Association between Mannose-Binding Lectin 2 Gene Polymorphism and Liver Fibrosis in Patients with Chronic Viral Hepatitis

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ABSTRACT

Objective: Mannose-Binding Lectin (MBL) has become a popular molecule in investigations on basic and clinical Gastroenterology and contributed to new approaches to the understanding of infectious and immune diseases associated with intestine and liver. The aim of the present study was to investigate the association between codon 54 polymorphisms in MBL2 gene coding MBL and predisposition to fibrosis in patients with viral Hepatitis B and C.

Methods: One hundred patients with chronic hepatitis (70 hepatitis B, 30 hepatitis C) who underwent liver biopsy and 100 healthy controls with no known chronic disease were included in the study. Patients in both viral hepatitis groups were divided into two groups according to their fibrosis scores with Ishak scoring system. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to determine the MBL2 codon 54 polymorphism. For the statistical analysis, the level of significance was set at $P < 0.05$.

Results: No significant differences in allele frequencies for any polymorphism were observed between patients and controls, although the G allele was more frequent in the patient groups ($p>0.05$). In the comparison in terms of G and A alleles between two groups, hepatitis B patients in Group-II (group with high fibrosis score) were found to have a significantly higher frequency of A alleles ($p=0.027$).

Conclusion: Although it is accepted that MBL2 polymorphism play a part in the course of HBV and HCV infections, larger studies investigating the relation between MBL2 polymorphism and disease progression and treatment are required.

Keywords: Fibrosis, hepatitis B, hepatitis C, MBL2 gene, polymorphism

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INTRODUCTION

Mannose-Binding Lectin (MBL), which is the one of innate immune system pattern recognising molecules, is a C type serum Lectin. It is a molecule that recognising major soluble patterns and which plays a significant role in the innate immunity by activating the complement pathway synthesized by the hepatocytes and phagocytosis(1,2). Its specific structure consists of the collagenous region and Lectin domain(3) Lectin contributes to the elimination of many microorganisms through complement pathway and opsonophagocytosis(4). Due to genomic polymorphisms in *MBL2* gene, there are differences in the serum MBL levels of people(5). Therefore, polymorphisms influencing serum levels of protein may lead to infections and predisposition to autoimmune diseases.

MBL is coded by *MBL2* gene located on chromosome 10 and containing four exons. It is the only collection with the ability to activate complement system(1). Recently, it has become a popular subject in investigations on basic and clinical Gastroenterology and contributed to new approaches to the understanding of infectious and immune diseases associated with intestine and liver.

For *MBL2* codon 54 polymorphism, normal allele is called G, and the variant allele is called A. The aim of the present study was to investigate the association between codon 54 polymorphisms in *MBL2* gene coding MBL and predisposition to fibrosis in patients with viral Hepatitis B and C.

SUBJECTS AND METHOD

Study subjects

One hundred patients with chronic hepatitis (70 hepatitis B, 30 hepatitis C) who underwent liver biopsy in Gastroenterology Department of Uludağ University Faculty of Medicine and

100 healthy controls with no known chronic disease were included in the study. Patients in both viral hepatitis groups were divided into two groups according to their fibrosis scores with Ishak scoring system(6). Those with the fibrosis score of 3 or lower were defined as Group-I; and those with a score of 4 or over as Group–II. The study was conducted in accordance with the Declaration of Helsinki and Principles for Good Clinical Practice and was approved by the local Ethics Committee (2009-12/96). Prior to the study inclusion, all patients read and signed the informed consent form. After signing an informed consent from each patient, a 2 ml of blood taken into EDTA tubes for the *MBL2* gene polymorphisms and were stored at -20° C.

DNA Extraction and Genotyping

Blood samples from both the patient and the control groups were taken 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 until PCR.

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(7). To identify the *MBL2* gene codon 54 polymorphism among the products, the *Ban I* enzyme was used. In the analysis conducted in 2% agarose gel after cutting the enzyme, genotypes were determined as follows: if the 349 bp PCR product from the *MBL2* gene was cut into two distinct products of 260 bp and 89 bp, then the genotype was identified as G/G; if three distinct products were formed as 349 bp, 260 bp and 89 bp, then the genotype was identified as G/A; and if the product was 349 bp then the genotype was identified as A/A.

