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Genetic Polymorphisms of Monocyte Chemotactic Protein-1 and Risk of Spontaneous Bacterial Peritonitis in Post Hepatitis C Cirrhotic Patients

Rashed Mohammed Hassen[1], Sahar Abdelshafy Elnemr[1], Heba Fouad Pasha[2], Akram Said Ali Khafagy[1], Ahmed S. Mohammed[1]

Departments of [1]Tropical Medicine and [2]Medical Biochemistry, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:** Akram Said Ali Khafagy, **Email:** khafagyakram790@gmail.com

ABSTRACT

Background: Monocyte chemotactic protein-1 (MCP-1) is efficient chemokine for activated lymphocytes, macrophages as well as monocytes during infections, which directly or indirectly affects neutrophil infiltration.
Objective: This study aimed to determine whether genetic variants of the MCP-1 are associated with spontaneous bacterial peritonitis (SBP) in individuals with post hepatitis C.

Patients and methods: Sixty patients with post-hepatitis C virus liver cirrhosis and ascites were included in the study. They were divided into two groups: 30 patients with SBP in Group I & 30 patients who had not been diagnosed with SBP in group II. Thirty healthy participants were included in the control group. PCR-RFLP was used to detect the MCP-1gene polymorphism, and all patients had their medical histories, physical examinations, and laboratory tests completed, including ascitic sample analysis.

Results: According to MCP-1 2518 genotypes, there was a statistically significant difference in alleles and MCP-1 2518 between the groups analyzed ($p \leq 0.001$ & $p=.003$ respectively). Both SBP and non-SBP groups studied showed statistically significant differences in MCP-1 2518 genotypes and alleles ($p=0.037$& $p= .038$ respectively). Moreover, SBP and control groups had significantly different MCP-1 2518 genotypes and alleles, ($p \leq 0.001$ & $p=.003$ respectively). Ascitic individuals with AG genotype had a 5.24-fold increased chance of developing SBP, compared to those with the GG genotype (COR=0.46). Ascitic patients who carry the G allele are less likely to develop SBP (COR=0.45)**.**

Conclusion: Patients with spontaneous bacterial peritonitis have statistically significant difference in MCP-1 2518 genetic polymorphism and AG genotype increases risk of SBP while G allele significantly decreased risk of it.

Keywords: Spontaneous bacterial peritonitis, Hepatitis C virus, Genetic polymorphisms, Monocyte chemotactic protein-1.

INTRODUCTION

Cirrhosis is the last stage of various chronic hepatic disorders, and it is marked by fibrosis, morphological conversion from normal hepatic architecture to structural aberrant nodules, and a disruption of normal liver vasculature. Compensated and decompensated cirrhosis are the two clinical forms. Signs of decompensation include jaundice, ascites, variceal bleeding, spontaneous peritonitis, and hepatic encephalopathy **(1).** More than a quarter of people with decompensated cirrhosis are at risk of acquiring bacterial peritonitis, an infection that can be fatal if left untreated. A neutrophil ascites counts more than $250/mm^3$ is indicative of the condition. In this regard, it is critical to understand how macrophages and monocytes infiltrate cirrhotic patients' ascites and become activated **(2).**

Monocyte chemotactic protein-1 is efficient chemokine for activated lymphocytes, macrophages as well as monocytes during infections, which directly or indirectly affects neutrophil infiltration **(3).**

MCP-1 levels were shown to be higher in cirrhotic patients' ascites than in healthy controls in some investigations. It was also found that during SBP, MCP-1 levels in the ascites were much greater, showing that this strong chemokine is involved in the formation and progression of SBP **(4).** MCP-1 is a powerful mononuclear phagocyte activator. MCP-1 is a 76-amino acid protein with a molecular mass of 13 kDa that is found on human chromosome 17 (chr.17, q11.2). The distal regulatory region's transcriptional activity and monocyte MCP-1 production are both affected by the MCP-1 polymorphism -2518 G/A (rs 1024611). Since MCP-1 production by monocytes is affected by this polymorphism. Monocytes from people with the -2518 G allele generated more MCP-1 than those from A/A homozygous people. According to the results, cells from G/A homozygotes at -2518 produced significantly more MCP-1 than did G/G heterozygotes, which suggests that the G allele's activity is dose-dependent **(5).**

The aim of the work was to determine whether genetic variants of the MCP-1 are associated with spontaneous bacterial peritonitis (SBP) in individuals with post hepatitis C.

