Research article

Study of the association of toll like-4 receptor gene polymorphism (rs1927911) and susceptibility to urinary tract infection among Iraqi patients

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ABSTRACT

Introduction and Aim: Toll-like receptors (TLRs) are cell innate immune receptors that explore microbial molecules. A quantity of single-nucleotide polymorphisms (SNPs) within the *TLR* genes have been related with altered susceptibility to infectious, inflammatory, and allergic diseases suggesting: This study was conducted to investigate the relationship between genetic polymorphism in *TLR*4 gene (rs1927911) with susceptibility to UTIs in a sample of Iraqi patients.

Materials and Methods: Blood samples were collected from 220 patients who had UTIs and 69 healthy subjects as control group, during the period from October 2021 to March 2022, at the Rheumatology Department of Clinic Consultant at Al-Yarmouk Hospital. The genetic study included an extraction of genomic DNA and PCR technique, to detect the role of *TLR*4 gene in the variation site (rs1927911) as a risk factor for the incidence of UTIs.

Results: The results of genotype distribution and allele frequency of rs1927911 indicated, CC (18.6 %), CT (70 %) and TT (11.4 %) among the patients while 57.9 % for CC and 26.1%; 16% for CT, TT genotypes respectively of healthy control. Heterozygous (CT) may be considered as a risk factor among UTIs patients. The results revealed significant differences between UTIs patients and healthy controls in serum levels TLR4, IL-6 IL-2, TNF-α and IL-10 at $P \leq 0.005$. In addition, the data found a highly significant difference of serum level of TLR4 protein with patients who have a carrier CT genotype.

Conclusion: our findings indicated that the carriers of CT genotype of TLR4 (rs1927911) were significantly more prone to UTIs.

Keywords: UTIs; LPS; gene polymorphism; *TLR*4 gene.

INTRODUCTION

Urinary tract infections (UTIs) are the major common infectious disease that affects both the upper and lower urinary tracts and seriously impair the quality of life for many people, particularly the elderly (1,2). UTI cases of all ages and genders are reported daily in most hospitals. The severity of contamination relies upon the interaction of the invader pathogen and the host's protection mechanisms (3,4). The host immune system response in defense can become destructive with excessive innate immune activation in the absence of molecular control, resulting in chronic inflammatory disorders and infection-associated morbidity (5, 6). UTIs, are mainly caused by Gram-negative, facultatively anaerobic bacteria from the uropathogenic Enterobacteriaceae family members that are prevalent globally (7-11). Toll-like receptor 4 (TLR4) is a member of the toll-like receptor family, that serves an important role in adaptive and innate immune responses via the signalling pathways, leading to chronic and inflammatory disorders (12). TLR4 can specifically recognize the lipopolysaccharide (LPS) pattern of invading bacterial pathogens, eliciting a pro-inflammatory response as a first line of defence in bacterial eradication (13). Several accessory proteins such as LBP, CD14, and MD-2 are also known to be involved in the detection of LPS (14). Single-nucleotide polymorphisms (SNPs), found in several *TLR*4 and associated genes have been linked to several infection and inflammation disorders as well as cancer (15). Several *TLR*4 genes have also been studied to test the association between TLR4 polymorphisms to the risk of developing urinary tract infections (16). In this study, we aimed to investigate the association between polymorphism in SNP rs1927911 of the *TLR*4 gene and susceptibility to UTIs among Iraqi patients.

MATERIALS AND METHODS

Two hundred and eighty-nine participants of both sexes who visited the Urology Unit at the Al-Yarmouk Teaching Hospital in Baghdad between

October 2021 and March 2022 were enrolled in the study. Based on their symptoms, signs and clinical diagnoses, the people were split into two groups. The initial cohort had 220 patients (127 females, 93 males). The second group contained 69 healthy people (33 females and 36 males).

