Molecular Science

AIMS Molecular Science, 4(1): 103-109.
DOI: 10.3934/molsci.2017.1.103
Received 29 December 2016,
Accepted 6 March 2017,
Published 13 March 2017

http://www.aimspress.com/journal/Molecular

Research article

Association of interleukin-2 gene variants (positions +114 and −384) and susceptibility to brucellosis in Iranian population

Sohrab Najafipour[1], Abdolreza karami[2], Manoochehr Rasouli[3], Mehdi Kalani[3], Seyed-Jalal-Aldin Ashraf-Mansoori[2], and Ali Moravej[1,*]

[1] Department of Microbiology, Fasa University of Medical Sciences, Fasa, Iran
[2] Department of Microbiology, Jahrom Islamic Azad University, Jahrom, Iran
[3] Department of Immunology, Prof. Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

* **Correspondence:** Email: Amoravej@gmail.com; Tel: +98-917-1066029.

Abstract: *Brucella* is an obligate intracellular gram negative bacterium and the causative agent of brucellosis. Interleukin-2 (IL-2) is a cytokine involved in cell-mediated immunity response secreted by activated T-cells and considered as the growth factor for T-cells. Previous reports have revealed that gene polymorphisms of cytokines can affect susceptibility to *Brucella* infection. The goal of this study was to investigate the relationship between IL-2 gene polymorphisms (positions +114 and −384) and susceptibility to brucellosis. A total of 173 brucellosis patients and 75 healthy animal husbandmen who had *Brucella* infected animals and consumed their contaminated dairy products, as control group, were included in this study. All participants were genotyped for IL-2 gene polymorphisms at positions +114 (G/T) and −384 (G/T), using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The frequency of TT genotype at the position +114 was significantly higher in the controls, compared with the patients. But, there was no significant difference between the groups regarding TG and GG genotypes as well as T and G alleles. Furthermore, at position −384, the frequencies of G allele and GG genotype were higher in the controls compared with patients, however, they were not significantly different Additionally, TT/TT haplogenotype (+114/−384) was significantly higher in the controls, compared with the patients. Conclusively, it is suggested that the inheritance of TT genotype (position +114) and TT/TT haplogenotype (+114/−384) of IL-2 gene could be considered as one of the genetic factors responsible for resistance to brucellosis.

Keywords: IL-2; polymorphism; *Brucella*; allele; genotype

1. Introduction

Brucella is a gram-negative aerobe and static bacterium without capsule and spore causing brucellosis in human and abortion in the cattle. Some species of these bacteria infecting human include *Brucella melitensis*, *Brucella suis* and *Brucella canis*. Generally, those strains of *Brucella* with smaller lipopolysaccharide in their external membrane are less pathogenic [1]. *Brucella* is mostly transferred from domesticated animals to human, but human-to-human transfer of these bacteria was also reported [2]. Dairy products from infected milk like cheese and yoghurt may contain a high amount of this bacterium, and their consumption could be the main cause of brucellosis [3]. Taking raw traditional dishes like liver [4], skin abrasion [5] and inhaling animal fertilizer particles suspending in the air [6] are among other causes of brucellosis. This infectious disease is also considered to be an occupational disease experienced more frequently in shepherds, slaughterhouses, dairy industries workers and microbiology laboratory personnel [7]. The endemic regions of this infectious disease are Southern America, Middle East and Mediterranean countries.

Brucella is an intercellular organism mostly living inside macrophages, monocytes in human body organs like lymph glands, spleen, bone marrow and reticoloendothelial system [8]. Eradication of the *Brucella* from these cells requires macrophages activation as well as T-helper cells activation and proliferation [9-14]. Interleukin-2 (IL-2), secreted by T-cells, is one of the major factors contributing to the control of *Brucella* infection by the induction of T-cells proliferation and activation of cellular immunity. IL-2 is also considered as the activating factor of NK cells. Activated NK cells secrete IFN-ɣ which in turn activates macrophages and increases their antimicrobial activity [15]. Interestingly, this cytokine not only plays a significant role in activating different immune system cells but also a key role in controlling the immune responses. It is shown that long term or permanent stimulation of T-cells by IL-2 leads to the cell death or apoptosis [7]. Indeed, extensive functions of IL-2 have made this cytokine a critical factor for activation or suppression of cell mediated immunity.

