http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (21): 1036-1041, 2010
ISSN 1028-8880

Interferon-Gamma Low Producer Genotype +5644 over Presented in Patients with Focal Brucellosis

[1]A. Hedayatizadeh-Omran, [1]A. Rafiei, [2]M. Hajilooi and [3]M. Haghshenas
[1]Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[2]Dental Research Center, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Microbiology and Immunology, Razi Hospital, Faculty of Medicine,
Mazandaran University of Medical Sciences, Sari, Iran

Abstract: Genetic polymorphisms that affect production levels of certain cytokines may determine the risk, severity or protection in some infectious diseases like brucellosis. IFN-γ plays a key role in the defense mechanism against brucella infection. This study aimed to determine the influence of the polymorphism within the +5644 position of IFN-γ gene on the susceptibility to brucellosis. We investigated the allelic and genotypes distribution of A5644G polymorphism in IFN-γ gene in an Iranian population comprising 259 patients with brucellosis and 238 healthy controls. The single nucleotide polymorphism was determined using the polymerase chain reaction in association with sequence-specific primers (PCR-SSP) incorporating mismatches at the 3'-end. Allelic and genotype frequencies of G5644A polymorphism of IFN-γ gene were not significantly differed between patients with brucellosis and controls ($p>0.05$). Stratification of patients to focal and non focal diseases revealed a significant increased of 5644A allele in patients with focal brucellosis (79.31% vs. 61.94%, $p = 0.0005$). Moreover, multivariate logistic regression models showed patients harboring the INF-γ G5644A genotype were significantly more likely to develop focal infectious complications (OR = 3.45, $p = 0.0004$, 95% CI = 1.26-7.94). The present study suggests that the variant genotypes of G6544A of IFN-γ might be associated with focal form of brucellosis and play as a genetic risk factor in brucellosis.

Key words: Brucellosis, polymorphism, IFN-gamma, focal, genotype

INTRODUCTION

Brucellosis caused by *Brucella* sp. is one of the five common bacterial zoonoses in the world and represent a public health problem especially in developing countries including Middle East (Cutler *et al*., 2005). The immune defense against *Brucella* is complex and involves the interaction between CD4+T, CD8+T lymphocytes, macrophages and monocytes along with the production of cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-a (TNF-a) and IL-12 (Rafiei *et al*., 2006). These cytokines produce at the onset of infection that activates macrophages and lymphocytes for induction of anti-brucella activities. On the other hand, cytokines are important mediators that regulate immune and inflammatory reactions. Upon animal studies, it has demonstrated that Th1 cytokines confer resistance (Zhan and Cheers, 1993; Baldwin and Parent, 2002; Rafiei *et al*., 2006), while Th2 cytokines predispose to brucellosis (Fernandes and Baldwin, 1995). IFN-γ, a prominent Th1 cytokine, has been showed to play a major role in macrophage activation (Jiang and Baldwin, 1993; Rafiei *et al*., 2006) and stimulating antitumor and antimicrobial activities, as well as the induction for the synthesis and expression of MHC-II (Basham and Merigan, 1983; Baldwin and Parent, 2002). It has reported that IFN-γ knock out mice are unable to control the infection (Murphy *et al*., 2001). Serum levels of IFN-γ is significantly higher in patients with brucellosis compared with healthy persons (Ahmed *et al*., 1999).

In addition to environmental factors and virulence of the pathogen, host genetic factors are also major determinants of susceptibility and development of infectious diseases like brucellosis (Caballero *et al*., 2000; Cooke and Hill, 2001; Rezazadeh *et al*., 2006; Hajilooi *et al*., 2006). Regarding the host protection mechanisms against brucella, investigating the possible influence of gene polymorphisms on susceptibility to brucellosis has recently been a focus of interest (Bravo *et al*., 2003). Expression and secretion of cytokines are dependent, at least in part, on genetic polymorphism within the promoter region or other regulatory sequences of cytokine genes. On the other hand, polymorphisms in the regulatory regions of cytokine genes can affect the

Corresponding Author: A. Rafiei, Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran Tel: +98-151-3543088 Fax: +98-151-3543087

level of cytokine production and may be associated with predisposition to infectious diseases as well as different clinical outcomes. Association between the genetic background of the human host and the susceptibility or resistance to brucellosis has been shown (Bravo *et al*., 2003, 2008; Hajilooi *et al*., 2006; Rafiei *et al*., 2007). Low synthesis of INF-γ has been associated with chronicity and longevity of brucellosis (Rafiei *et al*., 2006). Several polymorphisms in the gene of IFN-γ have been identified to influence on susceptibility, development or clinical features of diseases. A G to A polymorphism at position 5644 in the 3' untranslated region of the IFN-γ gene (Accession No. M37265) might affect gene expression and IFN-γ production by governing mRNA stability, localizing mRNA and regulating translation (Wu *et al*., 1998).

