Association of promoter polymorphism of the CD14 C (-159) T endotoxin receptor gene with chronic hepatitis B

Amir Houshang Mohammad Alizadeh, Mitra Ranjbar, Mehrdad Hajilooi, Farahnaz Fallahian

AIM: To investigate whether single-nucleotide polymorphisms in the promoter regions of endotoxin-responsive genes CD14 C (-159) T is associated with chronic hepatitis B.

METHODS: We obtained genomic DNA from 80 patients with established diagnosis of chronic hepatitis B and 126 healthy subjects served as a control population. The CD 14 C (-159) T polymorphism was investigated using an allele specific PCR method.

RESULTS: Twenty seven percent of chronic hepatitis B patients and 75% of controls were heterozygous for CT genotype. The difference between the chronic hepatitis B and control groups was statistically significant (P < 0.0001; Odds ratio (OR) = 2.887; 95% CI: 1.609-5.178). Twenty four point six percent of chronic hepatitis B and control groups was statistically significant (P < 0.004; OR = 0.416; 95% CI: 0.256-0.658; 95% CI: 0.229-0.755). The frequency of allele C was 61.9% and allele T was 38.1% in hepatitis B patients group. The difference between groups was not statistically significant (P = 0.256; OR = 0.658; 95% CI: 0.319-1.358). Forty eight point four percent of chronic hepatitis B and control were heterozygous for CT genotype. The difference between the chronic hepatitis B patients and 72.7% of control were homozygote for CC genotype (P < 0.004; OR = 0.416; 95% CI: 0.229-0.755). The frequency of allele C was 61.9% and allele T was 38.1% in hepatitis B patients group. The frequency of allele C was 55.2% and allele T was 44.8% for the control group (P = 0.179; OR = 1.319; 95% CI: 0.881-1.977).

CONCLUSION: The TT heterozygous genotype was not a risk factor for chronic hepatitis B. CC homozygote genotype is protective for hepatitis B. Lack of heterozygosis of genotype CT is a risk factor for chronic hepatitis B. Alleles C or T were not risk factors for chronic hepatitis B. These findings show the role of a single-nucleotide polymorphism at CD14/-159 on the development of chronic hepatitis B. Endotoxin susceptibility may play a role in the pathogenesis of chronic hepatitis B.

© 2006 The WJG Press. All rights reserved.

Key words: CD14 C (-159) T gene; Single nucleotide polymorphism; Chronic hepatitis B; Endotoxin susceptibility

Mehrdad Hajilooi, Amir Houshang Mohammad Alizadeh, Mitra Ranjbar, Farahnaz Fallahian. Association of promoter polymorphism of the CD14 C (-159) T endotoxin receptor gene with chronic hepatitis B. World J Gastroenterol 2006; 12(35): 5717-5720

http://www.wjgnet.com/1007-9327/12/5717.asp

INTRODUCTION

An estimated 350 million persons worldwide are infected with hepatitis B virus (HBV). Hepatitis B carriers are at risk for development of cirrhosis and hepatocellular carcinoma. Persons with chronic hepatitis B infection need life-long monitoring to determine when intervention with antiviral therapy is needed and to observe for serious sequels[1].

The mechanism by which HBV establishes a persistent infection is at present still unclear. Evidence suggests that the clinical manifestations and outcomes after acute liver injury associated with viral hepatitis are determined by the immunologic responses of the host[2]. CD14, a key gene of the innate immune system, functions as a receptor for lipopolysaccharide (LPS), a constitutive element of the bacterial cell wall. CD14 cannot bind to LPS directly. A protein termed LBP (lipopolysaccharide binding protein) must first bind to LPS. The LPS-LBP complex then binds to CD14 and the receptor-ligand complex is internalized. In addition, CD14 is associated with a protein known as Toll-like receptor 4 (TLR-4). As a consequence of the CD14-LBP/LPS interaction at the level of the membrane, TLR-4 becomes activated. TLR-4 plays an important role in signal transduction. Importantly, TLR-4 is now known to activate a transcription factor known as NFκB. Viruses have targeted cellular cytokine production and cytokine receptor-signaling pathways, apoptotic pathways, cell growth and activation pathways, MHC-restricted antigen presentation pathways and humoral immune responses[3].

