Mannose-binding lectin gene codon 54 polymorphism susceptible to brucellosis in Turkish children

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Genetic factors are as important as environmental factors in susceptibility to brucellosis. Among these genetic factors, mannose-binding lectin (MBL) deficiency contributes to susceptibility to animal brucellosis. The aim of the study is to determine the influence of codon 54 polymorphisms in the MBL gene on susceptibility to brucellosis.

Forty-three patients diagnosed with brucellosis and 106 healthy children were admitted in the study. In the patient group, 19 (44.2%) subjects had AA, 22 (51.1%) subjects had AB and 2 (4.6%) subjects had BB genotypes for codon 54 polymorphism. Eighty-two (77.4%) of the healthy children had AA genotype, while 24 (22.6%) had AB genotype.

Our results revealed that genotype frequencies carrying MBL variant allele at codon 54 among the patients were significantly higher compared to those found in the control group (55.8% and 22.6%, respectively; p=0.0001, odds ratio [OR]=4.316, 95% confidence interval [CI]: 2.030-9.177). Our data suggest that children with MBL codon 54 AB or BB genotype are more susceptible to brucellosis.

Key words: mannose binding lectin, brucellosis, susceptibility.

Brucellosis is an infectious disease caused by Gram-negative coccobacilli of the genus *Brucella*. It is the most common zoonotic disease worldwide, with over half a million new cases annually[1]. Human brucellosis is endemic in many countries, including Turkey[2]. *Brucella* species are intracellular acting pathogens that express a set of factors -including lipopolysaccharides, virulence regulator proteins and phosphatidylcholine- to ensure full virulence. They allow the bacteria to survive and proliferate within its replicative vacuole and enable the bacteria to escape detection by the host immune system[3].

Recent studies have shown that host genetic factors are as important as environmental factors in susceptibility to infectious diseases. The polymorphic nature of the genes involved in the immune response seems to be the ideal candidate for these studies. It is likely that identification of these genetic polymorphisms will provide a significant contribution to the pathogenic and protective mechanisms in infectious diseases. Among the genetic factors in susceptibility to brucellosis, the mannose-binding lectin (*MBL*) gene has been investigated only in animal studies, and it has been shown that MBL deficiency significantly contributes to the susceptibility to animal brucellosis[4].

Mannose–binding lectin (MBL) is a calcium-dependent lectin that plays an important role in innate immunity by activating the complement pathway[5]. MBL recognizes the pathogens, acts as an opsonin and independently triggers the lectin pathway complement systems of antibodies. It is encoded by the *MBL2* gene on chromosome 10. A number of functional polymorphisms have been described in the *MBL2* gene[6]. Single nucleotide polymorphisms in exon 1, at codon 52 (allele D), codon 54

(allele B) and codon 57 (allele D), result in reduced serum levels of functional MBL[7].

There have been a number of studies proposing an association between the certain MBL genotypes in humans and susceptibility for bacterial, fungal and viral infections; however, the relationship between human brucellosis and *MBL* gene polymorphisms has not been studied yet.

The aim of the current study was to determine the influence of codon 54 and codon 57 polymorphisms in the *MBL* gene on susceptibility to brucellosis in Turkish children affected with brucellosis and in healthy control subjects. This study is the first to investigate the association between MBL alleles and human brucellosis.

Material and Methods

Patients and Controls

The study group consisted of 43 children diagnosed with brucellosis and 106 healthy age-matched children. The patient group was recruited from the Pediatric Infectious Diseases Department of Ege University Medical School Hospital, Turkey, between 2005 and 2008. One hundred and six children who had no history of brucellosis or chronic symptoms and negative standard tube agglutination (STA) test formed the control group. The subjects in both groups were randomly selected and were not biologically related.

The diagnosis of brucellosis was based on the clinical findings compatible with brucellosis and the result of STA (titers ≥1:160) or Coombs agglutination test (titers ≥1:320). Clinical signs and symptoms at admission were fever, sweating, chills, malaise, arthralgia, arthritis, weight loss, anorexia, and neurological, liver and hematological complications.

All the subjects in the study and control groups were questioned for any other problems affecting their general health and those considered to have other serious problems were not included in the study.

Approval for the study was obtained from the ethical committee of Ege University Medical School Hospital. Informed consent was taken from the parents of the children according to the declaration of Helsinki.

