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Serum Homocysteine as an Early Diagnostic Marker of Spontaneous Bacterial Peritonitis in Patients with Hepatic Cirrhosis

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is a term used to describe acute infection of ascites, an abnormal accumulation of fluid in the abdomen, without a distinct or identifiable source of infection.

Objective: This study aimed to assess serum homocysteine as a novel reliable early diagnostic marker for spontaneous bacterial peritonitis in patients with hepatic cirrhosis. As the diagnosis of SBP depends primarily on a polymorphonuclear leukocyte cell (PMN) count ≥ 250 mm^3, however this method is invasive and sometimes not diagnostic. **Patients and methods:** This study was conducted on 50 cirrhotic patients with ascites. Patients were divided into 2 groups: Group (A) included 30 cirrhotic patients with SBP on the basis of PMN count in the ascitic fluid ≥ 250 cells/μL with or without positive ascitic fluid culture. Group (B) included 20 cirrhotic patients with ascites but without SBP (control group). **Results:** There was a significant difference between the two studied groups regarding C-reactive protein (CRP) (P=0.001) and erythrocyte sedimentation rate (ESR) (P=0.008). There was also significant difference between the two studied groups regarding ascitic fluid analysis parameters; as ascitic glucose and albumin were significantly lower in SBP group (P=0.002 & P=0.027, respectively) while ascitic lactate dehydrogenase (LDH) and PMN count were significantly higher in SBP group (P < 0.001, for both). Serum homocysteine was significantly higher in SBP group compared to non-SBP group (18.43 ± 6.95 vs. 12.13 ± 5.54 μmol/l; P=0.001). Serum homocysteine was significant at a cutoff level of 17.65 μmol/l with a sensitivity of 88.6% and 95.2% specificity for diagnosing SBP with an area under the curve (AUC) = 0.928.

Conclusion: Serum homocysteine could serve as a convenient novel and reliable noninvasive early diagnostic marker for SBP in cirrhotic patients with ascites.

Key words: Homocysteine, Spontaneous bacterial peritonitis, Cirrhosis; Ascites.

INTRODUCTION

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of liver cirrhosis (LC). According to the Global Burden of Disease 2010 study, LC is the 11th most common cause of death globally, and accounting for 1.6% of the worldwide burden [(1)]. LC results from progressive fibrosis and is the final outcome of all chronic liver disease. Cirrhosis can result in portal hypertension and/or hepatic dysfunction. Both of these either alone or in combination can lead to many complications, including ascites, varices, hepatic encephalopathy, hepatocellular carcinoma, hepatopulmonary syndrome, and coagulation disorders. Cirrhosis and its complications not only impair quality of life but also decrease survival [(2)].

SBP is a well-recognized and prevalent complication seen in cirrhotic patients with ascites, occurring in 10–25% of these patients [(3)]. It leads to more severe liver function damage, sepsis and multi-organ failure thus affecting the prognosis of such patients [(4)]. Once SBP is diagnosed, appropriate antibiotic treatment must be started as soon as possible, without prior knowledge of the causative organisms or their in vitro drug sensitivities [(5)].

Bacterial translocation is the major cause of SBP; therefore, no intra-abdominal source of infection can be found. Ascites culture is the gold standard for SBP diagnosis, and a high ascites PMN count is accepted as an early indicator of SBP. An ascites PMN count ≥ 250/mm^3 is considered to indicate empirical antibiotic therapy based on the current guidelines [(6)].

Ascetic fluid culture (AF) is the gold standard for the diagnosis of SBP. However, cultures have been negative in about 60% of patients despite the clinical manifestations suggestive of SBP and the increased ascites neutrophil count. Lysis of the ascetic fluid PMNs during transport to the laboratory may lead to false negative results. Manual measurement of the ascetic fluid PMN count is operator-dependent makes quality control difficult and can delay the diagnosis [(7)].

