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ORIGINAL ARTICLE

Association of polymorphisms in TLR3 and TLR7 genes with susceptibility to COVID-19 among Iranian population: a retrospective case-control study

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ABSTRACT

Background and Objectives: Host genetic changes like single nucleotide polymorphisms (SNPs) are one of the main factors influencing susceptibility to viral infectious diseases. This study aimed to investigate the association between the host SNP of Toll-Like Receptor3 (TLR3) and Toll-Like Receptor7 (TLR7) genes involved in the immune system and susceptibility to COVID-19 in a sample of the Iranian population.

Materials and Methods: This retrospective case-control study evaluated 244 hospitalized COVID-19 patients as the case group and 156 suspected COVID-19 patients with mild signs as the control group. The genomic DNA of patients was genotyped for TLR7 (rs179008 and rs179009) and TLR3 (rs3775291 and rs3775296) SNPs using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: A significant association between rs179008 SNP in the TLR7 gene and the susceptibility of COVID-19 was found between case and control groups. The AT genotype (Heterozygous) of TLR7 rs179008 A>T polymorphism showed a significant association with a 2.261-fold increased odds of COVID-19 (P=0.003; adjusted OR: 2.261; 99% CI: 1.117-4.575). In addition, a significant association between TC genotype of TLR7 rs179009 T>C polymorphism and increased odds of COVID-19 (P< 0.0001; adjusted OR: 6.818; 99% CI: 3.149-14.134) were determined. The polymorphism frequency of TLR3 rs3775291 and rs3775296 genotypes were not significantly different between the case and control groups (P> 0.004167).

Conclusion: SNPs in TLR7 rs179008 and rs179009 genotypes are considered host genetic factors that could be influenced individual susceptibility to COVID-19. The SNPs in TLR3 (rs3775296 and rs3775291) showed no significant association with COVID-19 in Iranian population.

Keywords: TLR3; TLR7; Genetic polymorphism; COVID-19; Susceptibility

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INTRODUCTION

COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Disease appeared first in late December 2019 in Wuhan Province, China. Then the virus quickly spread around the world; in March 2020, COVID-19 was declared the first pandemic of the century by WHO (1-3). Unfortunately, before the extensive National Immunization Program against COVID-19, Iran's official COVID-19 mortality rate was significantly higher than the global average (4, 5).

The effective antiviral response of both innate and adaptive immune systems has a vital role in clearing and controlling the virus and subsequent disease; conversely, the impaired immune system could lead to the severe pathological phenomenon encountered with the pathogen. It could be regarded that there is strong scientific evidence that host genetic factors play an essential role in determining the outcome of viral infections, especially in respiratory diseases (6-8). Numerous molecules involved in innate immunity have a pattern recognition feature through which they can recognize a specific class of microbial molecules. These patterns-recognizing molecules may be soluble, or, such as TLRs (Toll-Like Receptors), they can be cell membrane-related receptors (9). TLRs play a vital role in the pathogen recognition process and innate immunity activation, which are expressed in various immune cells, including macrophages, dendritic cells (DCs), mast cells, Natural killer cells (NKs), monocytes, neutrophils, basophils, regulatory T cells, and respiratory, intestinal, and so forth. TLR1, TLR2, TLR4, TLR5, and TLR6 are located on the cell surface; TLR3, TLR7, TLR8, and TLR9 are located predominantly within the endosomes and lysosomes (10-13).

TLRs' role in triggering the antiviral immune responses is vital. The presence of polymorphisms in TLRs genes could be associated with the susceptibility to various viral infections. SNPs can induce amino acid changes, promoter activities dysregulation, affecting gene expression and mRNA composition, and may consequently influence the stability or structure of proteins and their functions (12, 14, 15).

TLR3 is a transmembrane receptor type-1 with a molecular weight of 125-kDa located on chromosome 4 and plays a prominent role in inflammation and innate immunity against viral pathogens. Stimulating this receptor activates the numerous pathways that produce inflammatory cytokines and type I interferons (IFNs). The induction of this cascade facilitates the delivery of antigen to MHC class I molecules and accelerates cytotoxic T cells activation. Accordingly, TLR3 signaling is essential for inducing immune responses in the T cell to encounter viruses. Therefore, due to the significant effect of TLR3 on antiviral response, any polymorphisms in this gene could affect susceptibility and intensity to viral infections (11, 13). A TLR3-depending signaling pathway is activated by recognizing dsRNA viruses such as retrovirus, polyinosinic-polycytidylic acid (poly I: C), and dsRNA produced during replication of ssRNA viruses (16).

