SHORT PAPER

Interleukin-10 Gene Polymorphisms and Susceptibility to Brucellosis in Iranian Patients

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ABSTRACT

Background: Interleukin-10 (IL-10) is a Th2-type cytokine that inhibits macrophage activation. It is known that production of IL-10 is affected by its gene promoter polymorphisms. **Objective:** To investigate the relationship between IL-10 gene promoter polymorphisms and susceptibility to brucellosis. **Methods:** One hundred and ninety patients with brucellosis and 81 healthy animal husbandmen who owned infected animals and consumed their contaminated dairy products were included in this study. All individuals were genotyped for three bi-allelic IL-10 gene promoter polymorphisms at positions -1082(G/A), -819(T/C), and -592(A/C) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** The distribution of C alleles at positions -592 and -819 of IL-10 were significantly higher in patients than in the healthy animal husbandmen (p=0.034 and p=0.0086, respectively). IL-10 ATA single and double haplotypes were significantly higher in controls, compared to the patients (p=0.0278 and p=0.013, respectively). **Conclusion:** According to the results higher frequency of C alleles at positions -592 and -819 of IL-10 in patients may be considered as genetic factors for susceptibility to brucellosis.

Keywords: Brucella, Interleukin-10, Genetic Polymorphism, haplotype

INTRODUCTION

Th2 cytokines antagonize the macrophage activity effects of IFN-γ and inhibit the cell-mediated immune reaction. Interleukin-10 (IL-10), which is a Th2 cytokine, reduces production of IFN-γ and down regulates the Th1 effective mechanisms.

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Fernandes showed that the production of IFN-γ and the lytic capacity of the spleen cells against *Brucella abortus* increase due to neutralization of IL-10 by monoclonal antibodies (1). Similarly, down regulation of immune system in patients infected with intracellular bacteria (2) and parasites (3) have been shown.

There are striking differences between individuals in terms of their abilities to produce IL-10 following the lipopolysaccharide (LPS) stimulation of whole–blood cultures (4). In addition, there is evidence that the production rate of many cytokines is under genetic control and the polymorphisms of cytokine genes are potentially important as genetic predictors of disease susceptibility or clinical outcomes (5).

Functional polymorphisms were described for IL-10 gene promoter. The single-nucleotide polymorphisms (SNPs) at positions -1082(G/A), -819(T/C) and -592 (A/C) from the transcriptional start site of IL-10 gene are in linkage disequilibrium and they are responsible for different haplotypes (6). Association of at least one of these SNPs with various infectious diseases has been reported (5-8). Based on the important role of IL-10, we determined three functional biallelic polymorphisms in IL-10 promoter gene at positions -1082 G/A, -819 T/C and -592 A/C to find relationship between IL-10 gene polymorphisms and the susceptibility to *Brucella* in the Iranian population living in Fars province, southern Iran.

MATERIALS AND METHODS

Subjects. The study included 190 patients (82 males and 108 females, between 7-80 years of age) with active brucellosis and 81 non-relative healthy animal husbandmen (39 males and 42 females, between 5-74 years of age). Patients were selected randomly among 600 cases registered in the Health Center of Fars Province, Iran. Blood samples were collected in their places of residency following obtaining their informed consent. All patients were either farmers keeping animals (including diagnosed infected animals) or had a history of consuming raw milk and unpasteurized dairy products. Brucellosis was diagnosed according to the clinical findings (e.g., fever, night sweating, weakness, malaise, weight loss, splenomegaly, lymphadenophathy, myalgia and arthralgia), positive blood cultures and serological tests, defined as high titers of standard agglutination test (SAT ≥ 1/160) and confirmed by a titer of ≥1/160 in 2-mercaptoethanol test at the time of infection.

The control group was selected randomly among healthy animal husbandmen who had close contacts with animals infected with *Brucella* and consumed their milk and dairy products. They did not show any clinical manifestations after a six-month follow up. Brucellosis in their animals was confirmed by serological tests carried out in the laboratory of Fars Province Veterinary Administration. The ethnicity and place of residency of the controls were the same as the patients.

