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Investigation of Monnose-Binding Lectin gene Polymorphism in Patients with Erythema Multiforme, Stevens-Johnson Syndrome and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis Overlap Syndrome

Mutlu Karkucak[1], Emel Başkan Bülbül[2], Hakan Turan[3], Tahsin Yakut[1], Sevil Toka[2], Hayriye Sarıcaoğlu[2]

[1]*Department of Medical Genetics, Faculty of Medicine, Uludağ University, Bursa, Turkey*
[2]*Department of Dermatology, Faculty of Medicine, Uludağ University, Bursa, Turkey*
[3]*Department of Dermatology, Faculty of Medicine, Düzce University, Düzce, Turkey*

ABSTRACT

Objective: Monnose-Binding lectin (MBL) appears to play an important role in the immune system. The genetic polymorphisms in the MBL2 gene can result in a reduction of serum levels, leading to a predisposition to recurrent infection. The aim of this study is to investigate the influence of a polymorphism in codon 54 of the MBL2 gene on the susceptibility to Erythema Multiforme, Stevens-Johnson Syndrome and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis Overlap Syndrome (EM, SJS and SJS/TEN overlap syndrome).

Material and Methods: Our study included 64 patients who were clinically and/or histopathologically diagnosed with EM, SJS, and SJS/TEN overlap syndrome and 66 healthy control subjects who were genotyped for the MBL2 gene codon 54 polymorphism using the PCR-RFLP method. For all statistical analyses, the level of significance was set at $p<0.05$.

Results: The prevalence of the B allele was 18% in the EM, SJS and SJS/TEN patient groups and 13% in the control group. No significant differences in allele frequencies of any polymorphism were observed between the patient and control groups, although the B allele was more frequent in the patient groups ($p=0.328$).

Conclusion: Our results provide no evidence of a relationship between MBL2 gene codon 54 polymorphism and the susceptibility to EM, SJS and SJS/TEN overlap syndrome. However, these findings should be confirmed in studies with a larger sample size.

Key Words: Polymorphism, MBL2 gene, Erythema multiforme, Stevens-Johnson syndrome, Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome

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Introduction

Monnose-Binding lectin (MBL) is a major soluble pattern-recognition molecule that plays a significant role in the natural immune system by activating the complement pathway, and is synthesized by the hepatocytes and results in phagocytosis (1, 2). MBL typically consists of a collagenous site and a lectin domain (3). It contributes to the elimination of a large number of pathogenic microorganisms through the lectin-complement pathway and opsonophagocytosis (4). It recognizes the specific sugar groups on the surface of the microorganisms. In addition, it can bind to the phospholipids, nucleic acids and non-glycosylated proteins (2). Serum MBL levels in humans vary as a result of genetic polymorphisms in the monnose-binding lectin gene (5). Thus, polymorphisms affecting the protein serum levels can lead to infections and autoimmune diseases.

Erythema multiforme, Stevens-Johnson syndrome and Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome are acute mucocutaneous diseases that can lead to severe morbidity and mortality (6). All three conditions are considered to be variants of the same process because of their similar clinical and histopathological features, differing only in the surface area involved (7). The etiology of the three diseases is closely associated with infection and the use of certain drugs. Infectious agents account for the majority of Erythema multiforme cases, nearly 50% of SJS cases, and 5% of SJS/TEN cases (8). Erythema multiforme, Stevens-Johnson syndrome and Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome have a tendency to recur with recurrent viral or bacterial infections. In addition, opportunist infections, such as those caused by *Staphylococcus aureus* and *Staphylococcus epidermidis,* have been reported to exist on the ocular surfaces of patients with SJS and SJS/TEN (9). This finding suggests that an association could exist between the EM, SJS, SJS/TEN overlap syndrome and the natural immune system.

This study was designed to investigate whether the MBL2 gene codon 54 polymorphism leads to a predisposition for

Address for Correspondence: *Dr. Hakan Turan, Department of Dermatology, Faculty of Medicine, Düzce University, Düzce, Turkey*
Phone: +90 380 542 13 90 Fax: +90 380 542 13 87 E-mail: drhakanturan@gmail.com

diseases such as EM, SJS, SJS/TEN overlap syndrome, which may have microbial etiologies and may be associated with a defective immune system.