Moreover, TLR7 is a polymorphic gene that theoretically can be represented in diverse TLR7 types with different functional features. The functional role of TLR7-related gene polymorphisms has been examined in several studies. TLR7 gene product has a fundamental role against single-stranded RNA viruses. Represented polymorphisms in the TLR-7 gene could change human immune system function and increase human susceptibility to specific viral pathogens. Finally, that can increase or influence the severity of diseases (17, 18). Clearance of several viral infections, for example, human cytomegalovirus (HCMV), vesicular stomatitis virus (VZV), and hepatitis B virus (HBV), is principally dependent on the activation of TLR7-dependent signaling (19).

To evaluate the mentioned hypotheses, the present study aimed to determine the relationship between TLR3 and TLR7 gene polymorphisms frequencies and the severity of the disease in hospitalized and suspected COVID-19 patients in the Iranian population.

MATERIALS AND METHODS

Study design and define population. This case-control study was conducted in the Medical Genomics Research Center, Tehran Medical Sciences, Islamic Azad University, in collaboration with the Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran. Pharyngeal and nasopharyngeal swab specimens from unvaccinated individuals were collected from the Ghods Clinics, Islamic Azad University, in March-June 2020. This study has approval number IR.TUMS.VCR.REC.1399.378 from the Ethics Committee of Tehran University of Medical Sciences.

Inclusion criteria. The hospitalized patients with a history of fever or respiratory symptoms who had confirmed as being positive by SARS-CoV-2 RT-PCR were categorized in the case group. A non-hospitalized patient group with a history of mild fever or respiratory symptoms with negative SARS-CoV-2 RT-PCR results was considered the control group. All specimens with improper sample collection techniques were excluded from the study.

Extraction, amplification, and determination of targeted segments. Genomic DNA from all defined specimens was extracted using a commercial kit (Sinacolon DNA Extraction Kit DNP, Iran) according to the manufacturer's instructions. The purity and concentration of extracted DNA were measured using a NanoDrop Spectrophotometer (Eppendorf BioPhotometer). All extracted DNA was stored at -20°C until use. For this study, single nucleotide polymorphisms in both genes TLR3 (rs3775291 and rs3775296) and the TLR7 (rs179008 and rs179009) in all specimens were genotyped by PCR-restriction fragment length polymorphism (PCR-RFLP) methods.

All target fragments containing the polymorphism site of TLR3 and TLR7 were amplified using specific primers. The amplification conditions and the sequences of primers are listed in Table 1.

Digestion of PCR product by restriction enzymes to determine SNPs frequencies. Amplified products of TLR3 SNPs (rs3775291 and rs3775296) and TLR7 SNPs (rs179008 and rs179009) were digested using related restriction enzyme: HpyF3I (Thermo Scientific™ ER1881) for enzymatic digestion of rs3775291, MboII (Thermo Scientific™ ER0821) for digestion of rs3775296, XapI (Thermo Scientific™ ER1381 Inc., USA) and *Hin1II* (Thermo Scientific™ ER0471 Inc., USA), for digestion rs179008 and rs179009, respectively. Digestion condition for each enzyme was performed according to the manufacturer's instructions.

Then the 10 µl of digested DNA products were loaded in 2.5% agarose gels to properly separate fragments of digested DNA and visualized by ultraviolet light. The genotypes were determined based on the size of migrated DNA (well-defined pattern) on the gel compared with a 50 bp DNA ladder (Yekta Tajhiz Cat No. YT8501) as a DNA size marker. The results are interpreted based on the size pattern in (Table 1).

