Polymorphism in the First Intron of Interferon-Gamma Gene (+874T→A) in Iranian Patients with Brucellosis

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ABSTRACT

Background: *Brucella* is a gram-negative bacterium, causing acute and chronic infection in humans and animals. Cell-mediated immunity is the main protective immune response against *Brucella* spp. Activation of macrophages by IFN-γ and generation of reactive oxygen intermediates and nitric oxide are the main immunologic mechanisms responsible for control of *Brucella* infection. **Objective:** To investigate the correlation between IFN-γ gene polymorphism and brucellosis. **Methods:** 195 patients with brucellosis, 186 healthy patients' family members and 82 healthy farmers who kept infected animals and consumed their contaminated dairy products were selected to take part in the study. IFN-γ genotyping at position +874 (T→A) was carried out by allele specific polymerase chain reaction (AS-PCR) method. **Results:** The frequency of AT and TT genotypes significantly increased in farmers compared to patients with brucellosis (P=0.03) while there was no significant difference in genotype distribution between patients and their healthy family members. **Conclusion:** IFN-γ (+874) AA genotype is probably a genetic factor that contributes to the susceptibility of the individuals to brucellosis.

Keywords: Brucella, Interferon Gamma, Genetic Polymorphism

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INTRODUCTION

Brucellosis is a zoonotic disease caused by *Brucella* spp, which can be transmitted to humans. *B. melitensis*, *B. abortus*, *B.canis* and *B.suis* are pathogenic species for humans. The disease has a worldwide distribution, especially in the Mediterranean basin, Middle East, India and Central and South America (1,2). *Brucella* species often invade the reticuloendothelial system and can be sequestered in the infected macrophages at some specific parts of the body (e.g. spleen, brain, heart, liver and bone marrow) (3). Antibody-mediated immunity contributes to protection against virulent strains of *B.abortus* less effectively than attenuated ones. In contrast, type 1 immune response does contribute to control of both virulent and non-virulent strains and is more effective against higher dose challenge than antibodies (4, 5). One of the most important type 1 cytokines is interferon-γ (IFN-γ) which plays a pivotal role in macrophage activation and control of *Brucella* infection both *in vitro* and *in vivo* (6, 7). Activation of macrophages can promote the killing process of bacteria by induction of superoxide anions and hydrogen peroxide (7). Considering the crucial role of IFN-γ in protective immune response against *Brucella* spp infection, any increase or decrease in production of this cytokine can affect the outcome of the disease.

The IFN-γ expression level is related to polymorphism in the first intron of its gene (8). Pravica and his colleagues have reported that 12 CA repeat mirosatellite allele at the first intron of the IFN-γ gene is associated with a higher level of *in vitro* cytokine production (9). They have also demonstrated an absolute correlation between the 12 CA repeat allele and the presence of the T allele at the +874 position (T→A) that may be important in the induction of constitutively high IFN-γ production (9). In this study, we evaluated the relationship between IFN-γ (+874) gene polymorphism and susceptibility to brucellosis.

MATERIALS AND METHODS

Subjects: 195 patients with acute brucellosis living in Fars province, South of Iran were selected. Brucellosis was diagnosed based on the clinical signs and symptoms (e.g., fever, night sweating, weakness, malaise, weight loss, splenomegaly, lymphadenopathy, myalgia and arthralgia), serological tests, and/or positive blood cultures. The diagnostic criteria for positive serological test were single high titers (≥1/160) of standard agglutination test (SAT), confirmed by a titer of ≥1/160 in 2-mercaptoethanol test (2ME).

Two different control groups were selected and matched for age, sex, and their place of residence. One control group was composed of 186 healthy patients' family members and the other consisted of 82 healthy animal husbandry men who had close contact with animals infected with brucellosis and consumed their milk and dairy products. Brucellosis in animals was confirmed by serological tests in laboratory of Fars Province Veterinary Administration.

