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Association Between Cytokine Gene Polymorphisms and Human Susceptibility to Brucellosis

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Abstract

Background: Allelic single nucleotide polymorphisms (SNPs) in cytokine-encoding genes can affect the degree of cytokine production, and may be related to the tendency to infectious illnesses as well as various clinical consequences.
Objectives: The aim of this work was to evaluate the possible role of SNPs in the regions of the IL-10 (-592), IL-15 (-367), IL-18 (-656), IL-12 (+1188), IFN-γ (+874), TNF-α (-308), and TNF-β (+252) genes in susceptibility or resistance to brucellosis and its crucial complications.
Methods: In a period of one year, 125 patients with acute brucellosis referring to 3 large public teaching hospitals were enrolled in this study. We studied the SNPs of IL-10, IL-15, IL-18, IL-12, IFN-γ, and TNF-α/β genes using the allele specific polymerase chain reaction (AS-PCR) with sequence-specific primers.
Results: Frequency of GG genotype in the TNF-α and TNF-β-encoding genes increased significantly by 52% and 31.2% in patient and control groups, respectively. For IFN-γ, TA genotype was found highly enhanced in patients (60%), while the frequency of AA and TT genotypes were higher in controls (23.2% and 26.4%, respectively). The AA and CC SNPs in IL-12 were dominant in both patient (78.4%) and control (14.4%) groups. In the patient group, the GG and TT genotypes had a higher frequency for genes encoding IL-15 (33.6%) and IL-18 (89.6%).
Conclusions: Based on the present study, some SNPs within the several cytokine genes, including TNF-α/β (-308/+252), IFN-γ (+874), IL-15 (-367), IL-18 (-656), and IL-12 (+1188) are related to the susceptibility or resistance to brucellosis. In order to approve the biological consequence of our results, additional investigations should be carried out in larger population groups.

Keywords: Brucellosis, Cytokines, Single Nucleotide Polymorphisms

1. Background

The *Brucella* species are small facultative intracellular gram-negative aerobic bacteria with ability to infect both humans and animals (1). Brucellosis is usually a common zoonotic infection that causes worldwide disease afflicting more than half a million individuals annually (2). *B. melitensis* and *B. abortus* are the most common agents of human brucellosis in many countries (3). This illness is endemic in many Asian areas, such as Iran, Turkey, the Arabian Peninsula and Mediterranean countries, Indian subcontinent and Central and South America (4, 5). The actual prevalence of brucellosis in the world is indefinite because of poor reporting systems and lack of access to reliable diagnostic tests in many developing countries (4). More than 500,000 cases with brucellosis are reported to the world health organization (WHO) globally every year, most of whom are from developing countries (6). According to the center for disease control (CDC), the incidence rate of brucellosis in Iran was 39 per 100,000 and 30 per 100,000 populations in 2005 and 2007, respectively. Also, the incidence of brucellosis has increased to 130 per 100,000 populations in western Iran in recent years (6).

The *Brucella* spp. enter macrophage-monocyte lineage cells, survive, multiply within them, and spread in mononuclear phagocytes (7). Consequently, acquired cell-dependent immune, determined by the T-helper1 (Th1) lymphocyte activation and subsequent activation of macrophages, plays a crucial role in the protection against this infectious disease (7). Production of cytokine index can be considered as T-helper- immune cell, interleukin-2 (IL-2), interferon gamma (IFN-γ), or T-helper-cell-class-2 (Th-2) responses inducing humoral type of immunity (IL-

4/5/6/10) and Th-3 type determined by TGF-β (8).

Apart from environmental elements and pathogen strain differences, host genetic determinants are also major causes of susceptibility to or consequence of infectious illnesses. Expression and release of cytokines are dependent, at least in part, on genetic variation within the promoter region or other regulatory sequences of the cytokine genes (8, 9). It has been demonstrated that some single nucleotide polymorphisms (SNPs) in the genes encoding certain cytokines could not only rise susceptibility to some infectious diseases, but also change the course and prediction of the disease (9). For example, it is possible that TNF-α gene SNPs have an impact on the rate of TNF-α synthesis, which in turn might have an effect on inflammatory responses. The substitution of Guanine (G) or Adenine (A) at position +252 of TNF-β and -308 (A/G) genotype of TNF-α is associated with the susceptibility to autoimmune disease and microbial infections (10).

2. Objectives

There are limited data about the effects of polymorphisms on the outcome of brucellosis in the infected patients in Iran. In the present study, we aimed to determine the possible association between cytokine gene SNPs and either susceptibility to or development of focal complications of brucellosis, and also demonstrate that the systemic immune response to *Brucella* antigens might be enhanced by mutation in the cytokine encoded genes.

