DIAGNOSTIC AND PROGNOSTIC VALUE OF SERUM PRESEPSIN IN CIRRHOTIC PATIENTS WITH SPONTANEOUS BACTERIAL PERITONITIS

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INTRODUCTION

Hepatitis C virus (HCV) is a major health problem affecting 170 million people worldwide. The WorldHealth Organization (WHO) estimated in 2011 that Egypt comes first worldwide in HCV prevalence,with chronic infection leading to fibrosis of the liver and ultimately to cirrhosis. SBP (Spontaneous Bacterial Peritonitis) is one of the most frequent and life-threatening complications of patients with cirrhosis. It is an infection of ascitic fluid that cannot be attributed to any intra-abdominal, ongoing inflammatory, or surgically correctable condition. More than 92% of all cases of SBP are monomicrobial. Gram-negative bacteria remain the most common pathogens in SBP.

Pathophysiology:
Bacterial translocation (BT) is the most common cause of SBP. Limited BT to mesenteric lymph nodes (MLN) is a physiological phenomenon, whereas any increase in the rate and severity of BT should be termed 'pathological BT'. Only a few intestinal bacteria are able to translocate into MLN, including *Escherichia coli*, *Klebsiella pneumoniae* and other Enterobacteriaceae.

According to the International Ascites Club classification of ascitic fluid infections
-Spontaneous bacterial peritonitis (SBP) :Is defined by an absolute neutrophil count (ANC) ≥250 cells/mm³and because of the difficulties in culturing the pathogen, the criteria do not require a positive culture.
Diagnosis:
i. The diagnosis of SBP is based on PMN leucocytes count (≥ 250 mm^3) in ascitic fluid.
ii. A "clinical diagnosis" of infected ascitic fluid without a paracentesis is not adequate to rule out infection.
iii. peritoneal fluid culture (although yield is poor).
Treatment:
•Empirical antibiotic therapy must be initiated immediately after the diagnosis of SBP, without the results of ascitic fluid culture.
•Cefotaxime or a similar third-generation cephalosporin appears to be the treatment of choice for SBP.
•The use of prophylactic antibiotics must be strictly restricted to patients at high risk of SBP as those with:
• Acute gastrointestinal hemorrhage or low total protein content in ascitic fluid less than 1.0g/dl with no prior history of SBP (primary prophylaxis) or patients with a previous history of SBP (secondary prophylaxis).

Thus an early start of antibiotic therapy is important for the successful treatment of SBP and has been shown to reduce mortality and improve survival.Therefore, there has been considerable interest in the development of a new test that can diagnose SBP rapidly.
In the past few years, **Presepsin:** A new biomarker (sCD14-ST) has been proposed in the field of sepsis.

Biological characteristics of presepsin:

CD14 has two forms: membrane-bound CD14 (mCD14) and soluble CD14 (sCD14). mCD14 co-localizes with toll-like receptor 4(TLR4). Activating the TLR4-specific proinflammatory signaling cascade thereby starting the inflammatory reaction of the host against infectious agents. The complex of LPS-LPBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding soluble CD14 (sCD14). However, plasma protease activity generates also another sCD14 molecule called sCD14 subtype (sCD14-ST) or presepsin.
Presepsin lacks the ability of LPS-binding and cannot be detected by anti-CD14 antibodies.
Presepsin is currently under investigation in clinical practice as a reliable marker of adult and neonatal sepsis and for the postmortem diagnosis of sepsis-related death .
On the basis of these premises, we designed a prospective study to evaluate the usefulness of presepsin in the early diagnosis of SBP in cirrhotic patients with ascites as well as its predictive value in those patients.

MATERIALS AND METHODS

This study was conducted on 30 chronic HCV cirrhotic patients admitted to hepatobiliary unit, internal medicine department, Alexandria Main University Hospital during the period from December 2012 to June 2013 and they were divided into two groups based on clinical criteria and ascitic fluid analysis.
Group I: 10 patients with liver cirrhosis and ascites (as a control group).
Group II: 20 patients with liver cirrhosis and SBP.
Patients with other infection conditions were excluded from the study.
All patients were subjected to the following:
-Full history taking including risks for acquiring SBP, any received treatment, investigations done before and any associated medical diseases.
-Clinical evaluation
-**Laboratory investigations including**
Complete blood picture.
Liver function tests .
Serum alanine aminotransferase (ALT).
Serum aspartate aminotransferase (AST).
Total and direct serum bilirubin.
Serum albumin.
Prothrombin time and INR.
Urine analysis.
Widal test.
Ascitic fluid analysis to estimate the polymorph nuclear leukocyte (PMNL) counts in ascitic fluids. It was repeated in group II after 10 days of starting treatment.
Ascitic fluid culture.
Presepsin level. It was measured again in group II after 10 days of starting treatment.
Plain chest x ray.
To exclude any chest infection.
Paracentesis :
Paracentesis was done after taking Informed consent from the patient.
Collected ascitic fluid samples were sent for:
-Pathological assessment for PMN counts.
-Microbiological assessment where the specimen was centrifuged and deposit subcultured on blood, chocolate and MacConkey's agar at 37ºC for 48hours.

