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

Salama MK *et al.* *MCP-1* polymorphism in SBP 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 virus (HCV) liver cirrhosis and ascites categorized into two groups, group I: 25 patients with SBP, group II: 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, IL-10 level in both blood and ascetic fluid.

**RESULTS:** Significant *MCP-1* gene polymorphism was detected in group I and II (*P* = 0.001 and 0.02 respectively). SBP group I was significantly associated with AG genotype [control 8 (40%) *vs* SBP 19(76.0%), *P* <0.001] and cirrhotic group II with GG genotype when compared to healthy volunteers [control 1 (5%) *vs* cirrhotic 16 (64%), *P* < 0.001]. Accordingly, 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-1*0 and *MCP-1* gene expression were significantly higher in group I than that of group II. Group I showed a significant reduction 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 spontaneous bacterial peritonitis. Patients’ carrier should be under supervision for prophylactic or to restrict further complications.

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**Keywords:** 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 the higher susceptibility of cirrhosis and ascites patients to bacterial infections and 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 more progressive disease course while AG genotype may be a risk factor to spontaneous bacterial peritonitis in patients with decompensated post hepatitis C cirrhosis. MCP-1 expression and IL-10 elevated levels may due to the development of spontaneous bacterial peritonitis (SBP). HCV cirrhotic and SBP patients carrying genotypes should be under supervision and monitoring.

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**INTRODUCTION**

Patients with cirrhosis and ascites show a higher susceptibility to bacterial infections, mainly because of the inadequate defence mechanisms[[1](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)-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 ascetic fluid of patients[8,9]. In addition, hepatitis C virus (HCV) infection is associated with increased hepatic expression of monocyte chemotactic protein-1 (MCP-1)[[10](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

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-γ 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](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)]. TNF-γ has been shown to be elevated in the ascitic fluid of SBP patients, stimulating the release of interleukin-8 (IL-8), growth-related protein-8 (GRO-8), and MCP-1 by mononuclear cells or endothelial cells. This release propagates the inflammatory reaction[[13](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)]. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation[[14,15](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

The aim of this work was to study the association of the functional *MCP-1* promoter polymorphism (A-2518G) with SBP and investigating the expression of the 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 Boards (IRBs) of Kasr El-Aini hospital, the present study was conducted on 50 patients with post-HCV liver cirrhosis and ascites attending to the Kasr El-Aini Cairo university hospital in the period from February 2012 to September 2012. The study population was illustrated in Figure 1. Patients were categorized into two groups according to the presence of SBP as follow, group I (*n* = 25) includes patients with SBP as proved by ascitic fluid polymorphonuclear leukocyte (PMN) count ≥ 250 cells/mm3, group II (*n* = 25) includes patients with no SBP. Patients with alcoholic liver cirrhosis, Wilson disease, hemochromatosis, glycogen storage disease and malignant or tuberculous ascites were excluded from this study. As an additional control group III, 20 healthy volunteers (15 males and 5 females) with mean age 48.28 ± 4.56 years were included in our study, 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 (CBC, 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 group I and II was analysed for IL-10 level and the quantitative assessment of *MCP-1* gene expression. Hospitalized appropriate antibiotics medication therapy were prescribed for patients of group I and after the ascitic fluid PMN count becomes less than 250 cells/mm3, 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 samples on EDTA 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 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 polymerase chain-reaction (PCR) using the Forward*:* 5′-CCGAGATGTTCCCAGCACAG-3′ and Reverse: 5′-CTGCTTTGCTTGTGCCTCTT-3′[[16](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

PCR was performed using buffer 10× (10 mmol/L Tris-HCl pH 9, 2.0 mmol/L MgCl2, 50 mmol/L KCl), 200 µm dNTPs, 2.5 pmoles of each primer, 5 μL of DNA, 0.5 U Taq polymerase (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and ddH2O up to a final volume of 40 μL. 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 in 2% agarose gels stained with ethidium bromide previous dilution in blue juice buffer to check the expected 930-bp band. After checking, 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 gel-electrophoresis in 1.5% agarose gels, containing ethidium bromide in a final concentration of 0.5 g/mL Samples showing only a 930 bp band were assigned as A/A, samples showing two bands of 708 and 222 bp were considered G/G and samples showing three bands at 930, 708 and 222 bp were typed 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) wae used to extract RNA.