Material and Methods

The patient group included 64 patients (26 males, 38 females, mean age: 40.06±17.59 years) treated at the Uludag University Medical Faculty Dermatology Department between January 2000 and December 2010. The control group consisted of 66 healthy individuals (25 males, 41 females, mean age: 40.19±13.56 years) with no previous dermatological, systemic or allergic disorders. The patients were classified by clinical and/or histopathological findings using the clinical classification of Bastuji Garin et al. (7).

Blood samples from both the patient and control groups were collected in EDTA tubes. DNA isolation was performed according to the procedures of the Dr. Zeydanlı (DZ) DNA isolation kit, and samples were stored at -20°C.

The MBL2 gene codon 54 polymorphism was determined using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. For the MBL2 gene codon 54 polymorphism, forward (5'- TAGGACAGAGGGCATGCTC -3') and reverse (5'- CAGGCAGTTTCCTCTGGAAGG -3') primers were used to amplify a 349 bp region of the MBL2 gene (10). To identify the MBL2 gene codon 54 polymorphism among the products, the Ban I (Genemark, Russia) enzyme was used. The restriction fragments were separated using a 2% agarose gel. Genotypes were determined as follows: genotype A/A were two distinct products of 260 bp and 89 bp; genotype A/B was three distinct products of 349 bp, 260 bp and 89 bp; and genotype B/B was one 349 bp fragment. For MBL2 gene codon 54 polymorphism, the normal allele is called A, and the variant allele is called B.

Data were recorded with±standard deviations. A chi-square (χ^2) test was used to compare genotypes. P values less than 0.05 were considered statistically significant.

Results

The mean age was 40.06±17.59 years for the patient group (26 males and 38 females) and 40.9±13.56 years for the control group (25 males and 41 females). There was no difference in the age distribution between the patient and control groups (Table 1).

Of the 64 individuals in the EM, SJS and SJS/TEN patient groups, 44 individuals were identified with the A/A genotype; 17 with the A/B genotype; and 3 with the B/B genotype. Among the 66 individuals in the control group, 51 individuals were identified with the A/A genotype; 13 with the genotype A/B; and 2 with the B/B genotype. Using the A/A homozygous subjects as the reference group, we found no association between the A/B and B/B genotypes and the risk of EM, SJS and SJS/TEN (p=0.273). The prevalence of the B allele was 18% in the EM, SJS and SJS/TEN patient groups and 13% in the control group. No significant differences in allele frequencies for any polymorphism were observed between patient and control groups, although the B allele was more frequent in the patient groups (p=0.328) (Table 2).

Discussion

The rare occurrence of Erythema multiforme, Stevens-Johnson syndrome and Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome suggests the possibility of an individual genetic predisposition to these diseases. The herpes simplex virus, adenovirus, influenza, mycoplasma pneumonia and fungal infections were reported to be potentially involved in the etiology of EM, SJS and SJS/TEN overlap syndrome. The pathophysiological mechanism underlying this association is not clearly established (8). In patients with Erythema multiforme, the DQB1*0301 allele was reported to be observed more commonly, the HLA-B12 (HLA-Bw44) allele was more common in SJS patients with ocular involvement, and the HLA-A*0206 allele was more common in patients with SJS/TEN (11-14). In addition to the association with HLA antigens, cases were triggered by diphtheria, tetanus and hepatitis B vaccinations and the tuberculin skin test. These observations support the hypothesis that EM, SJS and SJS/TEN are the result of an immune response that develops against infectious antigenic stimuli in genetically predisposed individuals (15-17).

