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CASE CONTROL STUDY

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

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Abstract

AIM: To investigate the association of the functional monocyte chemotactic protein-1 (*MCP-1*) promoter polymorphism (A-2518G) with spontaneous bacterial peritonitis (SBP).

METHODS: 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 group of 20 healthy volunteers were included. We assessed the *MCP-1* gene polymorphism and gene expression as well as interleukin (IL)-10 levels in both blood and ascitic fluid.

RESULTS: A significant *MCP-1* gene polymorphism was detected in groups Ⅰ and Ⅱ (P = 0.001 and 0.02 respectively). Group Ⅰ was associated with a significantly higher frequency of AG genotype [control 8 (40%) *vs* SBP 19 (76.0%), P < 0.001], and group Ⅱ was associated with a significantly higher frequency of GG genotype when compared 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* gene expression were significantly higher in group Ⅰ than in group Ⅱ. Group Ⅰ showed significant reductions in the levels of *MCP-1* gene expression and IL-10 in the whole blood and ascetic fluid after therapy.

CONCLUSION: *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 prevent or restrict further complications.

Key words: Monocyte chemotactic protein-1; Genotype; Spontaneous bacterial peritonitis; Liver cirrhosis; Ascites; Gene expression; Interleukin-10

Core tip: Monocyte chemotactic protein-1 (*MCP-1*) polymorphism was investigated in hepatitis C virus (HCV) infected patients because of the higher susceptibility of cirrhosis and ascites patients to bacterial infections. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation. 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 may be a risk factor for spontaneous bacterial peritonitis (SBP) in patients with decompensated post-hepatitis C cirrhosis. MCP-1 expression and elevated IL-10 levels may be related to the development of SBP. HCV cirrhotic and SBP patients carrying the above genotypes should be under supervision and monitoring.

Salama MK, Sabry D, Al-Ghussein MAS, Ahmed R, AbdAllah S, Taha FM, Fathy W, Wadie MS, Nabih M, Abul-Fotouh A, Darwish T. Molecular detection of monocyte chemotactic protein-1 polymorphism in spontaneous bacterial peritonitis patients. *World J Gastroenterol* 2014; 20(33): 11793-11799 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i33/11793.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i33.11793

INTRODUCTION

Patients with cirrhosis and ascites show higher susceptibility to bacterial infections, mainly because of the inadequate defense mechanisms[1-3]. Factors influencing the development 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[4,5]. 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[6,7]. Consequently, elevated concentrations of pro-inflammatory cytokines are found in ascitic fluid of these patients[8,9]. In addition, hepatitis C virus (HCV) infection is associated with increased hepatic expression of monocyte chemotactic protein-1 (MCP-1)[10].

MCP-1 acts as a chemotactic factor for monocytes/macrophages, activated lymphocytes and neutrophils during infections[11,12]; thus, these cells migrate to the ascitic fluid. Monocytes and macrophages release TNF-α and other cytokines, which in turn induce the expression of adhesion molecules on endothelial cells, thereby mediating a systemic reaction to the infection[11,12]. TNF-α has been shown to be elevated in the ascitic fluid of SBP patients, stimulating the release 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[13]. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation[14,15]. Furthermore, a previous study showed elevated MCP-1 levels in ascitic fluid of cirrhotic patients with SBP compared to patients without SBP[13].

The aim of this work was to study 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.

MATERIALS AND METHODS

The case-control study protocol was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. After being approved by the Institutional Review Board of Kasr El-Aini Hospital, the present study was conducted on 50 patients with post-hepatitis C liver cirrhosis and ascites attending the Kasr El-Aini Cairo University Hospital from February 2012 to September 2012. The study population is illustrated in Figure 1. Patients were categorized into two groups according to the presence of SBP or not as follows; group Ⅰ (n = 25) included patients with SBP proved by ascitic fluid polymorphonuclear leukocyte (PMN) count ⩾ 250 cells/mm^3, and group Ⅱ (n = 25) included patients without SBP. Patients with alcoholic liver cirrhosis, Wilson's disease, hemochromatosis, glycogen storage disease and malignant or tuberculous ascites were excluded from this study. As an additional control group (group Ⅲ), 20 healthy volunteers (15 males and 5 females) with a mean age of 48.28 ± 4.56 years were included in the study, and they were recruited from the members of the Medical Biochemistry Department, Faculty of Medicine.

