Microbes and Infectious Diseases 2023; 4(4): 1383-1395

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

The study of monocyte chemoattractant protein-1 gene expression and ascetic fluid calprotectin biomarker levels in spontaneous bacterial peritonitis patients with liver cirrhosis

*Amany Mohammed Adwy [1], Ahmed Mostafa Ashmawy [2] , Mohammed Saad Badary [1] , Yossra Mahmoud Mohammed kamel [3], Mai Ahmed Mustafa [*4], Amal Ahmed Mahmoud Elkhawaga [1]*

1- *Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt*
2- *Department of Internal Medicine, Gastroenterology and Hepatology Unit, Faculty of Medicine, Assiut University, Assiut, Egypt*
3- *Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt*
4- *Department of Microbiology and Immunology, Faculty of pharmacy, Assiut University, Egypt.*

ARTICLE INFO

Article history:
Received 3 August 2023
Received in revised form 19 September 2023
Accepted 21 September 2023

Keywords:
ascitic fluid
calprotectin
Monocyte chemoattractant protein-1
(MCP-1)

ABSTRACT

Background and rationale: Spontaneous bacterial peritonitis (SBP) stands as a primary complication and the most common bacterial infection in individuals suffering from decompensated liver cirrhosis. It is linked to substantial morbidity and mortality rates. The study aimed to determine the level of MCP-1 gene expression in the ascitic fluid of liver cirrhotic patients with or without SBP to evaluate its role in SBP diagnosis and to evaluate the role of ascitic fluid (AF) calprotectin as a diagnostic biomarker for SBP in liver cirrhosis (LC) patients. **Methods:** A cross-sectional hospital-based study was conducted at Internal Medicine, Gastroenterology and Hepatology Unit at AL-Rajhi Hospital in the period between 2020 and 2022. Participants were divided into two groups, a SBP group (n = 37) and a non-SBP group (LC patients with ascites only) (n = 48). AF levels of MCP-1, calprotectin and blood chemistry tests were estimated in all patients. **Results:** Both studied groups had no significant differences as regards demographic and laboratory data, except lower potassium levels among the SBP group. Monocyte chemoattractant protein-1 (MCP-1) was significantly higher among the SBP group (p= 0.000) compared to the non-SBP group. Also, both groups had significant differences as regards calprotectin (p= 0.01). Only three patients in the SBP group had positive ascitic fluid cultures (*Escherichia coli, Klebsiella pneumonia,* and *Acinetobacter baumannii*) while the non-SBP groups were negative for bacterial growth. Only in the control group, did MCP-1 have a positive correlation with serum albumin and total bilirubin. **Conclusion:** Ascitic fluid MCP-1 and calprotectin are considered promising biomarkers for early diagnosis of SBP.

Introduction

Spontaneous bacterial peritonitis (SBP) denotes an infection in the ascitic fluid, developing without any perforation in the hollow organs or intra-abdominal inflammation. Early identification of the condition and timely administration of suitable antibiotics have led to a decrease in mortality rates to approximately 20-30% [1].

However, due to the diverse clinical manifestations of SBP, delaying the initiation of

DOI: 10.21608/MID.2023.227066.1580
* *Corresponding author:* Mai Ahmed Mustafa
E-mail address: mai_92_mostafa@yahoo.com

antibiotic treatment is linked to a significant rise in mortality. While a positive AF culture remains the definitive method for diagnosing SBP, more than half of patients presenting symptoms suggestive of SBP and elevated AF polymorphonuclear leukocytic (PMNL) count yield negative cultures [1,2].

Numerous non-invasive approaches have been explored in various studies to serve as alternatives to diagnostic paracentesis, including clinical scoring systems, fecal calprotectin, and various serum inflammatory cytokines, e.g., monocyte chemoattractant protein-1 (MCP-1). Nevertheless, none of these approaches have demonstrated sufficient accuracy to replace diagnostic paracentesis [3,4].

The concentration of calprotectin is directly related to the severity of inflammation, both in plasma and stool. Plasma calprotectin levels increase significantly in infectious and inflammatory conditions [5].

Monocyte chemoattractant protein-1 (MCP-1) is an effective chemokine that plays a role in activating lymphocytes, macrophages, and monocytes during infections, influencing the infiltration of neutrophils, either directly or indirectly. Certain studies have indicated higher levels of MCP-1 in the ascites of cirrhotic patients compared to healthy individuals. Moreover, during SBP, the levels of MCP-1 in the ascites were found to be significantly elevated, suggesting the significant involvement of this potent chemokine in the development and progression of SBP [6,7].

The ascitic fluid of SBP patients contains the chemokine MCP-1, which plays a crucial role in activating lymphocytes, monocytes, macrophages, and natural killer cells. These cells not only secrete pro-inflammatory cytokines but also contribute to sustaining the ongoing inflammatory condition. Researchers have explored the role of MCP-1 polymorphism in various viral and inflammatory disorders, and its importance in inflammation associated with chronic liver disease has been extensively studied and documented. Moreover, following treatment, a decrease in its gene expression has been observed, underscoring its significance in the pathophysiology of SBP [8-10].

The study aimed to determine the level of MCP-1 gene expression in the ascitic fluid of liver cirrhotic patients with or without SBP to evaluate its role in SBP diagnosis and to evaluate the role of AF calprotectin as a diagnostic biomarker for SBP in LC patients and its correlation to MCP-1 gene expression.