Although, the immunogenetic aspects of chronic intracellular infections have been widely studied, little is known about the genetic influence of the host in human brucellosis. The present study investigates the hypothesis of an association of polymorphic variants of the IFN-γ gene, with different clinical forms of brucellosis.

MATERIALS AND METHODS

Study population: The study consisted of a retrospectively recruited cohort of 259 (142 men, 117 women) patients with brucellosis from rural areas of Northen Iran diagnosed between March 2006 and November 2008. A control group was composed of 238 (136 men, 102 women) healthy volunteers matched for age, sex and geographic area. The control group had the same epidemiological conditions as patients, but was negative in brucella-specific tests. Diagnosis of brucellosis was established by isolation of Brucella in blood or bone marrow cultures. In negative cultures, diagnosis was based on compatible epidemiological and clinical manifestations together with the presence of high titers of specific antibodies or a four-fold or greater increase of the initial titers in two paired samples drawn 2-4 weeks a part. High titers were considered to be a Standard Tube Agglutination (STA) ≥1/160 or a Coombs' antibrucella test ≥ 1/320 confirmed by a 2-merkaptoethanol test (2-ME) titer of ≥ 1/160. Focal forms or complications were defined as the presence of symptoms or signs of infection at particular sites which continued for at least 7 days in a patient with active brucellosis. Upon diagnosis, all the patients were placed on the same treatment regimen comprising of doxycycline for a period of six weeks, in combination with streptomycin for two weeks followed by rifampin for four weeks. The study protocol was approved by the Medical Ethics Committee of Mazandaran University of Medical Sciences and conformed to the ethic guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all study population.

Genotype analysis: For each individual enrolled in the study, a 7 mL sample of venous blood was collected in tubes containing 50 mM of EDTA. DNA was extracted from uncoagulated blood by a modification of the salting -out technique (Miller *et al*., 1988) and stored at a final concentration of 200 μg mL^{-1} in -20°C until used for the genotyping. The genotyping was performed using polymerase chain reaction-sequence specific primers (PCR-SSP) method. Internal control primers were included to control for false negative reactions. The control primers at concentration 0.2 μmol L^{-1} (5′TGC CAA GTG GAG CAC CCA A and 5′GCA TCT TGC TCT GTG CAG AT-3) were used. The IFN-γ polymorphism at position 5644 (A/G) was identified by the sequence-specific forward primers: 5'CCT TCC TAT TTC CTC CTT CG and 5'ACC TTC CTA TTT CCT CCT TCA in combination with the consensus reverse primer 5'GTC TAC AAC AGC ACC AGG C at a final concentration of 1 μmol L^{-1} with an expected product size of 298 bp. The amplification products were visualized after electrophoresis (100 V/45 min) in agarose gel at 2% in 1 TAE buffer (Tris-base 1.6 mol L^{-1}, Na acetate 0.8 mol L^{-1} and ethylenediaminetetraacetic acid-Na 2 40 mM L^{-1} deionized water) containing 5 μL of ethidium bromide (10 mg mL^{-1}), using transillumination with a source of ultraviolet light The agarose gels were reported by an investigator unaware from the samples. Amplification was carried out using a DNA Technology MTC 400 in a total volume of 15 μmol L^{-1} that contained 100 ng of genomic DNA, 1 μmol L^{-1} each allele-specific primer pair, 200 μmol L^{-1} dNTP, 10 moL^{-1} Tris-HCl (pH, 8.3), 50 mmol KCl, 1.5 $mmoL^{-1}$ $MgCl_2$, 0.5 IU Taq DNA polymerase. The reaction was carried out as follow; initial denaturation at 94°C for 2 min, followed by 10 cycles of amplification at 96°C for 20 sec and annealing at 64°C for 50 sec, with extraction for 40 sec at 72°C, followed by 20 cycles of denaturation at 96°C for 20 sec and annealing at 61°C for 50 sec, with extraction for 40 sec at 72°C.