PATIENTS AND METHODS

At the Tropical Medicine Department at Zagazig University Hospitals, we conducted this case-control study from July 2021 to December 2021. Sixty patients with post-hepatitis C virus liver cirrhosis and ascites were included in the study. They were divided into two groups: Group I included 30 patients with SBP & group II that included 30 patients who had not been diagnosed with SBP. Thirty healthy participants were included as control group.

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Exclusion criteria: Cirrhotic patients due to other causes than hepatitis C, peritonitis due to other causes than spontaneous bacterial, cirrhotic patient with concomitant diseases that suppress the immune function as DM, autoimmune diseases, malignancy, chronic kidney disease or with history of immunosuppressive drugs administration were excluded.

The patients were subjected to the following:

Preliminary evaluation of the patient's past medical history, a thorough physical examination as well as regular lab investigations (complete blood count, liver and kidney function tests, coagulation profile, viral markers for hepatitis C and hepatitis B and PCR for HCV RNA). Ascitic fluid sample analysis chemically and bacteriologically to diagnose spontaneous bacterial peritonitis through count polymorphonuclear leukocytes >\=250 cell/ml, culture for isolated single organism, and transabdominal ultrasound was done for all patients.

Detection of MCP-1 gene polymorphism: By PCR-RFLP method [6].

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

SPSS version 25 was used to analyze the data. The Shapiro Walk test was used to determine if the data were normal. There were two methods used to determine the difference between qualitative variables: Chi square (χ^2) and Fisher exact. ANOVA F-test was used to compare quantitative variables across many groups. Kruskall Wallis and Mann-Whitney tests were used. Using the Crude Odds Ratio (COR), researchers were able to determine whether exposure had any effect on the outcome. HSD (honestly significant difference) tests were used to identify means that are substantially different from one other, and Pairwise comparisons were used to assess numerous populations means in pairs to see if they are significantly different from one another. In order to be considered significant, the P-value needed to be equal to or less than 0.05 and highly significant when lower than 0.001.

RESULTS

Patient characteristics were represented in table **(1).** There was no significant differences in age or sex between the SBP, non-SBP, and control groups. Ascites and hepatic encephalopathy were not significantly different between SBP and non-SBP. Study participants were found to be predominantly child-Pugh class C. (76.7 percent), but there was no significant difference between SBP and non SBP groups as regard child-Pugh class ($p = 0.165$), which denoted that both groups were similar as regarding the severity of liver disease.

Patients' laboratory test results were listed in the table **(2).** A statistically significant variation in white blood cell counts existed across the groups examined. On doing pairwise comparison, the difference was significant between non-SBP group and each other group (significantly higher in non-SBP group), but the difference was non-significant between SBP and control groups. A substantial statistical difference existed between several groups in terms of AST, ALT, total bilirubin, hemoglobin, platelet count, and albumin as well as INR. While statistically there was no significant difference between the studied groups regarding serum creatinine.

On studying MCP-1 gene polymorphism **(tables 3 & 4),** there was statistically highly significant difference between the studied groups regarding MCP-1 2518 genotypes and statistically significant difference regarding alleles. Also, there was statistically significant difference between the studied groups (SBP and non-SBP groups) regarding MCP-1 2518 genotypes and alleles. Moreover, there was statistically highly significant difference between the studied groups (SBP and control groups) regarding MCP-1 2518 genotypes and statistically significant difference regarding alleles. Ascitic individuals with the AG genotype had a 5.24-fold increased chance of developing spontaneous bacterial peritonitis, compared to those with the GG genotype (COR=0.46). Ascitic patients who carried the G allele were less likely to develop spontaneous bacterial peritonitis (COR=0.45).