Statistical analysis. The frequency of genotype distributions of the case and control groups for all SNPs were evaluated for deviation from Hardy-Weinberg equilibrium (HWE) using the Pearson Chi-square goodness-of-fit test and if P-value <0.05 the equilibrium is not in agreement with HWE. The association between both TLR7 polymorphisms and allelic frequencies with the risk of COVID-19 disease between the case and control group were evaluated by calculate of Odds ratios (ORs) and 99% confidential intervals (99% CIs) by using Logistic regression. The Bonferroni correction was done to determine the fre-

Table 1. The corresponding data for each analyzed SNP

Gene Name	SNP (Enzyme)	Primer sequence (5'–3')	Genotype (Type)	Fragment's size (bp)	PCR Protocol (Denaturation, Cycles, Extension)
TLR3	rs3775291 (HpyF3I)	F: GGCTAAAATGTTTGAGCAC R; GATTTTATTCTTGGTTAGGCTGA	CC (Homozygous Reference) CT (Heterozygous) TT (Homozygous recessive)	31, 169 31, 169, 200 200	95°C/5 min (95°C/45s-56°C/45s-72°C/45s) × 35 72°C/10 min
TLR3	rs3775296 (MboII)	F: GCATTTGAAAGCCATCTGCT R: AAGTTGCGGCTGGTAATCT	CC (Homozygous Reference) CA (Heterozygous) AA (Homozygous recessive)	279 279, 257, 17 257, 17	95°C/5 min (95°C/45s-56°C/45s-72°C/30s) × 35 72°C/7 min
TLR7	rs179008 (XapI)	F: TAACAACGAATAGGAAAATGC R: GTTTTAGGAAACCATCTAGCC	AA (Homozygous Reference) AT (Heterozygous) TT (Homozygous recessive)	66, 137, 203 66, 137, 166, 203 166, 203	94°C/5 min (94°C/30s-54°C/30s-72°C/40s) × 35 72°C/5 min 72°C/5 min
TLR7	rs179009 (*Hin1II*)	F: TATTTGCTGCCTCTCTTTTGC R: GCTGCTTCTACCCTCTCGAA	TT (Homozygous Reference) TC (Heterozygous) CC (Homozygous recessive)	86, 104 86, 104, 190 190	95°C/5 min (95°C/45s-56°C/45s-72°C/30s) × 35 72°C/7 min

quency of genotypes-alleles in each group considered and to consider dependency in multiple tests (0.05/n). All experiments were tested in triplicate, and about 10% of samples were randomly retested to minimize error. The SPSS software version 24 (SPSS Inc., Chicago, USA) was performed for all statistical analyses.

RESULTS

The study population for TLR3 (rs3775291 and rs3775296) and TLR7 (rs179008 and rs179009) polymorphisms are shown in Table 2. Due to limitations in specimen collection at the beginning of the epidemic peak in country, the population numbers in each SNP differed.

The allele and genotype frequencies of SNP of TLR3 (rs3775291 and rs3775296) and TLR7 (rs179008 and rs179009) were determined by the PCR-RFLP-based method (Figs. 1-4).

Table 2. Number of study population based on gender in TLR3 (rs3775291 and rs3775296) and TLR7 (rs179008 and TLR7 rs179009) polymorphisms

	TLR3 rs3775291	TLR3 rs3775296	TLR7 rs179008	TLR7 rs179009
Patients	F: 113	F: 98	F: 103	F: 120
with COVID-19	M: 122	M: 120	M: 112	M: 124
(Case Group)	Total: 235	Total: 218	Total: 215	Total: 244
Suspected patients to	F: 42	F: 28	F: 46	F: 72
COVID-19	M: 64	M: 70	M: 76	M: 84
(Control Group)	Total: 106	Total: 98	Total: 122	Total: 156

F: Female, M: Male

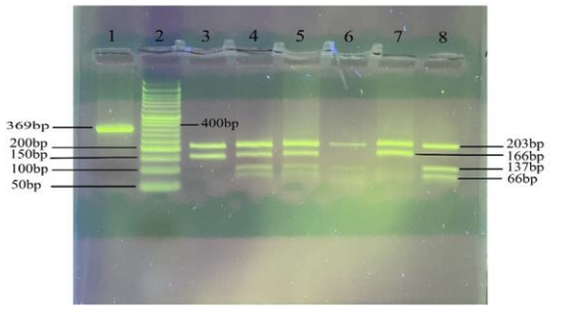

Fig. 1. Electrophoresis results of TLR7 SNPs (rs179008) after ApoI (Xpal) restriction enzyme cleavage on 2.5% agarose gel lanes 6 and 8, AA Wild Type, lanes 4 and 5, AT Heterozygous, lanes 3 and 7, TT Homozygous Recessive, lane 2 DNA ladder 50 bp and lane 1 PCR products.