3. Methods

3.1. Study Population

This cross-sectional study was performed during a one-year period of time in 2015 - 2016. The study population comprised two patient and control groups. 125 patients with confirmed acute brucellosis were admitted to three teaching therapeutic centers, including Imam Khomeini, Shariati, and Rasoul-e-Akram hospitals. The mean age of the study population was 49 $\pm$ 1.5 years, with a range of 17 to 76 years. The isolates were collected from the patients in various age groups: 12 - 22 years (n = 9), 23 - 32 years (n = 21), 33 - 43 years (n = 39), 44 - 54 years (n = 32), 55 - 65 years (n = 17), and 66 - 76 years (n = 7). 72 (57.6%) patients were male and 53 (42.4%) were female. 5 - 10 mL whole blood specimens were obtained from each participant and kept in EDTA-containing tubes for DNA extraction. Brucellosis was identified based on the clinical manifestation (e.g. Night sweats, fever, debility, arthralgia, weight loss, lymphadenopathy, splenomegaly, malaise, and myalgia), and/or positive blood cultures and serological methods. A single high titer ($\geq$ 1:160) of standard agglutination test (SAT) was considered as a prognostic standard for positive serological results that was approved by a high titer ($\geq$ 1:160) of 2-mercaptoethanol test (2ME) at the time of infection.

The control group comprised healthy blood donors with no history of brucellosis or genetic disorders. They were matched with the patients for sex, age, and geographic area; they had a similar history and were at the same risk of exposure to brucellosis. The study was approved by the institutional review board and the research ethics committee of Islamic Azad University, Qom branch.

3.2. Determination of Cytokine Gene Polymorphism

SNPs for TNF-α/β, IFN-γ, and IL-10/12/15/18 were analyzed by allele specific polymerase chain reaction (AS-PCR). Cellular DNA was obtained from EDTA-treated peripheral venous blood by salting out method (11). The concentration and the quality of the extracted DNA were assessed using a Nanodrop spectrophotometer (ND-1000; Thermo Scientific, Wilmington, DE, USA). Primer sequences used for gene amplification are shown in Table 1.

AS-PCR was conducted for the amplification of target genes; a volume of 1.0 μL of total extracted DNA was added to a whole volume of 25 μL PCR reaction mixture including 2.0 μL of 10$\times$PCR buffer, 1.3 μL $MgCl_2$ (50 mM), 0.5 μL dNTPs (10 mM), 1.0 μL of each primer, 0.5 μL of Taq DNA polymerase (5 U/μL) (Amplicon Co., Denmark), and 17.7 μL double-distilled water (ddH_2O). PCR was performed in a thermal gradient cycler (Eppendorf Co., Germany) according to the following procedure: initial denaturation at 95°C for 5 minutes, then 33 cycles with denaturation at 94°C for 30 seconds, annealing at 61°C for 55 seconds, extension at 72°C for 32 seconds, and final extension at 72°C for 40 seconds. The amplified products were visualized using a UV transilluminator, following electrophoresis on 1% agarose gel stained with Gel Red™.

3.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, ver. 23 (IBM). Chi-square test and logistic regression model were applied for analyzing data. Also, odds ratio (OR) and 95% confidence interval (CI 95%) were calculated. In addition, odds ratios were adjusted for age and sex variables in logistic regression model. Analyses were interpreted as statistically significant at $P < 0.05$.

4. Results

As shown in Table 2, all SNPs were calculated in the Hardy Weinberg equilibrium in the patient and control

Table 1. The Nucleotide Sequences of the Primers Used in This Study

Target Genes	Primer Sequence (5'→3')	References
TNF-α	F, TCTCGGTTTCTTCTCCATCG	(12)
	R, ATAGGTTTTGAGGGGCATGG	
TNF-β	F, AACCTGCTGCTCACCTTGTT	(13)
	R, CAGTGCAAAGGCTCCAAAGA	
IFN-γ	F, TGAACGCTACACACTGCATC	(14)
	R, CACTCGGATGAACTCATTGA	
IL-10	F, CCTAGGTCACAGTGACGTGG	(15)
	R, GGTGAGCACTACCTGACTAGC	
IL-12	F, TTTGGAGGAAAAGTGGAAGA	(16)
	R, AACATTCCATACATCCTGGC	
IL-15	F, TCT TCA ATA CTT AAG GAT TTAC	(17)
	R, CAA AAG AGT GGG ATA AGT GA	
IL-18	F, AGGTCAGTCTTTGCTATCATTCCAGG	(18)
	R, CTGCAACAGAAAGTAAGCTTGCGGAGAGG	

groups using chi-square test. Polymorphism in the TNF-α gene at position -308 showed that the frequency of GG genotype increased significantly in patients compared to the control group (52% vs. 27%; P = 0.016, OR = 0.789, 95% CI = 0.345 - 1.890). The patient group in comparison with the control group showed a higher frequency of the AA genotype at position +252 in TNF-β (34.4% vs. 27.2%; P = 0.038, OR = 1.455, 95% CI=0.091 - 1.734) and GG genotype of TNF-β (+252) (27.2% vs. 31.2%; P = 0.047, OR = 1.234, 95% CI = 0.764 - 2.986).