Patients and methods

Study setting and design

A cross-sectional hospital-based study was conducted at Department of Internal Medicine, Gastroenterology and Hepatology Unit at AL-Rajhi Liver Hospital, Assiut University, in the period between November 2020 and March 2022.

Participants

A total of 85 potentially eligible decompensated LC patients with ascites were recruited during the study period.

Sample size calculations

Sample size calculation was done using MS Excel Sample Size Calculator for Diagnostic Test Studies [11].

Ethical consideration

The current study was approved by the Ethical Committee of the Faculty of Medicine, Assiut University with IRB no: 1701291. Only those who consent to participate after descriptions were enrolled in the study according to the declaration of Helsinki.

Patients' criteria

Enrollment of the patients was based on the following criteria.

Inclusion criteria

Adult patients over 18 years old were hospitalized due to cirrhosis with moderate to severe ascites (Child class late B or C) [12], confirmed through ultrasonography. They were admitted for various reasons such as SBP (based on clinical suspicion), bleeding varices, and hepatic encephalopathy. The patients were split into two groups: the SBP group (n=37) and the non-SBP group (LC patients with ascites only) (n=48).

The diagnosis of SBP was established based on the ascitic fluid polymorphonuclear leukocyte count (AF-PMNL) being equal to or exceeding 250/mm3, with or without a positive culture for pathogenic bacteria in the ascitic fluid. If both criteria were not met, it indicated that the ascites were free from microorganisms [13,14].

Exclusion criteria

Patients with infections other than ascitic fluid infection.

Patients with hepatocellular carcinoma.

Patients who had received antibiotics within 10 days before their hospital admission.

Patients received anticoagulant medications, oral contraceptive drugs, and nonsteroidal anti-inflammatory drugs before hospital admission.

Other unrelated possibilities that may affect the levels of cytokines were excluded, e.g., inflammatory conditions and infection

Patients who declined to participate in the study or refused to provide their consent were also excluded from the study [13].

Blood chemistry test (routine work)

Participants underwent a thorough medical examination. Different Laboratory tests were measured for all participants including AST, ALT, bilirubin, serum albumin, and creatinine. Prothrombin time (PT) and international normalized ratio (INR) were analyzed. A complete blood count (CBC) was determined using the ABX Pentra XL 80 Hematology analyzer, China.

Diagnostic paracentesis

paracentesis was performed applying aseptic techniques and standards precautions after local anesthesia was applied.

Ascitic fluid (AF) tests (cellular and chemistry)

The aspirated AF was divided into 2 tubes; one tube was for culture and sensitivity testing, while the second containing ethylenediaminetetraacetic acid (EDTA), was subjected to biochemistry analysis and leukocyte counts [15].

The ascitic fluid was analyzed for color, turbidity, leukocyte count, PMN count, protein, and albumin levels, and subjected to microbiological examination.

Microbiological examination

Ten mL of the ascitic fluid was and inoculated at bed side into aerobic BACT /ALERT FA Plus (USA) blood culture bottles and was loaded in Bac-T/Alert 3D (bioMérieux, france). Identification of isolated pathogen was done by colonial morphology, Gram staining and different Biochemical tests [16].

Other investigations to set exclusion criteria

Urine analysis to exclude urinary tract infections

Chest radiograph to exclude chest infection.

Abdominal computed tomography with intravenous contrast with alpha-fetoprotein to exclude the presence of HCC.

Measurement of MCP-1 gene expression

mRNA extraction

RNA was extracted from collected ascetic fluid using the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany. The purity and concentration of the RNA fraction were assessed using the biotech nanodrop from the USA. Reverse transcription and cDNA synthesis was conduct¬ed with using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, MA, USA).

QRT-PCR analysis for RNAs

Quantitative real-time PCR (qRT-PCR) was performed on 7500 fast real-time PCR (Applied Biosystems, USA) for RNAs. 18s RNA acted as an internal control, and all primers were made by (Thermo Fisher Scientific, MA, USA) the following:

Primers

Human mcp-1 (ccl2)

Forward Primer:

5’-AGTCTCTGCCGCCCTTCT-3’

Reverse Primer:

5’-GTGACTGGGGCATTGATTG-3’17

Human 18s rRNA

Forward Primer:

5’-GTAACCCGTTGAACCCCATT-3’

Reverse Primer:

5’-CCATCCAATCGGTAGTAGCG-3’18

Marker bioassay: Measurement of AF calprotectin

A 5 ml from the second tube of AF was collected from patients and kept at −20°C until calprotectin analysis which was measured using a sandwich ELISA (Elabscience® Human CALP(Calprotectin) ELISA Kit, USA).

Statistical analysis

The analysis was conducted using SPSS, version 22.0, from SPSS Inc., Chicago, IL, USA. Numerical data were presented as means ± standard deviation (SD), while categorical data were expressed as numbers and percentages. The appropriate tests, such as t-test, Mann–Whitney U test, Pearson's chi-squared test, and Pearson's correlation test, were utilized as deemed suitable. A result was considered significant if the *p*-value was less than or equal to 0.05.

Results

The current study enrolled 85 patients with liver cirrhosis in both SBP group (n = 37) and a non-SBP group (n = 48).

Baseline characteristics

Both studied groups had no significant differences as regards age, sex, and weight (p> 0.05). The majority of the studied patients were males **(Table 1).**

Blood chemistry test findings

Both groups did not have significant differences as regard different blood chemistry tests except for the SBP group had significantly lower potassium level in comparison to the non-SBP group (3.66 ± 0.50 vs. 4.00 ± 0.69 (mg/dl); p= 0.013) **(Table 2).**

Ascetic fluid analysis among the studied groups (Table 3).