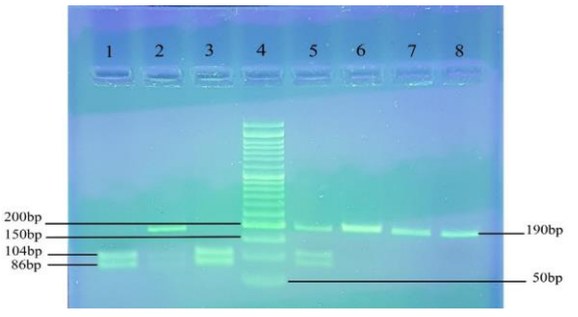

Fig. 2. Electrophoresis results of TLR7 SNPs (rs179009) after Hin1II (NlaIII) restriction enzyme cleavage on 2.5% agarose gel lanes 1 and 3, TT Wild Type, lanes 2 and 5, TC Heterozygous, lanes 6 and 7, CC Homozygous Recessive, lane 4 DNA ladder 50 bp and lane 8 PCR products.

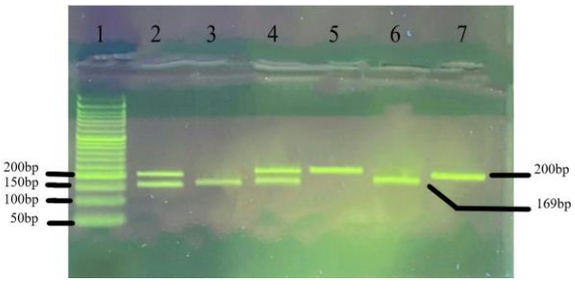

Fig. 3. Electrophoresis results of TLR3 SNPs (rs3775291) after HpyF3I (DdeI) restriction enzyme cleavage on 2.5% agarose gel lanes 3 and 6, CC Wild Type, lanes 2 and 4, CT Heterozygous, lane 5, TT Homozygous Recessive, lane 1 DNA ladder 50 bp and lane 7 PCR products.

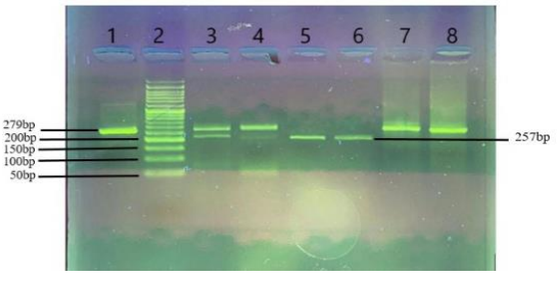

Fig. 4. Electrophoresis results of TLR3 SNPs (rs3775296) after MboII restriction enzyme cleavage on 2.5% agarose gel lanes 7 and 8, CC Wild Type, lanes 3 and 4, CA Heterozygous, lanes 5 and 6, AA Homozygous Recessive, lane 2 DNA ladder 50 bp and lane 1 PCR products.

According to the analyzed data presented in Table 3, the study results showed a significant difference in both frequencies of genotype and allele of TLR7 rs179008 and rs179009 loci between the studied case and control groups.

Regarding TLR7 rs179008 gene polymorphisms, AA,

Table 3. Distributions and association analysis of TLR3 SNPs (rs3775291 and rs3775296) and TLR7 SNPs (rs179008 and rs179009) analyzed genotypes and allele frequencies among case and control groups