Statistical analysis: The allele and genotype frequencies of the variants were obtained by direct counting. Hardy-Weinberg equilibrium and the differences between the allele and genotype frequencies between the brucellosis patients and the controls were determined by the χ^2 test with Yates correction, and Fisher's exact test, appropriately. A p-value<0.05 was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated if the χ^2 or Fisher's

exact tests were significant. All Statistical analysis was performed using the SPSS 10.0 software package (SPSS, Chicago, IL).

RESULTS

The mean age was 40.94±18.22 and 41.23±17.54 years in the patients and healthy individuals, respectively. The mode of transmission was consumption of dairy products (57%), close contact with infected animals (39%) and unknown origin (4%). The patients with brucellosis presented by fever (71.4%), anorexia (73.4%), myalgia (55.6%), delirium (5.1 %), headache (53.7%), weight loss (38.6%), arthralgia (71.4%), arthritis (14.7%), sweating (69.1%), back pain (73.4%), bone pain (61.8%), depression (17.8%), hepatobiliary complications (2.7%), hematological complications (3.1%). Of the 58 patients with focal forms of the disease, 45 (77.6%) had osteoarticular complications (38 arthritis, 4 sacroiliitis, 3 spondylitis), 4 (6.9%) had genitourinary complications, 7 (12.1%) hepatobiliary complications and 7 (12.1%) hematological disorders. Comparisons between the genotype and allelic frequencies in the brucellosis and control populations did not reveal significant frequency differences between the two groups.

Allelic and genotype frequencies of IFN-γ: DNA samples from 259 patients with brucellosis and 238 healthy individuals were analyzed for G5644A polymorphism of IFN-γ. The frequency of IFN-γ genotypes in control individuals was found in accordance with those expected by the Hardy-Weinberg equilibrium (p = 0.125). The allele and genotype frequencies of G5644A polymorphism of IFN-γ gene were not significantly differed between brucellosis and healthy control subjects (p = 0.053 and p = 0.073, respectively), although mutant homozygous genotype was slightly more common in patients than controls (48.3% vs. 38.2%, OR = 07, 95% CI; 0.43-1.17; p = 0.07) (Table 1). In order to investigate the influence of gender on allelic distribution we determined allele and genotype frequencies between patients and controls of male and female separately. There wan no significant difference between allelic and genotype frequencies of IFN-γ polymorphism between patient and control group based on gender distribution (data not shown). When the patients were divided according to the clinical features of the disease into focal brucellosis (22.4%) and non focal brucellosis (77.6%), a strong association was found in the focal disease and IFN-γ polymorphism (Table 2). Individuals with IFN-γ 5644 A allele had a higher risk of developing focal form of brucellosis compared to individuals with G allele (79.31% vs. 61.94%, OR = 2.355, p = 0.0005, 95% CI = 1.44-3.85). These differences were also seen in genotypes of IFN-γ among the patients with focal brucellosis and non-focal disease (67.2% vs. 42.8%, OR = 3.45, p = 0.0004, 95% CI = 1.26-7.94). Interestingly, as shown in Table 3 there was an increased of IFN-γ A/A genotype in patients with focal form compared to non focal form in both male and female groups. Otherwise, there was only a statistically significant difference in allelic and genotypes of G5644A polymorphism in female patients with focal brucellosis. The presence of 5644A allele was more associated with localization of the diseases in female patients (OR = 2.6, p = 0.015, 95% CI = 1.2-5.7).

Table 1: Distribution of allele and genotype frequencies of G5644A polymorphism of IFN–γ polymorphism in patients with brucellosis and controls

Results	Controls n = 238	Patients n = 259	p-value	OR	CI 95%
Allele- n (%)					
5644A	285 (59.87)	341 (65.83)	0.052	0.78	0.6-1.1
5644G	191 (40.13)	177 (34.17)			
Genotype-n (%)					
A5644A	91 (38.2)	125 (48.3)			
A5644G	103 (43.3)	91 (35.1)	0.073	0.7	0.43-1.17
G5644G	44 (18.5)	43 (16.6)			

Table 2: Genotype and allele frequencies of G5644A polymorphism of IFN-γ in Focal and non focal brucellosis