Homocysteine is a sulfhydryl-containing amino acid mainly produced and catabolized in the liver [(8)]. Plasma homocysteine (tHcy) is a marker of folate and cobalamin deficiency states and a risk factor for cardiovascular diseases, and is altered by renal insufficiency. Increased tHcy in liver diseases may also play a role in hepatic disorders [(9)].

Recently, **Ahmeda *et al.*** [(10)] demonstrated that homocysteine is considerably higher in SBP participants versus non-SBP patients. Serum homocysteine may have reliable diagnostic role in patients with SBP.

The aim of this study was to assess serum homocysteine as a novel reliable early diagnostic marker for SBP in patients with hepatic cirrhosis.

PATIENTS AND METHODS

This study was conducted on 50 Ascetic cirrhotic

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patients who were admitted at Internal Medicine Department, Benha University Hospital. Patients with established liver cirrhosis and ascites based upon clinical, laboratory and ultrasonographic findings.

Inclusion criteria: Age >18 years were included in this study. They were divided into 2 groups: Group (A) Included 30 cirrhotic patients with SBP on the basis of PMN count in the ascetic fluid ≥ 250 cells/μL with or without positive ascetic fluid culture, in absence of an intra-abdominal source of infection or malignancy [(11)]. Group (B): Included 20 cirrhotic patients with ascites and without SBP (control group).

Exclusion criteria: Patients with ascites due to other causes (malignancy, cardiac diseases, renal diseases, Budd-Chiari syndrome, tuberculosis or hypothyroidism). Patients with history of antibiotics 2 weeks prior to paracentesis. Patients with evidence of secondary bacterial infection, and patients with hepatocellular carcinoma (HCC) were excluded from the study. All patients were subjected to detailed history taking and complete clinical examination for stigmata of LC including ascites, biochemical investigations including complete blood count (CBC), ESR, CRP and markers of liver injury [alanine aminotransferase (ALT), aspartate amino-transferase (AST)]. Liver function tests [serum bilirubin (total, direct), serum albumin, prothrombin time and International normalized ratio (INR)]. Viral markers hepatitis C virus antibodies (HCV-Ab) and hepatitis B surface antigen (HBsAg). Renal function tests (serum creatinine and blood urea). Serum alpha fetoprotein (AFP), and serum homocysteine was evaluated by enzyme-linked immunosorbent assay (ELISA).

Diagnostic abdominal paracentesis and examination of ascetic fluid for PMN count, glucose, albumin and LDH. Pelvi-abdominal ultrasonography was done to detect radiological features of LC, spleen length, PV diameter, ascites and to exclude HCC. An assessment of disease severity using Child Pugh score [(12)] and a model for end-stage liver disease (MELD) score [(13)] was performed.

Ethical clearance:

An informed consent was obtained from every subject after taking approval of Institutional Review Board, Faculty of Medicine, Benha University. The work had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Statistical Analysis:

All data were collected, tabulated and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) and Fisher exact test were used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD for parametric and median and range for non-parametric data. Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. Receiver operating characteristic (ROC) curve was constructed to permit selection of threshold values for test results and comparison of different testing strategies. Areas under ROC curves and their standard errors were determined using the method of Cantor, and compared using the normal distribution, with correction for correlation of observations derived from the same cases. Value of area under a ROC curve (AUC) indicates: 0.90 – 1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair; 0.60-0.70 = poor; and 0.50-0.6 = fail. The optimal cutoff point was established at point of maximum accuracy. All statistical comparisons were two tailed with significance level of P-value ≤ 0.05 indicates significant, p < 0.001 indicates highly significant difference while, P > 0.05 indicates Non-significant difference.

RESULTS

There was no statistically significant difference between the two studied groups regarding age, sex and BMI **(Table 1)**.

