Research Article

Soluble CD١٦٣ is a Novel Diagnostic Marker for Spontaneous Bacterial Peritonitis in Patients with HCV Induced Liver Cirrhosis

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Abstract

Background/Aims: The diagnosis of spontaneous bacterial peritonitis (SBP) is based on a polymorphonuclear leukocytes (PMNs) exceeding ٢٥٠/μL in ascitic fluid. The aim of the study was to evaluate serum soluble CD ١٦٣ (sCD١٦٣) as an accurate diagnostic serum marker for detecting SBP. Methods: Forty patients with cirrhotic ascites were included. They were divided into a SBP group, including ٢٠ patients, and a non-SBP group of ٢٠ patients. Serum sCD١٦٣ was measured using an enzyme-linked immunosorbent assay. **Results:** Serum sCD١٦٣ was significantly higher in SBP patients than in non-SBP patients. At a cutoff value of ٣١٠٠ ng/mL, serum sCD ١٦٣ had ١٠٠٪ sensitivity and ٩٥% specificity for detecting SBP. Serum sCD١٦٣ was positively correlated with deterioration of liver function in patients with liver cirrhosis. **Conclusions:** According to our findings, determination of serum sCD١٦٣ level appears to provide satisfactory diagnostic marker for the diagnosis of SBP.

Keywords: Spontaneous bacterial peritonitis**,** Soluble CD ١٦٣, Liver cirrhosis

Introduction

Ascites is one of the most common complications of liver cirrhosis and it develops as a consequence of portal hypertension and splanchnic vasodilatation [١]. Spontaneous bacterial peritonitis (SBP) is one of the main infectious complications of cirrhosis and occurs in ٨–٣٠٪ of hospitalized patients with ascites[٢]. The ١-year probability of development of the first episode of SBP in end-stage liver disease patients with ascites is around ١٠٪ [٣]. SBP is defined as an infection of the previously sterile ascitic fluid in the absence of a visceral perforation and in the absence of an intra-abdominal inflammatory focus such as abscess, acute pancreatitis, or cholecyctitis[٤]. Also, the number of polymorphnuclear leukocytes (PMN) from the ascitic fluid obtained by paracentesis must exceed ٢٥٠ cells/ mm^3 and only one germ must be isolated in the bacteriological cultures[٥].

Infection and inflammation are often linked to the development of complications in cirrhosis, especially relating to portal hypertension. Portal hypertension increases bacterial translocation and endotoxaemia, ٤ which induces an inflammatory response in the liver and in the systemic circulation with subsequent activation of immune cells[٦]. CD١٦٣, a highly expressed macrophage membrane protein, belongs to the scavenger receptor cysteine rich (SRCR) domain family with a short cytoplasmic tail, a transmembrane segment, and an extracellular domain. This macrophage receptor is a specific marker for macrophage activation. It is shed into the circulation in a soluble form (sCD١٦٣) after Toll-like receptor activation and the serum concentrations of sCD١٦٣ are accordingly elevated during conditions of macrophage activation and proliferation [٧]. It is reported that CD١٦٣ showed anti-inflammatory effect in patients with liver failure[٨]. Fabriek et al.,[٩] founded that CD١٦٣ could enhance the host immunity as the innate immune receptor of gram-negative bacteria and gram-positive bacteria. Elevated circulating sCD١٦٣ has

been demonstrated in viral hepatitis, acute liver failure and cirrhosis[٧]. Liver cirrhosis with ascites is often complicated by a hyperdynamic circulatory state. It has been postulated that intestinal bacterial overgrowth, altered gut permeability, and bacterial translocation, all common in cirrhosis with ascites, may exert continuous pressure on the immune system. This disturbance is thought to lead to activation of monocytes and lymphocytes and increased serum levels of proinflammatory cytokines such as tumor necrosis factor α (TNF-α)[١٠]. To our knowledge, the roles of sCD١٦٣ have not been assessed in patients with SBP. We hypothesized that sCD١٦٣ would be associated with the development of SBP in cirrhotic patients with ascites. Also we assumed that soluble CD١٦٣ which is produced in serum as a result of macrophage activation in patients with SBP may be a promising diagnostic marker which is easily measured by ELISA. The aim of this study was to measure and compare the levels of s CD ١٦٣ in patients with SBP and patients without SBP and to assess their role in detecting patients at high risk of developing SBP.

