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COMPARATIVE STUDY OF ASCITIC FLUID AND SERUM LEVELS OF CALPROTECTIN, PROCALCITONIN AND ENDOCAN IN PATIENTS WITH LIVER CIRRHOSIS FOR EARLY DIAGNOSIS AND PREDICTION OF SPONTANEOUS BACTERIAL PERITONITIS

By

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is a serious, recurrent, and life-threatening condition developing in cirrhotic patients with a high mortality rate. Its diagnosis is based on ascitic fluid polymorphonuclear leukocytes (PMNs) to be more than 250/?L.

Objectives: polymorphonuclear leukocytes (PMNs) to be more than 250/?L. Objective: The aim of the study was to evaluate the ascitic fluid and serum levels of calprotectin, procalcitonin (PCT) and endocan as appropriate markers for predicting and diagnosing SBP.

Patients and Methods: This study was conducted on 90 patients with liver cirrhosis: 35 with decompensated cirrhosis and spontaneous bacterial peritonitis, 35 with decompensated cirrhosis without spontaneous bacterial peritonitis and 20 with compensated cirrhosis with no ascites. We evaluate the correlations of calprotectin, procalcitonin and endocan with indicators of infection and inflammation associated with spontaneous bacterial peritonitis in liver cirrhotic patients. Ascitic fluid and serum levels of Calprotectin, procalcitonin, endocan, and polymorphonuclear leukocytes, serum CRP, and blood leukocytes were analyzed. The control group (n = 30) composed of healthy blood donors with normal aminotransferase levels, normal complete blood counts and negative markers for viral hepatitis and HIV.

Results: Ascitic fluid and serum levels of Calprotectin, procalcitonin, endocan, and PMNs, serum CRP and blood leukocytes were statistically elevated in cirrhotic patients with SBP than the control group and cirrhotic patients without SBP. There were statistically significant correlations between the existence of SBP with serum calprotectin (r = 0.512), serum procalcitonin (r= 0.370), serum endocan (r = 0.501), ascitic calprotectin (r = 508), ascitic procalcitonin (r = 0.501), ascitic endocan (r = 0.496), ascitic PMNs (r = 0.562), and CRP (r = 0.492), for all $P < 0.001$. The diagnostic accuracies of calprotectin, endocan, procalcitonin, and PMNs were elevated in progressive disease stage. Ascitic PMNs $\geq 250/mm^3$ had a sensitivity of 97% and specificity of 94.7%, Serum calprotectin levels $\geq$ 45 μg/ml had a sensitivity of 96% and specificity of 94%, ascitic calprotectin levels $\geq$ 0.95 μg/ml had a sensitivity of 95% and specificity of 89.2%, serum endocan levels $\geq$ 2.03 ng/ml had a sensitivity of 90% and specificity of 83.7%, ascitic procalcitonin levels $\geq$0.33ng/ml had a

sensitivity of 89.9% and specificity of 83.3%, ascitic endocan levels ≥ 0.65 ng/ml had a sensitivity of 88.9% and specificity of 78.5%, and lastly serum procalcitonin levels ≥2.50 ng/ml had a sensitivity of 87.9% and specificity of 76.8% for the diagnosis of SBP in decompensated cirrhotic patients.

Conclusion: Ascitic fluid PMNs, serum calprotectin, ascitic calprotectin,serum endocan, ascitic procalcitonin, and serum procalcitonin, could be useful as powerful diagnostic markers to assess the progression of liver disease and early prediction of spontaneous bacterial peritonitis in cirrhotic patients.

Key words: Calprotectin, Procalcitonin, Endocan, Liver cirrhosis, Spontaneous bacterial peritonitis.

INTRODUCTION

Cirrhosis is considered an immunocompromised state that leads to a variety of infections, that can account for an approximately 30% of mortality *(Tendon and Garcia-Tao., 2008)*. Apart from early identification and preferable therapy of spontaneous bacterial peritonitis (SBP) leading to better survival, there has been little amelioration in overall mortality rates in recent decades: infections until now account for a 4-fold rise in mortality among patients with liver cirrhosis *(Arvaniti et al., 2010)*. Hospitalized patients with cirrhosis are at the intensive risk of having infection, especially in those with gastrointestinal (GI) bleeding. Bacterial infections accounting for 32% to 34% of in hospitalized patients with cirrhosis and for 45% of those with gastrointestinal hemorrhage *(Arvaniti et al., 2010)*. These rates are seriously higher than the usual 5% to 7% overall rate of infection in admitted patients *(Tendon and Garcia-Tao., 2008)*.

The prevalence of SBP in cirrhotic patients with ascites admitted to the hospital varies between 10% and 30%; nearly half of cases are existent at the time of hospital admission and the other half acquire the infection during hospitalization *(Dever and Sheikh., 2015)*. The acquired in-hospital mortality rate from SBP is nearly 32% *(Arvaniti et al., 2010)*. The predominance of these infections is caused by E. coli, streptococci, Klebsiella spp., other Enterobacteriaceae, P. aeruginosa, and enterococci *(Alexopoulou et al., 2013)*. The prevailing recommendation is to carry out a diagnostic paracentesis in almost all patients with ascites at the moment of hospital admission and in those who presented with symptoms of peritoneal infection, systemic manifestations of infection, hepatic encephalopathy, or rapid deterioration in renal function while hospitalized *(Alexopoulou et al., 2013, and Dever & Sheikh., 2015)*. The diagnosis cutoff of SBP is ascitic fluid polymorphonuclear (PMN) cell count of 250 cells/mm^3 while the highest specificity is reached at 500 cells/mm3 *(Yuan et al., 2013)*.

Calprotectin is an abundant, calcium- and zinc binding protein found mainly in neutrophils *(Lutz et al., 2015)*, and its presence in body fluids is proportional to the influx of neutrophils *(Soyfoo et al., 2009)*. It is an acute phase inflammatory reactant protein that exerts regulatory, antimicrobial and anti-proliferative functions. It can halt bacterial growth, playing an important role in non-specific immune reactions *(Alempijević et al., 2014)*. A study for measurement of ascitic fluid calprotectin by reagent strips as a bed side test for rapid diagnosis of SBP

showed high specificity (83%) and sensitivity (100%) *(Burri et al., 2013)*. Another study reported a significant correlation between elevated fecal calprotectin level and the occurrence of some complications in cirrhotic patients such as hepatic encephalopathy and SBP *(Gundling et al., 2011)*. However, the role of serum calprotectin in diagnosing SBP remains unexplored.

Procalcitonin, a calcitonin precursor, is a glycopeptides containing 116 amino acids and is produced by the C cells of thyroid gland. In the healthy population, pro- calcitonin levels are very low (<0.15 ng/mL) or undeterminable. Sepsis and serious invasive infections are the principal causes of increased procalcitonin levels and are rapidly decreased by appropriate antibiotic treatment *(Bode-Janisch et al., 2013)*. In contrast, viral infections, noninfectious inflammations, and malignant diseases have low or undetectable procalcitonin levels *(Becker et al., 2008, Milcent et al., 2016,* and *Schuetz et al., 2017)*. Studies have demonstrated that procalcitonin is a preferable diagnostic marker rather than white blood cell (WBC) count or C-reactive protein (CRP) concentration for the diagnosis of infection *(Cekin et al., 2013)*. In 2014, a systemic review indicated that procalcitonin assessment is a relatively sensitive and specific biomarker for the diagnosis of bacterial peritonitis *(Yang et al., 2014)*.