Polymorphism of IFN-γ gene (at position +874) indicated an increased prevalence of the TA genotype in the patient group (60%, P = 0.012, OR = 3.21, 95% CI = 0.452-3.167). However, the frequency of AA and TT genotypes was higher in the control than the patient group. SNPs in the gene encoding IL-10 (at position -592) showed no significant difference between patient and control groups for all three genotypes studied.

The AA genotype at position +1188 of gene encoding IL-12 was more common in the patients than control group, while the CC genotype was more prevalent in the control group. The most common genotypes in the gene encoding IL-15 (at position -367) were GG (in the patient group) and GA (in the control group). At position -656 in the gene encoding IL-18, TT genotype was more frequent in patients, while both GG and TG genotypes were more common in controls. According to our data, other cytokine genotypes studied showed no significant between-group differences.

5. Discussion

Research to detect SNPs within the genes encoding cytokines, and determining and comparing the cytokine genotype(s) in patients infected with *Brocella* spp. or healthy individuals can improve our knowledge regarding brucellosis immunology and facilitate the development of new therapeutic and vaccination strategies. Recently, several reports have shown that SNPs in cytokine genes could be an important factor in resistance and/or susceptibility to brucellosis (9-11). To better understanding of brucellosis immunology, we investigated the association of gene polymorphisms of cytokines with this infectious disease.

It has been reported a noteworthy relationship between AA genotype in the promoter region of IFN-γ at the position +874 and various human diseases, such as brucellosis, hepatitis B, and tuberculosis (19). According to study by Bravo et al., the IFN-γ AA genotype was substantially higher in brucellosis patients than healthy people (20). In another study conducted by Budak et al., no association was demonstrated between IFN-γ +874 polymorphism and the risk of acquiring brucellosis in humans (21). In agreement with Budak et al., our results indicated that IFN-γ polymorphisms (at positions +874) were notably prevalent in patients than the control group.

There are several genetic polymorphic sites which are known to be associated with the production of TNF, including TNF-α (-308 G/A, -238 G/A) and TNF-β (+252 A/G) (22, 23). In some previous studies, TNF-α polymorphisms at positions -308 and -238 have been associated with suscepti-

Table 2. Distribution of the Cytokine Gene Polymorphisms in Patients with Brucellosis

Cytokine Gene	Genotype	No. (%) of Patients	No. (%) of Healthy Control	Odds Ratio	95% CI	P Value
TNF-α (-308)	GG	65 (52)	34 (27.2)	0.789	0.345 - 1.890	0.016[a]
	AG	35 (28)	36 (28.8)	1.7	0.678 - 5.897	0.449
	AA	25 (20)	55 (44)	1.9	0.238 - 29.345	0.659
TNF-β (+252)	AG	57 (45.6)	43 (34.4)	0.871	0.677 - 1.984	0.578
	GG	34 (27.2)	39 (31.2)	1.234	0.764 - 2.986	0.047[a]
	AA	34 (27.2)	43 (34.4)	1.455	0.091 - 1.734	0.038[a]
IFN-γ (+874)	TA	75 (60)	63 (50.4)	3.21	0.452 - 3.167	0.012[a]
	AA	25 (20)	29 (23.2)	0.987	0.178 - 1.743	0.073
	TT	25 (20)	33 (26.4)	0.784	0.356 - 1.937	0.023[a]
IL-10 (-592)	AA	69 (55.2)	80 (64)	2.345	0.823 - 8.678	0.419
	AC	12 (9.6)	29 (23.2)	0.794	1.456 - 25.121	0.549
	CC	44 (35.2)	16 (12.8)	1.923	1.876 - 42.231	0.713
IL-12 (+1188)	AA	98 (78.4)	89 (71.2)	0.783	0.178 - 0.979	0.016[a]
	AC	16 (12.8)	18 (14.4)	2.765	1.345 - 12.654	0.457
	CC	11 (8.8)	18 (14.4)	1.351	1.981 - 13.451	0.017[a]
IL-15 (-367)	AA	43 (34.4)	39 (31.2)	1.231	0.567 - 1.986	0.246
	GG	42 (33.6)	37 (29.6)	1.278	0.987 - 2.642	0.039[a]
	GA	40(32)	49(39.2)	0.769	0.327 - 1.532	0.065
IL-18 (-656)	TT	112 (89.6)	104 (83.2)	0.945	0.231 - 1.763	0.017[a]
	TG	10 (8)	14 (11.2)	1.934	0.654 - 9.808	0.091
	GG	3 (2.4)	7 (5.6)	2.235	0.712 - 26.420	0.044[a]