Table (1): Info on the patients' demographics and clinical presentation

Parameter		Groups			Test	
		SBP group	Non-SBP group	Control group	F/χ^2	p
		N=30 (%)	N=30 (%)	N=30 (%)		
Age (year): Mean ± SD		58.97 ± 4.55	59.33 ± 4.76	58.6 ± 2.93	0.233	0.793
Sex:						
Male		19 (63.3%)	15 (50%)	15 (50%)	1.434	0.488
Female		11 (36.7%)	15 (50%)	15 (50%)		
Clinical finding	**Ascites:**					
	No	0 (0%)	0 (0%)	30 (100%)	92.644	<0.001**
	Moderate	14 (46.7%)	9 (30%)	0 (0%)		
	Tense	16 (53.3%)	21 (70%)	0 (0%)		
	p§	P_1 0.184	P_2 <0.001**	P_3 <0.001**		
	Hepatic encephalopathy:					
	No	14 (46.7%)	20 (66.7%)	30 (100%)	MC	<0.001**
	Grade I	5 (16.7%)	5 (16.7%)	0 (0%)		
	Grade II	7 (23.3%)	3 (10%)	0 (0%)		
	Grade III	3 (10%)	2 (6.7%)	0 (0%)		
	Grade IV	1 (3.3%)	0 (0%)	0 (0%)		
	p§	P_1 0.134	P_2 <0.001**	P_3 <0.001**		
	Child Pugh class:					
	B	7 (23.3%)	12 (40%)	-	-	-
	C	23 (76.7%)	18 (60%)			
		P1 0.165	-	-	-	-

F One way ANOVA test, χ^2Chi square test, MC Monte Carlo test §Chi square for trend test **p≤0.001 is statistically highly significant P1 difference between SBP and non-SBP groups P2 difference between non-SBP and control groups p3 the difference between SBP and control groups.

Table (2): Laboratory data of the patients

Parameter	Groups			Test	
	SBP group	Non-SBP group	Control group	KW	P
	Median(range)	Median(range)	Median(range)		
WBCs (10^3/ul)	8.85 (2.6 – 23.7)	5.15 (1.6 – 15.5)	5.75 (4 – 10.5)	9.192	0.01*
Pairwise	P_1 0.009*	P_2 <0.001**	P_3 0.139		
S creatinine (mg/dl)	0.96 (0.5 – 5.2)	1.05 (0.4 – 4)	0.8 (0.2 – 1.4)	0.233	0.793
AST (U/l)	46.5 (11.2 –196)	37 (15 – 205)	19 (10 – 34)	43.028	<0.001**
Pairwise	P_1 >0.999	P_2 <0.001**	P_3 <0.001**		
ALT(U/l)	26.5 (13 – 99)	21 (9 – 188)	18 (10 – 30)	14.156	<0.001**
Pairwise	P_1 0.454	P_2 0.065	P_3 0.001**		
T. bilirubin (mg/dl)	3.7 (0.6 – 13)	1.85 (0.5 – 13.5)	0.9 (0.4 – 1.6)	45.877	<0.001**
Pairwise	P_1 0.081	P_2 <0.001**	P_3 <0.001**		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**F**	**P**
Hemoglobin (g/dl)	9.61 ± 1.39	9.15 ± 2.08	13.25 ± 1.44	54.325	<0.001**
Turkey	P_1 0.532	P_2 <0.001**	P_3 <0.001**		
Platelet (10^3/ul)	68.07 ± 16.73	65.93 ± 18.68	218.97 ± 41.68	292.853	<0.001**
Turkey	P_1 0.953	P_2 <0.001**	P_3 <0.001**		
S albumin (g/dl)	2.31 ± 0.46	2.4 ± 0.37	4.04 ± 0.41	167.704	<0.001**
Turkey	P_1 0.643	P_2 <0.001**	P_3 <0.001**		
INR	1.82 ± 0.58	1.54 ± 0.35	1.01 ± 0.11	32.313	<0.001**
Turkey	P_1 0.019*	P_2 <0.001**	P_3 <0.001**		

KW Kruskal Wallis test ,F One way ANOVA test **p≤0.001 is statistically highly significant ,P1 difference between SBP and non-SBP groups, P2 difference between non-SBP and control groups, p3 the difference between SBP and control groups.

