559 (NS)			
Cigarette-smoking						
Yes	1.3 (0.1–22.2)	7.1 (4.1–10.8)	8.8 (1.9–25.1)	< 0.001 (S)	0.387 (NS)	< 0.001 (S)
No	1.6 (0.1–7.9)	8.5 (0.7–14.6)	10.1 (2.9–17.0)	< 0.001 (S)	0.134 (NS)	< 0.001 (S)
p (*pc*)	0.933 (NS)	0.754 (NS)	0.668 (NS)			
Alcohol-consuming						
Yes	2.7 (0.1–9.8)	7.0 (6.8.2–7.2)	4.6 (1.9–7.2)	0.309 (NS)	0.533 (NS)	0.173 (NS)
No	1.1 (0.1–22.2)	8.3 (0.7–14.6)	10.4 (2.0–25.1)	< 0.001 (S)	0.04 (NS)	< 0.001 (S)
p (*pc*)	0.023 (NS)	0.785 (NS)	0.007 (S)			
Family history						
Yes	1.0 (0.1–6.7)					
No	1.6 (0.1–22.2)					
p (*pc*)	0.397 (NS)					
Clinical stage						
I–II	1.3 (0.1–22.2)					
III–IV	2.1 (0.1–9.8)					
p (*pc*)	0.193 (NS)					
Tumor invasiveness						
Superficial	1.1 (0.1–6.7)					
Invasive	1.9 (0.1–22.2)					
p (*pc*)	0.086 (NS)					
Type of therapy						
Untreated	1.1 (0.1–6.7)					
Methotrexate	1.8 (0.1–22.2)					
BCG	1.3 (0.1–7.9)					
p (*pc*)	0.464 (NS)					

BCG Bacillus Calmette–Guérin, *C* control, *N* absolute number, *NS* not significant ($p > 0.05$), *p* Mann–Whitney *U* test probability, *pc* Corrected *p*, *S* significant ($p \leq 0.05$), *UBC* urinary bladder cancer, *UTI* urinary tract infection

sixth decade of age is considered as a crucial risk factor for UBC. Male gender is a further risk factor, and most studies agree that UBC occurs more frequently in men than in women [5]. Two important explanations may justify the male-gender dominancy in UBC; males are at a greater chance to be exposed to environmental risk factors, and a potential for sex steroid hormone regulation has also been suggested to play an active role in UBC development and progression [14]. Cigarette-smoking is also a further well-established risk factor for UBC worldwide [15]. Interestingly, smoker-men were reported to have UBC more frequently than smoker-women, and accordingly, cigarette-smoking and male gender have been regarded as co-risk factors for UBC [16]. Emerging data

Table 3 Single-nucleotide polymorphisms of *TLR7* gene in males of urinary bladder cancer patients and control

TLR7 gene SNP	Allele	UBC males (*N* = 58)		Control males (*N* = 24)		OR	95% CI	*p* (*pc*)
		N	%	*N*	%			
rs179018	*T*	46	82.1	21	87.5	0.66	0.2–2.6	0.745 (NS)
	C	10	17.9	3	12.5	1.52	0.4–6.0	
rs179019	*C*	42	76.4	12	50.0	3.23	1.2–8.8	0.034 (NS)
	A	13	23.6	12	50.0	0.31	0.1–0.8	
rs179020	*G*	42	73.7	12	50.0	2.80	1.1–7.4	0.069 (NS)
	A	15	26.3	12	50.0	0.36	0.1–1.0	
rs179021	*T*	46	82.1	20	83.3	0.92	0.3–3.2	1.000 (NS)
	G	10	17.9	4	16.7	1.09	0.3–3.8	

CI confidence interval, *N* absolute number, *NS* not significant ($p > 0.05$), *OR* odds ratio, *pc* corrected *p*, *p* two-tailed Fisher's probability, *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7, *UBC* urinary bladder cancer

suggest that nicotine exposure may enhance tumor growth and metastasis, and there is a growing body of evidence depicting that nicotine promotes cell proliferation, angiogenesis, and epithelial-to-mesenchymal transition through nicotinic acetylcholine receptors found in the urinary bladder, leading to enhanced tumor growth and metastasis [17].