Written informed consent to participate in the study was obtained from all participants. After that, they were subjected to a detailed medical history assessment and laboratory investigation (complete blood count, liver and renal function tests). Serum IL-10 level assessment, quantitative assessment of *MCP-1* gene expression in blood and detection of *MCP-1* gene polymorphism were performed. The ascitic fluid of patients of both groups Ⅰ and Ⅱ was analysed for IL-10 level and the quantitative assessment of *MCP-1* gene expression. Appropriate antibiotic medication therapy was prescribed for patients of group Ⅰ and after the ascitic fluid PMN count became less than 250 cells/mm^3, they were reassessed by measuring the *MCP-1* gene expression in the whole blood and in the ascitic fluid in addition to the IL-10 level in both serum and ascitic fluid.

Detection of MCP-1 polymorphism

Genomic DNA was prepared from venous blood sam-

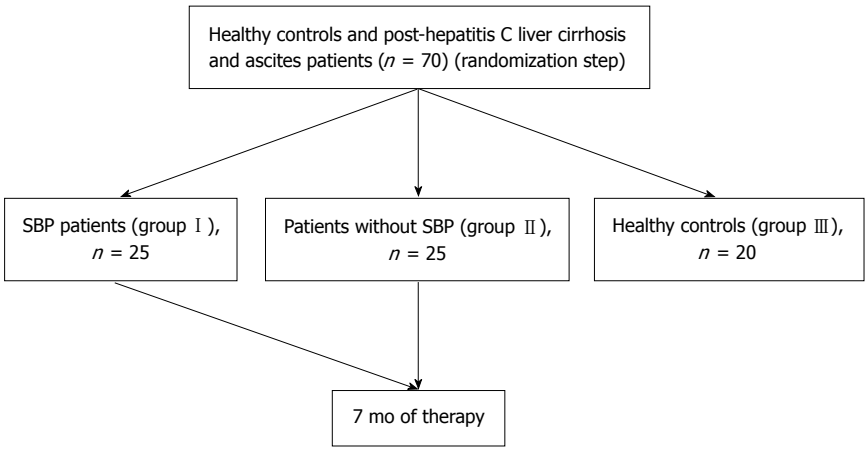

Figure 1 Algorithm for the study design. SBP: Spontaneous bacterial peritonitis.

ples 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 using a forward primer (5'-CCGAGATGTTCCCAGCACAG-3') and a reverse primer (5'-CTGCTTTGCTTGTGCCTCTT-3')[16].

PCR was performed in a 40 μL reaction system containing 10 × buffer (10 mmol/L Tris-HCl pH 9, 2.0 mmol/L $MgCl_2$, 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 ddH_2O. The following thermal profiles were run: 95 ℃ for 40 s, 56 ℃ for 30 s, and 72 ℃ for 4 min. After a final extension of 10 min at 72 ℃, 7 μL of the PCR products were resolved on 2% agarose gels and stained with ethidium bromide to visualize the expected 930-bp band. After visualization, 8 μL of the PCR products were digested with 10 U of PvuII in 10× buffer and H_2O up to a final volume of 20 μL at 37 ℃ 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.

Quantitative assessment of MCP-1 gene expression by real-time PCR

RNA extraction from blood and ascitic fluid samples: SV total RNA isolation system (Promega, USA) was used to extract RNA.

Primer design and selection: All primers were designed based on target sequences obtained from the reference[17].

cDNA synthesis: The extracted RNA was reverse transcribed into cDNA using RT-PCR kit (Stratagene USA)[18].

