

Diagnostic and Prognostic Values of Monocyte Chemotactic Protein-1 in Ascitic Fluid of Patients with Spontaneous Bacterial Peritonitis

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Spontaneous bacterial peritonitis (SBP) is a severe complication in cirrhotics with ascites. Monocyte chemotactic protein-1 (MCP-1) is a chemotactic factor for monocytes/macrophages, and it activates lymphocytes and neutrophils during infection. This study aimed to evaluate the role of MCP-1 in the pathogenesis of SBP and assess its prognostic value and correlation to disease severity. The study included ninety patients with liver cirrhosis and ascites. Patients were divided into 2 groups: Group I including 45 ascitic patients with SBP (polymorph nuclear cell count (PMN) ≥ 250 cell/mm³ in ascitic fluid), and Group II including 45 ascitic patients without SBP. Assessment of the severity of liver cirrhosis was done using the modified Child-Pugh and model for end stage liver disease (MELD) scores. Ascitic fluid samples were subjected to total leucocytic count and differential, albumin, protein, glucose, and serum-ascitic albumin gradient analysis. Ascitic fluid levels of MCP-1 was measured by ELISA. Higher level was detected in patients with SBP as compared to those without SBP. The number of polymorph nuclear cell count (PMN) ≥ 250 cell/mm³ in ascitic fluid) was used as gold standard for diagnosis of SBP. The diagnosis sensitivity and specificity of MCP level test were 86.7% and 95.4% respectively at cutoff of 122.5ng/ml with accuracy 91%. MCP-1 level showed positive significant correlation with TLC, PMN leucocytes and MELD score. In conclusion, ascitic fluid MCP-1 level could be a reliable test for diagnosis of SBP, and could be used as a prognostic marker due to its positive correlation with the severity of liver disease.

Spontaneous bacterial peritonitis (SBP) is the most frequent bacterial infection in cirrhosis, accounting for 10 to 30% of all reported bacterial infections in hospitalized patients (Wiest *et al.*, 2012).

It is also one of the potential life threatening complications in ascitic cirrhotic patients with a mortality rate ranging between 30 and 50% (Thevenot *et al.*, 2004).

In-hospital mortality for the first episode of SBP ranges from 10% to 50%, depending on various risk factors (Nobre *et al.*, 2008). One-year mortality after a first episode of SBP has been reported to be 31% and 93% (Wiest & Garcia-Tsao, 2005).

In clinical practice the diagnosis is based on a polymorph nuclear cell count (PMN) that must be greater than or equal to 250 cell/mm³ in ascitic fluid in absence of intra-abdominal cause of infection (Thuluvath *et al.*, 2001; Cardenas *et al.*, 2012). However, total

leukocytic and PMNs counts in ascitic fluid are not always readily available, the account of 250 cells/mm³ or more of PMN is highly indicative of SBP and is an indication for antibiotic therapy (Runyon, 2009; Castellote *et al.*, 2010).

Because inflammatory and immune reactions are altered by hepatic cirrhosis, the efficacy of innate reactions is limited. In these patients, spontaneous bacterial protein is recognized, and proinflammatory cytokines are released to the blood and ascites (Kim *et al.*, 2007).

Monocyte chemotactic protein 1 (MCP-1) is produced by many cell types, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells (Deshmane *et al.*, 2008).

Monocyte chemotactic protein 1 (MCP-1) is the most representative member of α and β

chemokines, respectively (Baggiolini *et al.*, 1994; Fuentes *et al.*, 1995).

Monocyte chemoattractant protein-1 (MCP-1), also called Chemokine (C-C motif) ligand 2 (CCL2), is a 76-amino acid protein and a member of the C-C subfamily of chemokines, was first purified by Matsushima from serum-free culture supernatant of human myelomonocytic cells in 1989 (Matsushima *et al.*, 1989). It acts as a chemotactic factor for monocytes/macrophages, activated lymphocytes and neutrophils during infections thus, these cells migrate to the ascitic fluid. Monocytes and macrophages release TNF- α and other cytokines, which in turn induce the expression of adhesion molecules on endothelial cells, thereby mediating a systemic reaction to the infection. TNF- α has been shown to be elevated 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 (Kolattukudy & Niu, 2012)

Aim of the work this study assesses the level of ascitic fluid monocyte chemoattractant protein-1 level in cirrhotic patients with and without SBP and to evaluate its role in the pathogenesis of SBP and its prognostic value and correlation to disease severity.