Table (3): Comparison between the studied groups regarding MCP-1 2518- genotypes and alleles

MCP-1 2518–	Groups			Test	
	SBP group	Non-SBP group	Control group	χ^2	P
	N=30 (%)	N=30 (%)	N=30 (%)		
Genotype: AA AG GG	3 (10%) 22 (73.3%) 5 (16.7%)	5 (16.7%) 7 (23.3%) 18 (60%)	16 (53.3%) 12 (40%) 2 (6.7%)	38.147	<0.001**
Alleles: A G	28 (46.7%) 32 (53.3%)	17 (28.3%) 43 (71.7%)	44 (73.3%) 16 (26.7%)	8.487§	0.003*

Table (4): Comparison in between the studied groups regarding MCP-1 2518- genotypes and alleles with risk assessment

MCP-1 2518–	SBP group N=30 (%)	Non-SBP group N=30 (%)	P	COR (95% CI)
Genotype: AA AG GG	3 (10%) 22(73.3%) 5 (16.7%)	5 (16.7%) 7 (23.3%) 18 (60%)	0.037*	1 (reference) 5.24 (0.99 – 27.69) 0.46 (0.08 – 2.63)
Alleles: A G	28(46.7%) 32(53.3%)	17 (28.3%) 43 (71.7%)	0.038*	1 (reference) 0.45 (0.21 – 0.96)*
	SBP group N=30 (%)	**Control group N=30 (%)**		
Genotype: AA AG GG	3 (10%) 22(73.3%) 5 (16.7%)	16 (53.3%) 12 (40%) 2 (6.7%)	<0.001**§	1 (reference) 9.78 (2.36 – 40.4)* 13.3 (1.71 – 103.8)*
Alleles: A G	28(46.7%) 32(53.3%)	44 (73.3%) 16 (26.7%)	0.003*	1 (reference) 3.14 (1.46 – 6.75)*
	Non-SBP group N=30 (%)	**Control group N=30 (%)**		
Genotype: AA AG GG	5 (16.7%) 7 (23.3%) 18 (60%)	16 (53.3%) 12 (40%) 2 (6.7%)	<0.001**	1 (reference) 1.87 (0.47 – 7.35) 28 (4.89 – 169.55)*
Alleles: A G	17 (28.3%) 43 (71.7%)	44 (73.3%) 16 (26.7%)	<0.001**	1 (reference) 6.96 (3.12 – 15.51)*

DISCUSSION

A common consequence of decompensated cirrhosis, spontaneous bacterial peritonitis occurs in about 25% of patients and is linked with considerable mortality [2]**.** Monocyte chemotactic protein-1 (MCP-1) is efficient chemokine for activated lymphocytes, macrophages as well as monocytes during infections, which directly or indirectly affects neutrophil infiltration [3, 4]. An investigation of the relationship between genetic variation of monocyte chemotactic protein-l and the risk of peritonitis in decompensated post-hepatitis C cirrhotic patients was carried out in this work.

This study found no significant differences in age or sex between the SBP, non-SBP, and control groups. Ascites and hepatic encephalopathy were not significantly different between SBP and non-SBP. Study participants were found to be predominantly child-Pugh class C. (76.7 percent), but there was no significant difference between SBP and non SBP groups as regard child-Pugh class (p = 0.165), which denoted that both groups were similar as regarding the severity of liver disease. The findings of **Cirera and his colleagues** [7] were consistent with this one**,** they conducted a study on 136 SBP patients and reported that about 70% of patients who developed SBP had Child class C. Also, similar to **Mohammed *et al.*** [8] who reported that the majority of SBP patients (66.7%) were children Pugh class C, as were 23.4% of those in the non-SBP group, and no statistical significance could be seen between the two groups. Regarding hemoglobin and platelet count, liver enzymes, total bilirubin, serum albumin and INR, SBP and non-SBP groups had no statistically significant difference, according to our research. Concerning creatinine, we could not find statistically significant difference among the three studied groups. These findings are consistent with **Zalam *et al.*** [9] unlike to **Mohammed *et al.*** [8]**,** they found that patients with SBP had considerably higher serum creatinine levels than non-SBP patients.