Real-time quantitative PCR using SYBR Green I

Real-time quantitative PCR (qPCR) amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The qPCR assay with the primer sets were optimized at the annealing temperature. All cDNA including previously prepared samples, internal control (for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene expression as housekeeping gene), and non-template control (water to confirm the absence of DNA contamination in the reaction mixture), were in duplicate. The sequences of the primers used for amplification of the *GAPDH* gene were forward, 5'CGCTCTCTGCTCCTCCTGTT 3' and reverse, 5' CCATGGTGTCTGAGCGATGT 3'[19].

Estimation of serum and ascitic fluid IL-10 by enzyme linked immunosorbent assay

IL-10 was analysed using kits produced by Orgenium Laboratories Business Unit (Vantaa, Finland)[20].

Statistical analysis

The results were analysed using the SPSS software package version 9.0 (Chicago, IL, USA). Quantitative data are expressed as mean ± standard deviation (SD). Differences between two groups were compared by the Student's *t*-test. Genotype and allele frequencies were reported as percentages and the differences between groups were determined by χ^2 test. Correlations between data were performed using Pearson and Spearman correlation tests as required. Differences were considered significant at $P < 0.05$.

RESULTS

The demographic and clinical data of the studied groups are presented in Table 1. Patients of both groups and the healthy controls were age and sex matched. There was no statistically significant difference between both studied

Table 1 Baseline demographic and clinical characteristics of the studied groups

	Control group (n = 20)	Group Ⅰ (SBP) (n = 25)	Group Ⅱ (cirrhotic) (n = 25)
Age (yr)	48.28 ± 4.56	51.24 ± 9.3	47.08 ± 12.9
Sex (male)	15 (75)	18 (72)	15 (60)
BMI (kg/m^2)	28.42 ± 2.33	27.94 ± 2.1	28.2 ± 1.8
DM (Yes)	0	5 (20)	8 (32)
GIT bleeding (Yes)	0	5 (20)	5 (20)
Hepatic encephalopathy (Yes)	0	9 (36)	7 (28)
Duration of liver cirrhosis (yr)	0	4.46 ± 5	4.04 ± 3.26
Duration of ascites (yr)	0	1 ± 2.01	1.71 ± 1.54
Hemoglobin (g/dL)	12.6 ± 1.6	10.27 ± 1.95[b]	9.5 ± 2.22[b]
Platelets (10^3/μL)	158.4 ± 12.8	146.6 ± 90.2	118.6 ± 35.9[b]
TLC (10^3/μL)	6.3 ± 0.97	5.82 ± 3.05	6.7 ± 2.58
Serum albumin (g/dL)	4.34 ± 0.62	2.26 ± 0.39[b]	2.3 ± 0.46[b]
Total bilirubin (mg/dL)	1.036 ± 0.064	5.42 ± 8.7[b]	2.69 ± 2.65[b]
Direct bilirubin (mg/dL)	0.176 ± 0.078	3.07 ± 5.3[b]	1.44 ± 1.58[b]
Urea (mg/dL)	17.4 ± 3.3	59.45 ± 26.6[a,b]	42.8 ± 24.49[b]
Creatinine (mg/dL)	0.86 ± 0.208	1.96 ± 1.79[b]	1.5 ± 0.95[b]
AST (IU/L)	47.96 ± 7.7	69.86 ± 38.03[a]	85.8 ± 52.99[b]
ALT (IU/L)	23.79 ± 7.5	39.7 ± 16.03[b]	40.17 ± 24.76[a]
ALP (IU/L)	95.5 ± 19.8	167.9 ± 69.49[b]	101.4 ± 39.67
INR	0.996 ± 0.13	1.84 ± 0.59[b]	1.66 ± 0.40[b]
MCP-1 gene expression in whole blood	0.131 ± 0.0367	1.04 ± 0.119[b,d]	0.112 ± 0.046
Serum IL-10 (pg/mL)	14.48 ± 3.29	29.26 ± 7.037[b,d]	15.91 ± 4.53
PMN count in ascites (cells/mm^3)	-	1194.6 ± 1187.6[d]	110.3 ± 60.89
Serum-Ascites Albumin gradient (SAAG) (g/dL)	-	1.34 ± 0.107[d]	1.67 ± 0.32
Ascitic IL-10 (pg/mL)	-	60.07 ± 12.67[d]	16.86 ± 5.2
Ascitic *MCP-1* gene expression	-	2.251 ± 1.039[d]	1.5 ± 0.59