Endocan, formerly called endothelial cell specific molecule-1 is expressed by endothelial cells, which are in the lung, liver and kidney. It has been shown that, the synthesis and secretion of endocan are up-regulated by tumor necrosis factor, interleukin-1, and lipopolysac-charides. Also it is over expressed in all human tumors and plasma levels of endocan are elevated in late-stage lung cancer and experimental tumor models. These results suggest that endocan is a biomarker of inflammatory disorders and tumor progression besides as a validated therapeutic target in cancer *(Balta et al., 2014,* and *Helmy et al., 2017)*. Endocan gene expression levels may be due to the inflammatory cytokines as well as the lipopolysaccharides (LPS) of the gram-negative bacterial cell wall, and thus increases *(Yilmaz et al., 2014, Cox et al., 2015,* and *Dallio et al., 2017)*. The inflammatory response to infection as manifested by elevated serum values of TNF-α and IL-6 is augmented in patients with liver cirrhosis *(Cazzaniga et al., 2009* and *Lee et al., 2014)*. A small number of studies have demonstrated that endocan can be accepted as a good marker of endothelial dysfunction and in sepsis with multi-organ failure *(Mihajlovic et al., 2014,* and *Pauly et al., 2016)*. In clinical setting, however, the usefulness and expression of endocan in cirrhotic patients with bacterial infections have not been investigated.

The aim of this study was to evaluate the potential role of ascitic fluid and serum levels of calprtectin, procalcitonin and endocan as an appropriate marker for prediction and early diagnosis of spontaneous bacterial peritonitis in patients with liver cirrhosis.

PATIENTS AND METHODS

In this prospective case-control study, we recruited consecutive 90 patients with liver cirrhosis with and without decomposition (20 patients without ascites

and 70 patients with ascites) admitted to the Gastroenterology Unit of Internal Medicine Department, Al-Husein University Hospital, Cairo, Egypt from August 2016 to December 2017. The control group (Group 1) comprised thirty healthy blood donors (20 males / 10 females, mean age 52.3, range from 43-63 years) with normal aminotransferase levels, normal complete blood counts and negative markers for viral hepatitis and HIV. Patients were divided into three groups based on morphological and bacteriological results: compensated patients without ascites at admission (Group 2 - n = 20), patients with decompensated liver cirrhosis without spontaneous bacterial peritonitis (Group 3 - n = 35), and patients with decompensated liver cirrhosis and spontaneous bacterial peritonitis (Group 4 - n = 35).

The Ethical Research and Review Committee of the Hospital approved the study protocol, and informed consents were obtained from the participants.

All patients were exposed to the following evaluations: full medical history and clinical examination, abdominal ultrasound of hepatobiliary system, laboratory measurements, and examination of ascitic fluid (PMNs, bacteriologic culture and sensitivity). As a routine measure, diagnostic aspiration (paracentesis) of ascitic fluid was done for all patients with liver cirrhosis and ascites who were accepted for admission to our department, unrelated to the clinical suspicion of ascitic fluid infection (AFI). Thus, its diagnosis was based on the presence of at least 250 cells/mL PMN in the ascitic fluid, accompanied with or without positive ascitic fluid culture in the lack of secondary peritonitis and hemorrhagic ascites.

Inclusion criteria:

Patients with liver cirrhosis with and without ascites, Age ≥ 18 years old.

Exclusion criteria:

Included patients who were immunocompromised and patients who had received antibiotic on prophylactic basis for SBP or prior to hospital admission. Moreover, patients with malignant lesions, with clinically overt thyroid dysfunctions or with autoimmune disorders, hematological disorders, diabetes mellitus, peripheral vascular disease, hypertension, hyperlipidemia, heart failure, and sepsis as well as other bacterial infection among patients with cirrhosis were also eliminated from this study. None of the study participants had exposed to non-steroidal anti-inflammatory drugs (NSAIDs), oral contraceptive drugs, and anticoagulant therapy before hospital admission.

Diagnosis of cirrhosis was proved by the combination of clinical, biochemical and ultrasound imaging data, presence of irregular margins on ultrasound, portal hypertension with laboratory evidence of chronic liver disease compatible with such a diagnosis. Patients were categorized as regard to Child-Turcotte-Pugh classification [three biochemical variables (serum albumin, bilirubin,

and prothrombin time (international normalized ratio, INR) in addition to the presence or absence of ascites and clinical signs of encephalopathy]. Patients were scored as follows: 5 – 6 as class A (10 patients = 14.4%), 7 – 9 as class B (30 patients = 42.8 %) and 10 – 15 as class C (30 patients = 42.8 %). At the time of the study no Child-Pugh class A patients showed clinical signs of decompensated liver cirrhosis (ascites or hepatic encephalopathy).

Ascites and hepatic encephalopathy were present by physical examination in 70 (77.8%) and 21 (23.3%) patients, respectively. Presence of ascites was assessed by ultrasonography and revealed that 20 patients (22.2 %) have no ascites, 35 patients (38.9 %) have slight ascites, and 35 patients (38.9 %) have moderate ascites. The etiology of liver cirrhosis in our patients revealed that 78 patients (86.7 %) have chronic HCV infection, 10 patients (11.1 %) have chronic HBV infection, and 2 patients (2.2 %) have a history of alcohol intake.

Peripheral venous blood from overnight fasting healthy subjects and cirrhotic patients was collected in separate tubes, one containing the anticoagulant EDTA and the other was a plain tube for serum collection. Serum was then separated and aliquot into tubes for storage. The tubes were stored frozen at –80°C until they were used to study different parameters.

Laboratory determinations:

Biochemical parameters were measured before the use of antibiotics at admission. Calprotectin levels were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by (Shanghai Sunred Biological Technology Co) with normal assay range: 0.15-40 ?g/ml ((Sunred Human (PMN Calprotectin) *(Elisa kit, 2014),* and *(Gad et al., 2015).* Procalcitonin (PCT) was analyzed using an immunoluminometric assay (LUMI test R PCT; BRAHMS Diagnostica, Berlin, Germany). Detection limit was 0.05 ng/ml. Endocan levels were determined by ELISA analyses (JDIEK H1) (Lunginnov SAS, Lille, France). The assay range of the ELISA kit was 0.15 ng/ml to 10 ng/ml. Serum C-reactive protein (CRP) level was assessed with a high-sensitivity nephelometric method using the Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, CA, USA), which has a minimum level of detection of 0.2 mg/L.

Ascitic fluid (AF) from patients included in this study was prospectively collected. Upon admission, a paracentesis was done using a standard sterile method in all patients with ascites. Within 30 minutes, the specimen obtained was centrifuged for 15 minutes and stored at–80 ?C until analysis was performed.

Ascitic fluid cultures were done with conventional cultures methods. Specimens were also inoculated in aerobic and anaerobic blood culture bottles. On the bases of previously defined criteria, patients were classified to two groups; the sterile ascitic fluid group (38 patients = 54.3%), and the SBP group (33 patients = 47.1 %). Ascitic calprotectin in ascites was assayed using a commercially-available ELISA (Bühlmann Laboratories AG, Sch?nenbuch, Switzerland). The measuring range of the test was 0.2-12 ?g calprotectin/mL ascites with an intra- and interassay coefficient of 4.7% and 11.3%, respectively. Ascitic endocan levels were determined by ELISA analyses (JDIEK H1) (Lunginnov SAS, Lille, France). The assay range of the ELISA kit was 0.15 ng/ml to 10 ng/ml. Ascitic procalcitonin was analyzed using an immunoluminometric assay (LUMI test R PCT; BRAHMS Diagnostica, Berlin, Germany). Detection limit was 0.05 ng/ml.