Subjects and Methods

Subjects

The study was carried out at Hepatology, Gastroenterology and Infectious diseases at Benha University Hospital & Medical Microbiology and Immunology Department of Benha faculty of Medicine, A written informed consent (in Arabic language) was obtained from the patients before Participation. This study included ninety patients with liver cirrhosis and ascites. Patients were divided into 2 groups: Group I including 45 patients with SBP (a polymorph nuclear cell count (PMN) that must be greater than or equal to 250 cell/mm³ in ascitic fluid) Cardenas *et al.*, 2012, Group II including 45 ascitic patients without SBP served as control group. Because

many cases of SBP are culture-negative, the isolation of the responsible organisms was not considered essential for the diagnosis.

- Inclusion criteria:

Cirrhotic Patients with spontaneous bacterial peritonitis not receiving antibiotics in the last one week.

- Exclusion criteria:

- Ascites secondary to bacterial peritonitis.

- Ascites secondary to mycobacterial or fungal peritonitis .

- Ascites secondary to (HCC) hepatocellular carcinoma.

Each patient was subjected to:

I- Complete medical history taking including demographic, clinical and laboratory data, etiology and history of complications were recorded and available for analysis.

II- Full general and local examination, assessment of the severity of liver cirrhosis was obtained in all patients with modified Child-Pugh score (Pugh *et al.*, 1973), MELD (model for end stage liver disease) score (Kamath *et al.*, 2001) and the updated MELD (u MELD) score (Sharma *et al.*, 2008).

III- Full laboratory investigations including:

Complete blood picture, renal function tests, liver profile, and serological tests for viral markers (Hepatitis B surface antigen and Hepatitis C virus antibody by enzyme – linked immunosorbent assay technique (ELISA))

Diagnostic paracentesis was carried out at the bedside under complete aseptic condition using a sterile 22-G needle attached to a 20-cc syringe after local anesthesia with lidocaine. Ascitic fluid (AF) was immediately drawn from the peritoneal fluid after the sterile needle was attached to a syringe for paracentesis. Then, aspirated AF was collected into ethylenediaminetetraacetic acid tubes and analyzed for biochemistry and leukocyte counts within 3 h tests for ascitic fluid includes:

(1) Biochemical tests including: Total protein content, albumin and glucose.

(2) WBCs (total and differential):

SBP is diagnosed when PMN count in ascitic fluid is > 250 cell/mm in the absence of data compatible with secondary peritonitis (i.e gastro-intestinal perforation).

(3) Serum - ascites albumin gradient (SAAG): SAAG is the best single test for classifying ascites into portal hypertensive (SAAG >1.1 g/dL) and non-portal hypertensive (SAAG <1.1 g/dL) causes. It was calculated by subtracting the ascitic fluid albumin value from the serum albumin value, it correlates directly with portal pressure, (Caldwell & Battle, 1999).

(4) MCP-1 level in ascitic fluid (Kim *et al.*, 2007):

a- Sampling aliquots of approximately 1 mL ascites were centrifuged for 15 min at 500 ×g. The supernatant phase was transferred to a fresh tube and stored at -70°C until analysis by ELISA

b- Principle of the test:

It was done using the commercially available Human MCP-1 ELISA Kit (BD OptEIA™ Biosciences, Pharmingen, USA). It is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for MCP-1 coated on a 96-well plate. Standards and samples are added to the wells, and any MCP-1 present binds to the immobilized antibody. The wells are washed and streptavidin-horseradish peroxidase conjugate mixed with biotinylated anti-human antibody is added, producing an antibody-antigen-antibody “sandwich”. The wells are again washed and TMB substrate solution is added, which produces a blue color in direct proportion to the amount of MCP-1 present in the initial sample. The Stop Solution changes the color from blue to yellow, and the microwell absorbances are read at 450 nm