Blood (5 ml) was drawn through venipuncture from everyone using a plastic disposable syringe. Blood collected was distributed into 2 tubes; 2 ml of the blood was transferred to a tube containing EDTA for subjecting to molecular analysis, while the remaining was transferred to another tube, centrifuged at 5000 rpm 15 mins for separating out serum and proceeded for the identification of TLR4, IL-6, IL-2, TNF-α, and 1L-10.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted using the Blood DNA Miniprep System (Genaid/Korea) according to the manufacturer's instructions. DNA extracted was checked for its purity by measuring its absorbance at 260/280nm using a nanodrop. DNA extracted was subjected to amplification of SNP rs1927911 of the TLR4 gene using PCR, this technique used in the medicine area (17-19). The primers (Macrogen, Korea) given in Table 1. PCR reactions were carried out in 25µl PCR tubes consisting of 5 µl of master mix ($MgCl_2$ 1.5 mM, Taq polymerase 1 U, every dNTPs 200 µM), 1.5 µl each of inner primers, 1 µl each of outer primers, 3 µl of template DNA and 12 µl of nuclease free water**.** The PCR reaction was carried out in a thermocycler (Eppendorf/Germany) with the following amplification conditions: Initial denaturation at 95°C for 3 minutes, followed by 35 cycles of amplification including denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 min., and a final extension at 72°C for 10 minutes. The amplified products were subjected to electrophoresis on 1% agarose gel (50-volt current for 15 min. followed by 80 volts for 1:30 min.), stained with ethidium bromide and the bands visualized. All PCR products were independently validated by Sanger sequencing (Macrogen, Seoul, South Korea) and analysed for their genotypes.

Detection of TLR4, IL-6 IL-2, TNF-α, IL-2 and IL-6 levels

The Sandwich ELISA assay, which is a highly specific, flexible and sensitive technique, was used to estimate serum levels of TLR4, IL-6 IL-2, TNF-**α** and IL-2, IL-6 in UTI patients and healthy individuals.

Statistical analysis

Statistical analysis was performed to test whether group variance was significant or not. Genotypes of *TLR4* gene are presented as percentage frequencies and significant differences between their distribution in patients and control were assessed by two-tailed Fisher's exact probability. The mean and SE of mean were calculated by using the IBM SPSS version 27.0. Statistical significance was defined as * $p \leq 0.05$ or ** $p \leq 0.01$.

RESULTS

Genotypic studies

Genotyping of SNP rs1927911 of *TLR-4* gene yielded three product sizes of sizes 250 bp, 310 bp and 510 bp (Fig. 1) pertaining to the CC, TT and CT genotypes respectively.

Table 1: Primer sequences used to detect SNP rs1927911 of *TLR4* gene

SNPs	Product size	Primer	Sequence
rs1927911	C allele: 250 bp	Inner F	5'- CCAGATTTTGACAACTGCATTCTTGTC-3'
	T allele: 311 bp	Inner R	5'-CTCAAGGGTCAATGAGCCACGA-3'
	Size of two outer primer 510	Outer F	5'-GATACAGAGAAGATGAGCATGGCCC-3'
		Outer F	5'-CAAGGAATTTTGTTGGAGGAAATGA-3'

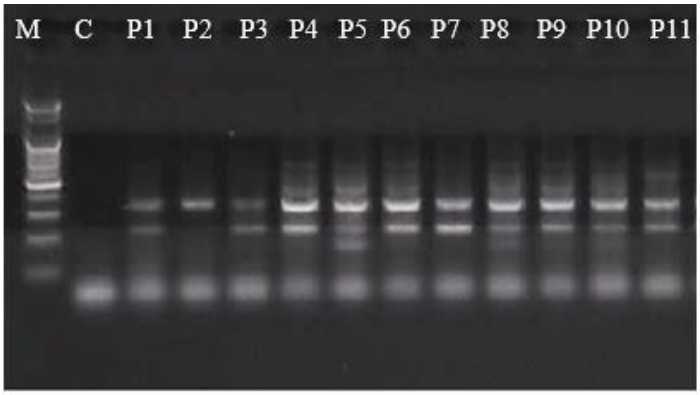

Fig. 1: PCR detection of rs1927911 in *TLR4* gene in UTIs patients M: 100bp DNA ladder; C: Negative control; C allele: (250 bp); T allele: (310 bp) and two outer primers: (510bp).