**Primer design and selection:**All primers were designed based on target sequences obtained from the reference[[17](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

**cDNA synthesis:**The extracted RNA was reverse transcribed into cDNA using RT-PCR kit (Stratagene USA)[[18](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

***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. Sequence of *GAPDH* gene: Forward primer: 5'CGCTCTCTGCTCCTCCTGTT 3'; Reverse primer:  5' CCATGGTGTCTGAGCGATGT 3'[[19](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

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

IL-10 was analysedusing kits produced by Orgenium Laboratories Business Unit (Vantaa, Finland)[[20](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

***Statistical analysis***

The results were analysed using the SPSS computer software package version 9.0 (Chicago, IL, USA). Quantitative data were expressed as mean ± SD. Differences between two groups were compared by Student’s *t* test Genotype and allele frequencies were reported with their group percentages and the difference between groups were determined by *x2* 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 represented 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 groups of patients regarding the studied laboratory data, except for a significantly higher level both the *MCP-1* gene expression in whole blood (control 0.131 ± 0.0367 *vs* SBP 1.04 ± 0.119, *P* < 0.001) and the serum IL-10 levels in SBP patients (control 14.48 ± 3.29 *vs* SBP 29.26 ± 7.037, *P* < 0.001).

*MCP-1* polymorphism in all studied groups is illustrated in Table 2, our results showed that the genotype frequencies in the healthy controls didn’t 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 significant association of the GG genotype was reported with cirrhotic patients with no SBP (group II)[control 1 (5%) *vs* cirrhotic 16 (64%), *P* < 0.001], while a significant association of the AG genotype was reported with cirrhotic patients with SBP (group I)[control 8 (40%) *vs* SBP 19 (76.0%), *P* < 0.001]. Moreover on comparing both groups of patients with each other, a significant higher frequency of the GG genotype was reported with cirrhotic patients with no SBP (group II)[SBP 4 (16) *vs* cirrhotic 16 (64%), *P* < 0.001], while a significant 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 significant association of the G allele and 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), while when comparing both groups of patients with each other it was revealed that the G allele represents 54% in those with SBP (group I) *vs* 74% in those with no SBP (group II) (SBP 27 (54.0%) *vs* cirrhotic 37 (74%), *P* < 0.001), while the A allele represents 46% in those with SBP (group I) *vs* 26% in those with no SBP (group II) [SBP 23(46.0%) *vs* cirrhotic 13 (26%), *P* < 0.001] and these differences are statistically significant.

***Ascitic fluid analysis***

Results represented in Table 1, our results revealed that the ascitic fluid levels of the *IL-10* and *MCP-1* gene expression was significantly higher in patients with SBP (group I) than those without SBP (group II).

Cirrhotic patients with SBP (group I) showed a significant reduction 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 detected between the *MCP-1* gene expression in the whole blood and the duration of liver disease with *r* = 0.46 and *P* = 0.02. Also, a significant positive relationship detected between the serum IL-10 and both the SAAG and the serum albumin level with *r* = 0.623 and 0.472 and *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 with *r* = 0.535 and *P* = 0.03. Contrarily, a significant negative relationship was detected between the ascitic *MCP-1* gene expression and the TLC count with *r* = 0.671 and *P* = 0.003. However these relationships were statistically insignificant in the other group of cirrhotic patients with no SBP. On the other hand, a significant positive relationship was detected between the serum IL-10 and the urea level with *r* = 0.449 and *P* = 0.036, as well as between the ascitic *MCP-1* gene expression and the serum creatinine level with *r* = 0.57 and *P* = 0.01. Other than, a significant negative relationship was detected between the ascitic IL-10 and the duration of the liver cirrhosis with *r* = 0.39 and *P* = 0.048. PCR products for *MCP-1* gene (930 bp) before cutting with restriction enzyme for different groups appeared in Figure 3. PCR products for *MCP-1* gene (930 bp) after cutting with restriction 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 undetectable in the healthy Egyptian volunteers. Further analysis reported that cirrhotic patients with no SBP were significantly associated with GG genotype, while those with SBP were significantly associated with AG genotype. Also it was reported 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 with no SBP than those with SBP. This agrees with Gäbele*et al*[[21](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)], 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). *In vitro* stimulated monocytes from individuals carrying a G-allele at -2518 produced more *MCP-1* than cells from A/A homozygous subjects[[22](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)]. Carriers of the G allele were significantly more frequent in HCV patients with more advanced fibrosis and severe inflammation[[15](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)].