The SBP group had significantly higher PMN in comparison to the non-SBP group (1259.05 ± 1898.88 vs. 77.15 ± 57.19 (cell/mm3); p= 0.000). Meanwhile, both groups had insignificant differences as regards AF albumin and protein

Correlation of MCP-1 gene expression and calprotectin biomarker among the studied groups (Table 4, Figures 1, 2)

It was noticed that MCP-1 gene expression was significantly higher among the SBP group (24.07 ± 26.76 vs. 1.68 ± 1.41; p= 0.000) and AF calprotectin also (70.65 ± 25.95 ng/ml vs. 88.87 ± 29.42 ng/ml; p= 0.01) as compared to the non-SBP group.

Ascetic fluid culture among the studied groups (Table 5)

Only three patients of the SBP group had a positive ascetic fluid bacterial culture in the form of *Escherichia coli* (was sensitive to meropenem, trimethoprim/sulfamethoxazole, and nitrofurantoin), *Klebsiella pneumonia* (was sensitive to tigecycline, ofloxacin, streptomycin, colistin, piperacillin/tazobactam) and *Acinetobacter baumannii* (was sensitive to colistin and tigecycline) while the non-SBP group had negative ascetic fluid culture.

Correlation of MCP1 and Calprotectin with variables in the Control Group (Table 6)

It was found that MCP-1 had a positive correlation with serum albumin (r= 0.321, p=0.026) and total bilirubin (r= 0.295, p= 0.042). Meanwhile, it has an insignificant correlation with other parameters (p> 0.05).

Correlation of MCP1 and Calprotectin with variables in study Group (Table 7)

It was found that MCP-1 had an insignificant correlation with different parameters in the SBP group (p> 0.05).

Table 1. Demographic data of the studied groups.

Personal data	Non-SBP patients (n= 48)	SBP patients (n= 37)	*P*-value
Age: (years)			
Mean ± SD	59.31 ± 12.61	59.32 ± 14.88	0.997
Sex: No. (%)			
Male	36 (75.0%)	33 (89.2%)	0.097
Female	12 (25.0%)	4 (10.8%)	
Weight: (kg)			
Mean ± SD	77.15 ± 15.50	80.22 ± 13.44	0.340

Data expressed as mean (SD), and frequency (percentage). *P* value was significant if < 0.05

Table 2. Blood chemistry data among the studied groups.

Liver functions	**Non-SBP (n= 48)**	**SBP (n= 37)**	***P*-value**
Albumin: (liver)(g/dl)			
Median (Range)	22.0 (1.1-45.0)	22.0 (1.3-40.0)	0.979
T. bilirubin: (mg/dl)			
Median (Range)	36.5 (0.7-457.0)	28.0 (0.6-416.0)	0.703
D. bilirubin: (mg/dl)			
Median (Range)	18.5 (0.3-325.0)	17.0 (0.3-304.0)	0.954
AST: (IU/L)			
Median (Range)	60.9 (16.0-1142.0)	73.4 (13.0-1058.0)	0.343
ALT: (IU/L)			
Median (Range)	39.5 (5.0-351.0)	43.0 (7.0-944.0)	0.828
ALP: (IU/L)			
Median (Range)	121.0 (56.0-783.0)	129.0 (53.0-631.0)	0.586
PT: (SEC)			
Mean ± SD	18.55 ± 5.84	18.99 ± 4.87	0.712
PC %:			
Mean ± SD	57.86 ± 20.97	51.15 ± 15.23	0.105
INR:			
Mean ± SD	1.59 ± 0.55	1.63 ± 0.43	0.754
Sodium (mmol/)			
Mean ± SD	134.76 ± 6.75	133.90 ± 7.07	0.573
Potassium (mg/dl)			
Mean ± SD	4.00 ± 0.69	3.66 ± 0.50	0.013*
Magnesium (mmol/l)			
Mean ± SD	1.91 ± 0.37	2.03 ± 0.54	0.220
Calcium (mg/dl)			
Mean ± SD	8.23 ± 0.79	8.11 ± 1.12	0.585
Phosphorus (mmol/l)			
Median (Range)	4.0 (0.0-9.8)	3.2 (1.9-8.9)	0.068
Creatinine (umol/L):			
Median (Range)	139.0 (14.0-637.0)	97.0 (33.0-508.0)	0.317
Urea (mg/dL):			
Median (Range)	13.8 (2.7-43.2)	10.3 (2.3-39.5)	0.306
Hemoglobin (g/dl)			
Mean ± SD	10.27 ± 2.66	10.87 ± 2.54	0.297
Platelets (10^3/ul)			
Median (Range)	136.5 (38.0-700.0)	155.0 (17.0-429.0)	0.873
Leucocytes (10^3/ul)			
Median (Range)	8.2 (0.6-33.0)	7.9 (1.3-27.0)	0.492

Data expressed as median (range), mean (SD). *P* value was significant if < 0.05. ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; PT: prothrombin time; PC: prothrombin concentration; INR: international randomized ratio

Table 3. Ascetic fluid analysis among the studied groups.