Genotyping. DNA was extracted from anti-coagulated blood samples. The genotypes of IL-10 promoter region at positions -1082, -819 and -592 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), (Table 1).

Table 1. Polymerase chain reaction (PCR) conditions for IL-10 gene amplifications

Locus	PCR condition	Primer Set	Restriction enzyme	Fragment Length (*Base pairs*)
A-592C	94°C(5m); 35 cycle: 94°C(1m), 63°C(70s), 72°C(1m); 72°C (10m) 250 ng DNA, 200 µmol dNTPs, 5.5 mM $MgCl_2$	F:5'-CCTAGGTC ACAGTGACGTGG-3' R:5'- GGTGAGCACTACC TGACTAGC-3'	*RsaI* (Fermentas)	AA: 236,176 CC:412
T-819C	94°C(5m); 30cycle : 4°C(45m),60°C(45s),72°C (1m), 72°C(1m);72°C(5m) 250 ng DNA, 200 µmol dNTPs, 3.2 mM $MgCl_2$	F:5'-TCATTCTATGTGCTGGAGATGG-3' R:5'-TGGGGGAAGTGGGTAAGA GT-3'	*MaeIIII* (Fermentas)	TT: 209 CC:125, 84
G-1082A	95°C(10m); 35 cycle: 95°C(30s), 61°C(45s), 72°C(60s);72°C(10m) 250 ng DNA, 200 µmol dNTPs, 2 mM $MgCl_2$	F:5'-CTCGCTGCAACCCAACTGGC-3' R:5'-TCTTACCTAT CCCTAC TTCC-3	Mn1I (Fermentas)	GG: 106,33 AA:139

Statistical Analysis. Allele and genotype frequencies were calculated on patient and control subjects by direct gene counting. Statistical analysis of the differences between groups was determined by x^2 test and t-test using EPI 2000 and SPSS software version 13. P values less than 0.05 were considered significant. We did Bonferroni correction wherever the data were tested for more than one independent hypothesis. The study power was calculated for each allele and genotype.

RESULTS AND DISCUSSION

In the present study, we investigated the relation between IL-10 gene polymorphisms at positions -592, -819 and -1082 and susceptibility to brucellosis. The results (Table 2) indicated that the frequencies of IL-10 -592 and -819 C alleles were significantly higher in patients, compared to the controls (p=0.0086, OR= 0.39, 95%CI= 0.84-0.97, study power=72%). Moreover, the CC genotypes of IL-10 gene at positions -592 and -819 were significantly more frequent in the patients, compared to the controls with a study power of 57% (p=0.0348, OR=1, 95%CI=1.18-1.38). However, this P value could not withstand a Bonferroni Correction. For IL-10 -592 and -819 gene polymorphisms, the frequencies of AA and TT genotypes, respectively were significantly higher in the controls than in the patients (p= 0.0138, OR=0.11, 95% CI= 0.62-1.04, study power=54%).

Regarding the frequency of IL-10 haplotypes (Table 3), IL-10 ATA/ATA double haplotype was significantly higher in the controls, compared to the patients (p=0.013, OR= 0.11, 95%CI=0.62-1.04, study power=54%). Also the single haplotype of ATA was less frequent in patients than in controls (p=0.0278, OR=0.42, 95%CI=0.64-0.97, study power= 32%). Again the P Value failed to reach a statistically significant level after Bonferroni Correction about ATA single and double haplotypes. It is worth mentioning that there was no significant difference in sex and age between the patients and the controls. (p=0.549 and p=0.164, respectively).

We found that the distribution of C alleles at positions -592 and -819, were significantly higher in the patients than in the healthy animal husbandmen who had been in close contact with infected animals (p=0.0086). It has been shown that IL-10 -819 and -592 C alleles are associated with high production of IL-10 (9,10). Then, based on the results, we speculate that individuals who inherited C alleles at the above mentioned positions of IL-10 gene can produce higher levels of IL-10 at a crucial point of *Brucella* infection and it can cause non-effective immune response against the bacteria and lead to full-blown disease.

