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Research Paper

Genetic Variants in the Vitamin D Receptor Gene Increase the Risk of Urinary Tract Infection in a Southeast Population of Iran: A Case-control Study

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ABSTRACT

Background and Aim: Urinary tract infection (UTI) is a common infection affecting children. Besides microorganism pathogens, genetic background plays a pivotal role in the pathogenesis of diseases. Vitamin D receptor (VDR) plays a crucial role in the pathogenesis of some cancers and infectious diseases. This study aimed to evaluate the association between polymorphisms in the *VDR* gene and risk of UTI.

Methods: In the current study, 127 children affected by UTI were referred to the pediatric nephrology clinic in Zahedan, and 100 subjects with no history of infection were genotyped. The genotyping was carried out by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). SPSS software, version 22.0 was used for statistical analysis.

Results: The results revealed that rs2228570C>T (FokI), rs7975232A>C (ApaI), and rs731236T>C (TaqI) increased the risk of UTI, whereas rs1544410G>A (BsmI) decreased the risk of the disease.

Conclusion: The results suggest that the genetic variants in the *VDR* gene may play a role in the pathogenesis of UTI.

Keywords: Urinary tract infection (UTI), Vitamin D, Vitamin D receptor (VDR), Single nucleotide polymorphism (SNPs)

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Introduction

Urinary tract infection (UTI) is a common infectious disorder among children, with an increased resistance to antimicrobial agents [1]. Two subtypes of UTI are classified as upper (acute pyelonephritis) and lower (cystitis) [2, 3]. In some cases, the etiology of UTI is identified, whereas in a minority of affected subjects, the specific risk factors remain unexplored. Studies have shown that several factors, including urinary system abnormalities, constipation, hypercalciuria, and vesicoureteral reflux, are risk factors for UTI [3, 4]. The most severe form of the disease is pyelonephritis, which is caused by the invasion of pathogenic organisms into the renal parenchyma. In most cases, *Escherichia coli* is the main cause of the disease, although many types of microorganisms have the potential to cause UTI [5].

Early diagnosis could lead to better efficiency and management of the disorder, while delayed diagnosis may result in severe complications, including renal scarring, hypertension, and chronic renal failure [6]. Meanwhile, genetic variations in some genes, including inflammatory-related genes, such as interleukin (ILs) 6 and IL8, are likely to increase the probability of UTI [7].

Vitamin D is a ketosteroid hormone that plays a role in immunological and inflammatory processes [8]. Some documents elucidate the participation of vitamin D receptors (VDRs) in some infectious diseases, such as tuberculosis and pneumonia [9, 10]. VDR is a nuclear receptor that can mediate the biological roles of vitamin D [11]. The results of the dimercaptosuccinic acid (DMSA) scan revealed that febrile UTI affects parenchyma in about half of children with UTI. In addition, permanent renal cell damage was observed in 20%-40% of these cases [12]. Epidemiological studies have shown an association between vitamin D deficiency and cancers, as well as autoimmune and infectious diseases. This association is supported by the benefits of solar radiation for tuberculosis patients [13]. Vitamin D suppresses the function of the adaptive immune system by decreasing the production of proinflammatory cytokines, including interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) while simultaneously increasing the production of cathelicidin in macrophages [14]. In vitro analysis revealed that endothelial cells treated with vitamin D suppressed the activation of cytokines and bacterial lipopolysaccharide (LPS)-mediated nuclear factor kappa-B (NF-kB) [15]. To date, several studies have pointed to the protective effect of serum vitamin D against different types of UTI [16, 17]. Moreover, several studies depict a correlation between vitamin D and the risk of UTI, reinforcing the potential of vitamin D and its receptor in the pathogenesis of UTI [17, 18].

In the management of infectious diseases, understanding genetic factors is a pivotal step in designing protective and curative targets. Therefore, the current study aimed to investigate the association between *VDR* gene polymorphisms and the risk of UTI in a southeast population of Iran.

Materials and Methods

Subjects and sample collection

The study included 127 individuals who were clinically diagnosed with UTI by healthcare professionals and visited the Pediatric Nephrology Clinic in Zahedan, Iran, between June 2022 and July 2023. The diagnostic criteria followed the European Association of Urology – European Society for Pediatric Urology (EAU-ESPU) instructions for UTIs in children [19]. The control group comprised 100 unrelated healthy samples of the same ethnicity, all without a history of inflammatory diseases, metabolic syndrome, cardiovascular conditions, renal or liver disorders, or cancer. To determine the sample size, in the first step, genotyping was initially carried out on 25 children affected by UTI and 25 unrelated healthy subjects. In the next step, the observed allele frequencies were introduced to the Sample Size Calculator server [20], and the appropriate sample size was determined.

