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Original article

Serum Endocan Levels in Patients with Spontaneous Bacterial Peritonitis

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a common severe complication in patients with liver cirrhosis. Endocan is a proteoglycan (PG) secreted by vascular endothelium, its serum level is elevated with an inflammatory or a malignant process. **Aim**: this study aimed to determine levels of serum endocan in cirrhotic patients with and without spontaneous bacterial peritonitis. **Methodologies**: A total of 83 patients with cirrhotic ascites were included in the study.41 of them had SBP . The severity of liver cirrhosis was assessed using the Child-Pugh score, the Model for End Stage Liver Disease (MELD), and its update (uMELD) scores . Ascitic fluid samples were collected for leucocytic count differentiation, albumin, glucose estimation, and the serum ascitic albumin gradient. . If the polymorph-nuclear leucocytic count in ascitic fluid was equal to or greater than 250/mm3, SBP diagnosed. **Results:** The serum endocan level was significantly higher in patients with SBP ROC analysis of serum endocan as a marker for SBP diagnosis revealed: sensitivity of 88.9%, specificity of 61.5% , and area under curve (AUC) of 0.792 at a cut-off value of 67.1(pg/ml). **Conclusion:** Although endocan level was higher in serum of SBP patients, it cannot be used as a diagnostic marker in SBP.

Keywords: Endocan, Cirrhosis, Ascites, Spontaneous bacterial peritonitis (SBP)

Introduction:

Ascites is the pathologic accumulation of fluid in the peritoneal cavity, and its most common cause is cirrhosis (1).It is the most common complication of cirrhosis and occurs in about (50%-60%) of patient with decompensated cirrhosis in ten years (2). Patients with cirrhotic ascites have a 3-year mortality rate of approximately 50% (3).

Spontaneous bacterial peritonitis is defined as a bacterial infection of ascitic fluid, without an evident source of sepsis in the peritoneum or adjacent tissues in patients with decompensated liver diseases (4).In ascitic patients, the frequency of spontaneous bacterial peritonitis may be as high as 18%,this number has grown from 8% over the past 2 decades, most likely secondary to an increased awareness of spontaneous bacterial peritonitis(5).

The prevalence of SBP in patients with liver cirrhosis ranges anywhere from twenty to fifty percent, depending on the study reviewed, with inpatient mortality rates as high as 32% (6), Survival after the first episode is estimated to be 40% at 1 year(7). Gram-negative bacteria were the main causative agents of spontaneous bacterial peritonitis, with *Escherichia coli* and *Klebsiella* spp. being the most frequently isolated organisms (8).

A rising prevalence of gram-positive bacteria was reported over the past years in North America, South America, and Europe representing at present 48%–62%of the isolated organisms.(9) The most frequent gram-positive isolates are *Streptococcus* spp., *Enterococcus* spp., and *Staphylococcus* spp.(10).

Clinical manifestations, though not always present, typically include fever, chills, and abdominal pain/discomfort, and may progress to mental status alterations and sepsis (11) SBP may be accompanied by other signs of decompensation such as jaundice, ascites, portal hypertension (with or without resultant gastrointestinal bleeding), hepatic encephalopathy, and hepatorenal syndrome (12).It is defined by presence of ≥250 cells/mm^3 polymorphonuclear cells (PMNs) in the ascitic fluid without evidence of an intra-abdominal treatable source *(13).*

Endocan (endothelial-cell-specific molecule-1 (ESM-1) is a soluble proteoglycan (50 kDa) (14), secreted by human vascular endothelial cells, mainly

liver, lung and kidney. Its secretion is increased in a variety of endothelium-dependent pathological states, such as inflammation, infections, tumor progression, and atherosclerosis (15).

The aim of this study was to determine the amount of serum endocan in liver cirrhosis patients with and without spontaneous bacterial peritonitis.

