Original Article

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Macrophage Inflammatory Protein Type 1 Beta as a Novel Diagnostic Marker for Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a severe complication of cirrhotic ascites. SBP diagnosis is based on the count of ascitic fluid neutrophils (>250/mm^3). This procedure is an invasive maneuver with many complications. This study aimed to find a sensitive diagnostic tool for SBP by examining ascitic fluid Macrophage Inflammatory Protein type 1 beta (MIP-1β) as a rapid bedside test for diagnosis of SBP. **Materials and methods:** The study included 53 cirrhotic ascitic patients (33 with and 20 without SBP). Patients were subjected to thorough medical history taking, clinical examination, and laboratory investigation. Two ascitic fluid samples were taken, the first at admission time for cell count and culture, and the second sample was taken 48 hours after treatment in patients with SBP. Ascitic MIP-1β was measured using ELISA technique at admission in both groups and 48 hours after treatment in the SBP group. **Results:** Ascitic MIP-1β and CRP levels were significantly higher in the SBP group versus non-SBP patients. There was a significant positive correlation between ascitic fluid MIP-1β and WBCs and serum CRP. Ascitic fluid MIP-1β at a cut-off value ≥ of 31.95 pg/ml had 79% sensitivity and 75% specificity for the diagnosis of SBP. Combined ascetic fluid MIP-1β at the cut-off value ≥31.95 pg/ml and CRP in serum at a cut-off value ≥of 36 mg/L had 61% sensitivity and 100% specificity. There was a significant decrease of both, ascitic MIP-1β and ascetic fluid PMN after treatment of SBP. **Conclusions:** Ascitic fluid MIP-1β is highly sensitive and specific in the diagnosis of SBP- especially when combined with CRP.

Introduction

Cirrhotic patients with ascites are immunocompromised with higher susceptibility to infections such as SBP, hospital-acquired infections, and a variety of other infections from uncommon pathogens, mainly owing to the inadequate innate and specific defense mechanisms [1]. SBP is defined as an ascitic fluid PMN >250/mm^3 after exclusion of intraabdominal surgically-treatable source [2]. SBP is a potential, life-threatening complication in cirrhotic patients with a mortality rate ranging between 30% and 50% [3]. Early diagnosis and appropriate treatment can reduce morbidity and mortality in SBP patients [1]. SBP may be symptomatic, therefore all cirrhotic patients with ascites should undergo diagnostic paracentesis at admission to diagnose SBP whatever the cause of admission, although SBP is less common in an outpatient setting, it is necessary to evaluate it especially in patients on the waiting list for liver transplantation because of high mortality [4]. Ascitic fluid PMN >250/mm^3 can diagnose SBP in absence of intra-abdominal surgical and inflammatory causes of secondary peritonitis [5]. Macrophage inflammatory protein type 1 beta (MIP-1β) belongs to the family of chemokines, best known for their chemotactic and proinflammatory effects**.** Synthesis of MIP-1p is stimulated by bacterial endotoxins. MIP-1β is responsible for the activation of PMN and is part of acute neutrophilic inflammation. The diagnostic importance of MIP-1β for bacterial infections is poorly recognized. The chemokines have significantly shorter half-lives than classical inflammatory biomarkers which theoretically make them more suitable for diagnostic and monitoring purposes [6]. The present study aims to assess the role of ascitic fluid MIP-1β as an early detector for the diagnosis of SBP and follow-up of these patients.

Patients and methods

This is a prospective case-control study that included 53 cirrhotic patients with ascites. The patients were classified into two groups; Group (1) included 33 patients with ascitic fluid PMN > 250/mm^3 (SBP group), and group (2) included 20 patients with an ascitic neutrophil count below 250 cells/mm^3 and -ve culture (Non-SBP group). Our patients were admitted to Specialized Medical Hospital, Mansoura University from January 2016 till the end of January 2017. All patients were above 18 years. Patients who had hepatocellular carcinoma, extrahepatic malignancy, recent abdominal surgery, hemoperitoneum, recent antibiotic therapy (within 48 hours before admission) chronic dialysis, acute pancreatitis, autoimmune diseases, organ transplant, HIV infections, or having other infections were excluded from the study. All patients underwent a thorough clinical evaluation (history taking, general and systemic examination). Laboratory tests, including complete CBC, serum creatinine, liver function tests, serum CRP, and urine analysis, radiological investigations (abdominal ultrasound, chest-X ray, and Triphasic CT when indicated) were done. Ascitic fluid analysis and culture were done at index

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admission in both groups and 48 hours after treatment in the SBP group.