Statistical Analysis:

Statistical analysis was performed using the statistical package SPSS version 23. The data were expressed as mean ± SD. They were compared by t- student test for comparison between two groups and ANOVA test when more than two groups were compared using Tukey test. The Mann-Whitney U test was used to analyze differences among two groups. The association between different variables was assessed by f- test. Also, Pearson's r correlation and chi – square test were used. Statistical significance was considered at $P \leq 0.05$. Receiver operating characteristics (ROC) curve was used to evaluate the performance of different tests, and DeLong test was used to compare between the areas under the curves (AUCs).

RESULTS

A total of ninty patients with liver cirrhosis were consecutively analyzed. The mean age was 52.8 ± 5.8 years (range 45 to 62 years) and a male predominance was observed (64.4%). The causes of liver cirrhosis were HCV infection (n = 78), HBV infection (n = 10) and alcohol abuse (n = 2). Chronic HCV infection was the predominant reason of cirrhosis (86.7%). Seventy patients (77.8%) had ascites, from whom 33 patients (47.1%) had spontaneous bacterial peritonitis, and 38 (54.3 %) had sterile ascetic fluid. Among the 33 patients with spontaneous bacterial peritonitis, 16 patients had positive ascitic fluid cultures, the micro-organisms isolated were Escherichia coli (7cases), Streptococcus pneumonia (5 cases), Listeria monocytogenes (2 cases) and Bacteroids fragilis (2 cases). The other patients with spontaneous bacterial peritonitis (16 patients) had a negative bacteriological culture of ascetic fluid (Table1).

Table (1): Clinical and biochemical characteristics of the study subjects

Groups / Parameters	Controls	Patients
No.	30	90
Male: Female Ratio (No.)	20/10	58/32
Age (Years) Mean ± SD, Range	54.5 ± 5.7 (43-63)	52.8 ± 5.8 (45-62)
Etiology. No. (%) **HCV** **HBV** **Alcoholic**	 0 (0%) 0 (0%) 0 (0%)	 78 (86.7%) 10 11.1%) 2 (2.2%)
CPT score, No. (%) **A** **B** **C**	- 	 20 (22.2%) 35 (38.9%) 35 (38.9%)
Ascites, No. (%) **None** **Slight** **Moderate**	- 	 20 (22.2%) 35 (38.9%) 35 (38.9%)
Encephalopathy, No. (%) **None** **Mild** **Moderate** **Severe**	- 	 69 (76.7%) 2 (2.2%) 2 (2.2%) 17 (18.9%)
SBP, No. (%)	-	33 (47.1%)
Ascites culture and sensitivity, No., % **Positive** **Negative** **Sterile**	-	60 (85.7%) 16(22.9%) 16 (22.9%) 38 (54.3%)
Ascites polymorphonuclear cells, No., % **PMNs ≥250 (/mm³)** **PMNs <250 (/mm³)**	-	70 (77.8%) 28(40%) 42 (60%)
Ascites Albumin (gm/dL), Mean, Range	-	2.01 (1.5-2.6)
SAAG, Mean, Range	-	0.7 (0.1-1)
Albumin (g/dL), Mean ± SD, Range	4.9 ± 0.2 (4.6-5.4)	2.9 ± 0.6 (2-3.7)
Bilirubin (mg/dL), Mean ± SD, Range	0.7 ± 0.1 (0.5-0.9)	2.9 ± 0.6 (1.2-7.0)
WBC (x 10^9/L), Mean ± SD, Range	5430.0 ± 1229.0 (4000-7500)	9594.6 ± 5064.9 (4400 - 18300)
Polymorphs (x 10^9/L), Mean ± SD., Range	2897.0 ± 91.8 (2700-3000)	9526.4 ± 4023.6 (3650 - 15300)
INR, Mean ± SD, Range	0.9 ± 0.1 (0.7-1)	2.1 ± 0.5 (1.2 - 2.8)
Creatinine (mg/dL), Mean ± SD, Range	0.7 ± 0.1(0.5-0.9)	1.5 ± 0.6 (0.7 - 4.0)
CRP, Mean ± SD, Range	2.6 ± 0.5 (2-3)	53.2 ± 0.3 (6 - 170)
ALT (U/L), Mean ± SD, Range	27.5 ± 3.1 (24-35)	77.3 ± 27.5 (45 - 137)
AST (U/L), Mean ± SD, Range	26.5 ± 2.2 (23-30)	71.8 ± 20.9 (35 - 95)

On comparing liver cirrhosis patients with SBP to patients without SBP, the prevalence of SBP particularly tended to increase with the progression of liver disease (0%, 22.9% and 71.4%, respectively for Child-Turcotte-Pugh class A, B and C). The other three factors tended to be related to SBP: presence of

ascites (100%), chronic HCV cirrhosis (82.9 %), and hepatic encephalopathy (60 %). Albumin and total bilirubin levels were elevated with advancement in liver disease among our patients. On the other hand, spontaneous bacterial peritonitis was associated with higher median values of white blood cells (WBC) counts, INR and creatinine (Table 2).

Table (2): Clinical and biochemical variables associated with spontaneous bacterial peritonitis at admission

Groups / Parameters	Healthy controls (Group I)	Compensated cirrhosis (Group II)	Decompensated cirrhosis Without SBP (Group III)	Decompensated cirrhosis with SBP (Group IV)
No.	30	20	35	35
Male: Female Ratio (No.)	20/10	14/6	19/16	25/10
Age (Mean, Range)	52.3 (43 - 63)	47.1 (45 - 50)	52.8 (45 - 62)	55.9 (45 - 62)
Etiology (No., %) **HCV** **HBV** **Alcoholic**	-	 20 (100%) 0 (0%) 0 (0%)	 33 (94.3%) 2 (5.7%) 0 (0%)	 25 (71.4%)° 8 (22.9%)° 2 (5.7%)
CPT score (Mean ± SD, Range)	-	5.4 ± 0.5 (5 - 6)	8.8 ± 0.5 (7 - 9)	13.9 ± 1.5 (10 - 15)°
CPT category A/B/C (No.)	-	 20/0/0	 0/35/0	 0/0/35
Ascites (No., %)	-	0 (0%)	35 (100%)	35 (100%)
SBP (No. , %)		0 (0%)	8 (22.9%)	25 (71.4%)°
Encephalopathy (No., %)	-	0 (0%)	0 (0%)	21 (60%)°
Albumin (g/dL), Mean ± SD, Range	4.9 ± 0.4 (4.6-5.4)	3.6 ± 0.1 (3.4-3.7)	3.1 ± 0.2 (2.8-3.5)*	2.4 ± 0.4 (2-3.5)*°
Bilirubin (mg/dL),Mean ± SD, Range	0.7 ± 0.1 (0.5-0.9)	1.7 ± 0.3 (1.2-2.2)	2.4 ± 0.3 (1.8-2.9)*	4.2 ± 0.9 (3-7)*°
WBC (x 10^9/L), Mean, Range	5430.0 (4000 - 7500)	4436.5 (4400 - 4480)	6888.0 (4490 - 9000)	15248.6 (9100 - 18300)*°
INR. Mean, Range	0.9 (0.7 - 1.0)	1.4 (1.2 - 1.6)	2.1 (1.7 - 2.3)*°	2.6 (2.1 - 2.8)*°
Creatinine (mg/dL), Mean, Range	0.7 (0.5 - 0.9)	0.9 (0.7 - 1.3)	1.5 (0.8 - 2.2)*	1.9 (0.85 - 4)*°

Continuous variables were expressed as mean ± SD and categorical variables as number (percentage). Significance between groups: * versus healthy controls; ? versus decompensated cirrhosis without infection. INR: normalized international ratio; WBC: white blood cells.