Results	Focal brucellosis n = 58	Non focal brucellosis n = 201	p-value	OR	CI95%
Allele-n(%)					
5644A	92 (79.31)	249 (61.94)	0.0005	2.355	1.44-3.85
5644G	24 (20.69)	153 (38.06)			
Genotype-n (%)					
A5644A	39 (67.2)	86 (42.8)	0.004	3.45	1.26-7.94
A5644G	14 (24.1)	77 (38.3)			
G5644G	5 (8.6)	38 (18.9)			

Table 3: Genotype and allele frequencies of G5644A polymorphism of IFN-γ in patients with or without focal brucellosis based on sex

	Male		Female	
Results	Focal (n=36)	Non focal (n=106)	Focal (n=22)	Non focal (n=95)
Allele –n (%)				
5644A	55 (76.4)	135 (63.7)	35 (79.5)	114 (60)
5644G	94 (23.6)	77 (36.3)	9 (20.5)	76 (40)
p-value	0.05		0.015	
OR (CI 95%)	1.84 (1.01-3.4)	2.6 (1.2-5.7)		
Genotype –n (%)				
A5644A	24 (66.7)	47 (44.3)	14 (63.6)	39 (41.1)
A5644G	7 (19.4)	41 (38.7)	7 (31.9)	36 (37.9)
G5644G	5 (13.9)	18 (17)	1 (4.5)	20 (21.1)
p-value	0.06		0.02	
OR (CI 95%)	2.6 (0.08-5.7)		7.4 (1.002-15.6)	

DISCUSSION

The key finding of this study is an association between the presences of 5644A allele with focal brucellosis susceptibility. Successful elimination of the brucella infection depends on the activation of

macrophages with the development of cell-mediated immunity (Zhan *et al*., 1996; Golding *et al*., 2001). Brucella antigens induce the production of Th1 cytokines in humans and adequate Th1 immune response is critical for the clearance of *Brucella* infection. IFN-γ is critical in modulating the IL-4, IL-10 and IL-12 cytokine network pathway and considered a proinflammatory cytokine because it augments (TNF) activity and induces nitric oxide (Dinarello, 2000). Cytokine producing potential may vary among individuals and it could be due to the polymorphisms observed in the cytokine genes (Pravica *et al*., 2000; Yilmaz *et al*., 2005). It has demonstrated that reconstitution of G to A at position 5644 of IFN-γ gene producing a homozygous genotype which correlates to low production of IFN-γ (Pravica *et al*., 2000; Anuradha *et al*., 2008). To investigate the association of G5644A polymorphism in the individual susceptibility to brucellosis and also to reveal the role of this polymorphism in development of active form to focal form, we studied allelic and genotypic frequencies of IFN-γ gene in healthy controls and brucellosis patients with different forms of the disease.

Our results were shown no significant differences in IFN-γ (G5644A) genotypes between healthy controls and overall patients with brucellosis. These results is line with a study in Iranian population (Davoudi *et al*., 2006) and also is according to other studies shown development of Th1 type response with cell proliferation and production of IFN-γ and IL-2 in acute brucellosis (Caballero *et al*., 2000; Giambartolomei *et al*., 2002). On the other hand, comparison of frequency of high producer of IFN-γ(5644 G) between patients with brucellosis and controls revealed no significant differences, albeit frequency of G allele was scarcely prominent in healthy controls. This finding confirmed our previous results demonstrated that CD3+CD4+T cells of patients with acute brucellosis produce IFN-γ in response to brucella antigen as same as controls (Rafiei *et al*., 2006). However, it has shown IFN-γ levels were significantly lower in patients with chronic brucellosis than in patients with acute brucellosis, showing a clear association of low IFN-γ plasma levels with an unfavorable immune response against brucella (Rafiei *et al*., 2006). It has been shown that neutralizing endogenous IFN-γ results in a decrease in control of brucellosis (Cooke and Hill, 2001; Baldwin and Parent, 2002).

An association between the presence of low protein producer allele of IFN-γ gene (5644A) and focalization of the disease in recent study is consistent with our previous report demonstrating that decreasing in IFN-γ has a fundamental role in prolongation of brucellosis (Rafiei *et al*., 2006). This analysis indicates that the insufficient production of IFN-γ results in the failure of macrophage activation, leading to the progression of chronic brucellosis (Rafiei *et al*., 2006; Skendros *et al*., 2007). It was also observed that patients with tuberculosis carrying the genotype +874A/A in homozygosis showed significantly lower IFN-γ plasma levels than those carriage +874 T allele, which is strong evidence in favor of the notion that this polymorphism reduces the production of IFN-γ (Anuradha *et al*., 2008; Vallinoto *et al*., 2010) and decreases the activation of cellular immunity, thereby increasing the chance of infection.