This study tested whether genetic factors, CD14 C (-159) T, has any role in molecular pathogenesis of chronic
hepatitis B and influences the individual susceptibility for chronic hepatitis B.

MATERIALS AND METHODS

Subjects
We analyzed 80 Iranian patients with chronic hepatitis B and 126 sex-matched control subjects. All chronic hepatitis B patients had visited at a liver clinic in Tehran for regular follow-up examinations. All participants signed the informed consent.

Chronic hepatitis B was based upon biochemical, virologic, histological activity and included patients still on interferon or lamivudine treatment; those who finished treatment course and nonresponders to treatment (lack of virologic and or histologic response by first treatment course in which sustained response was unlikely).

Asymptomatic carrier state defined as: chronically HBsAg positive patients who have anti-HBc in serum. anti-HBs is either undetected or detected at low titer against the opposite subtype specificity of the antigen regarded as inactive or asymptomatic carriers, HBV DNA load less than $10^5$ copies/mL, HBeAg (+, -), serum liver transaminases of normal range. Inactive hepatitis B surface antigen (HBsAg) carriers were monitored with periodic liver chemistries as liver disease may become active even after many years of quiescence. Controlled subjects had no evidence of hepatitis B infection. The serum of control subjects were evaluated for HBsAg, HBsAb, AST and ALT. Those who had negative HBsAg and HBsAb as well as normal AST and ALT were selected for control groups.

CD14 C (-159) T Genotype Determination
Ten milliliters were collected from each subject into tubes containing 50 mmol/L EDTA, and genomic DNA was isolated from anti-coagulated peripheral blood Buffy coat using Miller's salting-out method.

Total genomic DNA from peripheral blood leukocytes was extracted by standard methods. The CD14 C (-159) T polymorphism was investigated using an allele specific PCR method. PCR products were visualized by electrophoresis in 2% (w/v) agarose gel stained with ethidium bromide. The assay thus yields a 381-bp band for T allele and 227-bp band for C allele.

Statistical analysis
The differences in the frequencies of the CD14 genotypes and alleles and other risk factors were analyzed by the $\chi^2$ test. For comparing mean stage of liver pathology in different genotypes, Kruskal-Wallis test was used. Associations and differences with probability value less than 0.05 were considered significant. Statistical data was expressed as mean ± SD. All statistical analyses were performed with the use of SPSS software, version 11.05.

RESULTS

The mean age in hepatitis B group was 36.10 ± 13.78 and in control group was 43.60 ± 16.47. In the hepatitis B group 71.3% of patients were male and 28.8% were female. In the control group 62.7% were male and 37.3% were female. The two groups were matched by sex. In the hepatitis B group, 12% were HBeAg positive, 85% were HBeAg negative, 85% were HBcAb positive and 15% were HBcAb negative. Serum aspartate aminotransferase (AST) levels in 75.9% of hepatitis B patients were < 40 IU/mL and in 24.1% were ≥ 40 IU/mL. Serum alanine aminotransferase (ALT) in 68.4% of hepatitis B patients was < 40 IU/mL and in 31.6% was ≥ 40 IU/mL. The state of hepatitis B disease at the time of sampling was as follows: 53.8% were asymptomatic carriers, 31.3% were still on antiviral treatment, 6.3% again returned to treatment, 7.5% were in chronic state and 1.3% were cirrhotics. Liver biopsy was performed in 36 patients of hepatitis B group. Histological classification was measured by Modified Histological activity index (HAI) by Ishak score. Mean stage, grade and pathologic score were 1.85 ± 0.97, 5.28 ± 1.84, and 7.17 ± 2.58, respectively.

Twenty seven percent of hepatitis and 75% of control subjects were heterozygous for CT genotype. The difference between CT genotype was statistically significant ($P < 0.0001; OR = 2.887; 95% CI: 1.609-5.178$). The lack of heterozygosis for genotype CT was a risk factor for hepatitis B. 24.6% of hepatitis and 12.3% of control group subjects were heterozygous for the TT genotype. The difference between groups was not statistically significant ($P = 0.256; OR = 0.658; 95% CI: 0.319-1.358$). The TT homozygote genotype was not a risk factor for hepatitis B. 48.6% of hepatitis and 12.7% of control group subjects were heterozygous for the CC genotype ($P < 0.004; OR = 0.416; 95% CI: 0.229-0.755$). The CC homozygote genotype was protective for hepatitis B.