Patients and methods

In this prospective study, we recruited ٤٠ patients with ascites referred for paracentesis to the Internal and Tropical Medicine Departments of El-Minia University Hospital, Minia, Egypt, from March ٢٠١٤ to January ٢٠١٥. Twenty of these patients had cirrhotic ascites and had been admitted with SBP (cases) and ٢٠ patients had cirrhotic ascites with no existing evidence of SBP (controls). Diagnosis of SBP was made on the basis of the presence of at least ٢٥٠ cells/mL PMN in the ascitic fluid, with or without positive ascitic fluid culture, in the absence of secondary peritonitis and hemorrhagic ascites.

Exclusion criteria included patients with secondary causes of intra-abdominal sepsis (ascitic fluid protein >٢.٥ g/dl), patients with tuberculous peritonitis, patients with right-sided heart failure and diabetes mellitus, patients with renal impairment, rheumatoid arthritis, systemic lupus erythemotosus and hepatocellular carcinoma.

All patients were subjected to complete history taking and thorough clinical examination. Pelviabominal ultrasound examination was done for all patients. Liver profile and creatinine concentrations were measured on Hitachi ٩٠٢ Chemistry autoanalyzer (Roche Diagnostics, Basel, Switzerland) using its commercially available reagents, complete blood picture was measured on CELLDYN Emerald cell counter (ABBOTT, Wiesbaden, Germany), serum s CD١٦٣ was measured quantitatively by enzyme-linked immunesorbent assay (ELISA) technique using (Human Soluble CD١٦٣_sCD١٦٣_ ELISA Kit/ Glory Science Co., LTD).

This study was approved by the Ethical Committee of Minia University and all patients provided written informed consent prior to participation in any protocol-specific procedures. The study was conducted in accordance with the guidelines of the Helsinki Declaration

Statistical analysis

All statistical analyses were performed using the SPSS version ١٧.٠ software (SPSS Inc., Chicago, IL, USA). Kolmogorov- Smirnov test was used to test distribution of data. Parametric data were expressed in mean ± standard deviation. Student t-test and Mann-Whitney U test were used for intergroup comparisons.

Analysis of the receiver operator characteristics (ROC) and calculation of the area under the receiver operating characteristic curve (AUC) were used to evaluate serum s CD١٦٣ with maximum sensitivity and specificity for differentiation between ascitic patients with SBP and without SBP. Spearman correlation analysis was done between serum s CD ١٦٣ and other laboratory and radiological parameters. A p-value of less than or equal ٠.٠٥ indicated statistical significance.

Results

The present study was carried out on ٤٠ selected patients who presented ascites in patients with HCV induced liver cirrhosis; they were classified into two groups. Group I (cases group) included ٢٠ patients with cirrhotic ascites with SBP (١٥ males and five males) with their mean age was ٥٦.٣±٥.٨. Group II (control group) included ٢٠ patients with cirrhotic ascites without SBP (١٤ males and six males) with their mean age was ٥٥.٨±٥.٥. There was no significant differences regarding the age and sex between SBP and non-SBP patients. Serum levels of ALT, bilirubin, creatinine and prothrombin time exhibited significantly higher values in patients SBP when compared to ascitic patients without SBP. Serum albumin levels exhibited significant lower values in patients with patients with SBP when compared to patients with non-complicated ascites. Ultrasonographic measures of portal vein revealed that portal vein diameter was larger in patients of group I (SBP) compared to patients the control groups. These differences however were not significant statistically. The splenic longest axis diameter was significantly higher in patients with SBP compared to patients with non-complicated ascites with P value of ٠.٠٠٣.