SBP patients' ascitic fluid contained chemokine MCP1 that activates activated lymphocytes, monocytes, macrophages and natural killer cells [10]**.** In addition to secreting pro-inflammatory cytokines, these cells help to maintain the current inflammatory state. The role of MCP1 polymorphism in a variety of viral and inflammatory disorders has been investigated [11]. Its significance in chronic liver disease inflammation has been well-documented. After treatment, its gene expression has been demonstrated to decrease, highlighting its significance in the pathophysiology of SBP [4]**.**

Functional and disease-related differences between -2518 MCP-1 genotypes AA and non-AA have been previously documented by in vitro and epidemiological studies (i.e., carriers of the G-allele: genotypes AG and GG). Individuals with no G-allele (AA homozygotes) were compared to those who had the G-allele (G carriers) in later studies (genotypes GG as well as AG) [12].

Regarding MCP-1 2518- genotypes and alleles among the SBP and non-SBP, the prevalence of AG genotype in SBP patients' group (22, 73.3%) was higher than the prevalence among non-SBP group 7 (23.3%) with high statistical significance between the two groups. This might lead to that AG genotype increases risk of spontaneous bacterial peritonitis in ascitic patients by 5.24 folds while GG genotype was considered as a protective factor where the prevalence of GG among SBP (5, 16.7%) was lower than non-SBP group (18, 60%) and the odds ratio (COR=0.46). The current study's findings were in line with **Gäbele *et al.*** [12] and **Salama *et al.*** [4]**.** In cirrhotics, AG polymorphism was found to be associated with an increased risk of SBP. **Murthy *et al.*** [13] reported that SBP had a greater prevalence of the AG genotype (47.6% AG). **Murthy *et al.*** [13] mentioned that in people with SBP, the AG/GG variation was 60% more common than in people who did not have SBP (39%) (P=0.02; OR=2.41, 95% CI: 1.0964-5.3156). Because of this, the AG/GG genotype was associated with a greater chance of developing SBP.

The results of this study could conclude that G allele significantly decreased the risk of spontaneous bacterial peritonitis in ascitic patients (COR=0.45). Unlike to the results of this study, **Gäbele *et al.*** [12] disagree as SBP patients had a lower frequency of G-allele carriers, although this difference did not achieve statistical significance. However, they agree with the conclusion that SBP patients had a lower frequency of G-allele carriers than patients without SBP, and the statistical significance of the discrepancy was clearly evident. **Murthy *et al.*** [13] reported that patients with SBP were found to have a higher percentage of G alleles (36.5%) with statistically significant difference and odds ratio (OR) =1.955, 95% confidence interval (CI).

The current study revealed that the prevalence of AG genotype was higher in SBP (22, 73.3%) than those of control (12, 40%) indicating that AG genotype increased risk of SBP in healthy patients if they developed end-stage liver disease by 9.78 folds and GG genotype significantly increased that risk by 13.3 folds, with highly statistical significance between the two groups ($p<0.001$). We also concluded that G allele significantly increased risk of spontaneous bacterial peritonitis in healthy patients if they developed end-stage liver disease by 3.14 folds.

The comparison of genotypes and alleles prevalence between non-SBP and control groups showed that AG genotype increased risk of developing ascites in healthy patients if they developed end-stage liver disease by 1.87 folds and GG genotype and G allele significantly increased that risk by 28 folds and 6.96 folds respectively. **Mühlbauer *et al.*** [14] agree with the results when the 2518 MCP-1 polymorphism in chronic hepatitis C patients and healthy controls was compared, as they identified higher prevalence of AG

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polymorphism among hepatic patients 91 (44.2%) than those of healthy subjects 59 (43.1%). SBP risk can be identified in cirrhotic patients through the genotyping of monocyte chemotactic protein (MCP) in those who are at elevated risk [(13)]. As a result, early antibiotic treatment of individuals with liver cirrhosis could assist to reduce the mortality of this severe condition.

CONCLUSION

Patients with spontaneous bacterial peritonitis have statistically significant difference in MCP-1 2518 genetic polymorphism. AG genotype increases risk of spontaneous bacterial peritonitis. On the other hand G allele significantly decreased risk of SBP. This indicates that it may has a protective role against development of SBP. Further studies may be needed to confirm the relation between MCP-1 2518 polymorphism and patients with SBP.

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