The frequency of allele C was 61.9% and allele T was 38.1% in the hepatitis B group.

The frequency of allele C was 55.2% and allele T was 44.8% for control group ($P = 0.179; OR = 1.319; 95% CI: 0.881-1.977$). So alleles were not a risk factor for hepatitis B. There were no statistically significant associations between allele frequencies and genotypes frequencies in the hepatitis B group with state of disease; ALT (< 40, ≥ 40) IU/mL, mean stage of liver pathology, HBeAg (+, -). Mean stage of liver pathology was not statistically significant in different genotypes (CC, CT, CT) by Kruskal-Wallis test.

DISCUSSION

In a previous study, the effect of recombinant HBsAg (rHBsAg) on LPS- and IL-2-induced activation of monocytes was investigated. It showed that recombinant HBsAg particles, which contain the S protein only, bind almost exclusively to monocytes. Further it showed that recombinant HBsAg (rHBsAg) particles not only inhibit LPS-induced secretion of IL-1β and TNF-α, but also inhibit IL-2-induced secretion of IL-8. Their results suggested that monocytes express a receptor that is recognized by HBsAg and that HBV produces HBsAg in excess amounts to interfere with the normal function of antigen-presenting cells.

In our population, HBeAg negative chronic hepatitis B is more common than HBeAg positive. But difference
in associations of frequency of alleles and genotypes in HBcAg negative versus positive chronic hepatitis B patients were not statistically significant. Besides the viral role, we aimed to investigate the CD14 C (-159) T polymorphism as a host factor, which deteriorates the hepatitis course and outcome in our population.

The CD14 promoter genotype may affect inflammatory processes and be involved in atherogenesis, and it is therefore possible that this genotype might also be associated with other major forms of thrombotic disease, such as ischemic cerebrovascular disease, coronary artery disease. LPS is a structural component of gram-negative bacteria and is bound in plasma by the LPS binding protein. The LPS binding protein complex then binds to a glycosylphosphatidylinositol-anchored membrane protein, membranous CD14 (mCD14), on monocytes and macrophages and activates these cells. The activated phagocytes in turn secrete inflammatory cytokines through which LPS indirectly activates endothelial cells. Soluble CD14 (sCD14), which lacks a glycosylphosphatidylinositol anchor, can also be found in plasma. Endothelial cells and smooth muscle cells, lacking their own mCD14, are directly activated by LPS-sCD14 complex. Directly and indirectly activated endothelial cells express cell adhesion molecules and increased procoagulant activity, and they release free radicals, thereby mediating the initiation and development of atherosclerosis.

A previous study, demonstrated T allele frequency was significantly higher in myocardial infarction survivors and that the density of monocyte mCD14 was higher in T/T homozygotes than in other genotypes.

In another study, the possible association between the C (-260)→T polymorphism in the CD14 promoter and the occurrence of symptomatic ischemic cerebrovascular disease (CVD) was tested. They concluded that the C (-260)→T polymorphism in the CD14 promoter is not associated with an increased risk for CVD.

A previous study mentioned activated Kupffer cells release proinflammatory cytokines, a process that is regulated by the CD14 endotoxin receptor (CD14). Also, both clinical and experimental data suggest that Kupffer cell activation by gut-derived endotoxins and other bacterial products is an important pathogenic factor. In that study, the association of CD14 promoter polymorphism with different forms of alcoholic liver damage (ALD) was examined in 3 separate autopsy series. The overall age-adjusted risk for cirrhosis was 3.08 for the carriers of the CT genotype, and 4.17 for the homozygous TT genotype. Their results suggested that in the relatively isolated Finnish population, the T allele and in particular, TT homozygotes confers increased risk of alcoholic liver damage and are at a high risk to develop cirrhosis.