[a]P < 0.05, [b]P <0.01 *vs* control group; [d]P <0.01, group Ⅰ *vs* group Ⅱ. Results are expressed as mean ± SD or frequency (%) as required. BMI: Body mass index; DM: Diabetes mellitus; GIT: Gastrointestinal; TLC: Total lymphocyte count; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; INR: International normalized ratio; MCP-1: Monocyte chemotactic protein-1; SBP: Spontaneous bacterial peritonitis.

Table 2 *MCP-1* genotypes in the studied groups

	Control group (n = 20)	Group Ⅰ (SBP) (n = 25)	Group Ⅱ (cirrhotic) (n = 25)
Genotype			
AA	11 (55)	2 (8.0)[b]	4 (16)[b]
AG	8 (40)	19 (76.0)[b]	5 (20)[b,d]
GG	1 (5)	4 (16.0)	16 (64)[b,d]
Allele			
A	30 (75.0)	23 (46.0)[b,d]	13 (26.0)[b]
G	10 (25.0)	27 (54.0)[b]	37 (74.0)[b,c]

[a]P < 0.05, [b]P <0.01 *vs* control group; [c]P < 0.05, [d]P <0.01, group Ⅰ *vs* group Ⅱ. Results are expressed as frequency (%). MCP-1: Monocyte chemotactic protein-1.

groups of patients regarding the studied laboratory data, except for significantly higher levels of the *MCP-1* gene expression in whole blood (cirrhotic 0.112 ± 0.046 *vs* SBP 1.04 ± 0.119, P < 0.001) and serum IL-10 in SBP patients (cirrhotic 15.91 ± 4.53 *vs* SBP 29.26 ± 7.037, P < 0.001).

MCP-1 polymorphism in all the studied groups is presented in Table 2. Our results showed that the genotype frequencies in the healthy controls did not depart from those expected on the basis of Hardy-Weinberg equilibrium (P = 0.76). However, in cirrhotic patients without SBP (group Ⅱ) and those with SBP (group Ⅰ), the observed and expected frequencies were significantly different (P = 0.02 and 0.001, respectively). When compared to normal healthy volunteers, a significantly higher frequency of the GG genotype was reported in cirrhotic patients without SBP (group Ⅱ) [control 1 (5%) *vs* cirrhotic 16 (64%), P < 0.001], while a significantly higher frequency of the AG genotype was reported with cirrhotic patients with SBP (group Ⅰ) [control 8 (40%) *vs* SBP 19 (76.0%), P < 0.001]. When comparing the two groups of patients with each other, a significantly higher frequency of the GG genotype was reported with cirrhotic patients without SBP (group Ⅱ) [SBP 4 (16%) *vs* cirrhotic 16 (64%), P < 0.001], while a significantly higher frequency of the AG genotype was reported with cirrhotic patients with SBP (group Ⅰ) [SBP 19 (76.0%) *vs* cirrhotic 5 (20%), P <0.001]. Accordingly, there was a significantly higher frequency of the G allele in both groups of patients (Ⅰ and Ⅱ) when compared to healthy volunteers (control 10 (25%) *vs* SBP 27 (54%), P < 0.001 and *vs* cirrhotic 37 (74.0%), P < 0.001 respectively). When comparing both groups of patients with each other, it was revealed that the G allele represented 54% in those with SBP (group Ⅰ) *vs* 74% in those without SBP (group Ⅱ) [SBP 27 (54.0%) *vs* cirrhotic 37 (74%), P < 0.001], while the A allele represented 46% in those with SBP (group Ⅰ) *vs* 26% in those without SBP (group Ⅱ) [SBP 23(46.0%) *vs* cirrhotic 13 (26%), P < 0.001], and these differences were statistically significant.