Ascitic fluid MIP-1β

Ascitic fluid MIP-1β was measured by ELISA kit supplied by Sun RED (China). The unit utilizes a twofold counteracting agent sandwich catalyst connected immunosorbent examine (ELISA) to test the level of Human Macrophage Inflammatory Protein-1β (MIP-1β) in tests. Include MIP-1β to monoclonal counter acting agent Enzyme well which is precoated with MIP-1β monoclonal neutralizer, hatching; at that point, include (MIP-1β) antibodies marked with biotin, and joined with Streptavidin-HRP to shape resistant complex; at that point do brooding and washing again to evacuate the uncombined compound. At that point include Chromogen Solution A, B, the shade of the fluid changes into the blue, and at the impact of corrosive, the shading at last winds up yellow. The Chroma of shading and the grouping of the MIP-1β for example were decidedly connected. The sensitivity of this assay is defined as the lowest protein concentration which can be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. This was estimated to be 0.432pg/ml. Moreover, assay range was between 0.5pg/ml and 150pg/ml. The mean absorbance for each set of duplicate standards, controls, and samples was calculated. The average zero standard optical density was subtracted. The standard curve on log-log graph paper was the plot. The standard concentration was on the x-axis and absorbance was on the y-axis. The best-fit straight line through the standard points was drawn.

Statistical analysis

Correlation analysis between continuous data and ascitic fluid MIP-1β was done using the Spearman correlation test. Correlation between Categorical data and ascitic fluid MIP-1β was done using the Bi-seial correlation test. Association between potential predictors of SBP with SBP was done using logistic regression analyses. Comparison between different regressions models was done by -2 Log-likelihood (-2LL). The ability of ascitic fluid MIP-1β to discriminate between individuals with and without SBP was determined by the ROC curve. The best cutoff point was chosen according to Youden's index. Change of ascitic fluid PMN and MIP-1β before and after treatment determined by Wilcoxon signed-rank test.

Results

There was no significant difference between the two studied groups as regards, age, gender, gastrointestinal bleeding, hepatic encephalopathy serum creatinine, and albumin and hemoglobin level. Compared to patients without SBP, patients with SBP showed a significant increase as regards, fever, abdominal pain, serum bilirubin, INR, Child C score, and MELD score, and a significant decrease in platelet count as shown in **(Table 1)**.

Furthermore, there was a significant increase as regards, ascitic MIP-1β, ascitic fluid PMN, ascitic LDH and serum CRP levels, and peripheral WBC in the SBP group versus the non- SBP group as shown in **(Table 2)**.

There was a significant correlation between ascetic MIP-1β and peripheral WBCs, ascitic fluid LDH, and CRP level while, no statistically significant correlation between MIP-1β and other studied parameters **(Table 3)**. To determine predictive parameters for the diagnosis of SBP, variables with significant associations on Univariate analysis were subjected to logistic regression analysis.

The multiple logistic regression analyses of predictors of SBP were illustrated in **(Table 4)**. The presence of abdominal pain, fever and increase WBCs, serum CRP level, ascetic MIP-1β, Child-Pugh score, and MELD score were independent predictor factors for SBP.

(Table 5) shows that ascitic fluid MIP-1β at a cut-off value ≥ of 31.95 can discriminate individuals with SBP from those without SBP with sensitivity (79%) and specificity (75%), NPV (84%), PPV (68%) accuracy (77%). Also, CRP in serum at a cut-off value ≥ of 36 mg/L can distinguish individuals with SBP from those without SBP with sensitivity (73%) and specificity (95%), NPV (68%), PPV (96%) accuracy (81%). The addition of CRP in serum at a cut-off value ≥ of 36 mg/L to ascetic fluid MIP-1β at a cut-off value ≥ of 31.95 pg/ml increased specificity to (100%) with 61% sensitivity

Table 1. Demographic, clinical, and laboratory parameters of the studied groups.