Another study investigated whether single-nucleotide polymorphisms in the promoter regions of endotoxin-responsive genes CD14 and tumor necrosis factor-α (TNF-α) were associated with biliary atresia (BA) and idiopathic neonatal cholestasis (INC) in 90 patients with established diagnosis of BA and 28 patients with INC. Also, forty-two adult patients with hepatitis B-related cirrhosis and 143 healthy children served as control populations. According to that study the single-nucleotide polymorphism at CD14/-159 is associated with the development of BA and INC. They concluded that endotoxin susceptibility might play a role in the pathogenesis of infantile cholestasis.

In the present study, we did not compare risk factors of hepatitis B infection, its routes of exposure and transmission in hepatitis B and control groups. Although determining the exact time of hepatitis B infection was impossible. Whether specific polymorphisms of CD14 C (-159) T has any correlation with progression of liver pathology or whether it indirectly produces needs further study and a larger sample size.

CONCLUSIONS

Our study demonstrated that the role for CD14 C (-159) T polymorphism on the development of chronic hepatitis B. Although the number of subjects in the chronic hepatitis B subtypes was relatively small, differences in the genotype distributions for chronic hepatitis B subtypes was significant.

Lack of heterozygosity of genotype CT is a risk factor for chronic hepatitis B.

TT homozygote genotype was not a risk factor for chronic hepatitis B. CC homozygote genotype is protective for chronic hepatitis B.

These findings show a role for single-nucleotide polymorphisms at CD14/-159 C (-159) T on development of chronic hepatitis B. Endotoxin susceptibility may play a role in the pathogenesis of chronic hepatitis B.

REFERENCES

1 Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. Hepatology 2004; 39: 857-861
2 Dienstag JL, Isselbacher KJ. Acute viral hepatitis. In: Kasper DK, Fauci AS, Hauser SL, Longo DL, Jameson JL. Harrison’s Principles of Internal Medicine. 16th ed. New York: MacGraw-Hill, 2005: 1834-1930
3 Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL. Viral subversion of the immune system. Annu Rev Immunol 2000; 18: 861-926
4 Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor, 1989: 443-454
5 Karhukorpi J, Ikäheimo I, Karvonen J, Karttunen R. Promoter region polymorphism of the CD14 gene (C(-159T)) is not associated with psoriasis vulgaris. Eur J Immunogenet 2002; 29: 59-60
6 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The META VIR Cooperative Study Group. Hepatology 1996; 24: 289-293
7 Vanlandschoot P, Van Houtte F, Roodbrouck A, Farhoudi A, Leroux-Roels G. Hepatitis B virus surface antigen suppresses the activation of monocytes through interaction with a serum protein and a monocyte-specific receptor. J Gen Virol 2002; 83: 1281-1289
8 Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990; 249: 1431-1433
9 Frey EA, Miller DS, Jahr TG, Sundan A, Bazil V, Espevik T, Finlay BB, Wright SD. Soluble CD14 participates in the response of cells to lipopolysaccharide. J Exp Med 1992; 176: 1665-1671
10 Loppnow H, Steller F, Schönbeck U, Schlüter C, Ernst M, Schütz C, Flad HD. Endotoxin activates human vascular smooth muscle cells despite lack of expression of CD14 mRNA...
or endogenous membrane CD14. *Infect Immun* 1995; 63: 1020-1026

11 **Hubacek JA**, Rothe G, Piňha J, Skodová Z, Staněk V, Poledne R, Schmitz G. C(-260)-->T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999; 99: 3218-3220

12 **Ito D**, Murata M, Tanahashi N, Sato H, Sonoda A, Saito I, Watanabe K, Fukuuchi Y. Polymorphism in the promoter of lipopolysaccharide receptor CD14 and ischemic cerebrovascular disease. *Stroke* 2000; 31: 2661-2664

13 **Jarveläinen HA**, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. *Hepatology* 2001; 33: 1148-1153

14 **Shih HH**, Lin TM, Chuang JH, Eng HL, Juo SH, Huang FC, Chen CL, Chen HL. Promoter polymorphism of the CD14 endotoxin receptor gene is associated with biliary atresia and idiopathic neonatal cholestasis. *Pediatrics* 2005; 116: 437-441