MCP-1 acts as a chemotactic factor for monocytes and macrophages; thus, these cells migrate to the ascetic fluid. These monocytes and macrophages release TNF-α and other cytokines, which in turn induces the expression of adhesion molecules on endothelial cells, thereby mediating a systemic reaction to the infection[[14](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23),15],explaining the significant increase, reported in our study, of the mean level of the relative *MCP-1* gene expression in both blood and ascetic fluid of cirrhotic patients with SBP than in cirrhotic patients with no SBP which was in agreement with previous researches[[21-23](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793147/#B23)], suggesting 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 ascetic PMN count than those of cirrhotic patients with no SBP. Moreover, a significant positive correlation was detected between blood MCP-1 and duration of liver cirrhosis in SBP patients. While it worth mentioning that the mean level of *MCP-1* gene expression in blood was higher in control subjects than cirrhotic patients without spontaneous bacterial peritonitis but this difference was not statistically significant, this is in concordance with what was reported in Hans *et al*[24],where 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 that activity of the *MCP-1* gene promoter was inhibited byHCV core protein.

In agreement with the results of previous researches[15,25,26], our research reported a significant increase in the serum IL-10 level in the SBP patients than that of the healthy volunteers and cirrhotic patients with no SBP, also it was higher in cirrhotic patients with no SBP than that of the healthy volunteers but this results was not statistically significant, and this goes with the assumption that the role of the elevated IL-10 levels in both cirrhotic patients with and without SBP have a regulatory control of the inflammatory process *via* IL-10 in liver cirrhosis patients[25].

Our research study reported that mean level of serum ascites albumin gradient (SAAG) showed a significantly higher levels in cirrhotic patients than in SBP patients, this is in agreement with the results of [Jahangir](http://informahealthcare.com/action/doSearch?action=runSearch&type=advanced&result=true&prevSearch=%2Bauthorsfield%3A%28Khan%2C+J%29) *et al*[26] where it was shown that the serum ascites albumin gradient (SAAG) was higher in cirrhotic than SBP patients.

A change in various cytokines levels after the SBP treatment was previously observed where MCP-1 and IL-10 levels showed a significant decrease on follow up after treatment[13], and this is in agreement with the results of our study whereSBP patients showed a significant decrease in the mean level of blood and ascetic fluid *MCP-1* gene expression and serum IL-10 after SBP treatment. PCR images illustrated the *MCP-1* gene products and genotypes.

In conclusion, inheritance of *MCP-1* GG genotype and *MCP-1* G allele may predispose HCV infected patients to more progressive disease course while AG genotype may be a risk factor to spontaneous bacterial peritonitis 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 virus (HCV) patients with cirrhosis and ascites to bacterial infections correlated with the peritoneal macrophages that might contribute to the control of SBP or influence its associated pathology. Consequently, its 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 spontaneous bacterial peritonitis (SBP).

***Research frontiers***

The functional *MCP-1* promoter polymorphism (A-2518G) genotypes distribution and allele’s frequencies were demonstrated as markers for HCV patients’ cirrhosis and/or SBP susceptibility. Above and beyond, MCP-1 expression and value 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 to spontaneous bacterial peritonitis SBP in patients with decompensated post cirrhosis. Additionally, our investigations would propose MCP-1 expression and IL-10 levels in blood, and ascitic fluid to be correlated to the development and the course of SBP.

***Applications***

Monitoring HCV infected patients carries of the G-allele of the *MCP-1* polymorphism should be under intensive observation that *MCP-1* GG genotype carriers may develop cirrhosis while AG genotype can be with a higher risk for the spontaneous bacterial peritonitis SBP vulnerability. 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 level.