Ascetic fluid analysis	**Non-SBP (n= 48)**	**SBP (n= 37)**	***P*-value**
PMN/uL:			
Median (Range)	61.0 (4.0-202.0)	500.0 (280.0-8718.0)	0.000
Albumin(g/dL):			
Median (Range)	4.0 (0.0-31.0)	5.0 (0.2-31.0)	0.277
Protein(g/dL):			
Median (Range)	11.4 (0.0-53.0)	11.5 (1.0-53.0)	0.811

Data expressed as median (range),*P* value was significant if< 0.05 , PMN: polymorphonuclear cells

Table 4. Biochemical biomarkers among the studied groups

Biochemical biomarker	**Non-SBP (n= 48)**	**SBP (n= 37)**	***P*-value**
MCP1 expression (fold change)			
Median (Range)	1.3 (0.3-5.8)	12.6 (1.8-101.3)	0.000*
Calprotectin level (ng/dL):			
Median (Range)	85.5 (5.6-116.1)	90.2 (17.3-138.3)	0.01

Data expressed as median (range), *P* value was significant if < 0.05.

Table 5. Ascetic fluid culture among the studied groups.

	Non-SBP (n= 48)		**SBP (n= 37)**		***P*-value**
	No.	**%**	**No.**	**%**	
Culture:					0.079
Negative	48	100.0%	34	91.9%	
Positive	0	0.0%	3	8.1%	
Type of micro-organism:					--
E-coli	--	--	1	33.3%	
Klebsiella pneumonia	--	--	1	33.3%	
Acinetobacter baumannii	--	--	1	33.3%	

Data expressed as frequency (percentage). *P* value was significant if < 0.05

Table 6. Correlation of MCP-1 and calprotectin with variables in the control group

	MCP-1		**Calprotectin**	
	r-value	***P*-value**	**r-value**	***P*-value**
Age (years)	0.167	0.257	0.048	0.747
Weight (kg)	0.107	0.471	0.060	0.688
Albumin (liver)	**0.321***	**0.026***	0.027	0.857
T. bilirubin	**0.295**	**0.042***	-0.092	0.532
D. bilirubin	0.228	0.119	-0.127	0.389
AST	-0.145	0.327	-0.182	0.216
ALT	-0.050	0.738	-0.069	0.643
ALP	-0.100	0.499	0.069	0.640
PT	0.136	0.356	-0.109	0.460
PC %	-0.106	0.475	0.152	0.304
INR	0.100	0.500	-0.105	0.476
Creatinine	0.084	0.572	0.028	0.851
Urea	0.189	0.197	-0.041	0.781
Sodium	-0.096	0.514	0.001	0.996
Potassium	0.112	0.450	-0.183	0.212
Magnesium	0.171	0.244	-0.191	0.193
Calcium	0.081	0.582	-0.089	0.547
Phosphorus	0.179	0.223	-0.077	0.605
Hemoglobin	0.122	0.407	-0.144	0.328
Platelets	-0.142	0.334	0.067	0.649
Leucocytes	-0.132	0.370	-0.020	0.891
PMN	-0.178	0.225	0.161	0.275
Albumin(ascetic fluid)	0.019	0.896	0.022	0.884
Protein	-0.081	0.586	-0.131	0.376

Data is expressed as *r* value and *p* value. *P* value was significant if < 0.05. ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; PT: prothrombin time; PC: prothrombin concentration; INR: international randomized ratio; PMN: polymorphonuclear cells

Table 7. Correlation of MCP-1 and calprotectin with variables in study group

	MCP1		**Calprotectin**	
	r-value	***P*-value**	**r-value**	***P*-value**
Age (years)	0.178	0.291	0.196	0.245
Weight (kg)	0.168	0.319	-0.217	0.198
Albumin (liver)	0.064	0.705	0.044	0.794
T. bilirubin	0.269	0.107	-0.138	0.414
D. bilirubin	0.283	0.089	-0.179	0.290
AST	0.283	0.090	0.046	0.788
ALT	0.233	0.166	0.113	0.504
ALP	0.155	0.359	-0.071	0.676
PT	-0.030	0.858	-0.047	0.783
PC %	0.052	0.760	0.057	0.739
INR	-0.034	0.843	-0.051	0.764
Creatinine	-0.054	0.751	-0.139	0.413
Urea	-0.109	0.523	-0.142	0.402
Sodium	0.001	0.994	-0.003	0.987
Potassium	-0.244	0.145	0.132	0.435
Magnesium	-0.081	0.632	-0.117	0.490
Calcium	-0.181	0.284	-0.061	0.718
Phosphorus	-0.220	0.190	0.094	0.579
Hemoglobin	-0.184	0.275	0.057	0.737
Platelets	0.160	0.345	0.271	0.105
Leucocytes	-0.011	0.950	-0.131	0.441
PMN	0.154	0.364	-0.034	0.841
Albumin (ascetic fluid)	-0.121	0.474	0.200	0.235

Data is expressed as *r* value and *p* value. *P* value was significant if < 0.05. ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; PT: prothrombin time; PC: prothrombin concentration; INR: international randomized ratio; PMN: polymorphonuclear cells

Figure 1. MCP-1 gene expression in the SBP group compared to the non-SBP group.