Statistical Analysis

Descriptive statistics were presented as mean ± standard deviations (SD) for continuous variables and number and percentage for categorical variables (frequency distribution). Unpaired Student t-test (two sided) was used to test the significance of difference between the mean value of studied groups and chi-square test was used for comparison of categorical variables. The diagnostic value for MCP-1 by determining its sensitivity, specificity, positive (PPV) and negative predictive values (NPV). Receiver operating characteristic curves (ROC) (were constructed to assess the validity of the markers in predicting SBP by calculating the area under the curve (AUC). Pearson correlation test was used to identify the correlation between MCP-1 and different clinicopathological variables. All calculations were done using SPSS for windows version 20.0. For all tests, a *P*-value <0.05 was considered to be statistically

significant the diagnosis of SBP was based on a polymorph nuclear cell count (PMN) that is greater than or equal to 250 cell/mm³ in ascitic fluid in absence of intra-abdominal cause of infection (Thuluvath *et al.*, 2001; Cardenas *et al.*, 2012).

Results

Characteristics of the study patients

Characteristics of SBP patients and controls are provided in Table (1). There was no statistical difference between non-infected cirrhotic patients and patients with SBP with respect to age or sex and etiology of liver cirrhosis. Marked hepatic failure was present at time of diagnosis in most cases, as indicated by high Child, MELD and uMELD scores.

Renal impairment as indicated by higher mean value of serum creatinine levels were detected in patients with SBP.

Most cases of SBP were presented by abdominal pain and fever.

Ascetic fluid analysis

Parameters of AF analysis are shown in Table (2). SBP group showed higher ascetic fluid TLC, SAAG and lower protein and glucose levels.

Ascetic fluid MCP-1 levels: Patients with SBP showed increased AF concentration of MCP-1 Fig. (1).

MCP-1 as a marker for SBP

For MCP-1 the cutoff point that gives an area under curve of 91% was 122.5ng/ml with a sensitivity of 86.7%, a specificity of 95.4%, PPV of 95.1% and NPV of 87.5% table (3), fig. (2) Correlations of MCP-1.

There was statistical significant positive correlations between MCP-1 values and TLC, PMN leucocytes and MELD score table (4).

Table 1. Overview of the studied groups

Parameters	SBP N=45	Non-SBP N=45	P-value
Age(years)	47.69±7.43	50.36±7.78	NS
Gender			
Male	33(73.3%)	32(71.2%)	NS
Female	12(26.7%)	13(28.8%)	
Abdominal pain	45(100%)	9(20%)	0.000*
Bleeding	10(22.2%)	12(26.7%)	NS
Encephalopathy	20(44.4%)	13(28.9%)	NS
Fever	42(93.3%)	4(8.9%)	0.000*
Jaundice	18(40%)	15(33.3%)	NS
Hemoglobin(g/dl)	10.32±1.3	10.3±1.28	NS
TLC(mm ³)10 ³	8.6±4.5	5.26±2.4	0.02*
Platelets (mm ³)10 ³	100.6±36.54	80.9±34.85	0.01*
Creatinine(mg/dl)	1.67±0.7	1.1±0.4	0.001*
HCV	43(95.6%)	42(93.9%)	NS
HBV	2(4.4%)	2(4.4%)	-
Child score			
B	19(42.2%)	26(57.8%)	NS
C	26(57.8%)	19(42.2%)	NS
MELD	19.6±6.32	14.22±6.9	0.000*
uMELD	4.02±0.7	3.54±0.8	0.009*

SBP N: number of spontaneous bacterial peritonitis patients; Non-SBP N: number of non-spontaneous bacterial peritonitis patients. $P > 0.5$ is not Significant (NS). TLC=total leucocytic count, HCV=hepatitis C virus, HBV=hepatitis B virus, MELD=model for end stage liver disease, uMELD=updated MELD.

Table 2. Ascitic fluid analysis of the studied groups

Parameters	SBP N=45	Non-SBP N=45	*P-Value
	Mean \pm SD	Mean \pm SD	
TLC(mm ³)	1259.11 \pm 707.34	815 \pm 202.7	0.000
Polymorphs(mm ³)	7855 \pm 9.14	64.3 \pm 15.68	0.000
Albumin(g/dl)	0.67 \pm 0.28	0.74 \pm 0.25	NS
Protein(g/dl)	1.07 \pm 0.3	1.7 \pm 0.6	0.001
Glucose(mg/dl)	98.06 \pm 12.7	112.9 \pm 29.9	0.003
SAAG(g/dl)	1.7 \pm 0.31	1.63 \pm 0.35	NS
MCP-1(ng/ml)	743.14 \pm 488	38.13 \pm 56.8	0.000

SBP N: number of spontaneous bacterial peritonitis patients ; Non-SBP N: number of non-spontaneous bacterial peritonitis patients.
P > 0.5 is not Significant (NS). TLC=total leucocyticcount, SAAG=serum ascetic albumin gradient,
MCP-1=monocyte chemotactic protein-1.