Ascitic fluid analysis

Results are presented in Table 1. Our results revealed

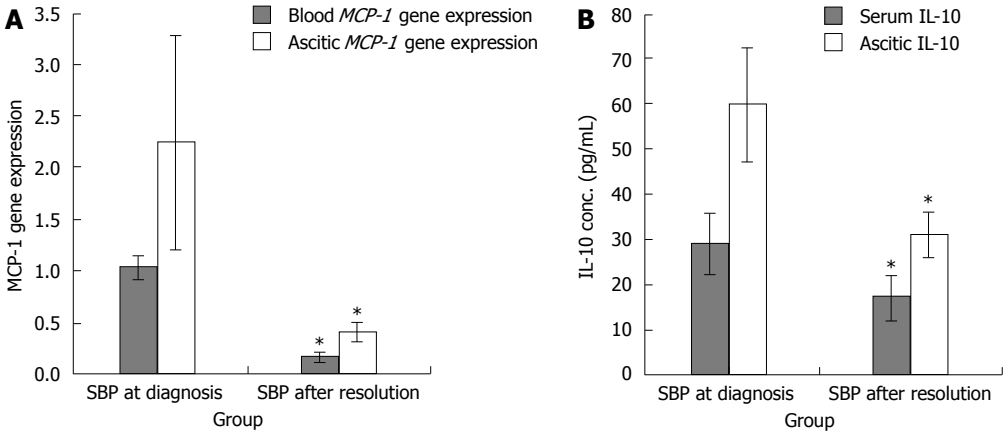

Figure 2 Cirrhotic patients with spontaneous bacterial peritonitis before and after therapy. A: Blood and ascitic *MCP-1* gene expression; B: Serum and ascetic IL-10 concentrations. Results are expressed as mean ± SD. Asterisk denotes a significant difference in measured parameters at diagnosis and after resolution. MCP-1: Monocyte chemotactic protein-1; IL-10: Interleukin 10; SBP: Spontaneous bacterial peritonitis.

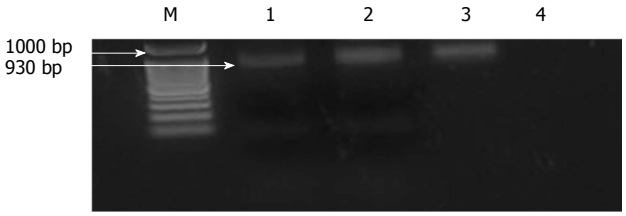

Figure 3 Agarose gel electrophoresis analysis of polymerase chain reaction products for *MCP-1* gene (930 bp) before digestion with restriction enzyme. Lane M: DNA ladder (100, 200, 300 to 1000 bp); Lane 1: polymerase chain reaction (PCR) product for *MCP-1* gene in a healthy control; Lane 2: PCR product for *MCP-1* gene in a cirrhotic patient with SBP; Lane 3: PCR product for *MCP-1* gene in a cirrhotic patient without SBP; Lane 4: Negative control. MCP-1: Monocyte chemotactic protein-1; SBP: Spontaneous bacterial peritonitis.

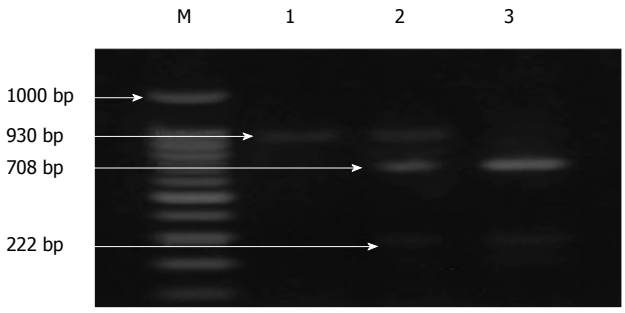

Figure 4 Agarose gel electrophoresis analysis of polymerase chain reaction products for *MCP-1* gene (930 bp) after digestion with restriction enzyme. Lane M: DNA ladder (100, 200, 300 to 1000 bp); Lane 1: polymerase chain reaction (PCR) product for A/A genotype (930 bp); Lane 2: PCR product for A/G genotype (930, 708 and 222 bp); Lane 3: PCR product for G/G genotype (708 and 222 bp). MCP-1: Monocyte chemotactic protein-1.