It was revealed that the level of cytokine secretion by the cells of immune system and also the functionality of the secreted cytokines in infected individuals, are affected by different factors such as the nature of the organism, the severity of infection as well as the host genetic background [11]. In this regard, previous studies showed that the production of IL-2 is influenced by their gene polymorphisms [12-19]. Given the key role of IL-2 in the immunity against *Brucella* and that cytokine genes are in control of their production [16-18], it seems that IL-2 gene variants can be among factors of susceptibility to this bacterium. So, in the present study, we aimed to investigate the association between the two most famous gene polymorphisms of IL-2 which are at positions +114 (G/T), located in the first intron, and −384 (G/T), located in promoter region (Figure 1) and susceptibility to brucellosis in Iranian population.

2. Materials and Methods

2.1. Study groups

A total of 173 brucellosis patients registered and approved by Fars Province health centers included in this study. Brucellosis was diagnosed based on clinical manifestations including fever, night sweat, weight loss, splenomegaly, positive bacterium culture and positive serology tests.

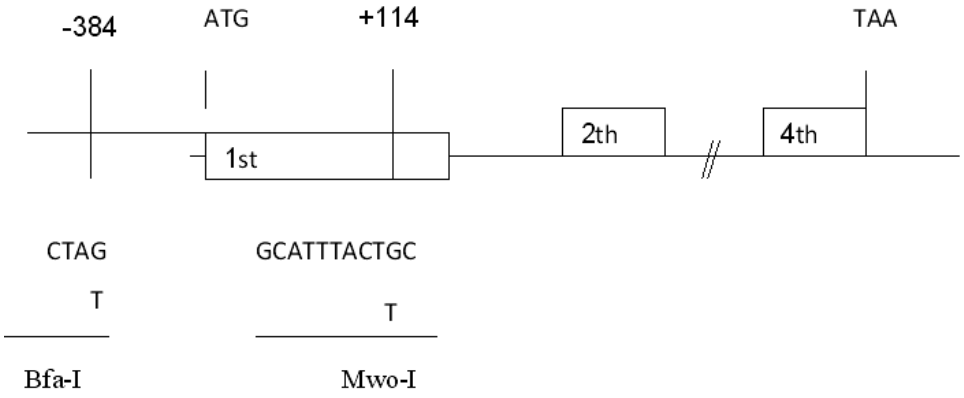

Figure 1. Location of IL-2 gene polymorphisms and restriction enzyme digestion sites. The two most famous gene polymorphisms of IL-2 are at positions +114 (G/T) which is located in the first intron and −384 (G/T) which is located in promoter region.

Serology tests included Standard Agglutination Test (SAT) and 2-Mercaptoethanol Test (2ME). Positive titer was taken to be > 1.160.

Seventy-five healthy stockbreeders living in endemic areas with infected cattle, having close contact with infected cattle, and using their dairy products yet not infected by brucellosis, were selected as control group. Cattle infection was approved by Fars Province Veterinary Office. Control group members were monitored for six continuous months and their health was confirmed. Blood sampling with EDTA anticoagulation substance was done in both control and patient groups. Then, samples were sent to the molecular laboratory for DNA extraction. This study was carried out under the supervision of ethics committee of Shiraz University of Medical Sciences (Approval number: 1389-712), and informed written consents were obtained from all patients and controls.

2.2. DNA extraction and genotyping

DNA extraction was carried out by salting out method [19]. To determine IL-2 gene polymorphism at position +114, the amount of 250 ng of extracted DNA was added to each microtube. Then, 9 µL of PCR mixture containing 1× buffer, 1.5 µL of $MgCl_2$ (CinnaGen, Iran), 500 pM direct and reverse primers (Forward: 5′-ATGTACAGGTGCAACTCCT-3′ and reverse: 5′-TGGTGAGTTTGGGATTCTTC-3′), 200 µM dNTP and one unit of Taq DNA Polymerase were added to each microtube, and placed in thermocycler. The temperature condition was set as follows: 2 min at 94 ºC and then 35 cycles at 94 ºC for 20 s, 52 ºC for 40 s, 72 ºC for 20 s, and finally 72 ºC for 10 min. Upon DNA sequence amplification, the Mwo-I (Fermentas, Lithuania) restriction enzyme was added to the tubes and kept at 37 ºC for 24 hrs. Products were separated on %2.5 of NA agarose gel (GE Halthcare, USA). After being dyed by ethidium bromide, the bands were examined by transilluminator. In the case of the existence of G allele in position of +114, the amplicon digested into two pieces with 111 and 151 bp after being exposed to restriction enzyme. But, If T allele existed, PCR product remained undigested and a 262 bp band appeared. To determine IL-2 gene polymorphism in position −384 of IL-2 gene, PCR was carried out using 5′-ATGTACAGGTGCAACTCCT-3′ and 5′-TGGTGAGTTTGGGATTCTTC-3′ primers based on the