Contrary to our findings, a study by Davoudi *et al*; found no significant differences in genotype of IFN-γ gene between patients with or without focal involvement (Davoudi *et al*., 2006). However, the second study suffered from low sample size for the detection of a significant difference between patients with or without focal form of the disease. They studied only 43 patients with brucellosis and no significant differences in the allele frequencies between patients with and without focal involvement were found (Davoudi *et al*., 2006).

The marked association between the IFN-γ (G5644A) allele and focal brucellosis in females strongly suggests that this relationship is significant. Therefore it seems that harboring IFN-γ-5644A low producer allele might be an increased risk of localization of brucellosis. The finding suggests a protective role for the +5644 G allele, in opposition to the role of the allele 5644 A which seems to be a predisposing factor to brucella infection. In fact, possession of the 5644 A allele seemed significantly to increase the probability of longevity of the disease. Thus, an IFN-γ genotype associated with low production of IFN-γ might be harmful for the control of brucella. It is worthy of note that allele 5644 A of the IFN-γ gene is associated with a low producing phenotype (Wu *et al*., 1998; Pravica *et al*., 2000) and IFN-γ 5644 A carriers have been described as having higher odds of suffering longevity of the disease and a worse course of disease outcome (Joannes *et al*., 2010; Prabhu Anand *et al*., 2010). Thus and taken together the results from others and our findings here, we may speculate that IFN-γ gene variations (G5644A) may have a role in determining the genetic susceptibility to elongation of disease and progress to chronic disorder that might occur over time in the illness.

CONCLUSION

Based on the importance of IFN-γ in the protective immunity against brucellosis, our results demonstrate an association of low IFN-γ producer allele (5644A) with

localized form of brucellosis, confirming that the protection against brucella depends on an intense cellular immune response to Th1 profile, characterized by the predominance IFN-γ production.

REFERENCES

Ahmed, K., K. Al-Matrouk, G. Martinez, K. Oishi, V.O. Rotimi and T. Nagatake, 1999. Increased serum levels of interferon-gamma and interleukin-12 during human brucellosis. Am. J. Trop. Med. Hyg., 61: 425-427.

Anuradha, B., S.S. Rakh, M. Ishaq, K.J.R. Murthy and V.L. Valluri, 2008. Interferon-gamma low producer genotype +874 overrepresented in Bacillus Calmette-Guerin nonresponding children. Pediatr. Infect. Dis. J., 27: 325-329.

Baldwin, C.L., M. Parent, 2002. Fundamentals of host immune response against Brucella abortus: What the mouse model has revealed about control of infection. Vet. Microbiol., 90: 367-382.

Basham, T.Y. and T.C. Merigan, 1983. Recombinant interferon-G increases HLA-DR synthesis and expression. J. Immunol., 130: 1492-1494.

Bravo, M.J., J. de Dios Colmenero, A. Alonso and A. Caballero, 2003. Polymorphisms of the interferon gamma and interleukin 10 genes in human brucellosis. Eur. J. Immunogenet., 30: 433-435.

Bravo, M.J., J.D. Colmenero, M.I. Queipo-Ortuño, A. Alonso and A. Caballero, 2008. TGF-beta1 and IL-6 gene polymorphism in Spanish brucellosis patients. Cytokine, 44: 18-21.

Caballero, A., M.J. Bravo, A. Nieto, J.D. Colmenero, A. Alonso and J. Martín, 2000. TNFA promoter polymorphism and susceptibility to brucellosis. Clin. Exp. Immunol., 121: 480-483.

Cooke, G.S. and A.V.S. Hill, 2001. Genetics of susceptibility to human infectious disease. Nat. Rev. Genet., 2: 967-977.

Cutler, S.J., A.M. Whatmore and N.J. Commander, 2005. Brucellosis-new aspects of an old disease. J. Appl. Microbiol., 98: 1270-1281.

Davoudi, S., A.A. Amirzargar, M. Hajiabdolbaghi, M. Rasoolinejad and A. Soodbakhsh *et al*., 2006. Th-1 cytokines gene polymorphism in human brucellosis. Int. J. Immunogenet., 33: 355-359.