DNA extraction and genotyping

Venous blood samples (5 mL) were collected from the subjects using tubes containing Ethylenediaminetetraacetic acid (EDTA). Genomic DNA (gDNA) was extracted utilizing the standard salting-out approach [21]. Genotyping was performed using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). The PCR reactions included 0.7 µL of each forward and reverse primer, 7 µL of 2X Taq PreMix Master Mix (Parstous Company, Iran), 0.7 µL of gDNA, and enough nuclease-free water to reach a final volume of 15 µL. Details of the designed primers are provided in a supplementary file. The prepared tubes underwent PCR under the following protocol: A pre-denaturation step at 95 ˚C for 5 minutes, followed by 30 cycles of denaturation at 95 ˚C for 30 seconds, annealing at a certain temperature for 30 seconds, and extension at 72 ˚C for 30 seconds, concluding with a post-extension step at 72 ˚C for 5 minutes. The PCR products received a specific restriction enzyme as instructed. Approximately 5-6 µL of

the final PCR products were separated on a 2% agarose gel stained with Safe DNA viewer dye (Sinaclone Co., Iran) using an electrophoresis system. The PCR products were visualized by a UV-transilluminator system.

Statistical analysis

Data analysis was done using SPSS software, version 22. The independent t-test or Mann-Whitney U test evaluated quantitative variables. Differences in genotyping between the groups were assessed using the chi-square (χ^2) test. Odds ratios (ORs) and the 95% confidence intervals (CI) were also determined. Additionally, the SHEsis online software analyzed haplotypes and linkage disequilibrium (LD).

Results

The case and control groups were homogeneous in terms of age and sex. The results of the genotyping of the studied variants in the *VDR* gene are shown in Table 1. The T allele of rs2228570C>T moderately increased the risk of UTI in our population (OR=1.5, 95% CI, 1.08%, 2.3%, P=0.018). The CT versus CC genotype and the CT+TT genotype resulted in an increase in the risk of UTI with an OR of approximately 2.6 (OR=2.66, 95% CI, 1.43%, 4.94%, P=0.002, and OR=2.63, 95% CI, 1.44%, 4.79%, P=0.001, respectively).

In addition, the T allele in the TT versus the CC genotype significantly enhanced the probability of the studied disorder (OR=2.51, 95% CI, 1.03%, 6.07%, P=0.040). Moreover, the CT versus CC+TT genotype model showed a slight increase in the risk of the disorder (OR=1.95, 95% CI, 1.14%, 3.34%, P=0.014). In terms of rs1544410G>A, the A allele significantly decreased the risk of UTI in our population (OR=0.69, 95% CI, 0.47%, 0.99%, P=0.047). Regarding this variant, the AA versus GG genotype significantly reduced the risk of the disorder (OR=0.45, 95% CI, 0.2%, 1%, P=0.048).

The C allele of rs7975232A>C was more frequent in UTI participants compared to healthy subjects and caused a significant increase in the risk of UTI (OR=1.47, 95% CI, 1.01%, 2.14%, P=0.044). The AC+CC genotypes, compared to AA, significantly enhanced the risk of UTI (OR=1.83, 95% CI, 1%, 3.34%, P=0.048). Furthermore, for rs731236T>C, the C allele was associated

