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Association of Mannose Binding Lectin Polymorphism with Hepatitis C Infection in Northwest of Iran

Mohammad H. Somi [1*], Mohammad Asgharzadeh [2], Sara Farhang [3], Rasoul Estakhry [4], Ali A. Pouri [3]

[1] Associate Professor of Gastroenterology, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Assistant Professor of Molecular Biology, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[3] General Practitioner, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences,Tabriz, Iran

[4] Assistant Professor of Anatomy and Pathology, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background and Aims: **Persistent infection with hepatitis C virus leads to liver cirrhosis and often to liver cancer. Mannose binding lectin is a C-type serum lectin, which plays an important role in innate immunity by activating the classical complement pathway. Variants of the mannose binding lectin have been shown to be associated with low serum concentrations of the protein and to predispose the subjects to bacterial, fungal and viral infections. This study was undertaken to investigate the association between hepatitis C virus infection and polymorphisms of mannose binding lectin gene.**

Methods: **We assessed the single nucleotide polymorphism of mannose binding lectin in exon 1, at codon 52, codon 54 and codon 57 in 100 patients infected with hepatitis C virus and 100 controls in Iranian population. Mannose binding lectin gene mutations were determined by means of polymerase chain reaction and restriction fragment length polymorphism analyses.**

Results: **The occurrence of the codon 54 mutation was significantly higher in patients (OR 3.53, CI 95%: 1.94-6.44, p<0.005). No significant difference in the frequency of codon 52 and 57 mutations was observed between patient and control groups.**

Conclusions: **Mannose binding lectin may be one of the factors that influence the course of HCV infection. Our results suggest that heterozygous carriage of the variant allele of codon 54 of mannose binding lectin is associated with hepatitis C virus infection in our cases. This may not be true about codons 52 and 57 mutations.**

Keywords: **Mannose Binding Lectin, Codon 54 Mutation, Hepatitis C**

Introduction

According to a report from WHO, an estimated 170 million persons are chronically infected with hepatitis C virus (HCV) and 3 to 4 million persons are newly infected each year. Prevalence of infection in healthy blood donors ranges from 0.01-0.02% in northern Europe, 1-1.5% in southern Europe to 6.5% in parts of equatorial Africa [(1)] and 0.12-0.59% in Iran [(2,3)]. It had been reported up to 76% in Iran, within high risk groups receiving blood products or dialysis[(4)]. Hepatitis C is the most frequent cause of chronic hepatitis. About 80% of newly infected patients progress to develop chronic infection which may step forward to cirrhosis and hepatocellular carcinoma with a high morbidity and mortality. The complications of cirrhosis due to chronic hepatitis C are the leading indications for liver transplantation [(5, 6)] while viremia persistence after transplantation may result in recurrent liver injury [(7)]. The natural history of HCV infection depends on a combination of viral-related and host-related

**** Correspondence:***
Mohammad Hossein Somi MD, Associate Professor of Gastroenterology, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Telefax: *+98 411 3367499*

E-mail: *mhosseinsina@yahoo.com*

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factors. Mannose binding lectin (MBL) is a calcium-dependent C-type lectin which is secreted by the liver as a part of acute phase response [8] and plays an important role in the first line host defense [9]. MBL functions as an opsonin [10], and its biological effect is mediated by direct killing via complement [11] through the lytic membrane attack complex or by promoting phagocytosis either by the MBL lectin pathway of complement or by direct binding to one or more cell surface receptors [12].

In human, one of the three structural mutations found within exon 1 of the mbl2 gene on chromosome 10, which encodes MBL, results in low functional serum levels [13, 14].

These single nucleotide polymorphisms (SNPs) at codons 52, 54, and 57 resulting in amino acid replacement, are believed to interfere with the stability of the protein [15, 16].

Deficiency of human MBL caused by mutations in the coding part of the mbl2 gene is associated with increased risk and severity of infections and autoimmunity in children and adults [17, 18].

There are few published studies about MBL mutations, risk of infection and course of the hepatitis C. We studied MBL gene mutations in Iranian patients with hepatitis C against healthy controls and investigated the possible relation between MBL structural mutations within exon 1 and susceptibility to be infected with hepatitis C virus.