There was a significant difference between the two studied groups regarding CRP (P=0.001) and ESR (P=0.008). However, there were no significant differences between the two studied groups regarding other laboratory investigations **(Table 2).**

Regarding ultrasonographic findings of the studied groups, there was a statistically significant difference between the two studied groups regarding splenomegaly as 60% of patients in group A (SBP group) had splenomegaly while 30% of those in group B (non-SBP group) had splenomegaly (P = 0.038). Hence, mean splenic diameter was significantly more in SBP group than in non-SBP group (P = 0.048) **(Table 3).** Regarding ascitic fluid parameters between the two studied groups, ascetic fluid glucose and albumin were significantly lower in SBP group (P=0.002 & P=0.027, respectively) while LDH and PMN count were significantly higher in SBP group (P<0.001, for both) **(Table 4).**

Concerning serum homocysteine, it was significantly higher in SBP group compared to non-SBP group (18.43 ± 6.95 vs. 12.13 ± 5.54 μmol/l; P=0.001) **(Figure 1).** Serum homocysteine was significant at a cutoff level of 17.65 μmol/l with a sensitivity of 88.6% and 95.2% specificity for diagnosing SBP with an area under the curve (AUC) = 0.928 **(Table 5, Figure 2).**

Serum homocysteine was positively correlated with TLC, ALT, AST, total bilirubin, CRP, ESR, ascitic fluid LDH, PMN count, Child–Pugh score, and MELD score. While, it was negatively correlated with serum albumin and ascitic fluid albumin and glucose **(Table 6).**

Table (1): Demographic data distribution between the two studied groups

		SBP (N=30)	Non SBP (N=20)	t / χ^2	P
Age (years) Mean ± SD		61.40 ± 7.54	59.73 ± 8.09	0.745	0.459
Sex	Male	14 (46.7%)	12 (60%)	0.855	0.355
	Female	16 (53.3%)	8 (40%)		
BMI (kg/m^2) Mean ± SD		24.82 ± 2.69	25.23 ± 2.74	0.524	0.603

Table (2): Laboratory investigation results between the studied groups

	Group A SBP (N=30)	Group B Non SBP (N=20)	t	P
Hb (g/dL) Mean ± SD	10.65 ± 1.68	10.34 ± 1.23	0.707	0.483
TLC (x 10^3/L) Mean ± SD	6.64 ± 1.34	7.05 ± 1.53	1.01	0.322
PLT (x 10^3/L) Mean ± SD	151.21 ± 36.45	138.5 ± 28.69	1.31	0.196
ALT (U/L) Mean ± SD	35.42 ± 1.38	29.65 ± 5.73	1.86	0.069
AST (U/L) Mean ± SD	47.54 ±9.44	49.26 ± 16.31	0.326	0.746
Total bilirubin (mg/dl) Mean ± SD	2.21 ± 0.745	2.09 ± 0.865	0.523	0.603
Albumin (g/dl) Mean ± SD	2.46 ± 0.239	2.54 ± 0.215	1.21	0.234
GGT (U/L) Mean ± SD	86.95 ± 17.97	79.5 ± 16.68	1.48	0.146
INR Mean ± SD	1.54 ± 0.294	1.44 ± 0.216	1.31	0.199
Creatinine (mg/dl) Mean ± SD	1.04 ± 0.176	0.958 ± 0.143	1.73	0.089
Urea (mg/dl) Mean ± SD	38.1 ± 7.54	35.21 ± 6.21	1.42	0.162
LDH (IU/L) Mean ± SD	229.24 ± 52.33	215.13 ± 51.42	0.919	0.363
CRP (U/L) Mean ± SD	29.46 ± 5.27	20.59 ± 4.35	**3.73**	**0.001**
ESR (mm/hr) Mean ± SD	32.26 ± 5.39	26.75 ± 5.82	**2.75**	**0.008**

Hb: hemoglobin, TLC: total leucocytes count, PLT: platelet count, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: gamma-glutamyl transferase

Table (3): Ultrasonographic findings of the studied groups

	Group A SBP (N=30)	Group B Non SBP (N=20)	x^2	P
Hepatomegaly	12 (40%)	4 (20%)	1.526	0.071
Splenomegaly	18 (60%)	6 (30%)	4.33	**0.038**
PV diameter$_{(mm)}$	14.9 ± 2.08	14.15 ± 2.30	0.981	0.635
Splenic diameter$_{(cm)}$	16.7 ± 2.12	14.72 ± 1.92	1.993	**0.048**