There was no statistically significant difference as regard to age and sex, but there were statistically significant differences as regard to temperature, total bilirubin, serum albumin, international normalized ratio of prothrombin time, AST, ALT, serum creatinine, blood leukocytes, blood polymorphonuclear leukocytes, serum CRP, serum calprotectin, serum procalcitonin, and serum endocan (Table 3).

Table (3): Clinical and laboratory parameters of our patients and controls (Mean±SD)

Parameters \ Groups	Mean ± SD		P value
	Controls	Patients	
Age	54.5 ± 5.7	52.8 ± 5.8	> 0.05
Temperature (°C)	36.8 ± 0.2	37.9 ± 0.7	0.001*
Bilirubin (mg/dL)	0.7 ± 0.1	2.9 ± 1.2	0.001*
Albumin (g/dL)	4.9 ± 0.2	2.9 ± 0.6	0.001*
INR	0.9 ± 0.1	2.1 ±0.5	0.001*
ALT (U/L)	27.5 ± 3.1	77.3 ±27.5	0.001*
AST (U/L)	26.5 ± 2.2	71.8 ± 20.9	0.001*
Creatinine (mg/dL)	0.7 ± 0.1	1.5 ± 0.6	0.001*
Polymorphs(x 10^9/L)	2897.0 ± 91.8	9526.4 ± 4023.6	0.001*
Leukocytes (x 10^9/L)	5430.0 ± 1229.0	9594.5 ± 5064.9	0.01*
CRP (mg/L)	2.6 ±0.5	53.2 ±40.3	0.001*
Serum Calprotectin (µg/mL)	3.96 ± 3.50	43.15 ± 18.76	0.001*
Serum Procalcitonin (ng/mL)	0.02 ± 0.01	2.7 ± 1.5	0.001*
Serum Endocan (ng/mL)	0.6 ± 0.4	4.3 ± 2.6	0.001*

* Statistically significant. INR = International normalized ratio, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, CRP = C-reactive protein.

The mean value for serum calprotectin was 43.15 µg/mL; range, 6.5 – 73.3 µg/mL. There was a statistically significant rise in serum calprotectin in SBP patients with or without decompensation (group IV and group III) versus non- SBP patients (group II). The serum procalcitonin main value was 2.7 ng/mL; range, 0 – 4 ng/mL. Serum endocan main level was 1.2 ng/mL; range, 0.15–2.15 ng/mL. There was a statistically significant elevation in serum endocan and serum procalcitonin in SBP patients with or without decompensation (group IV and group III) versus non- SBP patients (group II). As regard CRP, there was a statistically significant elevation in the levels of CRP between group III and group IV, group II and group IV, but non-statistically significant increase in CRP between group II and group III. There was a statistically elevated level of leukocytes between group III and group IV, and between group II and IV, but non-statistically significant elevation in blood leukocytes between group II and group III. As regards to blood polymorphonuclear leukocytes (PMNs), there was a statistically elevated blood level of PMNs between group III and group IV and between group II and IV, but non-statistically significant elevation in blood leukocytes between group II and group III (Table 4).

Table (4): Serum Calprotectin, Serum Endocan, Serum Procalcitonin, Serum CRP, Serum PMN and blood leukocytes in Patients with and without SBP

Groups / SPB		Group II (Compensated cirrhosis)		Group III (Decompensated cirrhosis without SBP)		Group IV (Decompensated cirrhosis with SBP)	
Serum Calprotectin (µg/dl)	Mean±SD	24.4 ± 9.3		34.4 ± 10.5		62.6 ± 9.5	
	Range	6.5 - 39		8.9 – 46.7		45 – 73.3	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P <0.000	P <0.000	P <0.000	P <0.000	P <0.000	P <0.000
Serum Procalcitonin (ng/dl)	Mean±SD	0.06 ± 0.02		2.9 ± 0.6		3.9 ± 0.06	
	Range	0.0 – 0.1		1.6 – 3.6		3.7 – 4.0	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P <0.001	P <0.000	P <0.000	P <0.000	P <0.000	P <0.000
Serum Endocan (ng/dl)	Mean±SD	1.2 ±0.7		3.4 ± 1.0		7.1 ± 1.3	
	Range	0.15 - 2.15		2.16 – 5.0		5.1 - 8.75	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P <0.001	P <0.000	P <0.000	P <0.000	P <0.000	P <0.000
Serum CRP (mg/L)	Mean±SD	9.4 ± 1.9		35.1 ± 14.6		96.2 ± 25.7	
	Range	6 - 12		13 - 58		59 - 170	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P > 0.05	P <0.001	P <0.000	P <0.000	P <0.000	P <0.000
Serum PMNs (x 10^9/L)	Mean±SD	4349.0 ± 548.0		8214.3 ± 2071.9		13797.1 ± 1265.5	
	Range	3650 - 5200		5300 - 11000		11200-15300	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P > O.05	P <0.001	P <0.000	P <0.000	P <0.000	P <0.000
Serum Leukocytes (x 10^9/L)	Mean±SD	4436.5 ± 27.3		6888.0 ± 1369.9		15248.7±3029.3	
	Range	4400-4480		4490 - 9000		9100-18300	
	Significance	Groups II &III	Groups II & IV	Groups III&II	Groups III&IV	Groups IV&II	Groups IV&III
		P = 0.496	P < 0.005	P < 0.005	P < 0.001	P < 0.001	P < 0.001

PMNs: Polymorphonuclear leukocytes; SBP: Spontaneous bacterial peritonitis.

As regard to the existence or absence of SBP, the ascitic fluid concentrations of calprotectin, procalcitonin and endocan were statistically significant higher in decompensated cirrhotic patients with SBP(group IV) in comparison to decompensated patients without SBP(group III). The association between the existence and absence of SBP with ascitic polymorphonuclear leukocytes (PMNs), revealed that, there was a statistically significant elevation in ascitic PMNs in patients with decompensated patients with SBP if compared to decompensated patients without SBP (Table 5).

Table (5): Ascitic Calprotectin, ascitic Endocan, Ascitic Procalcitonin, and Ascitic PMNs in group B and group C

Groups / SBP		Group III (Decompensated cirrhosis without SBP)	Group IV (Decompensated cirrhosis with SBP)	P value Mann-Whitney U- test
Ascitic Calprotectin (µg/ml)	Mean ± SD Range	1.26 ± 0.29 0.60 – 1.60	2.49 ± 0.77 1.61 – 3. 65	0.001
Ascitic Procalcitonin (ng/ml)	Mean ± SD Range	1.4 ± 0.1 1.21 – 1.55	1.9 ± 0.3 1.56 – 2.50	0.001
Ascitic Endocan (ng/ml)	Mean ± SD Range	2.5 ± 1.5 0.7 – 4.9	6.3 ± 0.6 4.8 – 6.75	0.001
Ascitic PMNs (/mm³)	Mean ± SD Range	42.2 ± 6.78 30 - 55	846.2 ± 833.2 40 -2663.5	0.001

SBP: Spontaneous bacterial peritonitis

There were statistically significant correlations between the existence of SBP with serum calprotectin (r = 0.512), procalcitonin (r = 0.370), serum endocan (r = 0.501), ascitic calprotectin (r = 508), ascitic procalcitonin (r = 0.501), ascitic endocan (r = 0.496), ascitic PMNs (r = 0.562), and CRP (r = 0.492) (Table 6).