Statistical analysis

The data were analyzed using SPSS 13.0 software (IBM Corp., NY, USA). The data was recorded in ± standard deviations. The Mann-Whitney U test used to compare the age between the two groups. The Chi-Square ($\chi 2$) test was used to compare genotypes. P values smaller than 0.05 were accepted as being statistically significant.

RESULTS

In this study, among the 100 cases in the patient group (53 males and 47 females), the mean age was 43.98±12.75, and among the 100 cases in the control group (54 males and 46 females), the average age was 43±12.46. There was no difference in the age and gender between the patient and control groups.

Regarding the codon 54 polymorphism of the *MBL2* gene in the study, among the 100 chronic Hepatitis B and C patients, 73 were identified with the G/G genotype, 16 with the G/A genotype, and 11 with the A/A genotype. Among the 100 individuals in the control group, 63 were identified with the G/G genotype, 19 with genotype G/A and 18 with the A/A genotype. Using the subjects with the G/G homozygote genotype as a reference group, we found no association between the G/A and G/A genotypes and the risk of chronic Hepatitis B and C with statistical analysis ($p>0.05$). No significant differences in allele frequencies for any polymorphism were observed between patients and controls, although the G allele was more frequent in the patient groups ($p>0.05$) (Table 1).

Chronic Hepatitis B

Overall 70 patients (40 male, 30 female) patients at the mean age of 43.17±11.94 who underwent liver biopsy with the diagnosis of Chronic hepatitis B were included in the study.

54 patients were found to have (77.14%) GG genotype, 8 (11.43%) GA genotype, and 8 (11.43%) AA genotype.

In the comparison of presence of A allele and absence of A allele groups, it was determined that patients in Group-II, high fibrosis score group, has a higher frequency of presence A allele with a difference approaching near significance ($p=0.052$). In parallel to these results, in the comparison in terms of G and A alleles between two groups, hepatitis B patients in Group-II, group with high fibrosis score, were found to have significantly higher frequency of A alleles ($p=0.027$) (Table 2).

Chronic Hepatitis C

Overall 30 patients (17 female,13 male) patients at the mean age of 45.8±14.5 were included in the study. 19 patients had (63.3%) GG genotype, 8 (26.6%) GA genotype, and 3 (10.1%) AA genotype. In terms of presence of A allele, no significant difference was found between three groups. Also, no difference was found between presence A allele and absence A allele groups also. No significant difference was found between two groups in terms of the frequency of G and A alleles($p>0.05$) (Table 3).

DISCUSSION

The functions of MBL are complement system activation, regulation of apoptosis and also opsonization and modulation of inflammation(5). MBL is coded by *MBL2* gene located on Chromosome 10 (10q11.2-q21) and contains four exons. Polymorphisms in exon 1 and promoter regions of *MBL2* gene were reported to be associated with lower MBL serum levels. It is known that structural polymorphisms in the first exon of the gene such as codon 52, 54 and 57 cause also functional deficiency by impairing the oligomerization of protein(8).

Therefore, these structural polymorphisms influencing MBL serum levels may lead to the predisposition to viral and bacterial diseases.

Personal factors influencing the development of liver damage in viral hepatitis are as follows: sex, age, ethnic origin, duration of infection, alcohol intake, dual infections with HIV/HBV/HCV and genotype of the virus. In addition, the importance of the immune state of the person is one of the issues recently addressed. In relation to this issue, polymorphisms of genes coding pro-inflammatory cytokines, vitamin D receptor and HLA types are being under consideration.

It is known that primarily Th1 response develops against hepatitis B infection and in cases when response remains inadequate; the disease enters the process of becoming chronic. In various studies, the importance of innate immune response in viral infections was stressed, as in all other infectious conditions(9). It has been suggested that MBL plays part in the clearance of virus through direct effect of complement activation in hepatitis B infection and in addition decreases inflammatory damage in liver tissue by reducing the release of pro-inflammatory cytokines(10,11).