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**Table 1 Baseline demographic and clinical characteristics of studied groups**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Control group****(*n* = 20)** | **Group I (SBP)** **(*n* = 25)** | **Group II (Cirrhotic) (*n* = 25)** |
| **Age** (years) | 48 .28 ± 4.56 | 51.24 ± 9.3 | 47.08 ± 12.9 |
| **Sex** (male) | 15 (75) | 18 (72) | 15 (60) |
| **BMI** (kg/m2) | 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 a, b | 9.5 ± 2.22 a, b  |
| **Platelets** (103/µL) | 158.4 ± 12.8 | 146.6 ± 90.2 | 118.6 ± 35.9 a, b |
| **TLC** (103/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 a, b | 2.3 ± 0.46 a, b  |
| **Total bilirubin** (mg/dL) | 1.036 ± 0.064 | 5.42 ± 8.7 a, b | 2.69 ± 2.65 a, b |
| **Direct bilirubin** (mg/dL) | 0.176 ± 0.078 | 3.07 ± 5.3 a, b | 1.44 ± 1.58 a, b  |
| **Urea** (mg/dL) | 17.4 ± 3.3 | 59.45 ± 26.6 a, b | 42.8 ± 24.49 a, b  |
| **Creatinine** (mg/dL) | 0.86 ± 0.208 | 1.96 ± 1.79 a, b | 1.5 ± 0.95 a, b  |
| **AST** (IU/L) | 47.96 ± 7.7 | 69.86 ± 38.03 a | 85.8 ± 52.99 a, b  |
| **ALT** (IU/L) | 23.79 ± 7.5 | 39.7 ± 16.03 a, b | 40.17 ± 24.76 a  |
| **ALP** (IU/L) | 95.5 ± 19.8 | 167.9 ± 69.49 a, b  | 101.4 ± 39.67 |
| **INR** | 0.996 ± 0.13 | 1.84 ± 0.59 a, b | 1.66 ± 0.40 a, b  |
| ***MCP-1*  gene expression in whole blood** | 0.131 ± 0.0367 | 1.04 ± 0.119 a, b, c, d | 0.112 ± 0.046 |
| **Serum IL-10** (pg/mL) | 14.48±3.29 | 29.26 ± 7.037 a, b, c, d  | 15.91 ± 4.53 |
| **PMN count in Ascites** (cells/mm3) | -- | 1194.6 ± 1187.6 c, d | 110.3 ± 60.89 |
| **Serum-Ascites Albumin gradient (SAAG)** (g/dL) | -- | 1.34 ± 0.107 c, d | 1.67 ± 0.32 |
| **Ascitic IL-10** (pg/mL) | -- | 60.07 ± 12.67 c, d | 16.86 ± 5.2 |
| **Ascitic *MCP-1* gene expression**  | -- | 2.251 ± 1.039 c, d | 1.5 ± 0.59 |
|  |  |  |  |
|  |  |  |  |

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 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* genotyping in the studied groups**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Control group****(*n* = 20)** | **Group I (SBP)** **(*n* = 25)** | **Group II (Cirrhotic) (*n* = 25)** |
| **Genotypes** |  |  |  |
| **AA** | 11 (55) | 2 (8.0) a, b | 4 (16) a, b |
| **AG** | 8 (40) | 19 (76.0) a, b, c | 5 (20) a, b, c, d |
| **GG** | 1 (5) | 4 (16.0) | 16 (64) a, b, c, d |  |
| **Alleles** |  |  |  |
| **A** | 30 (75.0) | 23 (46.0) a, b, c, d | 13 (26.0) a, b |
| **G** | 10 (25.0) | 27 (54.0) a, b, | 37 (74.0) a, 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 mean or frequency (%) as required. MCP-1: Monocyte chemotactic protein-1.

**Healthy and hepatitis C virus liver cirrhosis and ascites patients** (***n***=70) **(Randomization step)**

**7 month of Therapy**

**SBP patients (group I) 25 patient**

**Patients without SBP (group II) 25 patient**

**Healthy control (group III) 20 patients**

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



**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. () denotes high significant difference of 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 for PCR products for *MCP-1* gene (930 bp) before cutting with restriction enzyme.** Lane M: DNA ladder (100, 200, 300 to 1000 bp); Lane 1: PCR product for *MCP-1* gene in healthy control; Lane 2: PCR product for *MCP-1* gene in cirrhotic patient with SBP; Lane 3: PCR product for *MCP-1* gene in cirrhotic patient without SBP; Lane 4: Negative control. MCP-1: Monocyte chemotactic protein-1; SBP:Spontaneous bacterial peritonitis.

**Figure 4 Agarose gel electrophoresis for PCR products for *MCP-1* gene (930 bp) after cutting with restriction enzyme.** Lane M: DNA ladder (100, 200, 300 to 1000 bp); Lane 1: 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.