Genotyping of IFN-γ Gene Polymorphism at Position +874: DNA was extracted from EDTA-treated blood samples using salting out method (10). Polymorphism at position +874 of IFN-γ gene was identified using allele specific polymerase chain reaction (AS-PCR) as described by Pravica with some modifications(9). The PCR reaction was performed in total volume of 10 μL, containing 1X reaction buffer (Cinnagene-Iran), 200 μM (each) dNTPs (Cinnagene-Iran), 3.5 mM MgCl2 (Cinna-

gene-Iran), 0.5 U Taq DNA polymerase (Cinnagene-Iran), 0.5 μM each specific primers (antisense: TCA ACA AAG CTG ATA CTC CA; sense+874T: TTC TTA CAA CAC AAA ATC AAA TCT; or sense +874A: TTC TTA CAA CAC AAA ATC AAA TCA), 0.2 μM of each internal control primers, and 250 ng DNA template. Internal control primers amplify a human β-globin sequence (BGR1: ACA CAA CTG TGT TCA CTA GC; BGR2: CAA CTT CAT CCA CGT TCA CC). PCR amplification was performed using a touch down method that included initial denaturation at 95ºC for 5 minutes followed by two loops; loop 1 which consisted of 10 cycles with the following program: 95ºC for 30 seconds, 62ºC for 50 seconds, and 72ºC for 40 seconds and loop 2 included 20 cycles with the following program: 95ºC for 30 seconds, 56ºC for 50 seconds and 72ºC for 40 seconds and a final extension step at 72 ºC. The amplified products were run on 2% agarose gel that was in a buffer containing 0.5 μg/ml ethidium bromide (Fig.1).

Statistical Analysis: The data were analyzed by χ^2 test using EPI-Info 2000 and SPSS version 11.5 software. P-values less than 0.05 were considered significant.

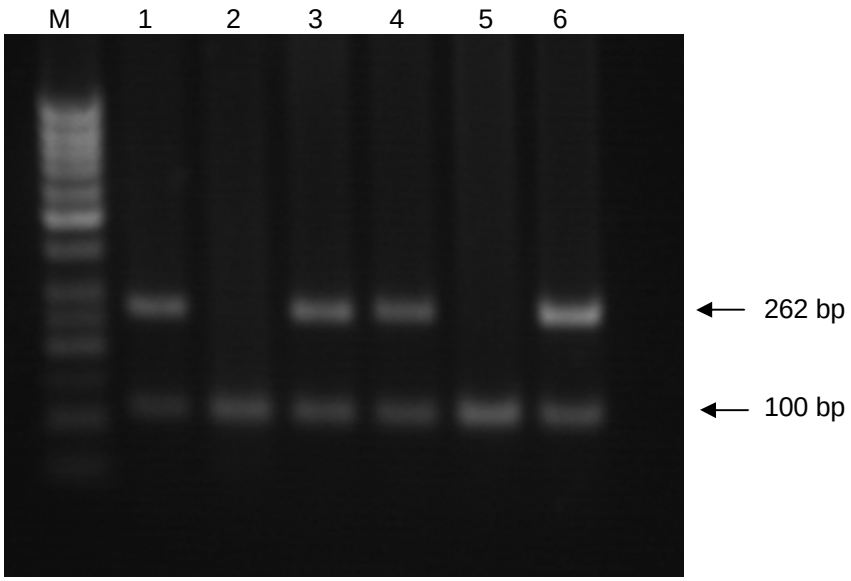

Figure 1. Agarose gel electrophoresis of AS-PCR products for IFN-γ (+874T→A) SNP; 262 bp bands correspond to IFN-γ A or T allele and the 100 bp bands correspond to internal controls. M: 50bp DNA size marker; lanes 1 and 2 show homozygosity for T allele; lanes 3 and 4 show heterozygosity for A and T alleles; lanes 5 and 6 show homozygosity for A allele.