PRESEPSIN
PATHFAST Presepsin
Principle of test:
The test principle of PATHFAST Presepsin is based on non-competitive CLEIA technology. During incubation of the sample with alkaline phosphatase labeled anti presepsin polyclonal Antibody and anti presepsin monoclonal antibody coated magnetic particles, the presepsin of the sample binds to the anti presepsin antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound substances, a chemiluminescent substrate is added. After a short incubation, the luminescence intensity generated by the enzyme reaction is measured. The luminescence intensity is related to the presepsin concentration of the sample which is calculated by means of a standard curve.
Sample collection:
We used a whole blood sample collected with qualified vacutainer collection tubes containing heparin and sent it immediately to the lab.
Procedure and preparation:
1-samples were centrifuged on arrival to the lab.
2- A reagent cartridge was set in cartridge rack, then approximately 100 µL of the sample was pipette into a sample well of a cartridge and the cartridge rack was loaded on PATHFAST.
3-It is rapid method, takes only 17 minutes.
Assay range of presepsin is from 20 to 20,000 pg/ml. A value >377 pg/ml was considered positive as indicated by manufacturers
Serum presepsin levels were measured in the two studied groups (T0) and it were measured again in group II (T1) after10 days of starting treatment for SBP.

RESULTS

Ascitic fluid analysis showed a significant increase in the neutrophilic count in ascitic fluid in patients with spontaneous bacterial peritonitis in comparison to cirrhotic patients with ascites (p=0.000)
As regard culture of ascitic fluid; negative culture results predominates by a ratio of 10:1 as patients were on prophylactic antibiotic therapy. The causative organisms of culture positive SBP were E.coli and klebsiella

Presepsin serum level measurements in the studied subjects ranged from 109 to 195 pg/ml with a mean of 148.6 and a median 145 in cirrhotic patients with sterile ascites (GroupI), while it was (T0) 598 to 5100 pg/ml with a mean of 3473 and a median of 4621.5 in cirrhotic patients with spontaneous bacterial peritonitis (GroupII) showing a statically significant difference between the two groups (p=0.000). Figure (1).

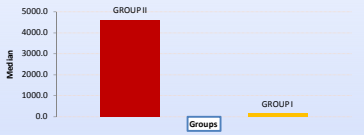

Figure (1): comparison between serum levels of presepsin in the two studied group.

Therapeutic outcome
By following up the patients with spontaneous bacterial peritonitis 16 patients were resolved while 4 patients deceased.
Ascitic fluid analysis in the resolved patients of group II:
As regards neutrophilic count in ascitic fluid in the survived patients of group II, it was significantly decreased with a mean of 107.7 and a median of 105 in comparison to the pretreatment values which were a mean of 1356 and a median of 430. Thus a statically significant difference was found between patients of group II before and after recovery (P=0.000) . Figure(2).
Presepsin level in the resolved patients of group II:
As regards presepsin level (T1) after 10 days of therapy in the resolved patients of groupII, it was obviously decreased with a mean of 673.4 ± 245 and a median of 705 in comparison to the pretreatment values which were a mean of 2364.8 ± 2221.5 and a median of 1180. Thus a statically significant difference was found between patients of group II before and after recovery (P=0.000). Figure(2).

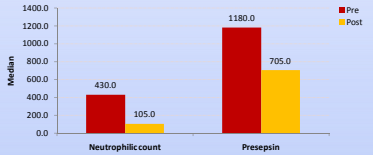

Figure (2): comparison between neutrophilic count and presepsin level in patients of group II before and after recovery.

Relation between presepsin level in patients with spontaneous bacterial peritonitis and their outcome:
Cirrhotic patients with SBP (GroupII) were classified according to their presepsin level (T0) into two categories :(according to decision thresholds by Spanuth E et al) .Figure (3).
a) Moderate risk for sepsis (presepsin level <1000pg/ml): they were 4 (20%) cases and all resolved.
b) High risk for sepsis (presepsin level>1000 pg/ml): they were 16 (80%) cases; 4 cases of them deceased and 12 resolved.
Thus mortality rate among moderate risk for sepsis patients was 0.0%, while it was 25% among high risk for sepsis patients (P=0.049).
Accordingly presepsin level might have a predictive value in the treatment outcome of cirrhotic patients with spontaneous bacterial peritonitis
Regarding presepsin level in the 4 deceased cases, the median was 4631 while the resolved cases had a median of 3915 (P=0.043).

Serum level of presepsin in resolved cases:
Regarding presepsin level after 10 days of therapy (T1) in the resolved cases, it can be classified into three categories according to decision threshold by Spanuth E et al.
a) Systemic infection (sepsis) possible (presepsin level <500 pg/ml): they were 4 cases (25%) .
b) Moderate risk of sepsis (presepsin level <1000pg/ml): they were 11 cases (68.8%).
c) High risk of sepsis (presepsin level>1000 pg/ml): only 1 case (6.3%).
Thus presepsin level can aid in the follow up and monitoring response to treatment in patients with spontaneous bacterial peritonitis.

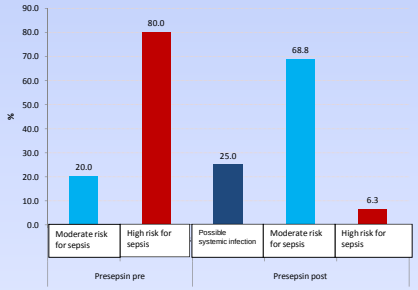

Figure (3): Comparison between serum level of presepsin before and after treatment

CONCLUSION

•Our previous data suggest that the measurement of serum presepsin can be a useful tool for early diagnosis of SBP in cirrhotic patients with ascites.
•Measurement of serum presepsin level may be a predictor for the treatment outcome of cirrhotic patients with SBP.
•Further studies that evaluate serum presepsin measurements in cirrhotic patients with SBP before and after treatment are needed.

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