Materials and Methods

HCV infected group and control population

Iranian hepatitis C infected cases were continuously recruited from Hepatitis Clinic, Tabriz University of Medical Sciences, which is the referral clinic of East Azarbaijan province, northwestern Iran. The mean age (±SD) of the patients (n= 100) was 42.5 ± 11.1 years, 57 % were male and all had Azeri ethnic background.

100 ethnically matched healthy individuals were randomly selected from Tabriz Blood Transfusion Center (mean age = 38.5 ± 10.5 years, 80 % male). The patients were informed about the purpose of the study and were enrolled with their consent.

DNA extraction and Genotyping of mbl2

DNA was extracted from peripheral blood mononuclear cells by the modified proteinase K, sodium dodecyl sulfate (SDS) and N-cetyl-N,N,N-trimethylammonium bromide (CTAB).

We analyzed SNPs in codons 52, 54 and 57 in the exon one of the MBL gene using previously described PCR-based methods (19, 20, 21). PCR reactions were performed in a volume of 20 µl that contained 500 ng of the genomic DNA , 0.5 µM of each specific primer (described in table1) in the presence of 1.5 mM MgCl2 ,100 mM each deoxynucleotide, 50 m M KCl, 20 m M Tris- HCl, pH 8.4, and 2.5 U recombinant DNA polymerase (Fermentas).

All PCR procedures were initiated by a 4-min denaturizing step at 94°C and completed by a 7-min extension step at 72°C .The temperature cycles for different types of PCRs were as follows: 32 cycles of 40 s at 94°C; annealing temperatures are described in table 1. In addition to SSP-PCR, B and C alleles were detected by BanI and MboII restriction enzyme digestions of the 328-bp product amplified by the allele P and Q primers , respectively (table 1), followed by a 2.5% agarose gel electrophoresis. BanI cleaves the A allele in to two fragments (245 and 83 bp) and Leaves the B allele undigested, while MboII specifically cleaves the C allele into two fragments (266 and 62 bp)(figure 1).

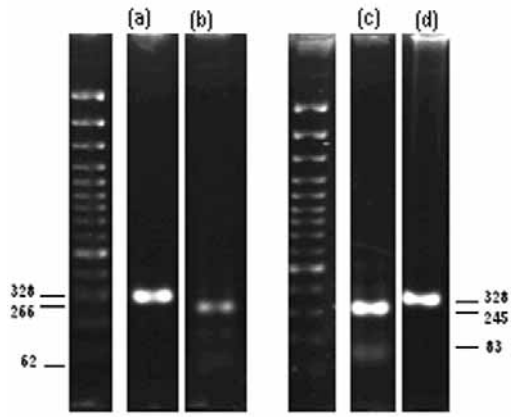

Figure 1. RFLP analysis of mbl2 in Iranian patients infected with HCV and healthy donors.
(a): Allele A undigested by Mbo II, (b) Allele C cleaved into two fragments, (c) Allele A cleaved by BanI into two fragments, (d) B allele; undigested.

Statistical analysis

Statistical analysis was performed using SPSS software, version 13.5. MBL genotype frequencies were compared by contingency table analysis by the 2 test. When significant a difference was obtained, logistic regression was used to calculate odds ratio (OR) with 95% confidence intervals (95%CI). A P value of 0.05 was considered to be significant.

Results

Genotype A/A was more frequent among healthy donors while genotype A/O and O/O were

Table 1. Oligonucleotides used in human MBL genotyping.

Primer name	Sequences (5'→ 3')		Annealing temperature °C
Codon 57 (wild type)	Forward	GAG GCT TAG ACC TAT GGG GCT AG	60
	Reverse	TAC CTG GTT CCC CCT TTT CTC	
Codon 57 (mutant)	Forward	GAG GCT TAG ACC TAT GGG GCT AG	63
	Reverse	TAC CTG GTT CCC CCT TTT CTT	
Codon 54 (wild type)	Forward	GAG GCT TAG ACC TAT GGG GCT AG	63
	Reverse	CCC CTT TTC TCC CTT GGT GC	
Codon 54 (mutant)	Forward	GAG GCT TAG ACC TAT GGG GCT AG	62
	Reverse	CCC CTT TTC TCC CTT GGT GT	
Codon52 (wild type)	Forward	CTT CCC AGG CAA AGA TGG GC	66
	Reverse	CAG GCA GTT TCC TCT GGA AGG	
Codon52 (mutant)	Forward	CTT CCC AGG CAA AGA TGG GT	63
	Reverse	CAG GCA GTT TCC TCT GGA AGG	
Allele Q	Forward	GTA GGA CAG AGG GCA TGC TT	67
	Reverse	CAG GCA GTT TCC TCT GGA AGG	
Allele P	Forward	GTAGGA CAG AGG GCA TGC TC	64
	Reverse	CAG GCA GTT TCC TCT GGA AGG	