Table (4): Ascitic fluid analysis parameters of the two studied groups

	SBP (N=30)	**Non SBP** (N=20)	**t**	**P**
Glucose (mg/dL) Mean ± SD	101.19 ± 12.54	115.37 ± 17.45	**3.35**	**0.002**
Albumin (g/dL) Mean ± SD	0.914 ± 0.131	0.824 ± 0.145	**2.28**	**0.027**
LDH (IU/L) Mean ± SD	162.41 ± 4.73	110.77 ± 20.59	**4.82**	**<0.001**
PMN count (cell/µL) Mean ± SD	341.36 ± 8.23	202.44 ± 7.47	**6.02**	**<0.001**

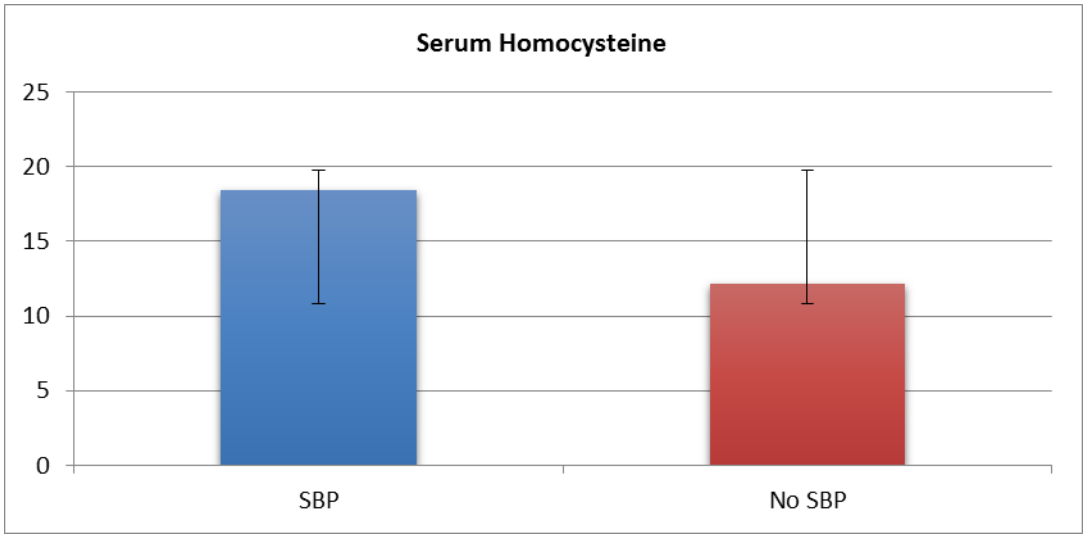

Figure (1): Serum homocysteine among the studied groups

Table (5): Validity of serum homocysteine in diagnosing SBP

Cutoff	**AUC**	**95% CI,**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**P value**
17.65 µmol/l	0.928	0.86 - 0.995	88.6%	95.2%	68.4%	98.1%	0.000