Serum sCD١٦٣ showed significantly higher levels in patients with SBP when compared to patients with non-complicated ascites (٣٢٧١±٦١٧vs. ١٤١٤±٣٠٠, P value <٠.٠٠١٠). Peripheral PMN showed significantly increased values in SBP group compared to control group (١٢.٥± ٣.٢vs. ٦.١٣±١.١, P value p<٠.٠٠١). Moreover there was a significant positive correlation between serum sCD١٦٣ and PMNs in cirrhotic patients with SBP (r ٠.٧١, P value ٠.٠٠٨) (figure ١)

Also a significant positive correlation was found between increased levels of sCD١٦٣ and worsening of liver function in patient with SBP. This was evident by significantly positive correlation between serum levels of sCD١٦٣ and serum levels of ALT, AST, serum bilirubin and INR and significantly negative correlation between serum levels of sCD١٦٣ and serum albumin in patients with SBP (Table ٢). ROC curve analysis of serum sCD١٦٣ showed that at a cutoff value of >٣١٠٠ ng/ml serum sCD١٦٣ had a diagnostic sensitivity of ١٠٠٪ and specificity ٩٥٪ for detecting SBP (AUC= ٠.٩٨٣). Concerning peripheral PMN cell count, it was found that at a cutoff value of ١٠,٣٠٠/mm٣, PMN cell count had ٨٠% sensitivity and ٣٥% specificity for detecting SBP (AUC= ٠.٥٢١)

Table (١): demographic, Laboratory and radiological findings in the studied groups

Variable	Group I (SBP) N=٢٠	Group II (Ascites without SBP) N=٢٠	P value
Age (years)	٥٦.٣ ± ٥.٨	٥٥.٨ ± ٥.٥	٠.٧٥
Gender Male/Female	١٥ (٧٥ %) / ٥ (٢٥ %)	١٦ (٨٠ %)/٤ (٢٠ %)	٠.٩٨٦
ALT (IU/L)	٦٦.٣٧ ± ٧٨.٠٤	٥١ ± ٢٦.٧٩	٠.٠٤
AST (IU/L)	٧٩.٢٤ ± ١١٠.٧	٦١.٤٢ ±٢٧.٤٥	٠.٦
Albumin (g/dl)	٢.٠٧ ± ٠.٩٤	٢.٩٢± ٠.٧٣	٠.٠٢٤
Bilirubin (mg/d)	٢.٥٤ ± ٥.٢٩	١.١٨ ±٠.٢٤	٠.٠٠١
Creatinine (mg/dl)	١.٣٧ ± ٠.٧٣	١.٠٨ ± ٠.٣٦	٠.٠٢
Prothrombin time (sec)	٢٠ ± ١.٤	١٧±١.١ ٠.٠٤	٠.٠٤
PMNs /mm٣	١٢.٥ ± ٣.٢	٦.١٣ ± ١.١	<٠.٠٠١
sCD١٦٣(ng/ml)	٣٢٧١ ± ٦١٧	١٤١٤ ± ٣٠٠	<٠.٠٠١
PV diameter (mm)	١٣.٤٣±٢.٠٧	١٢.٤٤±٢.٠٨	٠.٠٥٧
Splenic longest axis (mm)	١٧٢.٥٦ ± ٢٥.٣	١٤٨.٨٥±٣٢.٧٩	٠.٠٠٣

Data are presented as the mean ± SD or number (%).
SBP, spontaneous bacterial peritonitis; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PMN, Polymorphonuclear leukocytes; PV diameter, Portal vein diameter