Table (6): Correlation between serum Calprotectin, serum Endocan, serum Procalcitonin, serum Leukocytes, serum PMNs, Serum CRP, Ascitic Endocan, Ascitic Procalcitonin, and Ascitic PMNs with spontaneous bacterial peritonitis

SBP / Parameters	r	P value
Serum Calprotectin (µg/mL)	0.512	0.001
Serum Procalcitonin (ng/mL)	0.370	0.001
Serum Endocan (ng/mL)	0.501	0.001
Serum CRP (mg/L)	0.492	0.001
Ascitic Calprotectin (µg/mL)	0.508	0.001
Ascitic Procalcitonin (ng/mL)	0.501	0.001
Ascitic Endocan (ng/mL)	0.496	0.001
Ascitic PMNs (/mm³)	0.562	0.001

SBP: spontaneous bacterial peritonitis, r: Spearman's Rank Correlation Coefficient.

There were statistically significant correlations between serum and ascitic levels of calprotectin, procalcitonin, and endocan with mediators of inflammation in the form of ascitic polymorphonuclear leukocytes, blood leukocytes, blood polymorphs, and CR. Also, the serum, and ascitic levels of endocan correlated positivity with advanced stage of liver cirrhosis associated with spontaneous bacterial peritonitis (Table 7).

Table (7): Correlation between serum and ascitic fluid levels of calprotectin, procalcitonin, and endocan with mediators of inflammation

Parameters / Serum Calprotectin (µg/mL)	r	P value
Ascitic PMNs (/mm³)	0.904	0.001
Blood Leukocytes (x 10^9/L)	0.911	0.001
Blood polymorphs (x 10^9/L)	0.952	0.001
CRP (mg/L)	0.931	0.001
Acitic Calprotecrin (µg/mL)		
Ascitic PMNs (/mm³)	0.911	0.001
Blood Leukocytes (x 10^9/L)	0.934	0.001
Blood polymorphs (x 10^9/L)	0.997	0.001
CRP (mg/L)	0.957	0.001
Serum Procalcitonin (ng/mL)		
Ascitic PMNs (/mm³)	0.816	0.001
Blood Leukocytes (x 10^9/L)	0.925	0.001
Blood polymorphs (x 10^9/L)	0.955	0.001
CRP (mg/L)	0.954	0.001
Ascitic Procalcitonin (ng/mL)		
Ascitic PMNs (/mm³)	0.904	0.001
Blood Leukocytes (x 10^9/L)	0.936	0.001
Blood polymorphs (x 10^9/L)	0.999	0.001
CRP (mg/L)	0.974	0.001
Serum Endocan (ng/mL)		
Ascitic PMNs (/mm³)	0.904	0.001
Blood Leukocytes (x 10^9/L)	0.970	0.001
Blood polymorphs (x 10^9/L)	1.000	0.001
CRP (mg/L)	0.984	0.001
CPT Score	0.954	0.001
Ascitic Endocan (ng/mL)		
Ascitic PMNs (/mm³)	0.883	0.001
Blood Leukocytes (x 10^9/L)	0.918	0.001
Blood polymorphs (x 10^9/L)	0.981	0.001
CRP (mg/L)	0.972	0.001
CTP Score	0.897	0.001

r: Spearman's Rank Correlation Coefficient; HS: Highly significant; CTP: Child Turcotte Pugh Score

The compatibility between the outcomes of the existence or lack of SBP and the classification constructed on the variable cut-offs was analyzed for each variable and was manifested as the percentage of the samples that were correspondingly recognized (specificity and sensitivity). The receiver operator characteristic (ROC) analysis showed that

areas under the ROC curve (AUC) of ascitic PMNs, serum calprotectin, ascitic calprotectin, serum endocan, ascitic procalcitonin, ascitic endocan, and serum procalcitonin, were 0.825, 0.796, 0.794, 0.790, 0.790, 0.787, and 0.710, respectively. The ascitic PMNs level had a sensitivity of 97%, a specificity of 94.7%, positive predictive value (PPV) of 93%, and negative predictive value (NPV) of 71.9 % at the cut-off level of ≥ 250/ mm³.

The ascitic calprotectin level had a sensitivity of 95 %, a specificity of 89.2 %, PPV of 91.3 %, and NPV of 67.4 % at the cut-off level of 0.95 µg/ml.The serum endocan level had a sensitivity of 90 %, a specificity of 83.7%, positive predictive value of 90.9%, and negative predictive value of 67 % at the cut-off level of 2.03 ng/mL. The ascitic procalcitonin level had a sensitivity of 89.9%, a specificity of 83.3%, positive predictive value of 90.7%, and negative predictive value of 67 % at the cut-off level of 0.33 ng/mL. The serum calprotectin level had a sensitivity of 96 %, a specificity of 94 %, PPV of 91.6 %, and NPV of 67.7 % at the cut-off level of 45 µg/ml.

ascitic endocan level had a sensitivity of 88.9%, a specificity of 78.5%, positive predictive value of 90.3%, and negative predictive value of 66 % at the cut-off level of 0.65 ng/mL. The serum procalcitonin had a sensitivity of 87.9 %, a specificity of 76.8 %, PPV of 80.9 %, and NPV of 58.1 % at the cut-off value of 2.50 ng/mL. Furthermore, the specificity and sensitivity were lowest for ascitic procalcitonin, followed by ascitic endocan, and lastly for serum procalcitonin (Table 8 and Figures 1 & 2).

Table (8): The accuracy of ascitic PMNs, calprotectin, procalcitonin, and endocan for prediction and early diagnosis of spontaneous bacterial peritonitis in cirrhotic patients with decompensation

Presence or Absence of SBP	AUC	Cutoff value	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Ascitic PMNs (/mm³)	0.825	≥ 250/mm³	97	94.6	71.9	93
Serum Calprotectin (µg/mL)	0.796	≥ 45 µg/mL	96	94	67.7	91.6
Ascitic Calprotectin (µg/mL)	0.794	≥ 0.95 µg/mL	95	89.2	67.4	91.3
Serum Endocan (ng/mL)	0.790	≥ 2.03 ng/mL	90	83.7	67	90.9
Ascitic Procalcitonin (ng/mL)	0.790	≥ 0.33 ng/mL	89.9	83.3	67	90.7
Ascitic Endocan (ng/mL)	0.787	≥ 0.65 ng/mL	88.9	78.5	66	90.3
Serum Procalcitonin (ng/mL)	0.710	≥ 2.5 ng/mL	87.9	76.8	58.1	80.9

