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Effects of mannose-binding lectin and mannose-binding lectin polymorphisms on treatment response in patients with chronic hepatitis C

LIVER

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Original Article

ABSTRACT

Background/Aims: The natural course and clinical outcome of hepatitis C virus (HCV) infection is related to the interaction between HCV and the immune response of the host. Only a limited number of studies have investigated the role of mannose-binding lectin (MBL) levels in HCV infection. The aim of the present study was to explore the relationship between MBL levels and gene polymorphisms on treatment response in patients with chronic hepatitis C (CHC).

Materials and Methods: Serum MBL levels from 50 CHC patients who completed treatment at least 24 weeks before the present study and 75 healthy HCV-negative controls were measured. In addition, the presence of codon 54 mutations was investigated. Correlational analyses were performed to determine relationships between MBL levels and treatment response.

Results: In patients, mean serum MBL levels were lower and the rate of codon 54 mutations was higher. However, these differences were not statically significant. In both patients and controls, serum MBL levels were significantly lower in individuals with codon 54 mutations. Moreover, serum MBL levels and the rate of the codon 54 mutation were similar in patients regardless of treatment response.

Conclusion: Our findings suggest that low MBL levels do not increase the susceptibility for HCV infection. Furthermore, MBL levels were not found to have a significant effect on the course of the disease or treatment response.

Keywords: Hepatitis C, mannose-binding lectin level, treatment response

INTRODUCTION

Hepatitis C virus (HCV) infection is considered to be a serious and common health issue, with 170 million patients infected with HCV worldwide (1). In addition, severe complications, such as chronic liver disease, cirrhosis, and hepatocellular carcinoma, have been associated with chronic hepatitis C (CHC). The standard treatment for CHC consists of pegylated interferon (PEG-IFN) combined with ribavirin, and duration of therapy depends on the HCV genotype. Furthermore, treatment response can be influenced by several factors related to the virus (genotype and viral load) or host (fibrosis, age, sex, body weight, duration of the infection) (2).

Mannose-binding lectin (MBL) interacts with various microorganisms, including bacteria, viruses, fungi, and protozoa. The results from numerous studies have demonstrated that low MBL levels and MBL gene polymorphisms were associated with an increased risk of microbial infection (3). In addition, the results of other studies have indicated a potential relationship between MBL gene polymorphisms and the course, prognosis, and treatment response of CHC patients (4-6); .h However, the mechanisms are not clearunderlying such relationships have yet to be elucidated. Furthermore, tThe relationship between the MBL levels and the progression and complications of HCV infectionCHC, as well as the importance of serum MBL levels in clinical practice, remain unknown. In studies conducted in Japan, a relationship was found between MBL deficiency and nonresponse to PEG-IFN therapy, as well as the progression of cirrhosis. Other studies have yielded conflicting results as to whether relationships exist between MBL

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and the clinical course of CHC or treatment response. However these studies involved limited numbers of patients and the discordant results may have been related to diversity in the distribution of MBL mutations attributable to the ethnic origins of those studied (4-6).

Because the relationship between HCV and MBL remains unclear, the present study was conducted to investigate the effects of MBL levels and MBL gene polymorphisms on treatment response in CHC patients.

MATERIALS AND METHODS

Fifty CHC patients under regular follow-up at the Department of Bacteriology and Infectious Diseases of the Cukurova University Medical School were included in the study. The approval of the ethics committee at our institution and the written consent of the patients were obtained. Patients who were diagnosed with CHC and had completed treatment with PEG-IFN and ribavirin at least 6 months before study initiation were eligible for study inclusion. Patients with autoimmune diseases, diabetes mellitus or coinfection with other hepatitis viruses were excluded. Patient information was extracted from the outpatient files, and all patients were classified according to their treatment response. Sustained virological response (SVR) was defined as undetectable HCV-ribonucelic acid (RNA) 24 weeks after completion of antiviral treatment, and early virological response (EVR) was defined as undetectable HCV-RNA or a ≥2 $\log_{10}$ drop in HCV-RNA by week 12 of treatment (2).