Assessment of TLR7 serum level in UBC patients revealed that such serum marker was downregulated. Accordingly, the risk of UBC is suggested to be associated with a decreased serum level of TLR7, and its downregulation may serve as important biomarker for the progression of UBC. However, the potential-risk of TLR7 in UBC has not been well-investigated. So far, the expression of TLR7 has been determined in six human cancers, liver, cervix, pancreas, and lung cancers, as well as chronic lymphocytic leukemia and multiple myeloma. In agreement with the present study, downregulation of

Table 4 Single-nucleotide polymorphisms of *TLR7* gene in females of chronic urinary tract patients and control

TLR7 gene SNP	Allele/ Genotype	UTI females (*N* = 32)		Control females (*N* = 24)		OR	95% CI	*p* (*pc*)
		N	%	*N*	%			
rs179018	*T*	48	75.0	36	75.0	–	–	1.000 (NS)
	C	16	25.0	12	25.0	–	–	1.000 (NS)
	TT	18	56.3	12	50.0	1.29	0.5–3.7	0.788 (NS)
	TC	12	37.5	12	50.0	0.60	0.2–1.7	0.419 (NS)
	CC	2	6.2	ND	ND	4.02	0.2–82.5	0.510 (NS)
HWE *p*		1.000		0.102				
rs179019	*C*	44	68.8	44	91.7	0.20	0.1–0.6	0.005 (S)
	A	20	31.2	4	8.3	5.00	1.6–15.6	0.005 (S)
	CC	16	50.0	20	83.3	0.20	0.1–0.7	0.012 (NS)
	CA	12	37.5	4	16.7	3.00	0.9–10.6	0.135 (NS)
	AA	4	12.5	ND	ND	7.74	0.4–142.4	0.127 (NS)
HWE *p*		0.471		0.656				
rs179020	*G*	49	76.6	44	91.7	0.30	0.1–1.0	0.043 (NS)
	A	15	23.4	4	8.3	3.37	1.1–10.8	0.043 (NS)
	GG	20	62.5	20	83.3	0.33	0.1–1.2	0.135 (NS)
	GA	9	28.1	4	16.7	1.96	0.5–7.2	0.358 (NS)
	AA	3	9.4	ND	ND	5.81	0.3–111.3	0.252 (NS)
HWE *p*		0.221		0.656				
rs179021	*T*	46	71.9	36	75.0	0.85	0.4–2.0	0.830 (NS)
	G	18	28.1	12	25.0	1.17	0.5–2.7	0.830 (NS)
	TT	16	50.0	12	50.0	–	–	1.000 (NS)
	TG	14	43.8	12	50.0	0.68	0.3–2.2	0.788 (NS)
	GG	2	6.2	ND	ND	4.02	0.2–82.5	0.501 (NS)
HWE *p*		0.642		0.103				

CI Confidence interval, *N* absolute number, *HWE* Hardy–Weinberg equilibrium, *ND* not detected, *NS* not significant ($p > 0.05$), *OR* odds ratio, *pc* corrected *p*, *p* two-tailed Fisher's probability, *S* significant ($p \leq 0.05$), *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7, *UTI* urinary tract infection

Table 5 Impact of *TLR7* gene single-nucleotide polymorphisms on serum level of TLR-7 in males of urinary bladder cancer patients and control

TLR7 gene SNP	Allele	Median (range); ng/ml	
		UBC males	Control males
rs179018	*T*	1.5 (0.1–22.2)	6.9 (1.9–25.1)
	C	2.5 (0.1–7.9)	6.2 (5.0–8.2)
p (*pc*)		0.872 (NS)	0.805 (NS)
rs179019	*C*	1.7 (0.1–22.2)	5.1 (2.0–8.2)
	A	1.2 (0.1–7.2)	9.6 (1.9–25.1)
p (*pc*)		0.736 (NS)	0.024 (NS)
rs179020	*G*	1.7 (0.1–22.2)	5.1 (2.0–8.2)
	A	1.2 (0.1–7.2)	9.6 (1.9–25.1)
p (*pc*)		0.508 (NS)	0.024(NS)
rs179021	*T*	1.5 (0.1–22.2)	7.1 (1.9–25.1)
	G	2.5 (0.1–7.9)	5.6 (2.9–8.2)
p (*pc*)		0.872 (NS)	0.347 (NS)