Table 2: Distribution of genotypes and allele frequency within SNP rs1927911 of *TLR4* gene among the patient and healthy group

Genotype	Groups	
	UTIs patients (n=220)	**Healthy individuals (n=69)**
	No. (%)	**No (%)**
CC	41(18.6)	40 (57.9)
CT	154(70)	18 (26.1)
TT	25(11.4)	11(16)
Total	220(100)	69(100)
C	236(53.6)	98 (71)
T	204(46.4)	40 (29)
Total	440 (100)	138(100)

Table 3: Mean serum level of *TLR4* and other cytokines

Parameter	Mean serum level (Mean±SE) pg/ml		P-value
	Patients	**Healthy**	
TLR4	117.12±15.81	19.56± 2.76	0.001
CD14	83.75 ±6.39	18.73 ± 1.45	0.001
IL-2	55.57±2.97	17.71±1.08	0.001
IL-6	90.14±7.29	8.4±0. 45	0.001
TNF-α	56.74±2.25	15.31±0.81	0.001

Table 4: Association between genotypes of rs1927911 and serum level of *TLR4*

Genotypes	Mean serum level of TLR4 ± SE) pg/ml		Significant value between group	P-value
	Patients	**Healthy**		
CC	62.69 ± 5.52	19.61 ± 0.69	14.685 *	0.0027
CT	92.51 ± 3.57	27.38 ± 3.21	18.702 *	0.0001
TT	71.54 ± 6.54	17.57 ± 1.78	19.963 *	0.0001

*Significant

The genotypic and allelic frequencies of SNP rs1927911 investigated are presented in Table 2. The CC, CT, and TT genotype frequencies of rs1927911 were 18.6 %, 70 %, and 11.4 % respectively among the patients while it was 57.9 % 26.1%, and 16% for CC CT, TT genotypes respectively in healthy controls **(**Table 2). The allele C and T were observed in 53.6% and 46.4% respectively among the UT patients. Similarly, in healthy individuals the frequency of C and T was 71% and 29% respectively. The mean serum levels of TLR4, CD14, IL-2, IL-6, and TNF-α detected in UTI patients and healthy individuals are presented in Table 3. Increasing mean levels of parameters have been detected in patients in comparison to healthy individuals which was statistically significant (P value=0.001). The three different genotypes displayed significant differences between patients and controls for mean serum levels of *TLR4* and the genotypes (Table 4).

DISCUSSION

Our study showed the frequencies of CT and TT genotypes to be effective genotypes for urinary system infection as they present in patients more frequently in significant levels than control and more frequently than wild type genotypes. Testing for Hardy-Weinberg (H-W) equilibrium revealed that UTIs patients showed a significant variation in the distribution of rs1927911 genotypes ($p \leq 0.001$). Such a difference was observed due to variation between the observed and expected frequencies of CC, CT and TT. Toll-like receptors have been found as the most substantial class receptors between the host protections towards bacterial and viral infections. A complex signalling cascade begins when the ligand binds on TLR4, which leads to an activation of the dense encouraging genes. Genetic editions affect immune responses toward pathogenic challenges (20).

The expression of serum level of *TLR4* rs1927911 alters the interaction between TLR4 and its ligand thereby altering functions that affect the susceptibility to infections, which probably could be considered a risk factor for UTIs progression. Cytokines play an important role in the disease outcome. TLR4 polymorphisms and expressions have been associated with altering signalling leading to either agonist or antagonist effect to infection (21-23). Such associations with TLR polymorphisms have also been reported for Urinary tract infections (24, 25).

Single nucleotide polymorphisms (SNPs) between the *TLR4* genes have been related to changes at the amino acid level there by altering protein function (26). Our results showed a significant difference in serum level of *TLR4* between the patients and healthy group according to different genotypes of each investigated SNPs. The patients' carriers of the CT genotype with one mutant allele (T) produced more *TLR4* protein than carriers of CC (wild type) or TT (mutant type) respectively. The expression of serum *TLR4* was significantly associated with the SNP rs1927911 genotype, which indicates the association of this SNP to urinary tract infections. Hence SNP rs1927911 could be used as a biomarker in UTIs.

CONCLUSION

Our results confirm the role of SNP rs1927911 variation of *TLR4* gene in the susceptibility of UTIs. Thus, it shows that the TLR4 gene may additionally be used as a candidate gene to confirm the incidence of UTIs in Iraqi patients.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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