above thermal conditions. For this position Bfa-I (Fermentas, Lithuania) restriction enzyme was used. In the case of the present of G allele in position of −384 of Il-2, the amplicon digested into two pieces with 110 and 21 bp after being exposed to restriction enzyme. But, If T allele existed, PCR product remained undigested and a 131 bp band appeared (Table 1).

Table1. Polymerase chain reaction (PCR) primers and conditions for IL-2 gene amplifications.

Position	PCR primers	Annealing temperature (°C)	Restriction enzymes	Fragment sizes (bp)
+114	F: 5′-ATGTACAGGTGCAACTCCT-3′ R: 5′-TGGTGAGTTTGGGATTCTTC-3′	52	Mwo-I	T: 262 G: 151 + 111
−384	F: 5′-ATGTACAGGTGCAACTCCT-3′ R: 5′-TGGTGAGTTTGGGATTCTTC-3′	52	Bfa-I	T: 131 G: 110 + 21

2.3. Statistical analysis

The frequencies of alleles and genotypes were calculated in patient and control groups by direct gene counting, and compared using EPI2000 and SPSS16. Hardy-Weinberg equilibrium as well as haplotypes and haplogenotypes were determined using Arlequin3.1. Also, LD2SNPing program V2.0 (http://www.bio.kuas.edu.tw/LD2SNPing) was used to estimate linkage disequilibrium (LD) between the studied polymorphic sites. The study power was calculated via Power SSC software. *P* value less than 0.05 was considered significant.

3. Results

3.1. Allele and genotype distributions

The frequencies of the alleles and genotypes were determined in the control and patient groups. Genotypes of IL-2 in the control group met Hardy-Weinberg equilibrium. As shown in Table 2, at position +144, the frequency of TT genotype was significantly higher in control group, compared to patients (P = 0.016, OR = 0.16, 95%CI = 0.02–0.98). But, there was no significant difference between the groups regarding TG and GG genotypes as well as T and G alleles. Furthermore, at position −384, the frequencies of G allele and GG genotype were higher in the controls compared with patients, however, they were not significantly different (P = 0.44 and 0.68, respectively).

3.2. Haplotype and haplogenotype distributions

The frequencies of haplotypes and haplogenotypes were determined in the control and patient groups. As shown in Table 3, there was no significant difference between the groups in terms of four existing haplotypes (GG, TG, TT, and GT) in two loci of IL-2 gene (+114 T/G and −384 T/G). Furthermore, among 14 existing haplogenotypes, the frequency of TT/TT haplogenotype was significantly higher in controls, compared to patients (P = 0.049, OR = 0.14, 95%CI = 0.01–1.54). But, regarding other 13 haplogenotypes, there was no significant difference between groups.

Table 2. The frequencies of alleles and genotypes of IL-2 (positions +114 and −384) in patients with brucellosis and controls.

Genotypes and alleles	Patients, *n* (%)	Controls, *n* (%)	*P* value	OR (95%CI)
IL-2 (+114)				
Genotypes				
TT	2 (1.2)	5 (6.7)	0.016	0.16(0.02–0.98)
GG	1.3 (59.5)	47 (62.7)	0.64	0.88 (0.48–1.59)
TG	68 (39.3)	23 (30.7)	0.19	1.46 (0.79–2.72)
Alleles				
T	72 (21)	33 (22)	0.76	0.93(0.75–1.52)
G	274 (79)	117 (78)		
IL-2(−384)				
Genotypes				
TT	55 (31.8)	20 (26.7)	0.41	1.28 (0.67–2.45)
GG	33 (19.1)	16 (21.3)	0.68	0.87 (0.42–1.79)
TG	85(49.1)	39 (52)	0.67	0.89 (0.50–1.59)
Alleles				
T	195 (56)	79 (53)	0.44	1.16 (0.78–1.74)
G	151 (44)	71 (47)		

Table 3. IL-2 haplotypes and haplogenotypes distributions in patients with brucellosis and controls.