MBL Genotyping

DNA was extracted from blood samples using standard techniques. Codon 54 polymorphisms in exon 1 of the *MBL2* gene were genotyped in 43 patients and 106 controls using the polymerase chain reaction (PCR) and sequence-specific primers. The primer sequences were 5'-TAGGACAGAGGGCATGCTC-3' and 5'-CAGGCAGTTTCCTCTGGAAGG-3'. The PCR product (349 bp) was digested with BanI for codon 54. BanI digestion was performed at 50°C for 60 minutes (min) with 5U enzymes. The normal allele (allele A) was cut into two fragments with BanI, 260 bp and 89 bp. The variant allele (allele B) remained uncut. Products were projected through electrophoresis on 2% agarose gel.

Statistical Analysis

The consistency of MBL genotypes and allele frequencies were compared using the chi-square test. Results of the logistic regression analysis were expressed as odds ratio (OR), and 95% confidence intervals (CI) for the ORs were also calculated. P values of less than 0.05 (two-tailed) were considered to be significant. A chi-square test was used to query the appropriateness between the observed and expected genotype values and their fit to Hardy-Weinberg equilibrium. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) software package version 14.0.

Results

The mean age was 8.10 ± 4.4 (2.5–15.2) and 7.58 ± 4.1[1-16] years in the patient and healthy individuals, respectively. Gender frequency and mean age were similar in the two groups.

The most common complaints were fever (72.1%), malaise (62.8%) and osteoarticular complaints (53.4%). On physical examination, the most common findings were hepatomegaly and splenomegaly, which occurred in 51.2% of patients. Based on laboratory findings, anemia was detected in 39.5%, thrombocytopenia in 6.9%, leukopenia in 16.2%, increased erythrocyte sedimentation rate in 46.5%, C-reactive protein positivity in 13.9%, and increased alanine aminotransferase value in 32.5%. The most common focal complication was found as

musculoskeletal system involvement, at a rate of 39.5%.

Distribution of the genotypes in the control group was consistent with the Hardy–Weinberg equilibrium. Codon 57 polymorphism was not detected in any of the subjects in either group.

The genotype and allele frequencies of MBL codon 54 polymorphism in patients and controls are shown in Table I and Table II. Twenty-two of the 43 (51.1%) patients were found to have heterozygous variant (A/B) at codon 54 of the *MBL* gene. Two (4.6%) children from the patient group had the B/B genotype of codon 54 polymorphism. These subjects were included in the group with AB genotype during statistical analysis. Results showed that the genotype frequencies carrying MBL variant allele at codon 54 were significantly higher among the patients compared to the control group (55.8% and 22.6%, respectively; p=0.0001, OR: 4.316, 95%CI: 2.030–9.177) (Table I). None of the controls had BB genotype.

There was also a significant difference between the allele frequencies of the two groups (p=0.0001). The patient group had a lower incidence of allele A (69.8%) compared to the control group (88.7%). Allele B frequencies were also significantly higher in the patient group (30.2%) than the control group (11.3%) (Table II).

Genotype and allele frequencies of MBL codon 54 polymorphism were also analyzed for different clinical forms of brucellosis. However, no association was found between the frequency of AB or BB genotype and the clinical forms of the disease.

Discussion

In the present study, we investigated the influence of codon 54 and codon 57 polymorphisms in the *MBL* gene on susceptibility to human brucellosis in children. Data suggest that children who have MBL codon 54 AB or BB genotypes are more susceptible to brucellosis (Table I).

Brucella species cause a chronic infection, suggesting an ongoing interaction between the host and the pathogen. To advance long-term intracellular survival, *Brucella* minimizes activating host inflammatory mechanisms[8]. The specific mechanisms of intracellular survival by *Brucella* are not clearly understood, but bacteria often alter normal host functions to avoid immune detection. Host genetic factors play important roles in susceptibility to brucellosis. Different genetic polymorphisms involved in the immune response, such as tumor necrosis factor–α, interleukin (IL)–2, IL–6, IL–10, IL–4, interferon–γ, and major histocompatibility complex class I chain-related gene A, have previously been studied in human brucellosis, and controversial results were obtained[9-14]. Among these genetic factors, the *MBL* gene has been investigated in animal brucellosis and it has been shown that MBL deficiency is an important factor in animal brucellosis[4]. However, to our knowledge, no clinical study about the role of MBL polymorphisms in human brucellosis has yet been done.