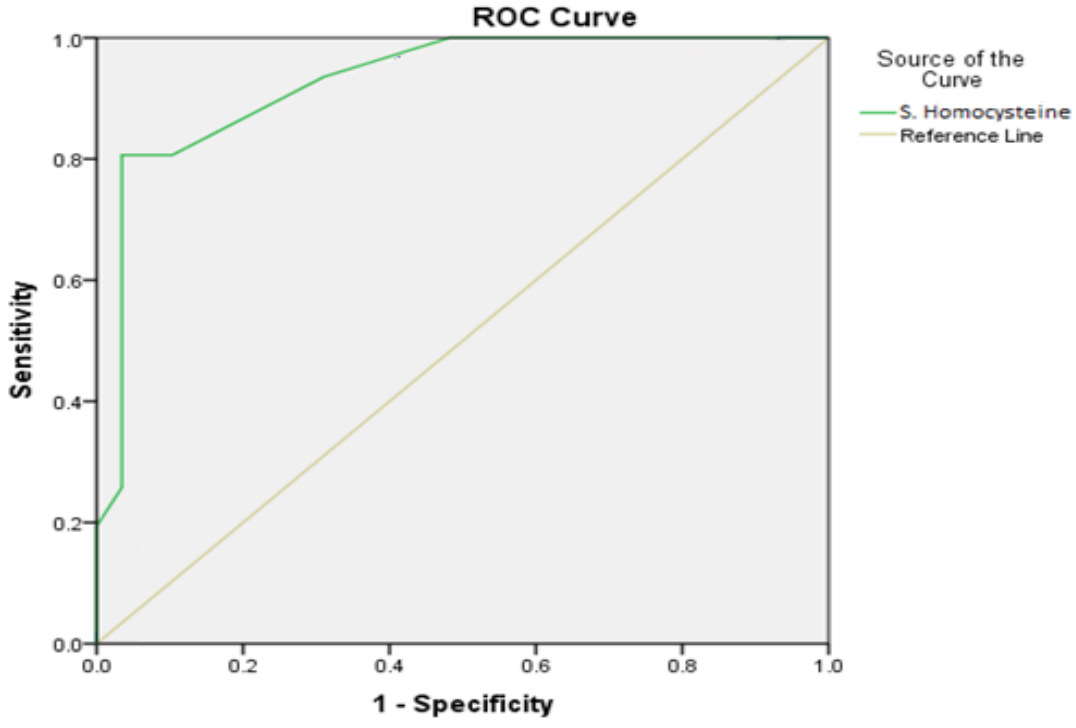

Figure (2): ROC curve of serum homocysteine as an early diagnostic marker for SBP in cirrhotic patients.

Table (6): Correlation between serum homocysteine and other parameters in SBP group

	Serum homocysteine	
	R	P
Hb	0.137	0.129
TLC	**0.351**	**<0.001**
PLT	0.135	0.132
ALT	**0.232**	**0.028**
AST	**0.190**	**0.034**
Total bilirubin	**0.221**	**0.013**
Albumin	**-0.312**	**0.001**
Creatinine	-0.172	0.125
LDH	**0.392**	**0.002**
CRP	**0.454**	**<0.001**
ESR	**0.333**	**<0.001**
AF Glucose	**-0.311**	**0.004**
AF Albumin	**-0.377**	**<0.001**
AF LDH	**0.412**	**<0.001**
AF PMN count	**0.430**	**<0.001**
Child–Pugh score	**0.520**	**<0.001**
MELD score	**0.478**	**<0.001**

DISCUSSION

SBP is a unique and a widespread complication in cirrhotic patients. The prevalence ranges from 10 to 30% in cirrhotic ascitic patients at the time of hospital admission and about 50% develop during hospitalization, with a mortality rate about 20–30% depending on several factors. In ascitic fluid, local and systemic immune dysfunction with bacterial translocation and reduced opsonic activity are the cornerstone mechanisms in the pathogenesis of SBP [(14)]. The diagnosis of SBP is still based upon diagnostic paracentesis [(15)]. It is an invasive maneuver with some complications. Therefore, there is a need for other noninvasive diagnostic methods.

Homocysteine (Hcy) is an amino acid that might be found in all cells in small amounts; it is a significant methionine metabolite quantitatively. In addition, Hcy can be present either in the form of disulfide proteins or freely in the body. Reduced or free form accounts for only 1–2%, in relation to a total Hcy quantity. However, about 80% is protein-bound Hcy, mostly to albumins [(16)].

The main aim of this study was to assess serum homocysteine as a novel reliable non-invasive early diagnostic marker for SBP in patients with hepatic cirrhosis.

