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Molecular detection of monocyte chemotactic protein-1 polymorphism in spontaneous bacterial peritonitis patients

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ABSTRACT

To investigate the association of the functional monocyte chemotactic protein-1 (MCP-1) promoter polymorphism (A-2518G) with spontaneous bacterial peritonitis (SBP). Fifty patients with post-hepatitis C liver cirrhosis and ascites were categorized into two groups; group I included 25 patients with SBP and group II included 25 patients free from SBP. In addition, a gaggle of 20 healthy volunteers were included. We assessed the MCP-1 gene polymorphism and organic phenomenon also as interleukin (IL)-10 levels in both blood and ascitic fluid. A significant MCP-1 gene polymorphism was detected in groups I and II (P=0.001 and 0.02 respectively). Group i used to be related to a significantly higher frequency of AG genotype [control 8 (40%) vs SBP 19 (76.0%), P < 0.001], and group II was related to a significantly higher frequency of GG genotype in comparison to healthy volunteers [control 1 (5%) vs cirrhotic 16 (64%), P < 0.001]. Accordingly, the frequency of G allele was significantly higher in both groups (I and II) [control 10 (25%) vs SBP 27 (54%), P < 0.001 and vs cirrhotic 37 (74.0%), P < 0.001, respectively]. The total blood and ascetic fluid levels of IL-10 and MCP-1 organic phenomenon were significantly higher in group I than in group II. Group I showed significant reductions within the levels of MCP-1 organic phenomenon and IL-10 within the blood and ascetic fluid after therapy. MCP-1 GG genotype and G allele may predispose HCV infected patients to a more progressive disease course, while AG genotype may increase the susceptibility to SBP. Patients carrying these genotypes should be under supervision to stop or restrict further complications.

Patients with cirrhosis and ascites show higher susceptibility to bacterial infections, mainly due to the inadequate defense mechanisms. Factors influencing the event of spontaneous bacterial peritonitis (SBP) in patients with liver cirrhosis are poorly understood. Previous studies have indicated that peritoneal macrophages of cirrhotic patients might contribute to the control of SBP or influence its associated pathology in human cirrhosis by producing high quantities of angiogenic peptides and nitric oxide. SBP can be caused by many reasons due to the alterations of the immune system that are very common in patients with end-stage liver disease and associated with an increased risk of infection and death. Consequently, elevated concentrations of pro-inflammatory cytokines are found in ascitic fluid of those patients. In addition, hepatitis C virus (HCV) infection is related to increased hepatic expression of monocyte chemotactic protein-1 (MCP-1).

MCP-1 acts as a chemotactic factor for monocytes/macrophages, activated lymphocytes and neutrophils during infections[; thus, these cells migrate to the ascitic fluid. Monocytes and macrophages release TNF-α and other cytokines, which successively induce the expression of adhesion molecules on endothelial cells, thereby mediating a systemic reaction to the infection. TNF-α has been shown to be elevated within the ascitic fluid of SBP patients, stimulating the discharge of interleukin-8 (IL-8), growth-related oncogene-α (GRO-α), and MCP-1 by mononuclear cells or endothelial cells. This release propagates the inflammatory reaction. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation. Furthermore, a previous study showed elevated MCP-1 levels in ascitic fluid of cirrhotic patients with SBP compared to patients without SBP.

The aim of this work was to review the association of the functional MCP-1 promoter polymorphism (A-2518G) with SBP and investigate the expression of MCP-1 in blood and ascites as well as serum and ascitic IL-10 levels.

Detection of MCP-1 polymorphism

Genomic DNA was prepared from blood samples using the Innu PREP blood DNA mini kit (Analytic Jena, Germany) following the manufacturer's instructions. The identification of the polymorphism was carried out using polymerase chain reaction (PCR), followed by a

restriction fragment length polymorphism (RFLP) assay, using a PvuII site, which is introduced by the presence of the G nucleotide. The regulatory region of the MCP-1 gene (from -2746 at -1817) was amplified by PCR employing a forward primer (5′-CCGAGATGTTCCCAGCACAG-3′) and a reverse primer (5′-CTGCTTTGCTTGTGCCTCTT-3′).

PCR was performed in a 40 μL reaction system containing 10 × buffer (10 mmol/L Tris-HCl pH 9, 2.0 mmol/L MgCl2, 50 mmol/L KCl), 200 μm dNTPs, 2.5 pmole of each primer, 5 μL of DNA, 0.5 U Taq polymerase (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and ddH2O. The following thermal profiles were run: 95 °C for 40 s, 56 °C for 30 s, and 72 °C for 4 min. After a final extension of 10 min at 72 °C, 7 μL of the PCR products were resolved on 2% agarose gels and stained with ethidium bromide to see the expected 930-bp band. After visualization, 8 μL of the PCR products were digested with 10 U of PvuII in 10× buffer and H2O up to a final volume of 20 μL at 37 °C for 2 h. The resulting products were separated by electrophoresis on 1.5% agarose gels, containing ethidium bromide at a final concentration of 0.5 g/mL. Samples showing only a 930-bp band were assigned as A/A, those showing two bands at 708 and 222 bp were considered G/G and those showing three bands at 930, 708 and 222 bp were typed as A/G.

In conclusion, inheritance of MCP-1 GG genotype and MCP-1 G allele may predispose HCV infected patients to a more progressive disease course, while AG genotype could also be a risk factor for SBP in patients with decompensated post-hepatitis C cirrhosis. MCP-1 expression and IL-10 levels in blood and ascitic fluid could also be associated with the event and therefore the course of SBP. Further randomized controlled trials with greater sample size are recommended.

Keywords: Monocyte chemotactic protein-1, Genotype, Spontaneous bacterial peritonitis, Liver cirrhosis, Ascites, Gene expression, Interleukin-10