Patients and methods

This cross sectional study was conducted on 83 patients (consecutive) with cirrhotic ascites with and without spontaneous bacterial peritonitis admitted to Department of Hepatology, Gastroenterology and Infectious Diseases of Benha University Hospital, from December 2019 to May 2020.

Patients were divided into two groups: Group I, which included 41 patients with SBP and Group II, which included 42 patients without SBP.

This study was reported to and approved by the committee of ethics of scientific research in Benha Faculty of Medicine in Benha University .All patients were verbally briefed about the study's procedures, with written consent obtained from all participants.

Criteria for inclusion: liver cirrhosis and ascites in patients over the age of 18 years.

Patients with ascites due to causes other than cirrhosis with portal hypertension, patients with hepatocellular carcinoma, patients with recent abdominal surgery, patients with chronic kidney disease, diabetes mellitus, cardiovascular disease, cardiac decompensation, patients treated with non-absorbable antibiotic in the preceding 6 weeks, patients with non-peritoneal infection (skin infection, chest infection, urinary tract infections, meningitis, dental infections, gastroenteritis, biliary tract infections) were all excluded.

Cirrhosis was diagnosed by combination of clinical, biochemical, and imaging data as abdominal ultrasound. The following procedures were performed on all patients: full medical history was taken, a full general and local examination was performed, , and complete blood samples, liver profile, kidney function tests, and serological tests for viral markers, abdominal paracentesis for diagnostic purposes, Child Pugh score(16), MELD score (17),uMELD score (18) and ultrasonography of the abdomen was used.

Peripheral blood samples were collected in serum separator tubes and were allowed to clot for 30 minutes before centrifugation for

10 minutes at approximately 3000×g. Serum was removed and samples were stored at -20°C. Endocan levels were determined by reagents for human Endocan ELISA Kit Cat.No.E-1909hu. Cloud-clone corp laboratory, Lot #202008 .

Statistical analysis:

SPSS program (statistical package of social science; SPSS Inc., Chicago, IL, USA) version 24 for Microsoft Windows was used to perform the statistical analysis .

The Independent t-test (t) was used to detect differences in the mean between two parametric data and the Mann-Whitney (MW) test was used to detect differences between two non-parametric data when comparing the different sample groups ,the Chi-square test (x2) was used to detect differences in proportions as required. The correlation between serum endocan and estimated parameters was evaluated using the Spearman Correlation coefficient (rho).

The diagnostic efficiency of serum endocan for SBP was evaluated using a Receiver Operating Characteristics (ROC) study. Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Area under the Curve (AUC) were calculated to determine the best cut-off point and its corresponding sensitivity and specificity. Statistical significance was accepted at P value <0.05 (S). A P value <0.001 was considered highly significant (HS) while a P value >0.05 was considered non-significant.

Results:

Patients with SBP had a higher mean age and more common in males than females, but there was no significant statistical difference. . While HCV is the most common cause of liver cirrhosis, there was no statistically significant difference in viral markers between the two groups. . Group I had substantially more abdominal pain, GIT bleeding, and fever than group II. Regarding jaundice was higher in group I than group II with no statistically significant difference. Hepatic encephalopathy was significantly higher in group I than in group II. Group I had significantly lower haemoglobin levels than group II. White blood cells were significantly higher in group I than group II. Group I had a higher levels of aspartate aminotransferase (AST) but with no significant statistical difference. Total bilirubin, international normalised ratio (INR), and serum creatinine were significantly higher in group I than group II. Group I had a higher child grade (C) than group II with significantly statistical difference. Group I had significantly higher

levels of model end stage liver disease disease (uMELD) than group II, as shown in table 1.Ascitic fluid Polymorph nuclear leucocytes were significantly higher in patients with SBP than in patients without SBP. Group I had significantly lower levels of ascitic fluid albumin and glucose. There is no significant statistical difference between two groups regarding Serum ascitic albumin gradient (SAAG) as shown in table 2.