that the ascitic fluid levels of the *IL-10* and *MCP-1* gene expression were significantly higher in patients with SBP (group Ⅰ) than those without SBP (group Ⅱ).

Cirrhotic patients with SBP (group Ⅰ) showed significant reductions in the levels of *MCP-1* gene expression and IL-10 in the whole blood and ascitic fluid after therapy (Figure 2A and B). In cirrhotic patients with SBP a significant positive relationship was detected between the *MCP-1* gene expression in the whole blood and the duration of liver disease ($r = 0.46$, $P = 0.02$). Also, a significant positive relationship was detected between the serum IL-10 and both the SAAG and the serum albumin level ($r = 0.623$ and 0.472, $P = 0.023$ and 0.02, respectively). In addition, a significant positive relationship was detected between the ascitic *MCP-1* gene expression and the total bilirubin level ($r = 0.535$, $P = 0.03$). Contrarily, a significant negative relationship was detected between the ascitic *MCP-1* gene expression and the total leucocytic count (TLC) ($r = 0.671$, $P = 0.003$). However, these relationships were statistically insignificant in cirrhotic patients without SBP. On the other hand, a significant positive relationship was detected between the serum IL-10 and the urea level ($r = 0.449$, $P = 0.036$), as well as between the ascitic *MCP-1* gene expression and the serum creatinine level ($r = 0.57$, $P = 0.01$). A significant negative relationship was detected between the ascitic IL-10 and the duration of the liver cirrhosis ($r = 0.39$, $P = 0.048$). PCR products for *MCP-1* gene (930 bp) before digestion with restriction enzyme for different groups are shown in Figure 3. PCR products for *MCP-1* gene (930 bp) after digestion with restriction enzyme in Figure 4 showed A/A genotype at 930 bp, A/G genotype at 930, 708 and 222 bp and G/G genotype at 708 and 222 bp.

DISCUSSION

Interestingly, a significant *MCP-1* genotype polymorphism was observed in cirrhotic patients with and without SBP in our study, which was not observed in the healthy Egyptian volunteers. Further analysis showed that cirrhotic patients without SBP were associated with a higher frequency of GG genotype, while those with SBP were associated with a higher frequency of AG genotype. Also, it was found that the G allele frequency was significantly higher in both the cirrhotic patients with and without SBP than the healthy volunteers as well as being higher in the cirrhotic patients without SBP than in those with SBP. This result is in agreement with the finding by

Gäbele *et al*[21], who reported that carriers of the G allele of the *MCP-1* polymorphism were more frequent in patients with alcohol induced cirrhosis than in heavy drinkers without evidence of liver damage (controls). Also, in a previous study, carriers of the G allele were significantly more frequent in HCV patients with more advanced fibrosis and severe inflammation[14]. *In vitro* stimulated monocytes from individuals carrying a G allele at -2518 produced more *MCP-1* than cells from A/A homozygous subjects[22]. Carriers of the G allele were significantly more frequent in HCV patients with more advanced fibrosis and severe inflammation[15].