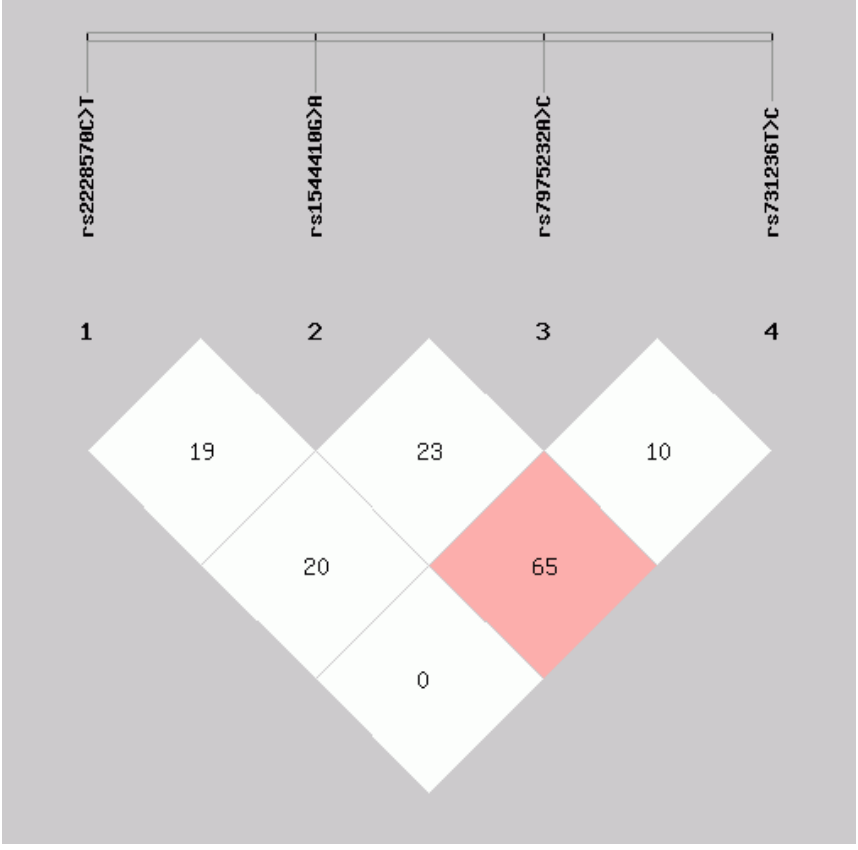

Figure 1. The results of linkage disequilibrium analysis of the studied variants in the *VDR* gene

Note: The results showed a strong correlation between rs1544410G>A and rs731236T>C.

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Table 1. The frequency of the alleles and genotypes of VDR variants in the UTI and control groups

SNPs		No. (%)		Genetic Model	OR (95% CI)	P
		UTI	Control			
rs2228570C>T	CC	24(18.9)	38(38)		1 [reference]	
	CT	84(66.1)	50(50)	Codominant 1	2.66 (1.43, 4.94)	0.002
	TT	19(15)	12(12)	Codominant 2	2.51 (1.03, 6.07)	0.040
	HWE			Dominant	2.63 (1.44, 4.79)	0.001
				Recessive	1.29 (0.59, 2.80)	0.519
				Over dominant	1.95 (1.14, 3.34)	0.014
	C	132(52)	126(63)	Allelic	1 [reference]	
	T	122(48)	74(37)	Allelic	1.57 (1.08, 2.30)	0.018
rs1544410G>A	GG	44(34.6)	23(23)		1 [reference]	
	AG	64(50.4)	55(55)	Codominant 1	0.61 (0.33, 1.13)	0.115
	AA	19(15)	22(22)	Codominant 2	0.45 (0.20, 1.00)	0.048
	HWE			Dominant	0.56 (0.31, 1.02)	0.056
				Recessive	0.62 (0.32, 1.23)	0.171
				Over dominant	1.02 (0.61, 1.69)	0.952
	G	152(59.8)	101(50.5)	Allelic	1 [reference]	
	A	102(40.2)	99(49.5)	Allelic	0.69 (0.47, 0.99)	0.047
rs7975232A>C	AA	26(20.5)	32(32)		1 [reference]	
	AC	76(59.8)	54(54)	Codominant 1	1.73 (0.93, 3.23)	0.083
	CC	25(19.7)	14(14)	Codominant 2	2.20 (0.95, 5.06)	0.062
	HWE			Dominant	1.83 (1.00, 3.34)	0.048
				Recessive	1.51 (0.74, 3.08)	0.260
				Over dominant	1.27 (0.75, 2.16)	0.377
	A	128(50.4)	118(59.9)	Allelic	1 [reference]	
	C	126(49.6)	79(40.1)	Allelic	1.47 (1.01, 2.14)	0.044
rs731236T>C	TT	35(27.5)	45(45)		1 [reference]	
	TC	81(63.8)	51(51)	Codominant 1	2.04 (1.16, 3.59)	0.013
	CC	11(8.7)	4(4)	Codominant 2	3.54 (1.04, 12.06)	0.035
	HWE			Dominant	2.15 (1.24, 3.74)	0.006
				Recessive	2.28 (0.70, 7.38)	0.160
				Over dominant	1.69 (0.99, 2.88)	0.053
	T	151(59.4)	141(70.5)	Allelic	1 [reference]	
	C	103(40.6)	59(29.5)	Allelic	1.63 (1.10, 2.42)	0.015

OR: Odds ratio; CI: Confidence interval; SNPs: Single Nucleotide Polymorphisms.