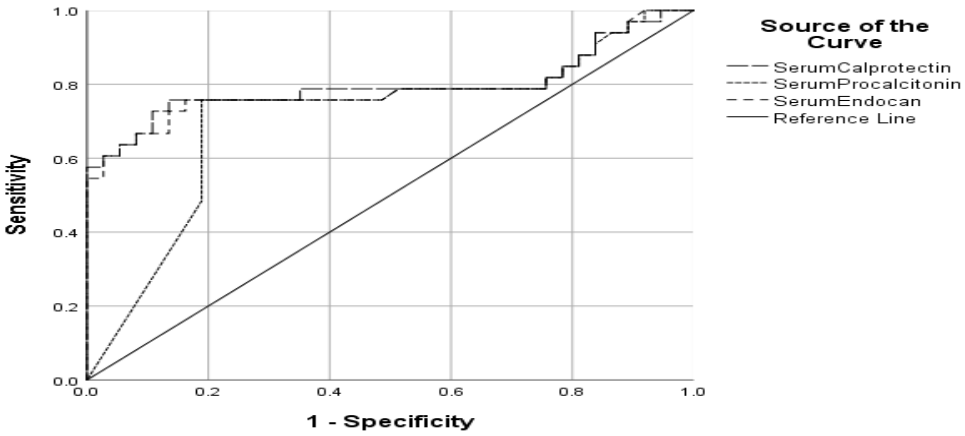

Figure (1): Receiver operating characteristics (ROC) curves of serum calprotectin, serum procalcitonin, and serum endocan for identification of SBP in cirrhotic patients with decompensation

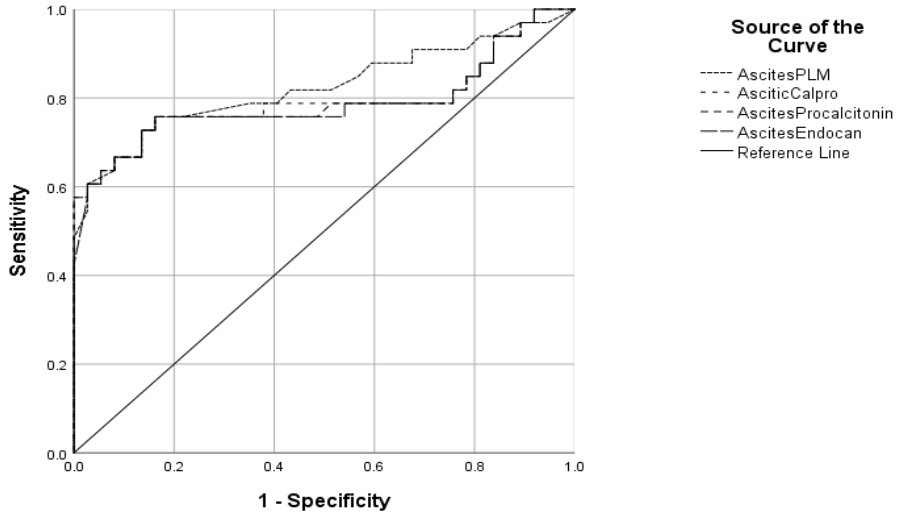

Figure (2): Receiver operating characteristics (ROC) curves of ascitic PMNs, ascitic calprotectin, ascitic procalcitonin, and ascitic endocan for identification of SBP in cirrhotic patients with decompensation

DISCUSSION

SBP is a very common bacterial infection in patients with cirrhosis and ascites *(Arvaniti et al., 2010).* Pathophysiology of SBP in patients with cirrhosis is regarded to be the main outcome of bacterial translocation (BT). The BT is the condition through which viable or nonviable bacteria and bacterial products (bacterial DNA or endotoxins) traverse across the intestinal lumen and get to the mesenteric lymph nodes or extraintestinal. Bacterial translocation also is participated in augmenting the hyperdynamic state of cirrhosis and in progression of hemostasis disorders *(Wiest*

et al., 2014). Although other methods using mean platelet volume, C-reactive protein, and white blood cell level assessments can be considered as accurate diagnostic tests in predicting SBP, possibly because of a continuous systemic inflammatory responses *(Abdel-Razik et al., 2014)*, the diagnosis of SBP is still depend on diagnostic paracentesis *(Wiest et al., 2011)*. It is an evasive maneuver with some complications. Therefore, there is a need for other noninvasive diagnostic tools. Liver cirrhosis patients are highly vulnerable to bacterial infections because of the acquired immune defects in both the cell-mediated and humoral immunity and bacterial translocation. Hepatic dysfunction is associated strongly with impeded defenses against bacteria, and with structural and functional alterations in the intestinal mucosa that result in an increase in the permeability to bacteria and bacteria-derived products, which worsens over time and with disease progression *(Bhat et al., 2013* and *Cai et al., 2015)*. In cirrhosis, systemic inflammatory response, in form of activated circulating immune cells and increased plasma levels of both proinflammatory cytokines (e.g. TNF-α, IL-6) *(Suliman et al., 2012,* and *Albillos et al., 2014)* and cell activation parameters, is the outcome of constant occasional activation of circulating immune cells from damage-associated molecular patterns, librated from necrotic liver cells and, as infection occurs, from pathogen associated molecular patterns, secreted from the leaky gut *(Albillos et al., 2014)*.

Early detection of SBP is very helpful for patients as the mortality rate among untreated patients is around 50% *(Cardenas et al., 2010)*. The diagnosis of SBP was established by ascitic fluid analysis. The most common marker of infection is an ascitic fluid PMN cell count of 250/mm3 or higher *(Runyon, 2009)* and in a meta-analysis, the negative likelihood ratio for SBP if the PMN cell count was greater than 250/mm3 was 0.2 *(Wong et al., 2008)*. *(Strauss, 2014)* reported that an ascitic fluid PMN count greater than 500/mm3 had specificity and sensitivity of 98% and 90%, respectively. Another study by *(El-Gendy et al., 2014)* reported that an ascitic fluid PMN cell count higher than 200/mm3 had a sensitivity and specificity of 100% in the diagnosis of SBP patients. These data were in agreement with our results as we found that, at a cutoff value of 250/mm³, ascitic PMN cell count had 97% sensitivity and 94.7 % specificity for detecting SBP.

In the current study, E.coli was the dominant organism (43.7%), followed by staphylococcus species (31.3%), then Listeria monocytogen in two patients (12.5 %), and Bacteroidis fragilis in two patients (12.5 %). E.coli was also the most common organism found in the study by *(Gill et al., 2012)*. Our results were in accordance with *(Lippi et al., 2014)*, who showed that E. coli was isolated in 9 patients (56.3 %), Streptococcus organisms in 4 patients (25 %), Listeria monocytogen in 2 patients (12.5 %) and Bacteroids fragilis in one patient (6.3 %). The study conducted in Khyber Teaching Hospital, Peshawar 2003, showed E. coli was isolated in 58.13%, Streptococcus organisms in 18.60%, Staphylococcus aureus in 9.13%, Klebsiella in 9.13% and Acinectobacter in 4.63% and this variation can be justified by different localities and

different sample sizes *(Iqbal and Alam, 2011)*.

In our study, we found significantly elevated levels of serum and ascitic fluid calprotectin in liver cirrhosis patients than in the controls, and also in SBP patients than in the non-SBP patients. This is in agreement with *(Ali et al., 2013)* who reported that ascitic calprotectin level showed significantly elevated value in patients with SBP than those without SBP. *(Lutz et al., 2015)* also concluded that ascitic calprotectin could be used as a test for SBP, but test performance was further improved by calculating the ratio between ascitic calprotectin and ascitic total protein. They also reported that this ratio could provide prognostic information on short term survival of SBP patients. Another study by *(Gundling et al., 2011)* who reported that fecal calprotectin levels were significantly elevated in cirrhotic patients with SBP if compared to those without SBP.