Table (٢): Correlation of mean sCD١٦٣ with laboratory and radiological findings

Variable	Correlation coefficient (r)	P. value
PMN/mm^3	٠.٧١	٠.٠٠٨
AST (U/L)	٠.٦٤	٠.٠٤
ALT (U/L)	٠.٥٣	٠.٠٣
Albumin (g/dl)	- ٠.٣٥	٠.٠٢
Billirubin (mg/d)	٠.٣٦	٠.٠١`
Prothrombin time (sec)	٠.٤٥	٠.٠١
Portal vein diameter (mm)	٠.٦١	٠.٠٤
Splenic longest axis (mm)	٠.٧١	٠.٠١

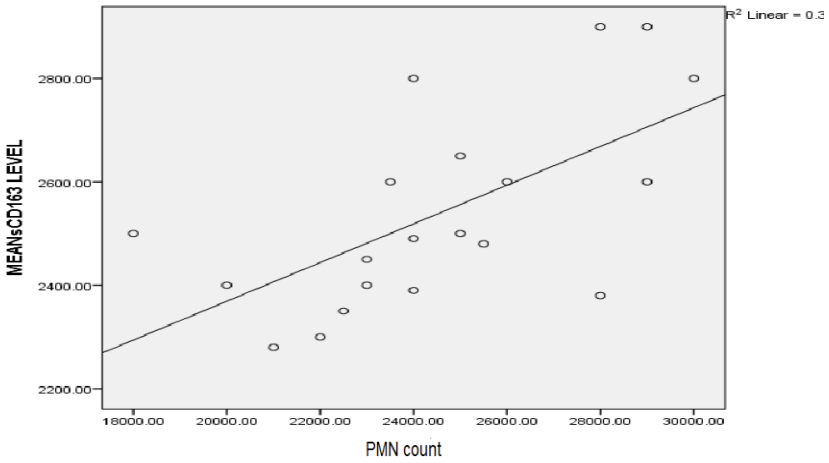

Figure (١): Correlation between PMN cell count and sCD١٦٣ level in patients with SBP

Table (٢): Receiver Operator Characteristics Curve Analysis of Serum s CD١٦٣ and PMN in patients with and without SBP

variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)
sCD١٦٣	٠.٩٨٣	>٣١٠٠	١٠٠	٩٥
PMNs	٠.٥٢١	>٢٥٠٠٠	٨٠	٣٥

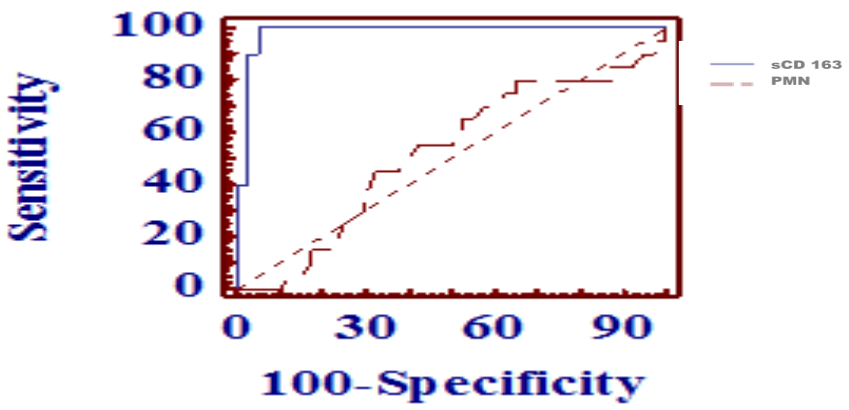

Figure (٢): Receiver operating characteristic curves of serum sCD١٦٣ and PMNs in detecting SBP in cirrhotic patients.

Discussion

SBP is one of the most common complications which occur in patients with cirrhosis and ascites. Complication of cirrhotic ascites arising most frequently in those patients with advanced liver disease, and it is characterized by spontaneous infection of the ascitic fluid without intra-abdominal sources of sepsis[١١].