Note: P<0.05 was considered statistically significant.

Table 2. Interaction analysis of the studied SNPs on the risk of UTI

rs2228570C>T	rs1544410G>A	rs7975232A>C	rs731236T>C	No. (%)		OR (95% CI)	Bonferroni P
				UTI	Control		
CT	AG	AC	TC	18(14.2)	11(11)	1 [reference]	
CC	AA	AA	TC	1(0.8)	5(5)	0.12 (0.01, 1.19)	0.042
CC	AG	AA	TC	5(3.9)	3(3)	1.02 (0.20, 5.13)	0.982
CC	AG	AC	TC	5(3.9)	6(6)	0.51 (0.13, 2.07)	0.343
CC	AG	AC	TT	1(0.8)	4(4)	0.15 (0.01, 1.55)	0.080
CC	GG	AC	TT	2(1.6)	4(4)	0.31 (0.05, 1.95)	0.195
CT	AA	AA	TC	2(1.6)	4(4)	0.31 (0.05, 1.95)	0.195
CT	AA	AC	TC	4(3.1)	2(2)	1.22 (0.19, 7.82)	0.832
CT	AG	AA	TC	7(5.5)	3(3)	1.43 (0.30, 6.96)	0.652
CT	AG	AC	TT	4(3.1)	7(7)	0.35 (0.08, 1.47)	0.145
CT	AG	CC	TC	7(5.5)	3(3)	1.43 (0.30, 6.96)	0.652
CT	GG	AC	TC	11(8.7)	2(2)	3.36 (0.63, 18.09)	0.144
CT	GG	AC	TT	17(13.4)	3(3)	3.46 (0.82, 14.59)	0.081
TT	AG	AC	TC	7(5.5)	3(3)	1.43 (0.30, 6.96)	0.652

OR: Odds ratio; CI: Confidence interval.

Note: P<0.0125 was considered statistically significant.

with an increase in UTI risk (OR=1.63, 95% CI, 1.1%, 2.42%, P=0.015). The greatest impact on the risk of UTI was observed with the CC vs TT genotype (OR=3.54, 95% CI, 1.04%, 12.06%, P=0.035). In addition, the C allele in the codominant model (TC compared to TT) and in the dominant model (TC+CC vs TT) caused a 2-fold increase in UTI risk (OR=2.04, 95% CI, 1.16%, 3.59,% P=0.013 and OR=2.15, 95% CI, 1.24%, 3.74%, P=0.006, respectively).

Table 2 depicts the results of the interaction analysis of the studied variants. The statistical analysis showed no significant interaction between the studied variants at the established statistical level.

Table 3 shows the haplotype analysis of the studied variants in the *VDR* gene. Based on the observed results, the rs2228570T/rs1544410G/rs7975232C/rs731236T haplotype was more common among healthy subjects and served as a reference. The rs2228570T/rs1544410A/rs7975232C/rs731236C haplotype significantly increased the risk of UTI (OR=8.10, 95% CI, 2.23%, 29.41%, P<0.001). Furthermore, the rs2228570T/rs1544410G/rs7975232A/rs731236T haplotype statistically raised the probability of UTI in the studied population (OR=3.29, 95% CI, 1.36%, 7.94%, P=0.007).

The results of the linkage disequilibrium analysis revealed that rs1544410G>A is highly correlated with rs731236T>C, with D=65% (Figure 1).