significantly more frequent among patients with hepatitis C (P <0.005).The genotype distribution of mbl2 polymorphisms at codons 54 (variant B), 57 (variant C) and 52 (variant D) were compared between the patients and controls (table 3). The occurrence of the codon 54 mutation was significantly higher in patients (OR 3.53, CI 95%: 1.94-6.44, P <0.005).

Table 2. mbl2 genotype in Iranian HCV infected patients and controls.

Coding genotype	%Patients (n=100)	%Donors (n=100)
A/A	29	61
A/B	45	23
A/C	5	3
A/D	11	9
Total A/O	61	35
B/B	6	2
B/C	1	1
B/D	2	1
C/C	1	0
Total O/O	10	4
Total with coding mutations(A/O or O/O)	71	39

No significant difference in the frequency of codon 52 and 57 mutation was observed between patient and control groups. Homozygosis mutation of the so-called codons was infrequent and did not influence the results.

Table 3. Iranian patients with hepatitis C and control divided by genotypes.

mbl2 genotype	Genotype frequency %		OR	95% CI	P
	HCV infected patients	Donors			
A/A	29	60	0.26	0.48-0.14	<0.005
A/O	61	35	2.86	1.6-5.0	<0.005
O/O	10	4	-	-	NS

Discussion

In this study we found that the occurrence of the codon 54 mutation of mbl2 may be a risk factor for infection with HCV in Iranian population. Because MBL is a well-characterized part of the immune defense system, identifying the mutations can help

to define susceptible individuals to infection, especially in high risk groups. Mbl2 variant alleles, which are known to decrease the serum MBL levels, are associated with increased risk and severity of viral hepatitis. These genotypes have been shown to be associated with viral persistence in HBV infection [22]. In another study, the frequency of mbl2 polymorphism and serum MBL levels didn't differ significantly in spontaneously recovered individuals from hepatitis B, nonprogressed carriers and controls whereas low MBL level was associated with occurrence of cirrhosis and hepatocellular carcinoma in progressed carriers [23].

A few other published studies (all from Asia) indexed on Pub Med, have examined mbl2 with respect to the course of HCV infection. In a study on fifty two patients, no significant relationship was observed between MBL polymorphisms and levels of HCV RNA [24]. In another study difference in the mutation rate and MBL levels between chronic hepatitis C patients and controls did not reach the significance level, but MBL levels in asymptomatic HCV carriers were significantly lower than those of the control population without codon 54 mutation [25]. In the present study we found that allele B carriers are more prone to HCV infection. Mbl2 polymorphism is frequent among general population, but differences have been observed in different ethnic backgrounds [26, 27, 28]. To the best of our knowledge, this is the first study from Middle East evaluating the mutations on mlb2, so we used a randomly selected group of blood donors from this region to perform a control group. This study gives an estimate of mbl2 polymorphism among people from East Azarbaijan. It is recommended to perform further studies with more samples from across the country.

Because MBL polymorphisms may play an important role in the further behavior of the disease as well as in susceptibility to the infection with HCV, studies have investigated the different characteristics of this system. One possible mechanism is through regulation of inflammatory cytokines by MBL. High MBL levels are believed to decrease the production of inflammatory cytokines, such as IL-6, IL-1β and TNF-α in response to meningococci by monocytes, while low MBL concentrations can enhance the production of IL-6 and IL-1β [29] and may promote chronic inflammation and oxidative stress. This can contribute to fibrogenesis and increase proliferation of hepatocytes [30, 31].

The major limitation of this study is the characteristics of the control group. Further studies comparing the mbl2 polymorphism between HCV infected patients and healthy subjects at a known risk for infection (e.g. undergoing hemodialysis or an immunosuppressive therapy) can be valuable especially in concordance of measuring functional MBL serum levels. Such studies can be improved by quantifying the viral load, as well.

In conclusion, occurrence of the codon 54 mutation of mbl2 is associated with the occurrence of hepatitis C in this selected Iranian population. It is worth assessing promoter SNPs in further studies because of multifaceted regulatory structure of MBL production.

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