[a] Statistically significant at 0.05 significance level.

bility to brucellosis (24). Rasouli et al., showed that the frequency of AA genotypes of TNF-β and distribution of A allele were significantly higher in patients than controls (25). It appears that both SNPs reported by Rasouli and coworkers are associated with low production of TNF-α, suggesting the increased susceptibility to infection. Reza et al., demonstrated that TNF-α -308 (A/A) genotype had a higher frequency in the population of patients in comparison with controls (26). Their results indicated that, although the frequency of allele in the two groups was not statistically remarkable, TNF-α polymorphism at nucleotide -308 (A/A) could be involved in the susceptibility to brucellosis (26). In addition, Caballero et al., found that there is no link between the TNF-α -308 (A/A) genotypes and brucellosis (27). Our results indicated that the GG genotypes of TNF-α and -β were significantly higher in patients than healthy people. It seems that individuals who inherit A allele as homozygous (AA) possibly produce lower levels of TNF-α, which could cause the lack of proper immune response at the early stages of *Brucella* infection, leading the incidence of a full-blown disease. Also, in persons with AG and GG haplotypes, due to more TNF-α production, the bacteria are controlled and the disease cannot develop.

IL-12 is a heterodimeric cytokine that has a key role in the promotion of type 1 immune response. Moreover, it has a protective effect against the *Brucella* infection (28). In a study conducted by Kamali-Sarvestani et al., IL-12 A allele, which is associated with higher production of IL-12, was significantly more frequent in the controls than the patients. Moreover, AA genotype was significantly more frequent in the controls than the patients (24). Based on these results, it can be concluded that individuals who acquire the AA genotype may produce higher levels of IL-12 which can lead to the initiation of CMI response to brucellosis. On the contrary, the results of the current study indicated that the AA genotypes of IL-12 (+1188) polymorphisms were significantly more frequent in patients with brucellosis compared to the healthy group.

IL-10 is a cytokine with anti-inflammatory action which can strongly prevent the release of cytokines, such as IL-2

and IFN-γ (27). Additionally, IL-10 has an obvious comitogenic effect on proliferation of B and T cells and enhances B cell antibody development (29). Karaoglan et al., showed that the rates of CT and CC genotypes of IL-10 (-819) were more likely in patient and control groups, respectively (30). In the study by Bravo et al., the association between IL-10 gene polymorphisms and susceptibility to brucellosis was not observed (20). Similar to the latter study, our results indicated no significant differences in the genotype distribution of IL-10 (-592) polymorphisms between the patients and controls.

IL-15 in synergy with IL-12 enhances IFN-γ production via NK and T cells and stimulates Th1 responses against the intracellular pathogens (31). In our study, three genotypes of IL-15 (-367), AA/GG and GA had nearly the same distribution in both groups of patient and control, while the GG genotype of IL-15 (-592) was significantly higher in the patient group compared to the control group. In the study by Kalani et al., no significant difference was observed between the frequency of alleles and genotype polymorphisms of IL-15 (-367) in the controls and patients, suggesting that there is not a significant relationship between IL-15 (-367) gene polymorphisms and susceptibility and/or resistance to brucellosis (22).

Some studies showed that SNPs of the IL-18 gene can lead to the increased rates of cytokine expression (23). Similar to findings by the Rasouli et al., our study showed that TT genotypes of IL-18 (-656) have a significantly increased frequency in the patients compared to the controls (32). These findings suggest that TT genotype of IL-18 (-656) could be involved as a potential risk factor in the susceptibility to brucellosis.

Our results also indicated that some genotypes of cytokine polymorphism, including IFN-γ (+874) TA, TNF-α (-308) GG, TNF-β (+252) GG, IL-12 (+1188) AA, IL-15 (-367) GG, and IL-18 (-656) TT are criteria for susceptibility to brucellosis. On the other hand, cytokine genotypes, such as IFN-γ (+874) AA and TT, IL-12 (+1188) CC, TNF-β (+252) AA, and IL-18 (-656) GG may play a protective role against this infection. It is important to evaluate the dependency of these SNPs in cytokine genes on expanding brucellosis in the Iranian population.

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Footnotes

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