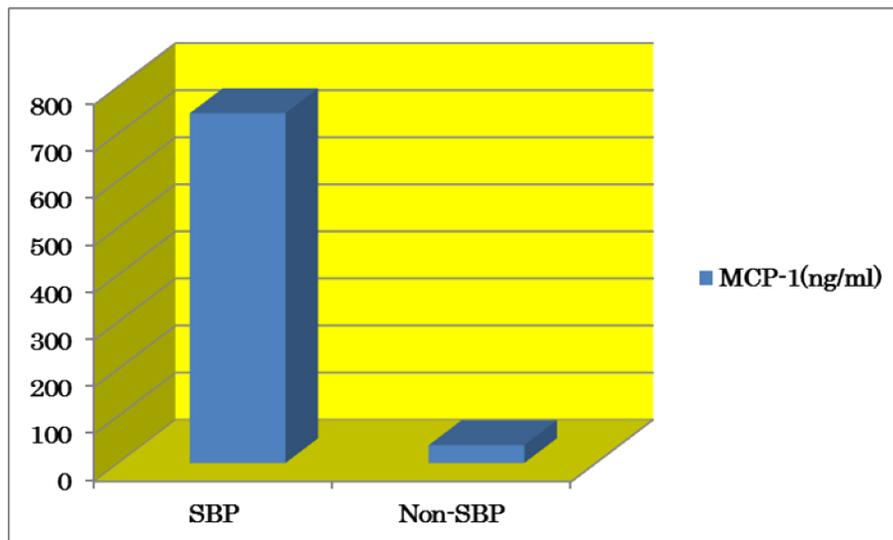


Figure 1. MPC-1 level in studied groups

Table 3. ROC curve analysis for MCP-1 as a marker for SBP.

MCP-1 cut-off point	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Area Under Curve (AUC)	P-value
122.5ng/ml	86.7%	95.4%	95.1%	87.5%	91	0.000*

$P \leq 0.5$ is significant.

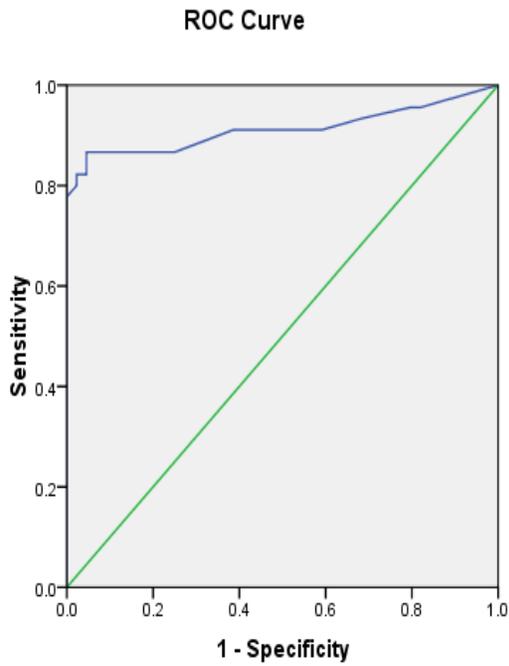


Figure 2. ROC curve analysis for MCP-1.

Table 4. Correlations between MCP-1 and different variables among SBP group

Correlations of MCP-1 with (ng/ml)	Pearson coefficient (r)	*P-value
TLC	0.5	0.000
Polymorphs	0.27	0.009
MELD score	0.22	0.042

$P \leq 0.5$ is Significant.

Discussion

The present study revealed that SBP was more frequent in males (73.3%) and it was not influenced by the age, these results were in agreement with the study done by Obstein *et al.*, (2007) in which 79% of SBP group were males and were not affected by the age.

As regards the cause of liver cirrhosis in our study, we found that chronic hepatitis C is the cause in 95.6% of patients and chronic hepatitis B is the cause in the remainder (4.4% of patients) with no significant difference in both groups and this goes in agreement with that was reported by Thomas & Strickland (2006) who found that the cause of liver cirrhosis does not affect incidence of SBP.