Characteristics	SBP (N=33)	Non-SBP (N=20)	P-value
Age (years) (mean±SD)	58.8±8.9	50.7±10.1	0.683
Gender (%)			
Male	16(48.5%)	7(35.0%)	0.337
Female	17(51.5%)	13(65.0%)	
Abdominal pain (%)			
Yes	28(84.8%)	8(40%)	0.001
No	5(15.2%)	12(60%)	
Fever (%)			
Yes	19(57.6%)	4(20%)	0.010
No	14(42.4%)	16(80%)	

Hepatic encephalopathy			
Yes	8(24.2%)	3(15%)	1.000
No	25(75.8%)	17(85%)	
GIT bleeding (%)			
Yes	7(21.2%)	1(5%)	1.000
No	26(78.8%)	19(95%)	
Serum Bilirubin (mg/dl)	3.8(1.75-7.2)	1.5(0.7-2.9)	0.002
Serum creatinine (mg/dl)	1.5(0.9-2.3)	1.1(1.0-1.6)	0.185
Serum albumin (gm/dl) (mean±SD)	2.3±0.5	2.5±0.5	0.474
INR (mean±SD)	1.9±0.5	1.4±0.4	0.003
Platelets (10^3/mm^3)	64.0(51.0-121.5)	114(65.3-152.3)	0.033
Hemoglobin (gm/dl) (mean±SD)	10.3±2.1	11.0±2.4	0.265
Child-Pugh classification	11.0(9.0-12.0)	9.0(8.0-10.0)	
Child A	2(6.1%)	2(10%)	0.002
Child B	6(18.2%)	13(65%)	
Child C	25(75.7%)	5(25%)	
MELD score	21.0(17.5-29.5)	12.5(10.0-17.5)	<0.001

MELD Score, *Model for end-stage liver disease*

Table 2. Comparison of MIP-1β, and other inflammatory markers between the studied groups.

Characteristics	SBP (N=33)	Non-SBP (N=20)	P-Value
Ascitic fluid MIP-1β (pg/ml)	44.1 (33.2-47.8)	18.4 (14.7-40.4)	0.002
Ascitic fluid PMN (10^3/mm^3)	2700 (1127-5864)	200.0 (102.0-250.0)	<0.001
Ascitic fluid LDH (IU/L)	160.0 (117.0-208.5)	86.0 (72.8-118.0)	<0.001
Serum CRP level (mg/dl)	72.0 (12.0-96.0)	24.0 (12.0-24.0)	0.002
Peripheral WBCs (× 10^3/mm^3)	8.7 (6.2-12.8)	5.9(3.5-7.1)	0.003

Table 3: Correlation between ascitic MIP-1 and Biochemical characteristics, MELD and Child-Pugh classification.

Variable	r	P-Value
Serum Creatinine (mg/dl)	0.143	0.307
Serum Albumin (gm/dl)	-0.042	0.766
Serum Bilirubin (mg/dl)	0.015	0.914
INR	0.207	0.136
Platelet (10^3/mm^3)	0.89	0.525
WBC (10^3/mm^3)	0.443	0.001*
Ascitic fluid neutrophils (10^3/mm^3)	0.198	0.270
Ascitic fluid glucose (mg/dl)	-0.036	0.800
Ascitic fluid albumin (gm/dl)	0.060	0.671
Ascitic fluid LDH (mg/dl)	0.312	0.023*
Serum CRP concentration (mg/L)	0.131	0.007*
MELD score	0.212	0.128
Child-Pugh classification	0.171	0.222

Table 4: Prediction of SBP by Clinical and laboratory predictors of SBP.