Patient peripheral blood samples were collected in both plain and ethylenediaminetetraacetic acid tubes. The presence of MBL codon 54 mutations was investigated in the Immunology Laboratory of Internal Medicine, and MBL levels were measured in the Central Laboratory. Serum MBL levels were analyzed using enzyme-linked immunosorbent assay (MICRO ELISA test kit; HBT human MBL, HyCult Biotechonology, Uden, Netherlands). Mutation at codon 54 of the MBL gene was detected using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method developed by Wang et al. (7), using a High Pure PCR Template Preparation Kit (Roche, Germany).

The control group included 75 healthy Turkish individuals seronegative for HCV, HBsAg, and HIV. MBL levels from the patient and control groups, as well as from patients with EVR, SVR, mutations, and fibrosis were compared.

Statistical analysis

Consistency of the variables with a normal distribution was tested using the Shapiro-Wilks test. Mann-Whitney U tests and Kruskall-Wallis tests were used in the analysis of variables without a normal distribution. Chi-Square or Fischer's exact tests were used for analyzing categorical variables. Spearman's correlation test was used for correlation analyses. SPSS version 11.5 (SPSS; Chicago, IL, US) was used for the analysis of the data and p values of <0.05 were considered significant. Data are presented as mean±standard deviation, median (range), and percentage.

RESULTS

Fifty CHC patients treated with PEG-IFN in combination with ribavirin who had completed the treatment at least 6 months before the present study and 75 healthy control patients were included. Demographic data from both groups are shown in Table 1. The mean duration of the treatment was 48.7±8.4 weeks.

The stage of fibrosis was determined according to the Ishak fibrosis score. The liver fibrosis stage was 1 in 15 (30%) patients, 2 in 14 (28%) patients, and 3 in 21 (42%) patients. None of the patients had been diagnosed with cirrhosis.

Sustained virological response was achieved in 31 (62%) patients. Among SVR nonachievers, relapse occurred in six patients and treatment failure was observed in 13 patients. EVR was observed in 86% patients. SVR was significantly higher among EVR achievers than among EVR nonachievers (p=0.0001).

We investigated whether mutations were present at codon 54 of the MBL2 gene in the both groups. Codon 54 mutations were detected in 16 (21.3%) healthy controls and 12 (24%) CHC patients. Although the rate of codon 54 mutations was higher in the patient group than in the healthy control group, the difference was not statistically significant.

Serum MBL levels were assessed in CHC patients and controls and were found to be higher in the control group, but difference was not statistically significant (Table 2). The relationships of MBL levels with codon 54 mutations (presence/absence), fibrosis levels, SVR, and EVR are shown in Table 2. Serum MBL levels were found to be lower in individuals with codon 54 mutations in both groups (p=0.0001). The relationship between MBL levels and codon 54 mutations is shown in Figure 1.

The rate of codon 54 mutations was assessed in SVR achievers and SVR nonachievers. No mutations were detected in 22 (71%) SVR achievers and in 16 (84.2%) SVR nonachievers. The rate of mutation was higher in SVR achievers; however, the difference between the groups was not statistically significant.

Early virological response achievers and nonachievers were compared with regard to the rate of codon 54 mutations. Although

Table 1. Demographic characteristics of the patients and healthy controls

		Patients (n=50)	Controls (n=75)
Age	Mean	51.06±9.7	40.8±8.4
	Median	52 (19-65)	40 (25-61)
Gender (M/F)		27/23	41/34

M: male; F: female

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Table 2. The relationships between MBL levels and codon 54 mutations, fibrosis levels, SVR, and EVR

	MBL (ng/mL) mean±standard deviation	p
Patients	65.1±61.4	0.493
Controls	72.1±52.4	
Codon 54 mutation (+)	33.1±55.9	0.0001
Codon 54 mutation (–)	79.7±51.8	
Fibrosis level		
1 (n=15)	86.8±69.5	0.228
2 (n=14)	62.7±64.1	
3 (n=21)	51.1±51.4	
SVR (+)	59.7±64.3	0.436
SVR (–)	73.8±57.1	
EVR (+)	67±61.5	0.586
EVR (–)	53.1±64.6	