In our study, we found a significant positive correlation between the existence of SBP with both elevated serum and ascitic levels of calprotectin. This is in accordance with *(Abdel-Razk et al., 2014)* who explained this finding by the fact that, serum calprotectin is an acute phase inflammatory reactant protein exerting regulatory, antimicrobial, and antiproliferative functions.

In this study, we found that elevated serum and ascitic fluid calprotectin levels were significantly correlated with mediators of inflammation in the form of ascitic fluid PMNs, blood leukocytes, blood PMNs, and CRP in cirrhotic patients with SBP. Our findings are in agreement with *(Ali et al., 2013), (Burri et al., 2013)*, and *(Abdel-Razik et al., 2016)* whose found that ascitic calprotectin levels were correlated well and reliably with ascitic PMN counts, and the samples with PMN > 250/mm³ also had elevated ascitic calprotectin levels than those with PMNs ≤ 250/mm³ in their studies. These findings can be explained by the presence of calprotectin in body fluids is proportional to the influx of neutrophils *(Soyfoo et al., 2009)*.

In the current study, a cutoff levels of ≥ 45 µg/ mL , and ≥ 0.95 µg/ mL for serum and ascitic fluid calprotectin in detecting SBP showed a highly significant performance with AUC 0.796 and 0.794, sensitivity 96 % and 95 %, specificity 94 % and 89.2 %, respectively. *(Rizk et al., 2014)* reported that at a cutoff value of 270 mg/dL, ascitic fluid calprotectin had 86 % specificity and 97.5 % sensitivity for detecting SBP with AUC = 0.924, whereas *(Lutz et al., 2015)* found that the ratio of ascitic calprotectin to ascitic total protein with acuoff value of 5.24 achieved a sensitivity of 90 % and specificity of 81 % in detecting SBP with AUC = 0.920. Lastly, *(Helmy et al., 2016)* found that a cutoff level of ≥ 46 µg/mL for serum calprotectin in detecting SBP showed an elevated performance with AUC 0.967, sensitivity 100 %, and specificity 92 %. The previous findings indicate that both serum and ascitic fluid calprotectin correlate well and reliably with the existence of SBP in liver cirrhosis patients.

In this study, there were significant elevations in ascitic fluid and serum Procalcitonin (PCT) levels in patients with liver cirrhosis than in controls and also in SBP versus non-SBP group. This is in

accordance with *(Cekin et al., 2013)* and *(Hamed et al., 2017)* whose reported the same results.

In our patients, we reported that at a cutoff value of 2.50 ng/mL, serum PCT had 87.9% sensitivity and 81.8% specificity for detecting SBP, AUC=0.710 with NPV and PPV values of 58.1% and 80.9%, respectively. Also, the ascitic fluid procalcitonin cut-off value was 0.33 ng/ml with sensitivity 89.9 %, specificity 83.3 %, NPV 67 %, and PPV 90.7 % with AUC of 0.790. Thus the ascitic fluid level of procalcitonin is more accurate for detecting spontaneous bacterial peritonitis in cirrhotic patients than the serum procalcitonin level. This is in agreement with *(Marciano et al., 2015)* who reported near the same results. Moreover, there is a statistically significant association between ascitic fluid and serum levels of procalcitonin with mediators of inflammation (CRP, blood leukocytes and serum PMNs) supported the hypothesis that the ascitic fluid and serum procalcitonin could indicate oncoming systemic inflammatory responses in cirrhotic patients with ascitic fluid infection. According to this finding, we could expect that ascitic fluid and serum PCT not only represents bacterial translocation and multiplications of microorganisms, especially gram negative species, but also systemic inflammation and infection through cascades of proinflammatory cytokine release in the course of disease progression. This finding may be in agreement with *(Alexopoulou et al., 2017)* who described simply the same findings. In our patients with liver cirrhosis and ascites, we noticed high specificity and sensitivity for ascitic fluid PCT followed by serum PCT levels in patients with SBP, as described before. From our point of view, these values may be reflected as a precise and an early method for diagnosing SBP in clinical practice. In contrast, *(Lesińska et al., 2014)* found that serum PCT levels did not distinguish between patients with and without SBP. In patients with decompensated cirrhosis signs of systemic inflammation are usually absent even in critical infections. So, PCT has very wide range of overlapping results and decreased sensitivity for SBP diagnosis especially in these groups *(Lin et al., 2014)*.

Our study provides several lines of evidence to suggest that endocan acts as mediator of inflammatory state associated with bacterial infection in liver cirrhosis. First, serum endocan levels in decompensated cirrhotic patients with spontaneous bacterial peritonitis were significantly elevated than in patients without spontaneous bacterial peritonitis. Second, and more importantly, a statistically significant correlation between endocan levels and Child-Turcotte-Pugh score and inflammatory markers (CRP, blood leukocytes, and blood polymorphonuclear leukocytes) was observed in cirrhotic patients with spontaneous bacterial peritonitis.

In our study, we noticed a significant correlation between serum endocan level and disease severity (indicated by Child-Turcotte-Pugh score) and a high level of circulating endocan was associated with the inflammatory mediator (CRP) in cirrhotic patients with spontaneous bacterial peritonitis. In a recent report, endocan appeared to indicate the severity of endothelial cell injury *(Su et al., 2014)*. Furthermore, endocan influences the

expression of proinflammatory cytokines, such as interleukin-8, monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α, which are involved in mechanisms of chronic liver inflammation *(Lee et al., 2014)*. Also, endocan expression in primary cultured human vascular endothelial cells is adjusted by TNF-α, and its secondary mediator CRP that is known to stimulate endothelial cell activation and injury *(Li et al., 2012)*, although the exact mechanism of endocan expression in infection still not yet elucidated. TNF-α is a known attractant mediator for leukocytes, augments the expression of adhesion molecules on endothelial cells, and, therefore, may play a significant role in hepatic inflammatory responses and cirrhosis progress. Thus, in this study there was a correlation between endocan concentration and mediators of inflammation in the form of CRP, blood polymorphs, and blood leukocytes in cirrhotic patients with spontaneous bacterial peritonitis. Endocan influences the expression of tumor necrosis factor-α and nuclear factor kappa β; these two proinflammatory cytokines activate hepatic inflammation and promote hepatic fibrosis by activating hepatic stellate cells *(Abhilash et al., 2014,* and *Liu et al., 2014)*. Although endocan has not been established to be specific for any systemic inflammatory diseases, it is known to initiate recruitment of circulating lymphocytes and monocytes to inflammatory sites *(Zuwala-Jagiello et al., 2017)*. As a result, these consequences of endocan on inflammatory status may result in deterioration of hepatic function in patients with progressive cirrhosis and bacterial infection. Collectively, these influences of endocan on liver inflammation and fibrosis may give rise to the occurrence of hepatic decompensation in patients with advanced liver cirrhosis.