RESULTS

The genotype distributions of the IFN-γ gene in patients and controls are shown in table 1. Distribution of the genotypes in all groups was consistent with the Hardy-Weinberg equilibrium. Analysis of our results showed that there was no significant difference in frequencies of IFN-γ genotypes between patients and their healthy family members (P=0.45) (Table 2) whereas as is indicated in table 3, there was a significant correlation in IFN-γ genotypes (AT + TT) between patients and animal husbandry men (P=0.03).

Table 1. IFN-γ genotype distribution in patients with brucellosis vs. controls

IFN-γ genotype	Patients (%)	Family members (%)	Farmers (%)
A/A	40 (20.5)	44(23.6)	8(9.8)
T/A	96(49.2)	105(56.5)	49(59.7)
T/T	59(30.3)	37(19.9)	25(30.5)
Total	195(100)	186(100)	82(100)

Table 2. IFN-γ genotypes distribution in patients with brucellosis vs. their healthy family members

Genotypes	Patients (%)	Family members (%)	P-value
AA	40(20.5)	44(23.6)	
TA + TT	155(79.5)	142(76.4)	
Total	195(100)	186(100)	0.45

Table 3. IFN-γ genotypes distribution in patients with brucellosis vs. healthy farmers

Genotypes	Patients (%)	Farmers (%)	P-value
AA	40(20.5)	8(9.8)	
TA + TT	155(79.5)	74(90.2)	
Total	195(100)	82(100)	0.03

DISCUSSION

Several factors can affect the outcome of host-parasite interactions including: quantity of parasites invading the host, existence of immunological memory due to previous host exposure to the parasite, genetic factors, etc. Cytokine genes can be candidate genes for studying and clarifying the genetic susceptibility to *Brucella* infection. In this study we investigated whether genetic variation in IFN-γ gene can be considered as a risk factor for brucellosis. It has been shown that IFN-γ has an important role in protective immune response against *Brucella* infection. Depletion of endogenous IFN-γ with anti-IFN-γ monoclonal antibody resulted in increasing the number of *Brucella abortus* in the spleen and the liver of infected CBA mice (11). Moreover, treatment of BALB/C mice with supplemental recombinant IFN-γ caused a 10-fold decrease in the number of bacteria at first week after infection (12). Rodriguez-Zapata *et al.* have reported that PHA-stimulated T lymphocytes from untreated patients with acute brucellosis have defective IFN-γ production (13), and finally, it is known that IFN-γ knockout mice die from brucellosis (14). IFN-γ plays its crucial role by inducing type 1 immune response. Conclusively, it seems that increase in IFN-γ production in response to *Brucella* infection may cause resistance in developing a full-pictured brucellosis and can probably control the organism. As is shown in Table 3, AA genotype which has association with low production of IFN-γ was significantly more frequent in patients compared to animal husbandry men who had been exposed to animals infected with brucellosis (9.8% vs. 20.5%; P=0.03) while, TT and TA genotypes which were associated with high and intermediate IFN-γ production, respectively were lower in patients than controls (P = 0.03) (9).

Considering Pravica's report about the association between IFN-γ (+874) gene polymorphism and cytokine production, we suggest that individuals inheriting TT and TA genotype can produce higher levels of IFN-γ in response to *Brucella* infection compared to those carrying AA genotype (9). Therefore, higher levels of IFN-γ can cause more effective cell-mediated immunity against brucellosis. Our results are in agreement with Bravo *et al.* (15) who have reported higher frequency of AA genotype in patients compared with controls (34% vs. 19%, p = 0.02). However, our control group had some differences with those of Bravo, et al (15). In their study, control group was composed of healthy volunteers who were from the same geographical area as the patients. While, we selected individuals who had close contact with infected animals and consumed contaminated dairy products but were still healthy.
Our results did not show any difference in IFN-γ (+874) genotype distribution between patients and their healthy family members (Table 2). This may be due to different genetic backgrounds of individuals, various interests in consuming milk and dairy products, and/or different exposures to infected animals or contaminated dairy products.

Conclusion

According to these findings, we suggest that IFN-γ (+874) AA genotype is a genetic factor which may contribute to the susceptibility to brucellosis.

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