N absolute number, *NS* not significant ($p > 0.05$), *p* Mann–Whitney *U* test, *pc* corrected *p*, *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7, *UBC* urinary bladder cancer

TLR7 expression has been demonstrated in these malignancies. In one study, the expression of TLR7 in cancerous and non-cancerous liver tissue from 87 patients with hepatocellular carcinoma was investigated, and the results demonstrated that TLR7 is significantly downregulated in neoplastic hepatocytes, especially in patients with hepatitis B virus infection [18]. TLR7 was also investigated in a human cell line of pancreatic adenocarcinoma (BxPC-3 cells). The cells were treated with TLR7 agonist (gardiquimod), and then proliferation, migration, cell cycle, and apoptosis of these cells were analyzed. It was demonstrated that TLR7 activation inhibited proliferation and migration, and induced apoptosis in pancreatic cancer cells [19]. Accordingly, agonists to TLR7 have been developed as a strategy for anti-tumor therapy. Among these are imidazoquinolines, which are suggested to have anti-tumor effect in urothelial cell carcinoma of the bladder. These agonists have a potent direct activity against UBC cells by decreasing cell viability and inducing cytokine production and apoptosis [8]. Further investigators formulated an immune-stimulating TLR7 agonist (TMX-202) in the liposomes and examined its immune activating potential in immune cells that included blood-derived monocytes, myeloid dendritic cells (DCs), and plasmacytoid DCs. These cells exhibited potent TLR7-specific secretion of some cytokines that have anti-cancer effects (IL-12p70, IFN-α, and IFN-γ) [20]. Based on these findings, two formulations of TLR7 agonists (TMX-101 and TMX-202) were investigated to determine their therapy potential for urothelial carcinoma in an orthotopic bladder cancer rat model. The results showed a lower number of tumor-positive rats after therapy with TLR7 agonists [21]. In line with these findings, MMC and BCG therapies were associated with an increased serum level of TLR7 compared to untreated patients. Although the differences were not significant, it is possible to suggest that both therapies enhanced production of TLR7. There is no direct evidence to support such findings, but it has been demonstrated that under treatment with MMC, urinary bladder cells showed increasing apoptosis through upregulation of TLR6 and connective tissue growth factor under hydrostatic pressure stimulation [22]. In a further study, it has been suggested that TLR8 expression can be upregulated in macrophages after exposure to BCG [23].

Table 6 Impact of *TLR7* gene single-nucleotide polymorphisms on serum level of TLR-7 in females of urinary tract infection patients and control

TLR7 gene SNP	Genotype	Median (range); ng/ml	
		UTI females (*N* = 30)	Control females (*N* = 24)
rs179018	TT	5.2 (0.7–14.5)	11.0 (5.6–16.5)
	TC	11.1 (3.3–14.0)	12.1 (7.2–17.0)
	CC	9.8 (9.6–10.0)	ND
p (*pc*)		0.052 (NS)	0.443 (NS)
rs179019	CC	7.4 (0.7–14.0)	11.5 (5.6–17.0)
	CA	11.5 (3.5–14.5)	12.3 (9.6–14.9)
	AA	2.7 (0.9–3.7)	ND
p (*pc*)		0.006 (S)	0.682 (NS)
rs179020	GG	7.4 (0.7–14.1)	11.4 (5.6–17.0)
	GA	10.8 (3.5–14.5)	12.1 (9.6–14.9)
	AA	2.0 (0.9–3.7)	ND
p (*pc*)		0.018 (NS)	0.680 (NS)
rs179021	TT	4.4 (0.7–14.5)	11.4 (5.6–16.5)
	TG	9.9 (3.3–14.0)	12.1 (7.2–17.0)
	GG	9.8 (9.6–10.0)	ND
p (*pc*)		0.092 (NS)	0.653 (NS)

N absolute number, *ND* not detected, *NS* not significant ($p > 0.05$), *p* Mann–Whitney *U* or Kruskal–Wallis *H* test, *pc* corrected *p*, *S* significant ($p \leq 0.05$), *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7, *UTI* urinary tract infection