		Control (%)	Case total: (%)	p-value*	¥OR (99%, CI)
TLR3 rs3775291 (HpyF3I)	CC	68 (64.2)	130 (55.4)	-	ref (1.00)
All subjects (106-235)	CT	32 (30.2)	88 (37.4)	0.154	1.438 (0.746-2.774)
	TT	6 (5.6)	17 (7.2)	0.429	1.482 (0.411-5.344)
	Total Genotypes	106	235	<0.0001#	---
	C allele	168 (79.2)	348 (74.0)	-	ref (1.00)
	T allele	44 (20.8)	122 (26.0)	0.144	1.339 (0.906-1.979)
TLR3 rs3775296 (MboII)	CC	82 (83.7)	160 (73.4)	-	ref (1.00)
All subjects (98-218)	CA	14 (14.3)	54 (24.8)	0.038	1.977 (0.847-4.616)
	AA	2 (2.0)	4 (1.8)	0.978	1.025 (0.107-9.803)
	Total Genotypes	98	218	<0.0001#	---
	C Allele	178 (90.8)	374 (85.8)	-	ref (1.00)
	A Allele	18 (9.2)	62 (14.2)	0.080	1.639 (0.942-2.854)
TLR7 rs179008 (XapI)	AA	88 (72.1)	120 (55.8)	-	ref (1.00)
All subjects (122-215)	AT	24 (19.7)	74 (34.4)	0.003#	2.261 (1.117-4.575)
	TT	10 (8.2)	21 (9.8)	0.291	1.540 (0.537-4.417)
	Total Genotypes	122	215	<0.0001#	---
	A Allele	200 (82)	314 (73.0)	-	ref (1.00)
	T Allele	44 (18)	116 (27.0)	0.009	1.679 (0.987-3.478)
TLR7 rs179009 (Hin1II)	TT	120 (76.9)	110 (45.1)	-	ref (1.00)
All subjects (156-244)	TC	16 (10.3)	100 (41.0)	< 0.0001#	6.818 (3.149-14.134)
	CC	20 (12.8)	34 (13.9)	0.047	1.855 (0.832-4.134)
	Total Genotypes	156	244	<0.0001#	ref (1.00)
	T Allele	256 (82.1)	320 (65.6)	-	ref (1.00)
	C Allele	56 (17.9)	168 (34.4)	< 0.0001#	2.400 (1.701-3.386)
Overall p-value*		…	…	< 0.0001#	---

*:calculated by Bonferroni correction for 12 (4 for SNP and 3 for genotype) or 8 (4 for SNP and 2 for allele) comparisons (P-value threshold considered at level 0.004167 and 0.00625)

Significant.

¥: confidence interval calculated as correspond to Bonferroni correction.

AT, and TT genotypes frequencies were 55.8%, 34.4 and 9.8% for the case group, 72.1%, 19.7%, and 8.2% for the control group, respectively, and showed the significant result in Heterozygous genotype (P-value = 0.003).

The frequency of TT, TC, and CC genotypes in rs179009 locus was 45.1%, 41%, and 13.9% in the SARS-CoV-2 positive cases and 76.9%, 10.3%, and 12.8% in the control group, respectively. A significant result was observed in frequency of heterozygous genotype between the case and control groups (P< 0.0001) but homozygous recessive genotype was not significant (P>0.004167).

The minor allele frequencies of the TLR7 SNPs rs179009 were compared between the case and control groups. In TLR7, the frequency of minor alleles in rs179009 locus was 65.6% and 34.4% in the SARS-CoV-2 positive cases and 82.1% and 17.9% in the control, respectively. Analysis of allele frequencies of TLR7 rs179009 locus in the case-control groups evaluated in this study showed that the C allele (Mutant Allele) compared with the T allele (Wild Allele) may be associated with increased risk of susceptibility to COVID-19 (P-value < 0.0001, OR: 2.400, 99% CI: 1.701-3.386). Also In TLR7, the frequency of major and minor alleles (A>T) in rs179008 locus were 73% and 27% in the SARS-CoV-2 positive cases and 82% and 18% in the control, respectively; but this distribution was not significant (P>0.006).

The genotype frequency of TLR3 rs3775291 locus showed no significant difference between the case and

control groups (P>0.004167). Frequency of CC, CT, and TT genotypes in TLR3 rs3775291 were 55.4%, 37.4% and 7.2% in the case and 64.2%, 30.2% and 5.6% in the control groups, respectively.

For TLR3 rs3775296 locus, frequency of CC, CA, and AA genotypes were 73.4%, 24.8%, and 1.8% in the case group and 83.7%, 14.3%, and 2% in the control groups, respectively. CA (Heterozygous) and AA (Homozygous) genotypes in TLR3 rs3775296 locus showed no association with increased risk of susceptibility to COVID-19 compared to CC genotype, between the case and control groups (P>0.004167).