Variable	OR (95% Cl)	-2LL	P-Value
Fever (YES)	5.42 (1.49-19.82)	62.7	0.010
Abdominal pain (YES)	8.4 (2.28-31.01)	58.7	0.001
WBC (10^3/mm^3)	1.34 (1.08-1.69)	59.1	0.009
Serum CRP level (mg/L)	5.65 (1.91-16.78)	52.8	0.002
Ascitic fluid MIP-β (pg/ml)	1.075 (1.03-1.12)	56.8	0.001
Child-Pugh Score	1.5 4(1.11-2.14)	61.8	0.008
MELD Score	1.25 (1.08-1.42)	51.9	0.001

Variable	Cut off point	AUC (95-CI)	Sensitivity	Specificity	NPV	PPV	Accuracy
Ascetic fluid MIP-1β	≥31.95pg/ml	0.76 (0.61-0.90)	79%	75%	84%	68%	77%
Serum CRP	≥36 mg/L	0.89	73%	95%	68%	96%	81%
Combined ascetic fluid MIP-1β and serum CRP			61%	100%	61%	100%	75%

Table 5. Level and area under ROC of ascetic fluid MIP-1β and serum CRP for detection of SBP.

Discussion

SBP is a serious cause of morbidity as well as mortality in patients with cirrhosis and ascites. Diagnosis of SBP should be early and accurate to avoid its complications [7]. The diagnosis of SBP is mainly dependent on a manual count of the ascitic fluid count of PMN which, is operator-dependent and the false-negative result may occur due to destruction of PMN during transport. Furthermore, ascitic fluid culture is positive nearly in 30% of SBP patients and time consuming, so searching for a more sensitive diagnostic tool for SBP is needed [8]. MIP-1β known also as CCL4 is an active protein, which is produced by macrophages. MIP-1β acts as a mitogen- inducible cytokine which stimulates inflammatory responses [7]**.** In the present study, ascitic fluid MIP-1β was statistically significantly higher in the SBP group in comparison to non-SBP ascitic cirrhotic patients. This result is following previous studies that demonstrated that, increasing ascitic MIP-1β inpatients with SBP [6,7,9-11]. We also found that, at cut-off value ≥31.95pg/ml, ascitic fluid MIP-1β had 79% sensitivity and 75% specificity to detect SBP with a positive predictive value (68%) and negative predictive value (84%). Lesinska et al found that, at cut-off value 69.4pg/ml, MIP-1β had 80% sensitivity and 72.7% specificity for prediction of SBP with PPV (57.1%) and N PV (88.9%) 6**.** Another study reported that, ascitic fluid MIP-1β with cut off value 121.9 pg/ml (76.1% sensitivity, 100% specificity, PPV 100% and NPV 80%, AUC=0.881) [7]. As regards acute inflammatory marker C reactive protein (CRP) in serum, the current study showed a significant increase in SBP versus none SBP group. This result matched with previous results [7, 9, 12-15].

In this study, we also found that, at cut-off value ≥ 36 mg/L, CRP had a sensitivity of 73% and a specificity of 95% with PPV 96%, NPV 68%, and accuracy 81% for the diagnosis of SBP with AUC 0.89. Previous studies demonstrated that CRP has a diagnostic value in SBP where is CRP at a cut-off value of 30 mg/ L had a sensitivity of 90% and specificity 96% with PPV 70% and NPV 95% (AUC=0.91) [12]. Mousa et al found that CRP >11.3 mg/dL had a sensitivity of 88.9% and specificity 92.6% [16]**.** Interestingly, in the current study combination of serum CRP at the cut-off value ≥ of 36 mg/L and ascitic fluid MIP-1β at the cut-off value ≥ of 31.95 pg/ml increase specificity up to 100%, however, sensitivity was 61%. The present study showed that both CRP and ascitic fluid MIP-1β were strong independent predictors of SBP. Another study found that CRP was an independent variable in SBP prediction[12,16] while others found that CRP act as a prognostic marker in cases of cirrhotic ascites with SBP [9, 14]**.** In this study, there was a positive correlation with total leucocyte count, serum CRP and ascitic fluid LDH in the SBP group. However, Khorshed et al found a positive correlation between MIP-1β, mean platelet volume, platelet distribution width, and PMN in ascites [7]. The current study revealed a significant decrease in ascitic fluid MIP-1β after 48 hours of proper intravenous antibiotic administration. A similar result was reported by El-Gindy et al, who found a significant decrease in MIP-1β in the ascitic fluid after MIP-1β [17].

Conclusion

Ascitic fluid MIP-1β is a highly sensitive and specific marker in the diagnosis of SBP- especially when combined with CRP.

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