For a further understanding of TLR7 in etiology and pathogenesis of UBC, four gene SNPs were investigated. Disease-association studies suggested that SNPs of TLR genes are associated with an increased risk to develop a wide range of different malignancies [24]. Among these are *TLR7* SNPs, and studies have suspected their potential in progression of different malignancies, and moreover, these SNPs are suggested to predict the response outcome to anti-cancer therapies [25]. In line with these suggestions, the present study examined an intron region of *TLR7* gene

in UBC and UTI patients to seek for novel SNPs that may have a role in predisposition to develop UBC and/or UTI in samples of Iraqi patients. DNA-sequence analysis revealed the existence of four informative SNPs that have minor allele frequencies greater than 10%, rs179018, rs179019, rs179020, and rs179021. Searching the PubMed for these SNPs came-up with five publications for rs179019 and one publication for rs179020, while rs179018 and rs179021 SNPs have not been investigated in any human disease.

For rs179019 SNP, the results suggest a susceptibility role for *C* allele in UBC male patients (OR = 3.23), while *A* allele may have a protective effect. No available evidence confirms or refutes these findings, but five previous studies evaluated the risk effect of rs179019 SNP in hepatitis C virus [26] and enterovirus 71 [27] infections and two autoimmune diseases, systemic lupus erythematosus (SLE) [28, 29] and Graves' disease [30]. No clear effect was presented regarding viral infections or Graves' disease, while contradicting results were found in SLE. However, among UTI patients, rs179019 SNP was associated with susceptibility for UTI but through *A* allele. Therefore, it is possible to suggest that *C* and *A* alleles of rs179019 SNP may have different predisposing effects in UBC and UTI.

For rs179020, it may also be a potential-risk SNP for UBC, and male patients having *G* allele were at a greater risk to develop UBC but the difference was not significant. This SNP appeared in one study, which investigated its association with vitiligo (a chronic disease characterized by distinctive lightening of the skin), and a strong association with the entire disease was observed; moreover, male, sporadic, and late onset vitiligo maintained a corrected significant association [31].

The presented findings highlighted the importance of an intron region in *TLR7* gene in predisposition and risk of UBC, and the SNP effect in this context cannot be underestimated but the available evidence is limited. In one published study, the relation between a promoter *TRL7* gene SNP (rs179008; not investigated in present study) and response to imiquimod (TLR7 agonist) was explored in cutaneous basal cell carcinoma patients. The results demonstrated that patients carrying at least one SNP allele (*T* allele) are at an increased risk to be resistant to such therapy [32]. Therefore, *TLR7* gene may harbor important functional SNPs that not just influence susceptibility to malignancies, but may also influence response to therapies. The results of present study agree with such conclusion, although some of the observed findings were statistically not significant. In terms of statistics, the sample size influences the level of significance effectively and represents an important limitation of present study.

Conclusions

Downregulation of TLR7 is suggested to have a role in etiology and pathogenesis of UBC, especially in the male, elderly, and smoker patients. MMC and BCG may enhance TLR7 production in the peripheral blood of UBC patients. *TLR7* gene SNPs are suggested to influence susceptibility to develop UBC, and their potential in impacting serum level of TLR7 is augmented.

Abbreviations

BCG: Bacillus Calmette–Guérin; C: Control; CI: Confidence interval; DC: Dendritic cell; HWE: Hardy–Weinberg equilibrium; NS: Not significant; OR: Odds ratio; *p*: Probability; *pc*: Corrected *p*; SLE: Systemic lupus erythematosus; SNP: Single-nucleotide polymorphism; TLR: Toll-like receptor; UBC: Urinary bladder cancer; UTI: Urinary tract infection

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Authors' contributions

AH wrote the manuscript and carried out the statistical analyses. MA contributed in writing and revising the manuscript. RA performed the laboratory assessments and manuscript writing, as well as handling the data of patients. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The participants provided their written informed consent to be included in the study. The study protocol was approved by the Ethics Committee at the Iraqi Ministry of Health on (No. 16265 on May 25, 2017).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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