Although, elevated levels of endocan in progressive liver disease had been formerly reported *(Nault et al., 2013, Tok et al., 2014* and *Toshikuni et al., 2015)*, its prospective value as a diagnostic tool has not been studied. To our knowledge, this study may be of little studies in which endocan levels in both serum and ascitic fluid demonstrated significant associations with the severity of liver disease within patients with spontaneous bacterial peritonitis, as evidenced by significant correlations with the Child-Pugh score. In addition, the accuracy of serum and ascitic fluid endocan for diagnosis of spontaneous bacterial peritonitis in cirrhotic patients increased in advanced liver disease. Diagnostic accuracies of serum and ascitic fluid endocan levels for identifying patients with spontaneous bacterial peritonitis were better for Child C stage cirrhosis, reaching a sensitivity of 90 %, and 88.9 % respectively with cut-off values of 2.03 ng/ml for serum endocan and 0.65 ng/ml for ascitic fluid endocan. These cut-off levels might useful in predicting spontaneous bacterial peritonitis in patients with decompensated cirrhosis. Serum endocan levels are detectable as early as 2 hours after starting the inflammatory response *(Dallio et al., 2017)*, which is earlier than the elevations in both PCT and CRP *(Zuwala-Jagiello et al., 2017*and *Voiosu et al., 2018)*. 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 *(Tok et al., 2014* and *Pauly et al., 2016)*. Endocan is still measurable when investigated on a

daily basis, whereas CRP or PCT will have already cleared from the blood. Our results propose that serum and ascitic fluid endocan are an independent variables able to differentiating cirrhotic decompensated patients with and without spontaneous bacterial peritonitis and may be used as biomarkers for early diagnosis of spontaneous bacterial peritonitis in these patients with high levels of specificity and sensitivity.

There are different limitations to the current study that virtue consideration. First, although the number of patients included might appear small, it adequately represents the sample size estimated to provide the specific power. Second, this study was not designed and powered to assess the ability of Calprotectin, procalcitonin and endocan to predict the incidence of spontaneous bacterial peritonitis in patients without apparent infections. Lastly, we excluded some diseases that may influence calprotectin, procalcitonin and endocan levels; however, some diseases may be unrecognized in our study group.

CONCLUSION

Ascitic PMNs, serum calprotectin, ascitic calprotectin, serum endocan, ascitic procalcitonin, ascitic endocan, and serum procalcitonin levels are sensitive markers for early diagnosis and prediction of SBP; cut-off values of 250/mm³, 45 µg/mL, 0.95 µg/mL, 2.03 ng/ mL, 0.33 ng/mL, 0.65 ng/mL, and 2.5 ng/mL, respectively. These results are suggested for distinguishing between SBP and sterile ascites and for the prediction, and early diagnosis of spontaneous bacterial peritonitis. However, the usefulness of ascitic fluid PMNs is superior to serum calprotectin, then ascitic calprotectin level is superior to serum endocan, then ascitic procalcitonin is superior to ascitic fluid endocan, and lastly ascitic fluid procalcitonin for early diagnosis of SBP. The survival of patients with liver cirrhosis is closely conjugated to the stage of liver dysfunction and the development of bacterial infection. Our study identified that ascitic PMNs, serum calprotectin, ascitic calprotectin, serum endocan, and ascitic procalcitonin may be adventageous as powerful diagnostic markers to assess the prognosis of liver disease and cirrhotic patients with spontaneous bacterial peritonitis. It may be beneficial to implement calprotectin, procalcitonin, and endocan in future diagnostic algorithms for assessing the prognosis of patients with advanced liver disease, prediction and early diagnosis of SBP in patients with liver cirrhosis. Larger prospective studies should be done to investigate the physiopathogenic, practical and clinical values of calprotectin, procalcitonin , and endocan measurements in cirrhotic patients with spontaneous bacterial peritonitis.

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دراسة مقارنة لمستويات السائل البريتونى والمصلى من الكالبروتكتين والبروكالسيتونين و الإندوكان في المرضى الذين يعانون من تليف الكبد من أجل التشخيص المبكر والتنبوء بالإلتهاب البريتونى الجرثومى العفوي

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خلفية البحث : التهاب الصفاق الجرثومي العفوي هو حالة خطيرة ومتكررة ومهددة للحياة تتطور لدى مرضى التليف الكبدي الذين يعانون من معدل وفيات مرتفع. ويستند تشخيصه على كريات العد البيضاء متعددة النسيلة للسائل البريتونى لتكون أكثر من ٢٥٠ / ميكروليتر.

الهدف من البحث: كان الهدف من هذه الدراسة هو تقييم مستويات السائل البريتونى والمصل من الكالبروتكتين والبروكالسيتونين والإندوكان كعلامات مناسبة للتنبؤ وتشخيص إلتهاب الصفاق الجرثومي العفوي.

المرضى وطرق البحث: أجريت هذه الدراسة على تسعين مريضًا يعانون من تليف الكبد: خمسة وثلاثون يعانون من تليف الكبد غير المعوَّض وإلتهاب الصفاق البكتيري العفوي ،و خمسة وثلاثون مع تليف الكبد غير المعوض بدون إلتهاب الصفاق البكتيرى العفوي وعشرين مع تليف كبدى معوض بدون إستسقاء. وقد تم تقييم إرتباطات الكالبروتكتين، البروكالسيتونين، وإندوكان مع مؤشرات العدوى والإلتهابات المرتبطة بإلتهاب الصفاق الجرثومي العفوي في مرضى التليف الكبدي. تم تحليل سائل الإستسقاء ومستويات المصل من الكالبروتكتين، البروكالسيتونين، وإندوكان ، والكريات البيضاء متعددة الأشكال النووية ، بروتين سي التفاعلي بالمصل ، والكريات البيضاء في الدم. المجموعة الضابطة (العدد = ثلاثين) المكونة من متبرعين بالدم أصحاء مع وجود مستويات طبيعية من أنزيمات الكبد ، عد الدم الكامل الطبيعي والعلامات السلبية لإلتهاب الكبد الفيروسي وفيروس نقص المناعة البشرية.

النتائج: كانت مستويات السائل الإستسقائى والمصلى من الكالبروتكتين و البروكالسيتونين والإندوكان ، والخلايا متعددة الأشكال النووية ، وبروتين سى التفاعلى وكريات الدم البيضاء مرتفعة إحصائيا في

مرضى التليف الكبدي مع إلتهاب الصفاق البكتيري العفوي اذا ما قورنت بالمجموعة الضابطة ومرضى تليف الكبد بدون إلتهاب الصفاق الجرثومي العفوي.

الاستنتاج: حددت هذه الدراسة أن السائل الاستسقائى للخلايا متعددة النواة ، والكالبروتكتين المصلى ، والكالبروتكتين بالسائل الإستسقائى ،والإندوكان المصلى ،والبروكالسيتونين بالسائل الإستسقائى والبروكالسيتونين المصلى على التوالي قد تكون مفيدة كعلامات تشخيصية قوية لتقييم تطور مرض الكبد في مرضى التليف الكبدي مع التهاب الصفاق الجرثومي العفوي وقد يكون من المفيد فرض مستويات كالبروتكتين المصلى ، ومستويات السائل الاستسقائى من الكالبروتكتين ،وإندوكان المصلى و بروكالسيتونين بالسائل الإستسقائى في خوارزميات التشخيص المستقبلية لقياس التنبؤ المرضى لدى مرض الكبد المتقدم. ان قياس الكالبروتكتين، البروكالسيتونين والإندوكان عند القبول قد تكون عوامل مختبرية جيدة للتشخيص المبكر وأيضاً التنبؤ بحدوث إلتهاب الصفاق البكتيري العفوي ، وقد يعبر عن درجة إصابة الخلايا البطانية المتأثرة بالإستجابة للإلتهاب النظامي ، وهي عملية فيسيولوجية مرضية يمكن أن تزيد من مسار التليف الكبدي المتقدم.