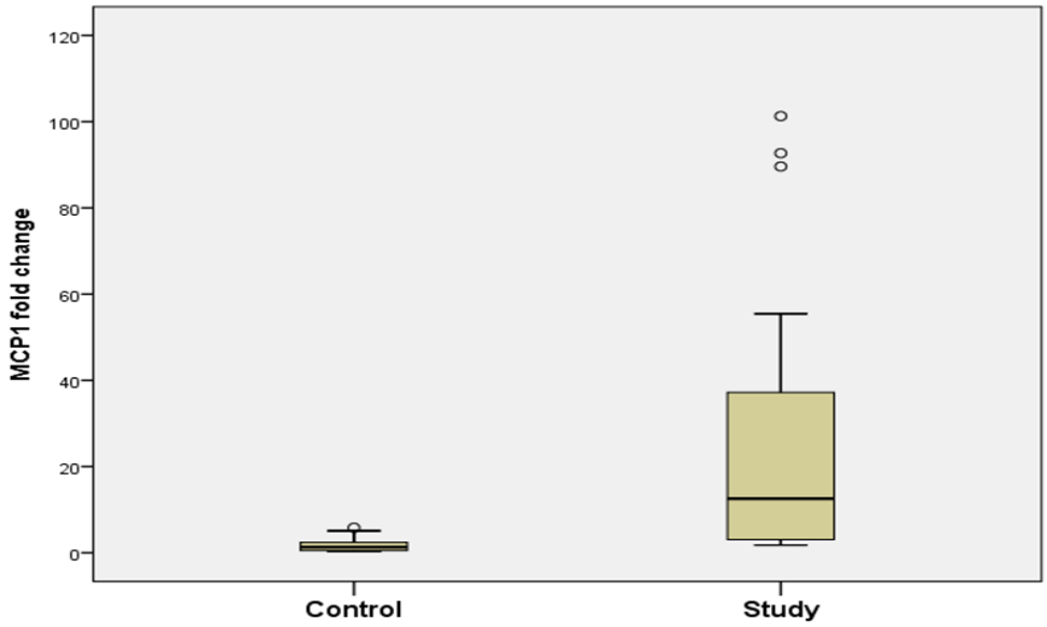
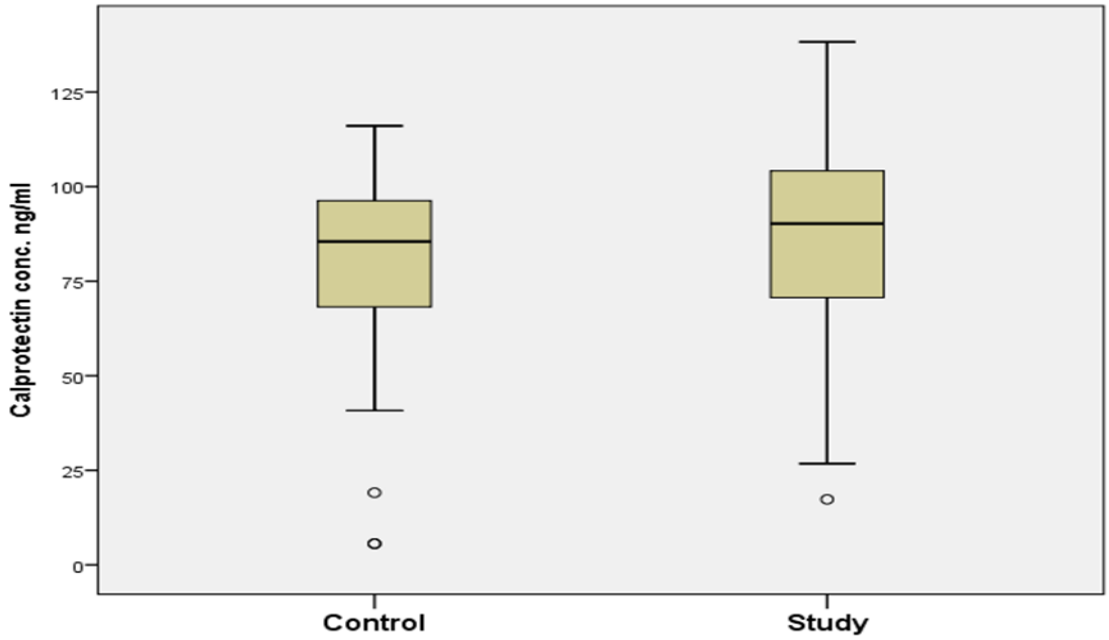


Figure 2. Ascetic fluid calprotectin levels in the SBP group compared to the non-SBP



Discussion

In previous decades, SBP was linked to a mortality rate exceeding 90%. However, with advancements in prompt diagnosis and appropriate therapy, the mortality rate has significantly decreased to approximately 20% in recent times. SBP causes abdominal pain with fever, but can also show other symptoms like vomiting and inflammatory signs. It can lead to deterioration of liver or kidney functions and cause hepatic encephalopathy [19,20].

The current study enrolled a total of 85 patients with decompensated LC, at Department of Internal Medicine, Gastroenterology and Hepatology Unit, 48 patients had ascites without manifestations of SBP (non-SBP group) and 37 patients with ascites with SBP (SBP group).

Baseline data as regards age, sex, and weight showed no significant differences between both groups with the mean ± SD age of SBP being 59 ± 32 years, In agreement with, **Farrugia et al.** [21]. Also, another study found that the majority of patients were males, moreover, SBP and non-SBP show no significant differences as regards age, sex, and amount of ascites [21]. **Mosalem et al.** found that, no significant differences between patients with SBP (n= 32) and those without SBP (n= 32) as regard age, sex, and etiology of liver cirrhosis [22].

SBP is more common in males (65%) than females (35%) with a mean age of 55.55±8.94 years, which is lower than non-SBP and higher than in SBP groups (p=0.000) [11]. This is similar to **Syed et al.** who found that higher ages have more chance of infection in those patients [23].