Haplotype and Haplogenotype	Patients	Controls	*P* value	OR (95%CI)
Haplotype				
GG	144 (41.6%)	66 (44%)	0.62	0.91(0.61–1.36)
TG	130 (37.6%)	51 (34%)	0.44	1.17(0.77–1.78)
TT	63 (18.2%)	28 (18.7%)	0.9	0.97(0.58–1.64)
GT	9 (2.6%)	5 (3.3%)	0.65	0.77(0.23–2.71)
Haplogenotype				
GT/TT	0	1 (1.3%)	0.12	0 (0–7.53)
TG/TT	12 (6.9%)	3 (4%)	0.37	1.79 (0.45–8.25)
TT/GT	0	1 (1.3%)	0.12	0 (0–7.53)
TT/TG	15 (8.6%)	4 (5.3%)	0.36	1.69 (0.5–6.25)
TT/TT	1 (0.6%)	3 (4%)	0.049	0.14 (0.01–1.54)

4. Discussion and Conclusion

Cellular immunity and its related cytokines play the main role in immunity against intracellular bacteria such as *Brucella* [20]. IL-2 modulates the activation and proliferation of T-cells and NK cells. It was revealed that IL-2 secretion level increases in the sera of patients with brucellosis [9]. Furthermore, some studies showed that the level of cytokine secretion is related to single nucleotide polymorphisms (SNPs) in cytokine genes [11]. Based on these studies, we aimed to investigate the potential associations between IL-2 gene polymorphisms and susceptibility to brucellosis in Iranian

population. The results of our study indicated that the inheritance of TT genotype in locus +114 of IL-2 significantly affects susceptibility to brucellosis and may be a protective factor against *Brucella* infection. It is likely that this genotype can increase IL-2 expression [21] and secretion by T-cells which in turn can result in *Brucella* resistance in individuals inheriting this genotype. Results also demonstrated higher frequency of TT/TT haplogenotype (+114 T/G and −384 T/G) in control group, as compared to brucellosis group. Hence, it can be suggested that inheriting TT/TT haplogenotype can affect individual's resistance against *Brucella* infection. However, our results showed that inheriting different genotypes (TT, TG, and GG) in loci −384 of IL-2 cannot affect resistance or susceptibility to brucellosis. To the best of our knowledge, there is no published article on the relationship between IL-2 gene polymorphisms and susceptibility to *Brucella* infection in Iranian population.

Some studies have shown the relationship between different cytokine polymorphisms and susceptibility to brucellosis. In this regard, a study on the IL-10 gene polymorphisms demonstrated that the distributions of C allele CC genotypes in loci −819 and −592 were significantly higher in brucellosis patients, compared to healthy stockbreeders [17]. Another study on IL-12 and TNF-β gene polymorphisms also showed that the frequencies of AA genotype and A allele related to locus +1188 of IL-12 were higher in the controls than that in the brucellosis patients, but the frequencies of AA genotype and A allele in locus +252 of TNF-β were significantly higher in control group, compared to brucellosis patients [18]. Results of a study on the polymorphisms of IFN-ɣ (interferon- ɣ) and IL-4 genes indicated that individuals with AA genotype in locus +874 of IFN-ɣ are further susceptible to brucellosis, while individuals with CC genotype in locus −590 of IL-4 are further brucellosis resistant [16].

Furthermore, some studies have shown the relationship between IL-2 gene polymorphisms and susceptibility to various diseases. Accordingly, Matsans et al. studied the IL-2 gene polymorphisms in multiple sclerosis (MS) patients, and showed an association between two TT and GT genotypes in locus −384 of IL-2 and susceptibility to progressive MS [19]. In another study, Woo et al. examined the relationship between IL-2 polymorphism and prostate cancer susceptibility. They showed that TT homozygote genotype has a higher frequency in patient, compared to control group [20].

Conclusively, the present study results showed significantly higher frequencies of TT genotype in locus +114 as well as TT/TT haplogenotype in loci +114 and −384 in control group as compared to brucellosis group. Hence, it can be suggested that, considering the key role of IL-2 in controlling *Brucella* infection, inheriting the above genotype and haplogenotype can probably lead to the higher production of IL-2 resulting in higher resistance to brucellosis.