The effect of *MBL2* gene polymorphism and MBL serum levels on the course of chronic hepatitis B has been reported so far in few studies. In spite of the differences in cohort characteristics, experimental approaches and in investigated polymorphisms, many of these studies found relation between the degree of disease caused by HBV and polymorphisms in *MBL2* gene and resultant low MBL levels. In various studies, an association was found between *MBL2* polymorphisms and viral persistence, advanced disease, HBV acquisition and survival in fulminant hepatic disease. However, there are also studies suggesting the contrary.

The relation between *MBL2* gene polymorphisms and persistence of HBV was first revealed in 1996(12). In this study, they showed in Caucasian patients an association of the codon 52 polymorphism of the *MBL2* gene with persistent HBV infection. In a study carried

out in 1999, it was maintained that codon 54 polymorphism was influential in the persistence and progression of disease and it was also shown that the probability of the development of symptomatic cirrhosis and spontaneous bacterial peritonitis was higher in adult HBV patients with codon 54 polymorphism. Thus, it was proposed that if people with polymorphism are identified, follow-up approaches may change, and prophylactic treatment against infections may be beneficial to these patients(13). In a study carried out in Vietnam, codon 54 polymorphism were found to be more frequent in the people who have acute Hepatitis B. In the same study, an association was found between codon 54 polymorphism and high viral load and transaminase values and it was suggested that MBL was directly effective in HBV clearance(14). Another study showed that MBL polymorphisms decrease survival in fulminant hepatitis caused by Hepatitis B virus. It was also thought that serum MBL levels may be used as a predictive factor for survival in these patients(15).

Overall, 527 patients were examined in an interesting study and it was demonstrated that in people without polymorphism, Hepatitis B infection resulted in natural immunity while in cases with codon 54 polymorphism and low serum MBL levels, persistent disease occurred significantly more common. Investigators suggested that low chronic MBL levels are related to persistence of disease(16). In another study, 320 HBsAg carriers, 199 HBV-related cirrhosis and hepatocellular cancer (HCC) patients, and 87 HBV infection patients with undergoing spontaneous seroconversion were compared with respect to *MBL2* gene polymorphisms(17). They did not find any relation between *MBL2* gene polymorphism and MBL level in those who are HBsAg carriers, who have spontaneous seroconversion and in healthy controls. However, in patients who have MBL genotypes with presence of A allele, the risk of advanced disease (cirrhosis, HCC, etc.) was found to be increased three fold.

In contrast, some publications resulted otherwise. In 1998, codon 52 and 54 polymorphisms were found to have no association with chronic hepatitis B in a German

study(18). Similarly in a study carried out in 2005 in Korea, no association was seen between codon 54 polymorphism and clearance of hepatitis B infection or progression of chronic Hepatitis B infection(19).

In spite of the presence of a few articles arguing for the opposite view, the idea that polymorphisms causing a decrease in serum level of MBL have an adverse effect on the prognosis of Hepatitis B infection is becoming more dominant.

In our study, 54 patients were found to have (77.14%) GG genotype, 8 (11.43%) GA genotype, and 8 (11.43%) AA genotype in hepatitis B group. High fibrosis, score group, has a higher rate of codon 54 polymorphism with a difference approaching significance ($p=0.052$). In parallel to these results, in the comparison in terms of G and A alleles between two groups, patients in Group-II, group with high fibrosis score, were found to have significantly higher prevalence of A alleles ($p<0.05$).

The role of MBL in Chronic Hepatitis C has not been clearly defined in studies performed to date. In a few studies, it was thought that high MBL levels have a positive correlation with pathology and response to treatment. These studies are different in terms of cohort characteristics, classification of cases and the investigated *MBL2* gene polymorphisms. In a study published in 1998, it was established that homozygote genotype in codon 54 was associated with weak response to interferon treatment in patients with chronic Hepatitis C(20). In another study performed in 2000, 52 patients with HCV infection were compared with 50 healthy controls and patients with codon 54 polymorphism were found to have more advanced disease and it was thought that MBL may be one of the factors influencing the course of HCV infection(21).

In 2006, 100 hepatitis C patients were compared with control group and codon 54 polymorphism was found to be more frequent in patient group and it was concluded that codon 54 polymorphism may be a risk factor for HCV infection in an another study(22).