Analysis of the results showed that patients with SBP commonly presented by abdominal pain (100%) with a highly significant difference compared to non SBP group (20%) and this goes in agreement with that elicited by Mchutchinson & Runyon, (1994), Liovet *et al.*, (1993), Filik & Unal (2004) and Wallerstedt *et al.*, (2007) who stated that abdominal pain was detected in (50%, 52%, 54.5% and 70%) of SBP cases respectively.

In the present study, 22.2% of SBP cases had gastrointestinal bleeding with no significant difference, this was close to that reported by Wallerstedt *et al.*, (2007) who stated that 30% of SBP cases had gastrointestinal bleeding.

Hepatic encephalopathy was detected in 44.4% of SBP and 28.9% of non SBP cases with statistically non significant difference, these results were close to that reported by Wallerstedt *et al.*, (2007) and Nobre *et al.*, (2008) who stated that hepatic encephalopathy was detected in 20% and 24.5% of SBP cases respectively and also this goes in agreement with Liovet *et al.*, (1993) who detected that hepatic encephalopathy was in 40% of SBP cases, also this is reported in (Paul *et al.*, 2015)

who detected that most patients of SBP have signs clearly suggestive of peritoneal infection, especially pain abdomen.

In clinical examination of studied patients fever was detected in 93.3% of SBP group with a highly statistical significant difference between both groups, this goes in agreement with that reported by Mchutchinson & Runyon (1994) and Wallerstedt *et al.*, (2007) as fever was detected in 66% of SBP, also this goes in agreement with (Paul *et al.*, 2015) who detected that most patients of SBP have signs clearly suggestive of peritoneal infection, especially fever, so fever is considered one of the characteristic clinical sign of SBP.

As regard clinical examination of studied patients Jaundice was detected in 40% of SBP group and this goes in agreement with that elicited by Filik & Unal (2004) who found that Jaundice was the most prominent physical signs 54.5% in SBP group, also this goes in agreement with (Paul *et al.*, 2015) who detected that jaundice was in 39% in SBP patients.

Laboratory investigations in this study revealed that higher leucocyte count in SBP patients, this result goes in agreement with that reported by Cholongitas *et al.*, (2006) as leucocytosis was higher in SBP patients.

As regard platelets count of studied patients, it revealed thrombocytopenia is higher in SBP group with highly significant difference between both groups, this result was in agreement with that reported by (Lata & Pribramska, 2004), also this result is in agreement with (Paul *et al.*, 2015), but this difference may not related to SBP, rather to the severity of the liver disease.

As regard hemoglobin value in studied patients showing no difference between two groups and this was documented by (Coşkun *et al.*, 2001) but the hemoglobin value may be

related to the severity of the liver disease which reported by (Paul *et al.*, 2015).

In this study, the level of serum creatinine was significantly increased in patients with SBP when compared to non SBP patients and this goes in agreement with Angeloni *et al.*, (2008), who showed that the hospital mortality in SBP is high due to hepato-renal syndrome and also reinforced by (Tsung *et al.*, 2013) who stated that renal dysfunction occurs in patients with SBP and it is independent predictor of mortality.

Regarding the relation between occurrence of SBP and severity of liver disease this study showed that 42.2% and 57.8 % of SBP cases had Child-Pugh class B and class C cirrhosis respectively.

This result matched with that reported by Navasa *et al.*, (1999), Liovet *et al.*, (1993) and Rodriguez –Romas *et al.*, (2001) who elicited that SBP patients usually had Child- Pugh class B or C cirrhosis, also Cirera *et al.*, (2001) reported that about 70% of patients who developed SBP had Child class C.

In the present study, it was found that a high MELD and uMELD scores was positively associated with a greater risk of incidence of SBP.

This goes in agreement with Obstein *et al.*, (2007) who suggested that MELD score was associated with SBP risk in a linear fashion and also this is reinforced by Evans *et al.*, (2003) who elicited that MELD score has been shown to be a good prognostic marker in cases of SBP.

The total leucocytic count > 500 cell/cm³ and PMN count >250 cell/cm³ in ascitic fluid is laboratory diagnostic marker in SBP patients (wiest *et al.*, 2012).