In patients with SBP, MCP-1 acts as a chemotactic factor for monocytes and macrophages; thus, these cells migrate to the ascitic fluid. These monocytes and macrophages release TNF-α and other cytokines, which in turn induce the expression of adhesion molecules on endothelial cells, thereby mediating a systemic reaction to the infection[11,12]. This explains the significant increase, reported in our study, of the mean level of the *MCP-1* gene expression in both blood and ascetic fluid of cirrhotic patients with SBP compared with cirrhotic patients without SBP, which was in agreement with previous studies[21-23]. These findings suggest that this potent chemokine plays a pathophysiological role during the development and the course of SBP. As well in our study, the SBP patients showed a significant increase in the mean level of PMN count compared with cirrhotic patients without SBP. In the present study, the mean level of *MCP-1* gene expression in blood was higher in control subjects than in cirrhotic patients without SBP. However, this difference was not statistically significant. This is in concordance with what was reported by Nischalke *et al*[24], who found that the MCP-1 was markedly lower in HCV-infected patients than in controls, and it was explained by the down-regulation of MCP-1 expression by viral proteins and the inhibition of activity of the *MCP-1* gene promoter by HCV core protein.

In agreement with the results of previous studies[13,25,26], our research reported a significant increase in the serum IL-10 level in the SBP patients than those in the healthy volunteers and cirrhotic patients without SBP. Also it was higher in cirrhotic patients without SBP than in healthy volunteers, but the difference was not statistically significant. This goes with the assumption that the elevated IL-10 levels in both cirrhotic patients with and without SBP have a regulatory role in the inflammatory process in liver cirrhosis patients[25].

Our study reported that mean level of serum ascites albumin gradient (SAAG) was significantly higher in cirrhotic patients than in SBP patients, and this is in agreement with the results of Khan *et al*[26] who found that the SAAG was higher in cirrhotic than SBP patients.

Changes in various cytokines levels after SBP treatment were previously observed, *e.g.*, MCP-1 and IL-10 levels showed a significant decrease during follow-up after treatment[13], and this is in agreement with the result of our study that SBP patients showed significant decreases in the mean levels of blood and ascitic fluid *MCP-1* gene expression and serum IL-10 after SBP treatment.

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 may 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 may be related to the development and the course of SBP. Further randomized controlled trials with greater sample size are recommended.

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COMMENTS

Background

The high susceptibility of hepatitis C patients with cirrhosis and ascites to bacterial infections correlates with peritoneal macrophages that might contribute to the control of spontaneous bacterial peritonitis (SBP) or influence its associated pathology. The chemotactic factor monocyte chemotactic protein-1 (MCP-1) secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation, and thus the functional *MCP-1* promoter polymorphism (A-2518G) can be associated with cirrhosis and SBP.

Research frontiers

The functional *MCP-1* promoter polymorphism (A-2518G) genotypes distribution and allele frequencies were demonstrated as markers for cirrhosis and/or SBP susceptibility in HCV patients. Above and beyond, MCP-1 expression and level along with IL-10 level were evaluated as pre- and post-treatment monitoring indicators for such cases.

Innovations and breakthroughs

Several reports have highlighted that carriers of the G allele of the MCP-1 polymorphism were more frequent in patients with alcohol induced cirrhosis and HCV fibrosis and severe inflammation. This is the first study to report that inheritance of *MCP-1* GG genotype and *MCP-1* G allele may predispose HCV infected patients to cirrhosis, while AG genotype may be a risk factor for spontaneous bacterial peritonitis SBP in patients with decompensated cirrhosis. Additionally, our investigations would propose MCP-1 expression and IL-10 levels in blood and ascitic fluid to be correlated with the development and the course of SBP.

Applications

HCV infected patients carrying the G allele of the *MCP-1* polymorphism should be under intensive observation, because *MCP-1* GG genotype carriers may develop cirrhosis and AG genotype can be a high risk factor for spontaneous bacterial peritonitis. MCP-1 expression and IL-10 levels in blood and ascitic fluid should be investigated during the development of these cases.

Terminology

MCP-1 is a signalling protein that acts as a chemotactic factor for monocytes and macrophages; thus, these cells migrate to the ascetic fluid. SBP is a peritoneal recurrent bacterial infection due to lower immunity state.

Peer review

This paper has high scientific and methodological levels.

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