The results of our study showed that there was a significant difference between the two studied groups regarding CRP (P=0.001) and ESR (P=0.008). However, there were no significant differences between the two studied groups regarding other laboratory parameters and demographic characteristics. Concerning ascitic fluid parameters between the two studied groups, ascitic fluid glucose and albumin were significantly lower in SBP group (P=0.002 & P=0.027, respectively) while LDH and PMN count were significantly higher in SBP group (P<0.001, for both). These results are in agreement with the results of the study of **Elsadek *et al.*** [(17)], which showed that comparison between SBP group (n = 60) and sterile ascites group (n = 118) regarding demographic, clinical, and laboratory characteristics revealed significantly higher ESR, and CRP, as well as significantly higher AF LDH and PMNs count in SBP group (P < 0.001, for all parameters). On the other hand, **Kim *et al.*** [(18)] reported that there were no significant differences in the laboratory findings, including the neutrophil count in the ascites, between the groups. The only exception was the white blood cell (WBC) count: the median WBC counts were 9150 and 6650cells/μL in the case and control groups, respectively (P<0.001).

The current study showed that serum homocysteine was significantly higher in SBP group compared to non-SBP group (18.43 ± 6.95 vs. 12.13 ± 5.54 μmol/l; P=0.001) and at a cutoff level ≥ 17.65 μmol/l, it had a sensitivity of 88.6% and 95.2% specificity for diagnosing SBP with an area under the curve (AUC) = 0.928). Our results are supported by study of **Ahmeda *et al.*** [(10)] as they reported that serum and ascitic homocysteine were considerably elevated in the SBP group than in the non-SBP group (17.94±7.57 vs. 11.75±5.68 μmol/l; P<0.001 and 14.70±5.45 vs. 9.75±4.55 μmol/l; P<0.001). At a cutoff value of 17.79 μmol/l, serum homocysteine had 89.3% specificity and 95.1% sensitivity for distinguishing SBP (area under the curve: 0.932) and, at a cutoff value of 16.1 μmol/l, ascitic homocysteine had 84.4% specificity and 92.7% sensitivity for distinguishing SBP (area under the curve: 0.901). A possible explanation for this finding may be the presence of bacterial endotoxins (lipopolysaccharide) in the blood, which induces a chronic inflammatory condition characterized by persistent innate immunity and cytokine synthesis activation. Circulatory dysfunction (hyperdynamic circulation) in cirrhotic patients may be through the enhancement of the systemic immune-inflammatory system. The cascade of proinflammatory cytokines and bacterial translocation activate nitric oxide (NO) production, increase the generation of vascular endothelial growth factor, and activate peritoneal macrophages, thus increasing peripheral vasodilation. Large amounts of circulating endotoxin were found in the ascitic patients that may persist even without obvious clinical manifestations of infection. These endotoxins are assumed to induce, indirectly or directly, an increase in NO synthesis and production, which increases the methionine–synthase inactivation, giving rise to Hcy accumulation in the extracellular space and cells [(19)].

On correlating serum homocysteine with other parameters in patients with SBP, tHcy was positively

correlated with TLC, ALT, AST, total bilirubin, CRP, ESR, ascitic fluid LDH, PMN count, Child–Pugh score, and MELD score. While, it was negatively correlated with serum albumin and ascitic fluid albumin and glucose. This is in agreement with the study of **Ahmeda *et al.* [(10)],** who reported positive correlation of serum and ascitic homocysteine with the polymorphonuclear count, C-reactive protein, Child-Pugh score, and model for end-stage liver disease score as well as negative correlation with the protein content in the ascitic fluid. So, there is an association between the severity of cirrhosis and serum Hcy as there was a significant correlation between the MELD, Child–Pugh scores and serum levels of Hcy. This relation may be linked to a reduction in NO, decreased vasodilation in the liver, and the effects of hyper-homocysteinemia on endothelial cellular damage. These results are in agreement with **Bhanji *et al.* [(20)],** who obtained the same findings. Moreover, **Bhanji and colleagues** also reported that hyperhomocysteinemia was related to ascites formation, potentially suggesting an exaggeration of portal hypertension secondary to impaired NO synthesis and impaired vasodilation.

Limitation of the study: The number of patients is small.

CONCLUSION

Serum homocysteine could be used as a reliable non-invasive early diagnostic marker for SBP in cirrhotic patients with ascites.

Data Availability: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Funding: Not available.

Conflicts of interest: Not available.

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