SBP is an important cause of morbidity and mortality in cirrhotic patients with ascites. SBP is estimated to affect ١٠٪ to ٣٠٪ of cirrhotic patients hospitalized with ascites[١٢]. Rapid and proper diagnosis of SBP can prevent death although mortality according to this complication is near ٣٠-٥٠٪[١٣].

The diagnosis of SBP is still based on diagnostic paracentesis[١٤]. It is an invasive maneuver with some complications. Therefore, there is a need for other noninvasive diagnostic tools.

SBP pathogenesis in patients with cirrhosis is considered to be the main consequence of bacterial translocation (BT). The BT is the process through which viable or nonviable bacteria and bacterial products (bacterial DNA or endotoxins) cross the intestinal lumen and come into the mesenteric lymph nodes or extra-intestinal. Bacterial translocation also is involved in increasing the hyperdynamic state of cirrhosis and in aggravation of hemostasis disorders[١٥].

Other alternative method using mean platelet volume, C-reactive protein, and white blood cell levels measurement can be considered as an accurate diagnostic test in predicting SBP, possibly because of a continuous systemic inflammatory response[١٦].

Soluble CD١٦٣ which is produced in serum as a result of macrophage activation is found to be increased in liver cirrhosis and portal hypertension but not yet assessed in patients with SBP. In this study, there is a significant increase in serum sCD١٦٣ in SBP versus non-SBP group.

Our study confirmed that there is positive correlation between the level of soluble cd١٦٣ and portal vein diameter and splenic longest axis, these finding may be in accordance with Waidmann et al.,[١٧] who showed sCD١٦٣ correlates strongly with the hepatic venous pressure gradient (HVPG) and is thereby a good indicator of portal hypertension. Holland-Fischer et al.,[١٨] also reported that Hepatic kupffer cells are activated in cirrhotic patients in parallel with their portal hypertension and sCD١٦٣ is a sensitive marker of macrophages activation that positively correlated with the degree of portal hypertension in cirrhotic patients.

The present results show that patients of SBP had significant higher biochemical parameters than patients without SBP and our results was in agreement with Rizk et al.,[١٩] who found that there was significant increase in, AST and creatinine in the SBP group versus the non-SBP group. And also with Wei et al.,[٢٠] who found similar results as regard increased levels of ALT, AST, bilirubin, INR in SBP group as compared with the non SBP group.

In our patients, we reported that at a cut off value of > ٣١٠٠ ng/ml serum s CD١٦٣ had a diagnostic sensitivity of ١٠٠٪ and specificity ٩٥٪ for detecting SBP. Moreover, there is a positive correlation between serum sCD١٦٣ and serum PMN cell count and deterioration of liver function in cirrhotic patients with ascites supported the hypothesis that, serum sCD١٦٣ , being a marker of macrophage activation, could reflect ongoing systemic inflammatory responses in cirrhotic patients with SBP.

The cornerstone for the diagnosis of SBP stills the ascitic fluid analysis with the PMN cell count. Albillos et al.,[٢١] reported that an ascitic fluid PMN count greater than ٥٠٠/mm٣ had specificity and sensitivity of ٩٨٪ and ٩٠٪, respectively. In our cirrhotic patients with ascites, we found high specificity and sensitivity for serum sCD١٦٣ levels in patients with SBP, as discussed before. From our point of view, these values may be considered as an accurate and rapid method for detection of SBP in clinical practice and have similar accuracy in the determination of the PMN cell counts in ascitic fluid.

Conclusion

This prospective study evaluated the diagnostic utility of measuring serum s CD١٦٣ to identify patients with SBP and conclude that patients with SBP had higher serum sCD١٦٣ than ascitic patients without SBP; this finding indicates that serum sCD١٦٣ correlate well and reliably with ascitic PMN cell count. Indeed, serum sCD١٦٣ may serve as a surrogate marker for PMN count and would be valuable for routine SBP screening as a noninvasive rapid and accurate assay.

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