As regards blood chemistry test, the current study revealed that both groups had no significant differences in liver function tests, kidney function tests, serum electrolytes, and complete blood count except for significantly lower serum potassium among the study group. In agreement with our study, **Mosalem et al.** found that, no significant differences between patients with SBP and those without SBP as regard baseline laboratory data [22, 24-28].

Another finding in this study was that the SBP group had significantly higher PMN in comparison to the non-SBP group (1259.05 ± 1898.88 vs. 77.15 ± 57.19 (cell/mm3); p= 0.000). Meanwhile, both groups had insignificant differences concerning ascetic fluid albumin (6.01 ± 6.10 vs. 6.68 ± 5.74 (g/dl); p= 0.277) and protein (14.37 ± 11.05 vs. 14.21 ± 9.99 (g/dl); p= 0.811). These findings were agreed with many previous studies [24-29].

The ascitic fluid of SBP patients contains the chemokine MCP-1, which plays a crucial role in activating lymphocytes, monocytes, macrophages, and natural killer cells. These cells not only secrete pro-inflammatory cytokines but also contribute to sustaining the ongoing inflammatory condition.

Researchers have explored the role of MCP-1 polymorphism in various viral and inflammatory disorders, and its importance in inflammation associated with chronic liver disease has been extensively studied and documented. Moreover, following treatment, a decrease in its gene expression has been observed, underscoring its significance in the pathophysiology of SBP [8,30,31].

The current study found that AF level of MCP-1 was significantly higher among the SBP group (24.07 ± 26.76 vs. 1.68 ± 1.41; p= 0.000) in comparison to the non-SBP group. Also, both groups had significant differences as regards calprotectin (70.65 ± 25.95 ng/ml vs. 84.87 ± 29.42 ng/ml ; *p*= 0.01).

The study by Mohamed et al. (2022) demonstrated that MCP-1 concentration in the ascitic fluid was significantly higher in SBP patients compared to non-SBP cases, and MCP-1 and PMNL had a significant correlation [32]. **Salama et al.** and **El-Toukhy et al.** reported similar results. They found that MCP-1 levels were greater in SBP compared to sterile ascites [24, 26]. Additionally, **Kim et al.** also observed higher levels of MCP-1 in SBP patients, and after treatment, these levels rapidly decreased.

Calprotectin is found in plasma and stool as well and becomes stimulated during inflammation, with its concentration directly linked to the severity of the inflammatory response. Studies have revealed that its level in plasma increases significantly, ranging from 5 to 40 times, in the presence of infectious and inflammatory conditions [5].

Hadjivasilis et al. also noted that ascitic calprotectin could serve as a highly effective alternative to PMN for diagnosing SBP, offering quicker processing time. However, its practical application in routine clinical practice is restricted due to its higher cost [5]. This result was also, reported by many previous studies that revealed elevated ascetic calprotectin among patients with SBP [33-36].

The current study noticed that MCP-1 had a positive correlation with serum albumin (r= 0.321, *p*=0.026) and total bilirubin (r= 0.295, *p*= 0.042). Meanwhile, it has an insignificant correlation with other parameters (*p*> 0.05).

The current study also noticed that AF calprotectin, in agreement with previous research, that there was a correlation between AF calprotectin and different parameters among the studied groups. There was a positive correlation between AF calprotectin and main parameters including ascetic fluid leucocytes, and there was a negative correlation between AF calprotectin and the concentration of prothrombin and serum albumin [32, 37].

Lastly; we found that only three (8.1%) patients of the study group had a positive ascetic fluid culture in the form of *Escherichia coli, Klebsiella pneumonia, and Acinetobacter baumannii* while the control group had a negative ascetic fluid culture. According to **Elsadek et al.** ascitic fluid culture was positive in 41.67% (25/60) of the SBP group, while no cases of culture positivity were found in the non-SBP group (0/60). The most commonly encountered pathogens in the culture-positive cases were *Escherichia coli* in 68% and *Klebsiella* in 16% [15]. Similar findings have been frequently reported in previous studies [38-41].

Murthy et al. reported that positive ascetic fluid culture was found in 15/63 (23.8%) patients with SBP. The isolated microorganism was *Escherichia coli* (7/11, 63.6%), *Klebsiella pneumoniae* (4/11,36.4%), Coagulase-negative *Staphylococci* (2/11, 18.2%), *Streptococcus pneumonia* (1/11, 9.1%) and *Brevundimonas vesicularis* (1/11, 9.1%) [42].

According to **Mosalem et al.** , the ascitic fluid culture using a BACT/ALERT blood bottle was positive in 23 cases (71.9%) of SBP, while it was negative in 9 cases (28.1%). The most commonly isolated bacteria in the positive culture cases were *Escherichia coli* (10 - 31.3%), followed by *Klebsiella pneumoniae* (4 - 12.5%), *Staphylococcus aureus* (3 - 9.4%), *Enterococci* (3 - 9.4%), CONS (2 - 6%), and *Pseudomonas* (1 - 3.1%) [22]. This variation in the results of culture with different studies may be due to different culture methods, timing of culture, and history of antibiotics use.

The main limitations of our study included being conducted in a single center , and also that we didn't perform a longer duration of follow-up to assess the value of such biomarkers on patient's survival. Yet, the current study is considered the first study to discuss such issues in our locality.

Conclusion

Based on the data presented earlier, it can be inferred that ascitic fluid calprotectin and MCP-1 levels were notably elevated in SBP patients when

compared to individuals with non-infected ascites. This suggests that ascitic calprotectin and MCP-1 have the potential to be used as diagnostic markers for SBP. However, to establish more definitive conclusions, further multi-center studies are needed. These future studies will provide more robust evidence to support the utility of ascitic calprotectin and MCP-1 as reliable diagnostic tools for SBP.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding was used for the study.