Acknowledgements

This study was financially supported by the grant No. 89290 awarded by Fasa University of Medical Sciences. Also, our thanks are due to Dr. Hassan Khajehei for linguistic copy editing of the manuscript.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Corbel MJ (1997) Brucellosis: an overview. *Emerg Infect Dis* 3: 213-321.

2. Mantur BG, Mangalgi SS, Mulimani M (1996) Brucella melitensis: Asexually transmissible agent? *Lancet* 347: 1763.
3. Eckman MR (1975) Brucellosis linked to Mexican cheese. *JAMA* 232: 636-637.
4. Malik GM (1997) A clinical study of brucellosis in adult in the Asir region of southern Saudi Arabia. *Am J Trop Med Hyg* 56: 375-377.
5. Glass WI (1970) Brucellosis as an occupational disease in New Zealand. *N Z Med J* 63: 301-308.
6. William E (1970) Brucellosis and the British public. *Lancet* 1: 1220-1222.
7. Pappas G, Akritidis N, Bosilkovski M, et al. (2005) Brucellosis. *N Engl J Med* 352: 2325-2336.
8. Kozukeev TB, Ajeilat S, Maes E, et al. (2006) Risk factors for brucellosis--Leylek and Kadamjay districts, Batken Oblast, Kyrgyzstan, January-November, 2003. *MMWR Suppl* 28: 31-34.
9. Refik M, Mehmet N, Duramz R, et al. (2004) Cytokine profile and nitric oxide levels in sera from patients with Brucellosis. *Braz J Med Biol Res* 37: 20.
10. Bassat H, Saadany S, Yamany A, et al. (2005) Role of T-helper-1 Cytokine and Nitric oxide production in patients with Acute Brucellosis. *Tanta Med* 3: 1087-1095.
11. Kariminia A, Kavossy G, Khatami S, et al. (2002) Study of interleukin-10 and interleukin-12 productions in response to lipopolysaccharides extracted from two different Brucella strains. *Comp Immunol Microbiol Infect Dis* 25: 85-93.
12. Zhan Y, Cheers C (1995) Endogenous interleukin-12 is involved in resistance to Brucella abortus infection. *Infect Immun* 63: 1387-1390.
13. Hoover DL, Crawford RM, Van De Verg LL, et al. (1999) Protection of mice against bruellosis by vaccination with Brucella melittensis WR201 (16M A Pur EK). *Infect Immun* 67:5877-5884.
14. Pasqual P, Adone R, Gasbarre LC, et al. (2001) mouse cytokine profiles associated with Brucella abortus RB51 vaccination or B. abortus 2308 infection. *Infect Immun* 69: 6541-6544.
15. Serre A, Bascoul S, Vendrell JP, et al. (1987) Human immune response to Brucella infection. *Ann Inst Pasteur Microbiol* 138: 113-117.
16. Rasouli M, Kiany S (2007) Association of interferon-gamma and interleukin-4 gene polymorphisms with susceptibility to Brucellosis in Iranian patients. *Cytokine* 38: 49-53.
17. Rasouli M, Kiany S, Behbin M (2008) Interleukin-10 Gene Polymorphisms and Susceptibility to Brucellosis in Iranian patients. *Iran J Immunol* 5: 131-135.
18. Rasouli M, Kiany S, Moravej A, et al. (2010) Interleukin-12 and Tumor Necrosis Factor- Gene Polymorphisms as Genetic Susceptibility Factors for Brucellosis in Iranian Patients. *Iran Red Crescent Med J* 12: 266-271.
19. Matesanz F, Fedetz M, Collado-Romero M, et al. (2001) Allelic expression and interleukin-2 polymorphisms in multiple sclerosis. *J Neuroimmunol* 119: 101-105.
20. Wu HC, Chang CH, Tsai FJ, et al. (2006) IL-2 gene C/T polymorphisms is associated with prostate Cancer. *J Clin Lab Anal* 20: 425-429.
21. Peng Q, Li H, Lao X, et al. (2014) Association of IL-2 polymorphisms and IL-2 serum levels with susceptibility to HBV-related hepatocellular carcinoma in a Chinese Zhuang population. *Infect Genet Evol* 27: 375-381.

AIMS Press