MBL: mannose-binding lectin; SVR: sustained virological response; EVR: early virological response

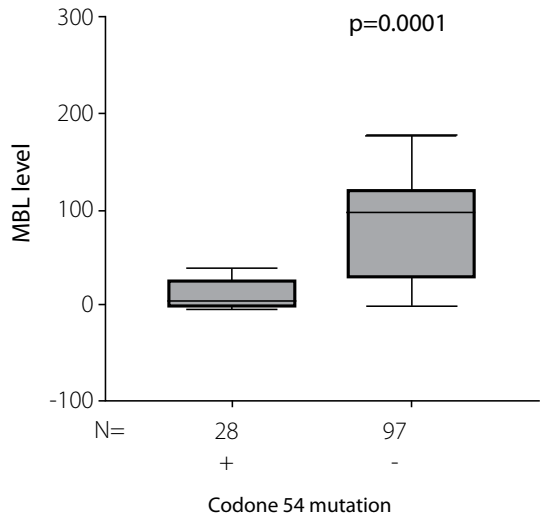

Figure 1. The relationship between codon 54 mutations and MBL levels

the rate of mutation was found to be 27.2% in EVR achievers, no mutations were detected in any of the patients EVR nonachievers; the difference between the groups was not statistically significant. Mean MBL levels were higher in SVR achievers than in SVR nonachievers; however, no statically significant difference was found between the groups. In addition, there were no significant differences between MBL levels from EVR achievers and EVR nonachievers.

DISCUSSION

In the present study, we investigated the relationship between CHC and MBL, considering the important role of MBL in the innate immune response. For example, MBL deficiency has been identified as the most common immune defect in humans (8). This deficiency was attributed to single point mutations at codons 52, 54, and 57 of exon 1 of the MBL gene. In the order of frequency, these mutations are defined as variants D, B, C, and A. These variant alleles lead to low MBL levels by causing structural changes in MBL. The results from several studies have indicated that low MBL levels and MBL gene polymorphisms were associated with increased risk of infections (9-12).

Although no relationship was detected between the carrier status of mutant alleles and serum MBL levels in Juliger's study (13), the present study revealed lower MBL levels in individuals with codon 54 mutations of the MBL2 gene, in all groups. In a study conducted by Babula et al. (14) in Lithuanian individuals, MBL levels were found to be decreased in carriers of mutant alleles of the MBL gene. In the present study, mean MBL levels were found to be lower in people with codon 54 mutations. When the patient and healthy control groups were assessed together, a statistically significant relationship was found between the presence of a mutation and low MBL levels (p=0.0001).

The prevalence of MBL2 mutations and the distribution of polymorphisms have been known to vary on the basis of different ethnic origins. Although the B allele has been reported at a rate of 22%-28% in the European population, the C variant was observed at a rate of 50%-60% in African populations. Furthermore, the prevalence of the D allele in European populations has been reported to be 14%, with a lower prevalence observed in other populations. Although the C and D alleles have not been observed in Japanese populations, the B allele has been detected at a rate of 37% (15). Because of the lack of a large study investigating MBL polymorphisms in the Turkish population, the distribution and the rate of MBL2 mutations remains unknown. Özbaş-Gerçeker et al. (16) assessed the polymorphisms at codons 54 and 57 of the MBL gene in 49 patients with tuberculosis and in 69 pediatric patients with otitis media; they determined that no mutant alleles were detected at codon 57 in either group and that the rate of the B allele, which is the codon 54 mutant allele, was 9% in patients with tuberculosis and 6% in pediatric patients with otitis media. In the present study, the rate of the B allele was 21.3% in the healthy control group and 24% in CHC patients. The difference between the rates of mutation was considered to be related to the small sample sizes of the studies. The rate of the B allele in the present study was similar to that reported for the European population.