Mannose-binding lectin (MBL) is a key molecule in the innate immune defense. Recent studies have shown especially that the presence of the different variant alleles in the *MBL* gene causing low MBL levels in serum is associated with an increased risk of extracellular infections, primarily in children[15-17]. In contrast, intracellular bacterial infections such as *Mycobacterium tuberculosis* occur more frequently in patients carrying the AA genotype and increased serum MBL levels [18-20]. Complement-mediated phagocytosis as a result of opsonization has been suggested to facilitate the intracellular infections. MBL has been proven to be involved in the clearance of

Table I. Genotype Frequencies of Mannose-Binding Lectin Codon 54 Polymorphism in Patients with Brucellosis and Controls

	Patient group *N=43* *n (%)*	Control group *N=106* *n (%)*	OR (95% CI)	*P*
Genotype AA	19 (44.2)	82 (77.4)	4.316 (2.030–9.177)	0.0001
Genotype AB + BB	24 (55.8)	24 (22.6)		

OR: Odds ratio. CI: Confidence interval.

Table II. Allele Frequencies of Mannose-Binding Lectin Codon 54 Polymorphism in Patients with Brucellosis and Controls

	Patient group *N=43* *n (%)*	Control group *N=106* *n (%)*	OR (95% CI)	*P*
Allele A	60 (69.8)	188 (88.7)	4.914 (2.998–8.650)	0.0001
Allele B	26 (30.2)	24 (11.3)		

OR: Odds ratio. CI: Confidence interval.

apoptotic cells and in controlling cytokines on inflammatory cells, thus making it particularly relevant in chronic and progressive phases of infections[21].

Brucella species are also considered as intracellular pathogens, but as shown in this study, the frequency of the B allele was significantly higher in the patient group (Table II), and individuals having variant low MBL coding allele are more susceptible to *Brucella* infections as opposed to the other intracellular-acting microorganisms (p=0.0001, OR: 4.316, 95%CI: 2.030–9.177). In a prior study of MBL promoter polymorphisms in water buffalo, the MBL haplotype pair LYD/LYD carrying low level coding was associated with susceptibility to *B. abortus* infection[4]. As indicated in the aforementioned study, the antibacterial activity present in the serum results from the interaction between MBL and *B. abortus*. Therefore, MBL deficiency may result in insufficient antibacterial activity in serum and lead to the development of the invasive bacterial burden in brucellosis detected in our study group.

In another study, the deposition of complement components and MBL on the bacterial surface of *Brucella* was detected by flow cytometry[22]. It was shown that MBL initiates antibody-independent complement activation and brucellacidal activity. Both the classical and the MBL-mediated pathways are involved in complement deposition and complement-mediated killing of *Brucella*. The bactericidal action of serum against *B. abortus* is due to the effects of the classical -not the alternative-pathway. As a result, the argument was that MBL may play a role in host defense against *Brucella*[22].

The symptoms and signs of brucellosis were consistent with those of other studies[23,24]. Fever is the most common feature of brucellosis, followed by osteoarticular involvement and constitutional symptoms of malaise, fatigue, sweating, and chills. Focal disease is common and most frequently localizes to the musculoskeletal system. However, there was no association between the frequencies of AB or BB genotypes or B allele and the different clinical forms of brucellosis. This result might partly be due to the limited number of patients.

The MBL codon 57 variant was not seen in our patient and control groups. This variant is common in sub-Saharan populations, whereas it is seen rarely in other populations[15]. Two previous MBL genotyping studies conducted in Turkish populations also showed that this variant is not found in Turkish populations[20,25].

In conclusion, we report that variant *MBL* gene polymorphisms are associated with the occurrence of brucellosis in children. Particularly, there are three limitations that need to be addressed in the scope of this study. The first limitation is that it covers a small number of patients. Secondly, the D variant or MBL2 promoter polymorphisms were not investigated. The third limitation is that the serum MBL levels in the population were not studied, although the association between the MBL levels and gene polymorphisms has been demonstrated previously in many other studies[18,19,26]. Further investigation is needed to define the role of MBL in the immune response to *Brucella*, and a larger number of subjects are required to confirm our results.

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