The alleles frequency of TLR3 rs3775291 and rs3775296 loci did not show any significant difference between the case and control groups (P>0.006).

Furthermore, the frequency of TLR7 rs179009, TLR7 rs179008, TLR3 rs3775291 and rs3775296 genotypes and alleles in both case and control groups were fine in agreement with HWE (P>0.05).

According to the analyzed data that presented in Table 4, in TLR7 SNP (rs179008) in AT genotype frequency within the male group were significant differences between the case and control groups (P= 0.001) and in TLR7 SNP (rs179009) in TC genotype frequencies within both male and female groups was a significant difference in the SARS-CoV-2 positive cases and control groups (P<0.0001 and P<0.0001 respectively). The minor allele frequency of the TLR7 SNP (rs179008) within male group showed a significant difference between the case and control groups (P= 0.006). In addition, a significant association in the frequency of minor allele of the TLR7 rs179009 within male and female groups between the case and control groups were determined (P<0.0001 and P= 0.006 respectively). Our results showed no significant differences in the frequency of genotype and allele of TLR3 (rs3775291 and rs3775296) within both male and female groups between SARS-CoV-2 positive cases and control groups (P > 0.004167).

DISCUSSION

TLRs are a crucial component in detecting viruses and stimulating immune responses (20-22). The current study is the first research to evaluate TLRs polymorphisms' effects on individuals' susceptibility to COVID-19 in Iran. We showed that TLR3 rs3775296 and rs3775291 polymorphisms have no influenced COVID-19 severity, this may be due to the main stimulator of TLR3 is dsRNA, but SARS-CoV-2 is a single strand RNA virus. In contrast to our results, several studies have shown the role of SNPs in TLR3 rs3775296 and rs3775291 loci in susceptibility to several viral infections caused RNA viruses. Sironi et al. performed a study to determine the effect of TLR3 polymorphisms on HIV-1 susceptibility in a population of 102 infected people and 124 healthy individuals as a control group in Spain; against our results, the role of TLR3 SNP rs3775291 was shown in people susceptible to HIV-1 infection (23). Habibabadi et al. found an influential association between TLR3 SNP rs3775296 polymorphism and sensitivity to HTLV-1 in a study of 100 HTLV-1 patients, and 118 healthy blood donors in the Iranian population (24). Barkhash et al. examined the effect of polymorphism in TLR3 rs3775291 and found that there is a direct relationship between the presence of minor TLR3 SNP rs3775291 allele with an increase in the incidence of Tick-borne encephalitis virus (25). Ishizaki et al. showed a direct correlation between the presence of polymorphisms in TLR3 SNP rs3775291 and the likelihood of developing acute sclerosing panencephalitis due to measles virus in the Japanese population (26). This may be due to TLR3 signaling could be triggered by other elements such as TNF, IFN-γ, or microbial ligands. Therefore, as a consequence of SARS-CoV-2 infection, inflammatory cytokines can trigger TLR3 receptors (22).

Similar to our study, Askar et al. studied TLR3 polymorphism rs3775291 and the clinical manifestations of liver patients with chronic hepatitis C and found no significant difference between the distribution of TLR3 rs3775291 genotypes in clinical manifestations or progression of liver disease (27). Zayed et al. showed that the frequency distribution of genotypes and alleles in TLR3 rs3775296 was not significantly different between HCV-positive patients and the control group. Therefore, TLR3 rs3775296 has no effect on disease progression (28).