MBL2 gene polymorphisms was found to occur at a significantly higher rate in cases with HCV infection than healthy controls in more recent study and it was also determined that response to Pegylated interferon (Peg-IFN) and Ribavirin treatment was lower in cases with polymorphism, even though the difference was not statistically significant(23). Another study published in 2008 stated that polymorphisms in *MBL2* gene exon-1 region were associated with low MBL levels and the progression of HCV infection towards liver inflammation and fibrosis (24). And also *MBL2* gene polymorphism was suggested to be directly related to progression of chronic hepatitis C and response to Peg-IFN therapy(25). In a most recent study; it was reported that *MBL2* variant alleles and hence low MBL levels increase the predisposition to HCV infection, and that HYO haplotype is associated with the severity of fibrosis(26). In a study comparing MBL related serine protease-1 complex (MBL/MASP-1) activity in patients with HCV infection with that in the healthy control group, positive correlation was found between the liver fibrosis and the MBL serum levels. In addition, a significant relation was found between the MBL/MASP-1 activity and the HCV-related liver fibrosis(9).

In a few studies, no significant association was found between *MBL2* gene polymorphisms and accordingly low MBL levels, and predisposition to HCV infection, disease progression and response to treatment(27,28)

In the present study, among patients in chronic hepatitis C group, 19 had (63.3%) GG genotype, 8 (26.6%) GA genotype and 3 (10.1%) AA genotype. There was no statistically significant difference between fibrosis groups in terms of the presence of A allele.

It is difficult to determine the role of MBL in viral hepatitis accurately since *MBL2* gene polymorphisms and MBL serum levels vary significantly between and within populations. To our knowledge, there is no study in the literature which investigates the association between *MBL2* gene polymorphisms and liver histopathology directly. In the

present study, among chronic hepatitis B patients, fibrosis was found to be higher in presence of A allele carrying group, suggesting that individuals with these polymorphisms should be examined earlier for liver damage and if necessary for treatment.

Although it is accepted that MBL plays a part in the course of HBV and HCV infections, larger studies investigating the association between MBL levels and disease progression and treatment are required. It is suggested that other single nucleotide polymorphisms in exon1 and promoter regions of *MBL2* gene should be investigated and accordingly it is hoped that with the determination of changing MBL serum levels, information predictive of the course of disease and response to treatment can be obtained.

AUTHORS' NOTE

All of the authors declare that they have no conflicts of interest regarding this paper.

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Table 1: *MBL2* gene codon 54 allele frequency and genotype distribution among chronic hepatitis patients and control group

Genotype/Allele	Patient Group (Chronic hepatitis B and C) (n =100)	Control Group (n =100)	p-value
G/G Genotype	73	63	1(Reference)
G/A Genotype	16	19	0.92
A/A Genotype	11	18	0.59
G allele frequency (%)	81	78	0.59
A allele frequency (%)	19	22	

Table 2: Analysis of genotypes and alleles according to chronic hepatitis B fibrosis groups

Hepatitis B	Group-I (Fibrosis Score ≤3)	Group-II (Fibrosis Score ≥4)	p-value
GG genotype (n) (%)	48 (81%)	6 (54%)	
GA genotype (n) (%)	6 (10%)	2 (18%)	0.11
AA genotype (n) (%)	5 (9%)	3 (28%)	
Absense A allele(GG) (n) (%)	48 (81%)	6 (54%)	
Presense A allele(GA+AA) (n) (%)	11 (19%)	5 (46%)	0.052
G allele (n)(%)	102 (86%)	14 (64%)	
A allele (n) (%)	16 (14%)	8 (36%)	0.027

Table 3: Analysis of genotypes and alleles according to chronic hepatitis C fibrosis groups

Hepatitis C	**Group-I (Fibrosis score ≤3)**	**Group-II (Fibrosis score ≥4)**	**p-value**
GG genotype (n) (%)	16 (64%)	3(60%)	
GA genotype (n) (%)	6 (24%)	2 (40%)	0.60
AA genotype (n) (%)	3(12%)	0 (0 %)	
Absense A allele(GG) (n) (%)	16 (64%)	3 (60%)	
Presense A allele(GA+AA) (n) (%)	9 (36%)	2 (40%)	0.86
G allele (n) (%)	38 (76%)	8 (80%)	
A allele (n) (%)	12 (24%)	2 (20%)	0.78