ROC review for serum endocan as a marker for SBP diagnosis showed the following: (MELD) and updated model end stage liver

Cut off value for endocan which gave optimum balance between sensitivity and specificity was 67.1 (pg/ml) with sensitivity 88.9% and specificity 61.5% and Positive Predictive Value 85.1 % and Negative Predictive Value 66.4% area under curve 0.792, as shown in table 3.

There is a positive correlation between Endocan and white blood cells, ascitic polymorph nuclear leucocytes, Child Pugh score , MELD and u-MELD Scores but not statistically significant as shown in table 4.

Table 1. Baseline data of the studied groups.

Parameters	Group I with SBP Number=41	Group II without SBP Number=42	P value
Age (mean±SD)	63.37± 8.51	61.10± 9.48	0.254
Gender:			
Male	27(65.9%)	27(64.3%)	0.881
Female	14(34.1%)	15(35.7%)	
HCV Ab	39(95.1%)	38(90.4%)	0.540
Abdominal pain	31(75.6%)	15(35.7%)	<0.001(HS)
GIT bleeding	30(73.2%)	15(35.7%)	0.001
Fever	31(75.6%)	5(11.9%)	<0.001(HS)
Jaundice	27(65.9%)	20(46.6%)	0.094
Hepatic encephalopathy	31(75.6%)	16(38%)	0.018
Haemoglobin(gm/dl) (mean±SD)	8.88± 1.57	10.2±1.42	0.037(S)
White blood cells (cells/Litre)	13465.85± 4008.22	7669.05± 2358.74	<0.001(HS)
(mean±SD)			
AST (IU/L)	70.17± 12.33	64.18± 15.09	0.076
(mean±SD)			
Total bilirubin (mg/dl)	4.18± 2.40	2.46±1.21	0.001(S)
(mean±SD)			
INR	1.63± 0.43	1.4± 0.36	0.001(S)
(mean±SD)			
Creatinine (mg/dl)	1.82± 1.12	1.14 ± 0.7	0.002(S)
(mean±SD)			
Serum endocan	45.00-226.80	0.00-200	<0.001(HS)
Range			
(pg/ml)			
Child Pugh score:			
Grade B	7(17.1%)	20(38.1%)	0.006
Grade C	34(82.9%)	28(52.4%)	
MELD score	22.00± 5.17	15.86± 5.66	<0.001(HS)

Mean±SD : mean±standard deviation. **(S**) =Significant, **P value (<0.05). (HS)=**Highly significant, **P value (<0.001). HCV Ab:** Hepatitis C virus antibody. **GIT: Gastrointestinal** bleeding. **MELD:** Model End Stage Liver Disease.**uMELD:** updated Model End Stage Liver Disease.

Table2. Ascitic fluid analysis of the studied groups:

Ascitic fluid analysis	Group I with SBP N=41	Group II without SBP N=42	P value
Polymorph nuclear leucocytes(cells/mm^3) (mean±SD)	837.07±710.5	132.85±45.54	0.001(S)
Albumin(gm/dl) (mean±SD)	0.49± 0.15	0.85± 0.21	<0.001(HS)
Glucose(mg/dl) (mean±SD)	87.93± 14.44	122.95± 27.77	<0.001(HS)
Serum ascitic albumin gradient(SAAG) (≥1.1mg/dl) (mean±SD)	1.73± 0.74	1.99± 0.64	0.093

(Mean±SD) =mean±standard deviation.

HS =highly significant, P value (<0.001).

Table 3: ROC analysis for serum endocan as marker for diagnosis of SBP.

Cut-off level	67.1(pg/ml)
Sensitivity (%)	88.9%
Specificity (%)	61.5%
Positive predictive value (%)	85.1%
Negative predictive value (%)	66.4%
AUC	0.792

Table 4: correlation between endocan and some parameters.