Interestingly, Dhangadamajhi et al. (2021), examined available data on the frequency of alleles and genotype of TLR3 gene fragment rs3775291 in populations of 40 different countries and their relationship with COVID-19 incidence and mortality and showed that the mutant allele is associated with disease susceptibility and mortality (29). However, by analyzing this reported data, Abhijit Pati et al. (2021) did not find any significant relationship between polymorphism at rs3775291 and susceptibility to COVID-19

Table 4. Frequencies of all analyzed genotypes and alleles within female and male among the case and the control groups

			Control (Total: 168) N (%)	Case (Total: 244) N (%)	p-value*	¥OR (99%, CI)
TLR3	Male (64-122)	CC	42 (65.6)	65 (53.2)	-	ref (1.00)
rs3775291		CT	16 (25.0)	49 (40.2)	0.051	1.979 (0.998-3.925)
(HpyF3I)		TT	6 (9.4)	8 (6.6)	0.796	0.862 (0.279-2.660)
		C allele	100 (78.1)	179 (73.4)	-	ref (1.00)
		T allele	28 (21.9)	65 (26.6)	0.314	1.297 (0.782-2.151)
	Female (42-113)	CC	26 (61.9)	65 (57.5)	-	ref (1.00)
		CT	16 (38.1)	39 (34.5)	0.946	0.975 (0.466-2.041)
		TT	0 (0.0)	9 (8.0)	0.107	1.138 (1.046-1.239)
		C allele	68 (81.0)	169 (74.8)	-	ref (1.00)
		T allele	16 (19.0)	57 (25.2)	0.256	1.433 (0.770-2.670)
TLR3	Male (70-120)	CC	62 (88.6)	86 (71.7)	-	ref (1.00)
rs3775296		CA	8 (11.4)	30 (25.0)	0.021	2.703 (0.901-7.247)
(MboII)		AA	0 (0.0)	4 (3.3)	0.146	1.047 (1.001-1.094)
		C Allele	132 (94.3)	202 (84.2)	-	ref (1.00)
		A Allele	8 (5.7)	38 (15.8)	0.005#	3.104 (1.404-6.862)
	Female (28-98)	CC	20 (71.4)	74 (75.5)	-	ref (1.00)
		CA	6 (21.4)	24 (24.5)	0.881	1.081 (0.389-3.004)
		AA	2 (7.1)	0 (0.0)	0.051	0.909 (0.797-1.038)
		C Allele	46 (82.1)	172 (87.8)	-	ref (1.00)
		A Allele	10 (17.9)	24 (12.2)	0.281	0.642 (0.287-1.437)
TLR7	Male (76-112)	AA	58 (76.3)	60 (53.6)	-	ref (1.00)
rs179008		AT	12 (15.8)	41 (36.6)	0.001#	3.303 (1.580-6.906)
(XapI)		TT	6 (7.9)	11 (9.8)	0.289	1.772 (0.615-5.106)
		A Allele	128 (84.2)	161 (71.9)	-	ref (1.00)
		T Allele	24 (15.8)	63 (28.1)	0.006#	2.087 (1.235-3.526)
	Female (46-103)	AA	30 (65.2)	60 (58.3)	-	ref (1.00)
		AT	12 (26.1)	33 (32.0)	0.431	1.375 (0.622-3.038)
		TT	4 (8.7)	10 (9.7)	0.724	1.250 (0.362-4.318)
		A Allele	72 (78.3)	153 (74.3)	-	ref (1.00)
		T Allele	20 (21.7)	53 (25.7)	0.460	1.247 (0.694-2.240)
TLR7	Male (84-124)	TT	64 (76.2)	54 (43.5)	-	ref (1.00)
rs179009		TC	12 (14.3)	50 (40.4)	<0.0001#	4.938 (2.388-10.214)
(Hin1II)		CC	8 (9.5)	20 (16.1)	0.018	2.963 (0.879-8.574)
		T Allele	140 (83.3)	158 (63.7)	-	ref (1.00)
		C Allele	28 (16.7)	90 (36.3)	<0.0001#	2.848 (1.760-4.609)
	Female (72-120)	TT	56 (77.8)	56 (46.7)	-	ref (1.00)
		TC	4 (5.5)	50 (41.7)	<0.0001#	12.500 (4.229-36.945)
		CC	12 (16.7)	14 (11.6)	0.724	1.167 (0.496-2.744)
		T Allele	116 (80.6)	162 (67.5)	-	ref (1.00)
		C Allele	28 (19.4)	78 (32.5)	0.006#	1.995 (1.218.3.267)

*:calculated by Bonferroni correction for 12 (4 for SNP and 3 for genotype) or 8 (4 for SNP and 2 for allele) comparisons (P-value threshold considered at level 0.004167 and 0.00625)

Significant.