Table 2. The frequencies of IL-10 genotypes and alleles in patients with brucellosis and controls

Genotypes and alleles	Patient group n (%)	Control group n (%)	P value*
IL-10 -1082			
Genotypes			
GG	28 (14.7)	6 (7.4)	0.095
GA	72 (37.8)	32 (39.5)	0.80
AA	90 (47.3)	43 (53)	0.388
Alleles			
G	128 (33.6)	44 (27.1)	0.135
A	252 (66.3)	118 (72.8)	
IL-10 -819			
Genotypes			
TT	8 (4.2)	10 (12.3)	0.0138
TC	78 (41)	38 (46.9)	0.37
CC	104 (54.7)	33 (40.7)	0.0348
Alleles			
T	94 (24.7)	58 (35.8)	0.0086
C	286 (75.3)	104 (64.1)	
IL-10 -592			
Genotypes			
AA	8 (4.2)	10 (12.34)	0.0138
AC	78 (41)	38 (46.9)	0.37
CC	104 (54.7)	33 (40.7)	0.0348
Alleles			
A	94 (25.7)	58 (36)	0.0086
C	286 (75)	104 (64)	

* In genotype, each *P* value is the result of comparing corresponding row with the sum of other related rows. Considering Bonferroni correction, *P* values less than 0.017 were considered significant for genotypes.

Table 3. IL-10 haplotypes distribution in patients with brucellosis and controls

Haplotypes (-1082/-819/-592)	Patient Group n (%)	Control group n (%)	P value*
Single haplotype			
GCC	128 (33.7)	44 (27.2)	0.135
ACC	158 (41.6)	63 (38.9)	0.559
ATA	94 (24.7)	55 (33.9)	0.0278
Double haplotype			
GCC/GCC	28 (14.74)	6 (7.41)	0.37
GCC/ACC	47 (24.74)	16 (19.75)	0.67
GCC/ATA	25 (13.16)	16 (19.75)	0.44
ACC/ACC	29 (15.26)	14 (17.28)	0.095
ATA/ATA	8 (4.21)	10 (12.35)	0.013
ACC/ATA	53 (27.89)	19 (23.46)	0.165

*Each *P* value is the result of comparing corresponding row with the sum of other related rows. Considering Bonferroni correction, *P* values less than 0.017 were considered significant for genotypes.

Bravo in Spain showed no significant differences in distribution of the IL-10 genotype variants between patients and controls (8). They concluded that the different genotypes of this cytokine may not influence the susceptibility or protection in human brucellosis or in developing disease complications. But Budak in Turkey indicated that high/intermediate producer IL-10 gene polymorphisms (GCC/ATA) are more common in patients with Brucellosis, compared to healthy controls but they did not find any difference between the two groups regarding allele frequencies (7). They suggested that individuals with these haplotyes may be more susceptible to the disease.

The discrepancies between our results and Bravo's may be due to racial differences. Besides, the selection of control groups may be responsible for these differences;

Bravo's control group consisted of healthy volunteers who were from the same geographical areas as the patients, while our subjects were similar to Bravo's except that they had close contacts with infected animals and consumed contaminated dairy products. Although the results of Budak's study showed a relation between IL-10 polymorphisms and susceptibility to brucellosis, they did not match ours. These differences may be related to the selection of control groups and racial differences; Budak's groups were Turkish. Also it may be due to the sample size of Budak's study (40 patients and 50 controls). Neither Bravo nor Budak showed the details of their genotypic and allelic information, so we can not make further comparison between our results and theirs.

In conclusion, according to the above finding, we suggest that IL-10 (-592 and -819) C alleles are influential factors in susceptibility to brucellosis. Therefore, we speculate that significantly higher frequencies of IL-10 -592 and -819 C alleles in the studied groups may have resulted in higher IL-10 production and have had a role in disease induction. We suggest further studies to clarify the independency of these polymorphisms involved in the development of brucellosis in the Iranian population living in other parts of the country. We also suggest more investigations into the relationship between these polymorphisms and the ability of IL-10 production in an independent and brucellosis-dependent population.

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