Correlation of endocan with	Correlation coefficient (rho)	P value
White blood cells	0.176	0.27
Polymorph nuclear cells (PNCs)in ascitic fluid	0.17	0.31
Child Pugh score	0.14	0.31
MELD score	0.15	0.38
uMELD	0.017	0.67

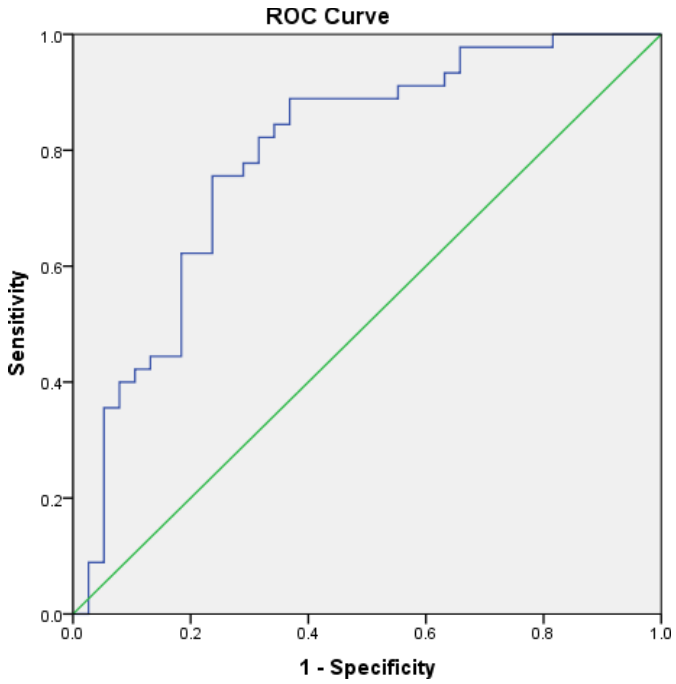

Fig1: ROC analysis for serum endocan as marker for diagnosis of SBP.

Discussion

Bacterial infections are a well-known cause of morbidity and mortality in cirrhotic patients, (*19*), Spontaneous bacterial peritonitis is the most frequent bacterial infection in cirrhotic patients with mortality rates reported at 20–40% among the cirrhotic patients with ascites (20). Patients with polymorphonuclear cells (PMNs) in the ascitic fluid ≥250 cells/mm^3 are diagnosed with SBP. Endocan, a newly recognized biomarker, has been investigated only with respect to its relationship with sepsis, regarding its role in SBP, a previous study aimed to identify the endocan as risk factor for development of decompensation event (i.e. SBP) to optimize stratification for primary prophylaxis and therapeutic strategies to improve survival (21). Serum endocan levels are detectable as early as 2 hours after starting the inflammatory response (22), which is earlier than the elevations in both PCT and CRP (23).

Endocan shows active kinetic properties that allow it to serve as an early diagnostic as well as a follow-up (72 hours and beyond) marker of inflammation and infection. Endocan is still measurable when investigated on a daily basis, whereas CRP

or PCT will have already cleared from the blood (24).

SBP patients had significantly higher endocan levels (45.00-226.80) than non SBP patients (00.00-200.00) in this sample. This was in accordance with a study showed that serum endocan levels in decompensated cirrhotic patients with spontaneous bacterial peritonitis [median (IQ) 3.4 (2.16–5.0)ng/ml] were significantly elevated than in patients without spontaneous bacterial peritonitis [median (IQ) 67.1 (5.1–8.75) ng/ml] (25) . also, another study found that serum endocan levels were significantly higher in patients with SBP [median (IQ) 3.2 (0.55–5.9)ng/ml] than patients without SBP [median (IQ) 6.2 (5.3–8.75)ng/ml] (26).

In the current research, the following ROC analysis was performed for serum endocan as a marker for SBP diagnosis; the cut-off value for serum endocan was 67.1 (pg/ml), the sensitivity was 88.9 percent, the specificity was 61.5 percent, and the area under the curve (AUC) was 0.792.

Conclusion: According to this study, although endocan level was higher in serum of SBP patients, it cannot be used as a diagnostic marker in SBP.

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