¥: confidence interval calculated as correspond to Bonferroni correction.

or mortality rate. They criticized the study conducted by Dhangadamajhi. Abhijit Pati et al. believed that the results of the study by Dhangadamajhi have deviated from the Hardy-Weinberg equilibrium (30).

The main reason for the controversy between the results of our study and the Dhangadamajhi study could be related to the differences Iranian among the ethnic groups, which could impact allelic and genotypic frequencies. On the other hand, the statistical population of the two studies basically is different.

Additionally, we evaluated the polymorphism associations in the TLR7 rs179008, TLR7 rs179009, and TLR7 rs179009 loci and the risk of susceptibility to SARS-CoV-2 infection among the Iranian population. This data indicates that TLR7 rs179008 AT genotype (2.261-fold) and T allele (1.679-fold) were linked to a highly increased risk of susceptibility to SARS-CoV-2 infection among the Iranian population. Moreover, TLR7 rs179009 TC was associated with a 6.818 -fold greater risk of susceptibility to COVID-19 disease among the Iranian population. The TLR7 rs179009 C allele may be a main variant responsible factor for the COVID-19 severity among the Iranian population.

The prominent functional roles of TLR7 included the viral RNA-sensing (ssRNA) and priming of the innate immune response. The rs179009 locus is located within intron 2 of TLR7, which does not correspond with any protein-coding function. Inversely, the TLR7 rs179008 locus is located within the signal sequence of the TLR7 gene; any degeneracy in the signal peptide may modulate the function of the affected protein (17, 18).

Because of the TLR7 gene linked to the X-chromosome, it is presumed that the gender-dependent variable must be displayed a statistically significant difference between males and females (31). Several studies indicated the sex-based differences presenting in the outcome of the pathogenesis of infections, which may reflect the influence of sex hormones on both innate and adaptive immune systems; for instance, estradiol can enhance IFN-a response by its regulatory function on TLR-mediated immune response of plasmacytoid dendritic cells (17).

Our results are similar to Xin-Su Wei et al. report that TLR7 and TLR9 polymorphisms are associated with HCV infection status. The results were shown that the TLR7 polymorphism (rs179009) might be used as a risk factor in susceptibility to chronic HCV in the female population (16). In 2009, the effect of TLR7 polymorphisms on AIDS was assessed by Djin-Ye Oha et al. in the case (734 HIV-positive) and control (545 healthy) studies. TLR7 polymorphism (rs179008) was indicated to be correlated with higher viral load and faster clinical progression in HIV patients (19).

Furthermore, several studies described that TLR7 rs179009 polymorphisms might be a risk factor for susceptibility to chronic HCV in the Egyptian female in 2019 (32), HCV infection in Chinese women in 2013 (33), associated with HIV in India in 2020 (34). Previously, the association of TLR7 polymorphism with susceptibility to enterovirus-71-related disease and protection against chronic HCV among the Chinese population had been revealed (35). Previously, based on the in-silico Trial study, Teimouri et al. have reported the critical role of TLR7 genetic polymorphism in COVID-19 infection (36).

Activation of the TLR7-dependent signaling pathway can trigger the antiviral cytokines production, including IFNs, secretion of IL-6 and IL-12, and TNFα, and subsequently could mediate the vigorous Th1-dependent immune response against viral diseases. However, released cytokines are the main cause of inflammation and can lead to organ injury after viral infection (37-40). A previous study showed that impaired TLR7 function could reduce IFN production and augment the secretion of inflammatory cytokines. Subsequently, it may lead to an increased risk for persistent and progression of the viral disease by chronic activation of the immune system, including, HIV-1, HBV, and HCV (41).

Urcuqui-Inchima et al. reported that both TLR3 and TLR7 have a key role in controlling dengue virus replication and provoking cellular antiviral response (35). Undoubtedly, sensing and the innate immune response triggering ability can affect any mutation formation incidence, and consequently, adverse effects on the immune response will occur.

CONCLUSION

There was a significant association between the TLR7 rs179008 and rs179009 polymorphisms with susceptibility to COVID-19 disease among the Iranian population. These results could clarify the mechanisms of SARS-CoV-2 pathogenesis and patients managements, consider the therapeutic strategies, and eligibility for antiviral treatment.

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