Monnose-Binding lectin, which is a pattern-recognition molecule of the natural immune system, is a C-type serum lectin. The functions of monnose-binding lectin include the activation of the complement system, regulation of apop-

Table 1. Demographic and clinical characteristics of the study groups

	Patient Group	Control Group
Number of subjects	64	66
Gender (Male/Female)	26/38	25/41
Mean age (in years)	40.06±17.59	40.19±13.56

Table 2. MBL2 gene codon 54 allele frequency and genotype distribution of patients with EM, SJS and SJS/TEN patients and the control group

	Patient Group (n=64)	Control Group (n=66)	p value	ORs (95% CI)
A/A Genotype	44	51	0.273	1.55 (0.66-3.63)
A/B + B/B Genotypes	20	15		
A allele frequency (%)	82	87	0.328	1.47 (0.64-3.42)
B allele frequency (%)	18	13		

tosis and opsonization, and modulation of inflammation (5). Monnose-Binding lectin is encoded by the MBL2 gene, which is localized on chromosome 10 (q11.2-q21) and consists of 4 exons. The polymorphisms in exon 1 and the promoter of the monnose-binding lectin gene are reported to be associated with MBL serum levels (18). Polymorphisms in the first exon of the gene, such as codons 52, 54 and 57, are known to decrease functionality by disturbing protein oligomerization (19). Therefore, these structural polymorphisms could predispose individuals to viral and bacterial diseases by affecting the MBL serum levels.

Decreased protein serum levels and an increased tendency to develop infections are associated with MBL polymorphic structural variants of interest and are increasing coninuously (20-22). In a previous study, Sappanen et al. (23) identified the genotypes for the MBL2 gene structural and promoter site polymorphism in 51 patients with recurrent herpes virus infection and 147 healthy individuals. They concluded that MBL2 gene structural variant genotype (A/B or B/B) could be a risk factor in patients with recurrent HSV-2 infection (10). Similarly, in a study investigating the effect of structural polymorphisms on the role of MBL in the prevention of mycoplasma infections, a predisposition to mycoplasma infection was demonstrated in patients with the MBL2 exon 1 polymorphism (24). Vardar et al. (10) reported that MBL2 exon 1 codon 54 polymorphism could be significantly involved in the predisposition to bacterial meningitis in pediatric patients. Hashimoto et al. (25) found no significant difference between patient and control groups in their study investigating the role of MBL2 codon 54 polymorphisms on the predisposition of patients with atopic dermatitis to cutaneous infections, such as the herpes virus and *Staphylococcus aureus*. The MBL2 gene codon 54 polymorphism was reported to exhibit no difference in preterm neonates with nosocomial fungal infection and the control group (26). Rantala et al. (27) reported that MBL2 promoter site polymorphism and low serum levels were associated with increased *Chlamydia pneumoniae* antibodies. In our study, in which we investigated MBL2 codon 54 polymorphism, we found no statistically significant difference between the genotype and allele frequencies of the patient and control groups.

Our study is the first to investigate the role of the single nucleotide polymorphism in the MBL2 gene in EM, SJS and SJS/TEN overlap syndrome. A literature review revealed a small number of studies with similar hypotheses. In these studies, TLR 3 and nine single nucleotide polymorphisms were evaluated in EM, SJS and SJS/TEN. Toll-like receptors are known to be involved in antiviral and antibacterial defense by activating the natural immune system against microbial pathogens. Ueta et al. (28) investigated a TLR 3 gene single nucleotide polymorphism in SJS/TEN patients with ocular surface complication and concluded that 299698T/G, 293248A/A and 299698T/T genotypes were strongly associated with SJS/TEN. Turan et al. (29) investigated a TLR9 polymorphism, which is significantly involved in antibacterial and antiviral immune defense, in their study on 52 patients with EM, SJS, SJS/TEN overlap syndrome and 50 healthy control subjects. No significant association was found.

Conclusion

While our study did not find a significant correlation between the MBL2 gene codon 54 polymorphism and EM, SJS, SJS/TEN overlap syndrome, we believe that a larger sample size and investigation of other single nucleotide polymorphisms in exon 1 and the promoter of this gene are required to reach a definitive conclusion.

Conflict of Interest

No conflict of interest was declared by the authors.

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