Discussion

Our data showed that rs2228570C>T, rs7975232A>C, and rs731236T>C increased the risk of UTI in our population, whereas rs1544410G>A was associated with a decreased risk of the disease. Further analysis revealed that the rs2228570T/rs1544410A/rs7975232C/

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Table 3. Haplotype analysis of the studied SNPs on the risk of UTI

rs2228570C>T	rs1544410G>A	rs7975232A>C	rs731236T>C	%		OR (95% CI)	Bonferroni P
				UTI	Control		
T	G	C	T	35	37	1 [reference]	
C	A	A	C	18	28	0.68 (0.32-1.44)	0.313
C	A	A	T	10	29	0.35 (0.15-0.86)	0.019
C	A	C	C	20	8	2.64 (1.03-6.77)	0.039
C	A	C	T	9	7	1.36 (0.46-4.04)	0.580
C	G	A	C	5	7	0.75 (0.22-2.6)	0.656
C	G	A	T	46	21	2.32 (1.16-4.63)	0.017
C	G	C	C	0	2	-	
C	G	C	T	24	24	1.06 (0.51-2.19)	0.881
T	A	A	C	21	12	1.85 (0.79-4.31)	0.152
T	A	A	T	0	11	-	
T	A	C	C	23	3	8.10 (2.23-29.41)	<0.001
T	A	C	T	0	2	-	
T	G	A	C	0	0	-	
T	G	A	T	28	9	3.29 (1.36-7.94)	0.007
T	G	C	C	15	0	-	

OR: Odds ratio CI: Confidence interval; UTI: Urinary Tract Infection. P<0.0125 was considered statistically significant.

rs731236C and rs2228570T/rs1544410G/rs7975232A/rs731236T haplotypes were more frequent in UTI individuals compared to healthy subjects, thereby increasing the risk of UTI.

VDR, also known as the calcitriol receptor, is a member of the nuclear receptor family (NR1l1; nuclear receptor subfamily 1, group L, member 1) located on chromosome 12q13.11. It consists of 12 exons and produces a protein of approximately 48 kDa. This receptor, upon binding to 1,25-dihydroxyvitamin D3, forms a heterodimer with the retinoid X receptor and binds to hormone-response elements on DNA, resulting in the expression of specific genes [22].

Sunlight radiation on the skin induces the conversion of 7-dehydrocholesterol (7-DHC) into a form of vitamin D called vitamin D3 [23]. The 1,25-dihydroxy vitamin D3 is the active form of vitamin D that is produced by hydroxylation of vitamin D3 by CYP2R1 and CYP27B1 enzymes of cytochrome P450 in the liver and kidney, respectively [24]. Vitamin D has various roles, such as regulating calcium-phosphorus homeostasis and contributing to the activity of immune cells, including monocytes and lymphocytes. *VDR* and *CYP27B1* are expressed on the membranes of monocytes and macrophages, confirming the role of *VDR* in both innate and adaptive immune systems [25]. These cells utilize 25-dihydroxyvitamin D3 in a signaling pathway that induces antimicrobial response. In addition, toll-like receptors (TLRs) on these cells recognize surface molecules of pathogens, resulting in the upregulation of genes coding VDR and CYP27B1 [26, 27]. It has been shown that some ILs, including IL6 and IL8, are differentially expressed between UTI patients and healthy subjects [28]. In the urinary tract system, 25-hydroxyvitamin D3

is converted to 1,25-dihydroxyvitamin D3 as a ligand of VDR in bladder cells, leading to the upregulation of CAMP and the production of cathelicidin, which has a conflicting effect on the pathogenicity of *E. coli* [29].

In a study performed on a population of affected children residing in central Iran, rs1544410G>A and rs7975232A>C increased the risk of UTI [3]. The results of an association study involving 92 children with UTI indicated that the rs2225870C>T variant of the VDR increased the risk of UTI, while the rs7975232A>C variant decreased the risk of the disorder [8]. In that study, rs1544410G>A and rs731236T>C showed no significant difference between the case and control groups. Additionally, in a study involving a population of subjects suffering from sepsis, the association between vitamin D levels, VDR genetic variants, and the incidence of sepsis was investigated. The results showed reduced levels of vitamin D in sepsis subjects compared to healthy participants. In addition, individuals with sepsis who experienced septic shock had lower levels of vitamin D compared to those with severe sepsis. Moreover, the minor allele of rs2228570C>T was more prevalent among sepsis patients compared to the reference group, suggesting that this allele may increase the risk of sepsis [30].

Conclusion

The findings of the current study revealed that rs2228570C>T (FokI), rs7975232A>C (ApaI), and rs731236T>C (TaqI) increased the susceptibility of UTI, whereas rs1544410G>A (BsmI) decreased the risk of the disease and showed a protective role in the pathogenesis of UTI in our population. These results suggest that VDR is likely to play a role in the etiology of UTI.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (Codes: IR.ZAUMS.REC.1399.158 and IR.ZAUMS.REC.1399/161). All subjects provided informed consent.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflicts of interest.

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