Acknowledgments

There is nothing to declare.

References

1-**Cao NH, Ho PT, Bui HH, Vo TD.** Non-Invasive Methods for the Prediction of Spontaneous Bacterial Peritonitis in Patients with Cirrhosis. Gastroenterology Insights 2023;14(2):170-177.

2-**Kim JJ, Tsukamoto MM, Mathur AK, Ghomri YM, Hou LA, Sheibani S, et al.** Delayed paracentesis is associated with increased in-hospital mortality in patients with spontaneous bacterial peritonitis. The American journal of gastroenterology 2014;109(9):1436-1442.

3-**Wehmeyer MH, Krohm S, Kastein F, Lohse AW, Lüth S.** Prediction of spontaneous bacterial peritonitis in cirrhotic ascites by a simple scoring system. Scand J Gastroenterol 2014;49(5):595-603.

4-**Abdel-Razik A, Mousa N, Elbaz S, Eissa M, Elhelaly R, Eldars W.** Diagnostic utility of interferon gamma-induced protein 10 kDa in spontaneous bacterial peritonitis: single-center study. Eur J Gastroenterol Hepatol 2015;27(9):1087-1093.

5-**Hadjivasilis A, Tzanis A, Ioakim KJ, Poupoutsi I, Agouridis AP, Kouis P.** The diagnostic accuracy of ascitic calprotectin for the early diagnosis of spontaneous bacterial peritonitis: systematic review and meta-analysis. European Journal of Gastroenterology & Hepatology 2021;33(3).

6-**Salama MK, Sabry D, Al-Ghussein MA, Ahmed R, AbdAllah S, Taha FM, et al.** Molecular detection of monocyte chemotactic protein-1 polymorphism in spontaneous bacterial peritonitis patients. World journal of gastroenterology 2014;20(33):11793-11799.

7-**El-Toukhy N, Emam SM.** Diagnostic and Prognostic Values of Monocyte Chemotactic Protein-1 in Ascitic Fluid of Patients with Spontaneous Bacterial Peritonitis. Egypt J Immunol 2016;23(2):17-27.

8-**Mohammed M, Ibrahim W, Salama M, Abd El Kader A.** The ratio of calprotectin to total protein as a diagnostic marker for spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites 2019;6(7):1-10.

9- **Mohamed AA, Salah W, Hassan MB, Eldeeb HH, Adaroas AS, Khattab RA, et al.** MCP1, CRP and Procalcitonin as Novel Diagnostic Markers in Cirrhotic Patients with Spontaneous Bacterial Peritonitis 2022;12(1).

10-**Omar M, Mohammed SA.** Monocyte Chemotactic Protein-1 Gene Expression in Blood and Ascitic Fluid of Cirrhotic Patients with Spontaneous Bacterial Peritonitis. Afro-Egyptian Journal of Infectious and Endemic Diseases 2016;6(4):166-174.

11-**Tsoris A, Marlar CA.** Use of the Child Pugh score in liver disease.2019.

12-**Abdel-Razik A, Mousa N, Elhammady D, Elhelaly R, Elzehery R, Elbaz S, et al.** Ascitic fluid calprotectin and serum procalcitonin as accurate diagnostic markers for spontaneous bacterial peritonitis 2016;10(4):624.

13-**Burri E, Schulte F, Muser J, Meier R, Beglinger C.** Measurement of calprotectin in

ascitic fluid to identify elevated polymorphonuclear cell count 2013;19(13):2028.

14-**Elsadek HM, Elhawari SA, Mokhtar AA** novel serum index for accurate diagnosis of spontaneous bacterial peritonitis in cirrhotic patients without other infections. Egyptian Liver Journal 2020;10(1):10.

15-**Sajjad M, Khan ZA, Khan MS.** Ascitic Fluid Culture in Cirrhotic Patients with Spontaneous Bacterial Peritonitis. J Coll Physicians Surg Pak 2016;26(8):658-661.

16-**Liu Y, Upadhyaya B, Fardin-Kia AR, Juenemann RM, Dey M.** Dietary resistant starch type 4-derived butyrate attenuates nuclear factor-kappa-B1 through modulation of histone H3 trimethylation at lysine 27. 2016;7(9):3772-3781.

17-**Ali NM, Yeap SK, Abu N, Lim KL, Ky H, Pauzi AZM, et al.** Synthetic curcumin derivative DK1 possessed G2/M arrest and induced apoptosis through accumulation of intracellular ROS in MCF-7 breast cancer cells 2017;17:1-12.

18-**Oblokulov A, Oblokulov A, Ergashov M.** Clinical And Laboratory Criteria For Spontaneous Bacterial Peritonitis In Liver Cirrosis Of Viral Etiology. Central Asian Journal of Medical and Natural Science 2021;2(1):172-177.

19-**Abudeif A, Elbadry MI, Ahmed NM.** Validation of the diagnostic accuracy of neutrophil to lymphocyte ratio (NLR) and mean platelet volume (MPV) in cirrhotic patients with spontaneous bacterial peritonitis. Egyptian Liver Journal 2023;13(1):1-7.

20-**Farrugia MA, Le Corvec M, Renou C, Nousbaum JB, Ouizeman DJ, Sire O, et al.** Mid-Infrared Spectroscopy as a New Tool for Ruling Out Spontaneous Bacterial Peritonitis: A Proof-of-Concept Study. Biomedicines 2023;11(3):838.