In this study we found that as regard to the ascitic fluid cell count, there were in higher levels of TLC, PMN count in SBP group compared to non SBP group, these results goes in agreement with Giron- Gonzzales *et al.*, (2001), also this goes in agreement with

Yildirim *et al.*, (2002) who reported higher ascitic TLC in SBP more than non SBP patients.

As regard to ascitic fluid total protein level was lower in SBP group ,this agrees with (Paul *et al.*, 2015) who denoted that patients with poor synthetic function have diminished level of protein in ascitic fluid that correlate with low level of opsonization and this play a role in SBP susceptibility and denoted also AF total protein < 1 g/dl is important predictor for SBP.

In this study,we found that the ascitic fluid glucose was with lower level in SBP patients , and this goes in agreement with (Tsung *et al.*, 2013), who reported that lower level of ascitic glucose level in SBP patients and also considered that lower ascitic glucose level is independent predictive factor of overall survival rates in cirrhotic patients with SBP.

As regard SAAG value, in our study had significantly higher in SBP patients than non SBP patients, this is in agreement with (Desai *et al.*, 2012) who denoted that regardless of the severity of liver disease, those with lower ascitic fluid protein levels, lower ascitic albumin and higher SAAG, are also less likely to mount a satisfactory immune response and poor clearance of infection.

Because inflammatory and immune reactions are altered by hepatic cirrhosis, the efficacy of innate reactions is limited. In these patients, SBP is recognized, and proinflammatory cytokines are released to the blood and ascites (Kim *et al.*, 2007).

Monocyte chemotactic protein-1 (MCP-1) is a cytokine involved in the chemotaxis of monocytes and activated T lymphocytes, and interleukin-10 (IL-10) is an anti inflammatory substance. These compounds partially control the degree of inflammation by regulating the production of proinflammatory cytokines in SBP (Kim *et al.*, 2007).

In this study we evaluate the difference in ascitic MCP-1 level in SBP and non SBP

patients which showed that higher levels in SBP patients than non SBP patients, this is in agreement with Giron-Gonzalez *et al.*, (2001) and Rodriguez-Ramos *et al.*, (2001) who mentioned that MCP-1 concentration is increasing in ascitic fluid in SBP patient and rapidly decreasing after treatment.

Also this result is in agreement with Kim *et al.*, (2007) who mentioned that patients with SBP had higher levels of MCP-1 in their ascitic fluid than non SBP patients and its concentrations had decreased after treatment. As the bacterial invasion stimulates the immune system, in SBP MCP-1 released in ascites and play as a potent chemotactic factor for monocytes and activated T lymphocytes Kim *et al.*, (2007).

In this study, MCP-1 is significantly a determining predictor for SBP positivity, this is in agreement with lutz *et al.*, (2015) who stated that there is higher level of the MCP-1 in both blood and ascitic fluid of cirrhotic patients with SBP than non SBP.

ROC curve analysis of MCP-1 as a marker of SBP of the studied cases revealed that a high MCP-1 was independently associated with a greater risk of SBP with cut off level of 122.5 ng/ml with sensitivity 86.7% and specificity 95.4% in diagnosis of SBP with area under the curve 91%.

This is in agreement with Giron-Gonzales *et al.*, (2001) who reported the higher concentrations of MCP-1 level in SBP patients with highly statistical difference than non SBP patients.

Also reinforced with Kim *et al.*, (2007) who showing the same higher concentration of ascitic MCP-1 in SBP patients as highly sensitive diagnostic marker.

In this study, there is a significant positive correlation between MCP-1 level and ascitic TLC, PMN among SBP patients. These findings agree with Kim *et al.*, (2007) who state that there is positive association between

ascitic MCP-1 concentration and ascitic total leucocytic count and PMN.

In this study, there is a significant positive correlation between MCP-1 level and MELD score among SBP patients, this is in agreement with (Tsung *et al.*, 2013) who stated that increasing MELD score was independently associated with greater risk of SBP and had important implications for increasing the suspicion of SBP.

In conclusion, according to this study, MCP-1 level was higher in ascitic fluid of SBP patients, so it can be used as a diagnostic marker in SBP and can be used as a good prognostic marker due to its positive relation with the severity of liver disease which indicated by high MELD and uMELD scores.

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