Dinarello, C.A., 2000. Proinflammatory cytokines. Chest, 118: 503-508.

Fernandes, D.M. and C.L. Baldwin, 1995. Interleukin-10 downregulates protective immunity to *Brucella abortus* infection. Infect. Immun., 63: 1130-1133.

Giambartolomei, G.H., M.V. Delpino, M.E. Cahanovich, J.C. Wallach and P.C. Baldi *et al*., 2002. Diminished production of T helper 1 cytokines correlates with T cell unresponsiveness to *Brucella cytoplasmic* proteins in chronic human brucellosis. J. Infect. Dis., 186: 252-259.

Golding, B., D.E. Scott, O. Scharf, L. Huang and Y. Zaitseva *et al*., 2001. Immunity and protection against *Brucella abortus*. Microbes Infect., 3: 43-48.

Hajilooi, M., A. Rafiei, M. Reza Zadeh and N. Tajik, 2006. Association of interleukin-1 receptor antagonist gene polymorphism and susceptibility to human brucellosis. Tissue Antigens, 68: 331-334.

Jiang, X. and C.L. Baldwin, 1993. Effects of cytokines on intracellular growth of *Brucella abortus*. Infect. Immun., 61: 124-134.

Joannes, M.O., G. Loko, J. Delourneaux, R. Chout and T. Marianne-Pepin, 2010. Association of the +874 T/A interferon gamma polymorphism with infections in sickle cell disease. Int. J. Immunogenet., 37: 219-223.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 16: 1215-1215.

Murphy, E.A., J. Sathiyaseelan, M.A. Parent, B. Zou and C.L. Baldwin, 2001. Interferon-gamma is crucial for surviving a *Brucella abortus* infection in both resistant C57BL/6 and susceptible BALB/c mice. Immunology, 103: 511-518.

Prabhu Anand, S., M. Harishankar and P. Selvaraj, 2010. Interferon gamma gene +874A/T polymorphism and intracellular interferon gamma expression in pulmonary tuberculosis. Cytokine, 49: 130-133.

Pravica, V., C. Perrey, A. Stevens, J.H. Lee and I.V. Hutchinson, 2000. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum. Immunol., 61: 863-866.

Rafiei, A., S.K. Ardestani, A. Kariminia, A. Keyhani, M. Mohraz and A. Amirkhani, 2006. Dominant Th1 cytokine production in early onset of human brucellosis followed by switching towards Th2 along prolongation of disease. J. Infect., 53: 315-324.

Rafiei, A., M. Hajilooi, R.J. Shakib and S.A. Alavi, 2007. Transforming growth factor-beta1 polymorphisms in patients with brucellosis: An association between codon 10 and 25 polymorphisms and brucellosis. Clin. Microbiol. Infect., 13: 97-100.

Rezazadeh, M., M. Hajilooi, A. Rafiei, M. Haidari and E. Nikoopour *et al*., 2006. TLR4 polymorphism in Iranian patients with brucellosis. J. Infect., 53: 206-210.

Skendros, P., P. Boura, D. Chrisagis and M. Raptopoulou-Gigi, 2007. Diminished percentage of CD4+ T-lymphocytes expressing interleukine-2 receptor alpha in chronic brucellosis. J. Infect., 54: 192-197.

Vallinoto, A.C., E.S. Graça, M.S. Araújo, V.N. Azevedo and I. Cayres-Vallinoto *et al*., 2010. IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to Mycobacterium tuberculosis infection and clinical manifestation of tuberculosis. Hum. Immunol., 71: 692-696.

Wu, S., D. Muhleman and D.E. Comings, 1998. G5644A polymorphism in the interferon-gamma (IFNG) gene. Psychiatr. Genet., 8: 57-57.

Yilmaz, V., S.P. Yentur and G. Saruhan-Direskeneli, 2005. IL-12 and IL-10 polymorphisms and their effects on cytokine production. Cytokine, 30: 188-194.

Zhan, Y. and C. Cheers, 1993. Endogenous gamma interferon mediates resistance to *Brucella abortus* infection. Infect. Immun., 61: 4899-4901.

Zhan, Y., Z. Liu and C. Cheers, 1996. Tumor necrosis factor alpha and interleukin-12 contribute to resistance to the intracellular bacterium *Brucella abortus* by different mechanisms. Infect. Immun., 64: 2782-2786.