21-**Mosalem M, Fayed SM, Ismail YM, Elfeky HM.** Ascitic Fluid Micro RNA 155 as a Biomarker for Spontaneous Bacterial Peritonitis. Egyptian Journal of Medical Microbiology 2022;31(4):45-52.

22-**Syed V, Ansari J, Karki P, Regmi M, Khanal B.** Spontaneous bacterial peritonitis (SBP) in cirrhotic ascites: a prospective study in a tertiary care hospital, Nepal. Kathmandu University Medical Journal 2007;5(1):48-59.

23-**Abu Rahma MZ, Mahran ZG, Shafik EA, Mohareb DA, Abd El-Rady NM, Mustafa MA, et al.** The role of serum procalcitonin level as an early marker of ascitic fluid infection in post hepatitic cirrhotic patients. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 2021;20(1):61-67.

24- **Filik L.** Monocyte chemotactic protein-1 gene polymorphism and spontaneous bacterial peritonitis. World Journal of Gastroenterology. 2010 Feb; 16(7):914. DOI: 10.3748/wjg.v16.i7.914.

25-**Kim JK, Chon CY, Kim JH, Kim YJ, Cho JH, Bang SM, et al.** Changes in serum and ascitic monocyte chemotactic protein-1 (MCP-1) and IL-10 levels in cirrhotic patients with spontaneous bacterial peritonitis. Journal of Interferon & Cytokine Research 2007;27(3):227-230.

26-**Elshayeb EIE, Helwa MA, Elenin MEMA, Badr MH.** Evaluation of serum and ascitic monocyte chemotactic protein-1 level in patients with and without spontaneous bacterial peritonitis. Menoufia Medical Journal 2021;34(3):798.

27-**Supriya U, Jinda A.** Diagnosis of Spontaneous Bacterial Peritonitis with Dipstick Test. The Journal of the Association of Physicians of India. 2023;71(1):1-1.

28-**El-Toukhy N, Emam SM.** Diagnostic and Prognostic Values of Monocyte Chemotactic Protein-1 in Ascitic Fluid of Patients with Spontaneous Bacterial Peritonitis 2016;23(2):17-27.

29-**Oblokulov A, Oblokulov A, Ergashov MM.** Clinical And Laboratory Criteria For Spontaneous Bacterial Peritonitis In Liver Cirrosis Of Viral Etiology 2021;2(1):172-177.

30-**M Singh S, Anshita D, Ravichandiran V.** MCP-1: Function, regulation, and involvement in disease. Int Immunopharmacol. 2021 Dec;101(Pt B):107598. doi: 10.1016/j.

31-**Nabiel Y, Barakat G, Abed S.** Serum CD64 and ascitic fluid calprotectin and microRNA-155 as potential biomarkers of spontaneous bacterial peritonitis. European Journal of Gastroenterology & Hepatology. 2019;31(8):1064-1069.

32-**Honar N, Nezamabadipour N, Dehghani SM, Haghighat M, Imanieh MH, Ataollahi, M, et al.** An evaluation of ascitic calprotectin for diagnosis of ascitic fluid infection in children with cirrhosis. BMC pediatrics. 2022;22(1):1-7.

33-**Shafik AO, Elgazzar AM, Elgazar AS, Mostafa MS, Elaraby M.** Evaluation of Ascitic Fluid Calprotectin as an Accurate Marker for Rapid Diagnosis of Spontaneous Bacterial Peritonitis in Patients with Chronic Liver Disease. Egyptian Journal of Medical Microbiology 2022;31(1):47-54.

34-**Ali ST, Mohamed NA.** The value of ascitic fluid calprotectin and calprotectin-to-albumin ratio in the diagnosis and prognosis of spontaneous bacterial peritonitis. The Scientific Journal of Al-Azhar Medical Faculty, Girls 2019;3(2):527-537.

35-**Ali ST, Mohamed NAE.** The value of ascitic fluid calprotectin and calprotectin-to-albumin ratio in the diagnosis and prognosis of spontaneous bacterial peritonitis. The Scientific Journal of Al-Azhar Medical Faculty, Girls 2019;3(2).

36-**Liver EAFTSOT.** EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. Journal of hepatology 2010;53(3):397-417.

37-**Runyon BA.** Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. Hepatology (Baltimore, Md) 2013;57(4):1651-1653.

38-**Lata J, Stiburek O, Kopacova M.** Spontaneous bacterial peritonitis: a severe complication of liver cirrhosis. World journal of gastroenterology: WJG 2009;15(44):5505.

39-**Wiest R, Krag A, Gerbes A.** Spontaneous bacterial peritonitis: recent guidelines and beyond. Gut 2012;61(2):297-310.

40-**Murthy KV, Subrahmanian M, Sairam T, Leelakrishnan V, Sankaran R.** Cirrhotics with Monocyte Chemotactic Protein 1 Polymorphism are at Higher Risk for Developing Spontaneous Bacterial Peritonitis - A Cohort Study. J Clin Transl Res 2021;7(3):320-325.

Adwy AM, Ashmawy AM, Badary MS, kamel YMM, Mustafa MA, Elkhawaga AAM. The study of monocyte chemoattractant protein-1 gene expression and ascetic fluid calprotectin biomarker levels in spontaneous bacterial peritonitis patients with liver cirrhosis. Microbes Infect Dis 2023; 4(4): 1383-1395.