A limited number of studies have examined the role of MBL in HCV pathogenesis (4,5,17-21). In the studies conducted in Japan, relationships were found between the codon 54 mutation and low MBL levels, disease progression, and treatment response. In a study of 52 CHC patients, Sasaki et al. (17) no difference was found between CHC patients and controls with regard to the B allele rate. In CHC patients with homozygous or

heterozygous B alleles, MBL levels were found to be lower than in those with A alleles. Furthermore, the presence of a B allele has been associated with chronic active hepatitis and progression to cirrhosis. On the other hand, lower MBL levels were detected in patients with chronic active hepatitis or cirrhosis than in those with chronic inactive hepatitis. It was suggested that MBL2 gene mutations and low MBL levels may be risk factors for progressive liver damage. In a study conducted by Vallinoto et al. (18), the A, B, C, and D alleles were investigated in 73 CHC patients and 93 healthy individuals, with no differences found between the groups. Moreover, the rate of mutation and the distribution of polymorphisms were found to be similar in patients with and without cirrhosis. In addition, unlike the study by Sasaki et al. (17), it was demonstrated that MBL mutations had no effect on CHC course. In the present study, only the B allele, which results from the mutation at codon 54, was investigated. Similar to the studies conducted by Sasaki and Vallinoto, no statically significant differences were found between the CHC patients and healthy controls with regard to codon 54 mutations of the MBL2 gene. In the present study, MBL levels were found to be lower in patients homozygous or heterozygous for the B allele than in individuals without a mutation. A negative correlation was found between the presence of the B allele and a good treatment response in two studies conducted by Matsushita et al. (4,5). In the present study, the mutation rate in SVR achievers was high; however, no relationship was found between treatment response and the presence of a mutation.

In a cohort study conducted by Kilpatrick et al. (19) in Europe, no relationship was found between MBL levels and disease progression or treatment response, and higher MBL levels were observed in the CHC patient group than in the control group. In a recent cohort study conducted in Europe, higher MBL levels were observed in the CHC patient group than in the control group (22). In the present study, mean serum MBL levels were higher in the control group than in CHC patients; however, the difference between the groups was not statistically significant. Mean MBL levels were higher in SVR nonachievers than in SVR achievers; however, no statistically significant relationship was found between SVR and MBL levels, similar to the findings of Kilpatrick et al. (19). Furthermore, the rate of mutation in the present study was higher in SVR achievers; however, no statistically significant difference was detected between the groups. These findings suggest that there is no relationship between MBL levels and treatment response.

On the other hand, no difference was found between CHC patients and controls in terms of MBL2 haplotypes and MBL levels in a study conducted by Pedroso et al. (21). Mutations leading to lower MBL levels were fewer in patients with advanced fibrosis than in patients with moderate fibrosis; however, no relationship was found between the HCV genotypes and the degree of fibrosis or the MBL2 genotypes. Furthermore, MBL levels were found to be higher in patients responding to PEG-IFN treatment than in nonresponders. MBL2 genotypes, including X or O mutations, were found to be associated with treatment failure, and MBL2 genotypes were reported as potentially beneficial in estimating treatment response (21). In the present study, lower MBL levels were detected as the degree of fibrosis increased; however, no significant relationship was found between fibrosis and MBL mutations as in the Pedroso study. Unlike the present study, MBL levels were higher in SVR nonachievers than in SVR achievers.

There present study had a few limitations. The absence of patients with cirrhosis and the small sample size may not have allowed for a representative distribution of CHC patients. In addition, there is a paucity of data pertaining to MBL polymorphisms in the Turkish population.

In conclusion, results from the present study demonstrated that MBL levels were not associated with a significant effect on disease course or treatment response in CHC patients. To date, the present study represents the sole attempt to investigate the relationship between MBL and CHC in the Turkish population. Therefore, more comprehensive studies conducted in different regions of Turkey and including larger patient groups may be required to acquire more precise results.

Ethics Committee Approval: Ethics committee approval was received for this study from Çukurova University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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