

Zinc deficiency correlates to spontaneous bacterial peritonitis in patients with cirrhotic ascites

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Received 3 July 2018

Accepted 11 August 2018

The Egyptian Journal of Internal Medicine
2019, 31:73–77

Background

Zinc deficiency in patients with liver cirrhosis has been frequently studied. Although spontaneous bacterial peritonitis (SBP) is one of the most serious complications of liver cirrhosis, few studies addressed the relationship between zinc deficiency and SBP.

Aim

This study aims to detect the effect of zinc deficiency in the development of SBP in patients with liver cirrhosis.

Patients and methods

The current study included 306 patients with liver cirrhosis and ascites who underwent diagnostic paracentesis for exclusion of SBP during admission to the Gastroenterology and Hepatology Unit, Ain Shams University Hospital, Egypt. Of these, 79 patients diagnosed with SBP by means of ascitic fluid polymorph nuclear leukocytic count more than or equal to 250 cells/mm³ were assigned to group 1. The remaining 227 patients with cirrhotic ascites were assigned to group 2. Routine laboratory tests, in addition to, serum zinc concentrations were assessed in all patients.

Results

Patients with SBP showed lower serum zinc in comparison to patients with cirrhotic ascites (49.11±11.84 vs. 79.27±9.58 µg/dl) with a statistically significant difference between the two study groups ($P<0.001$).

Conclusion

Zinc deficiency might be related to the development of SBP in patients with cirrhotic ascites.

Keywords:

ascites, liver cirrhosis, serum zinc, spontaneous bacterial peritonitis

Egypt J Intern Med 31:73–77

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1110-7782

Introduction

A variety of mechanisms result in necroinflammation and fibrogenesis of the liver tissue ending in cirrhosis. Cirrhosis is defined as diffuse nodular regeneration of hepatocytes with dense fibrous tissue septa, leading to hepatic parenchymal extinction and architectural collapse [1].

Regardless of the etiology of cirrhosis, individuals with chronic liver disease and cirrhosis are more prone to complications such as ascites, hepatic encephalopathy, variceal hemorrhage, and hepatocellular carcinoma [2].

Ascites is a hallmark of liver cirrhosis. It can be attributed to a series of pathophysiological events including portal hypertension and progressive vascular dysfunction. The presence of ascites is associated with a high 3-year mortality rate. Following the development of ascites, there is an increased risk of certain complications including hyponatremia, spontaneous bacterial peritonitis (SBP), and progressive renal impairment [3].

SBP is a frequent complication of ascites in patients with liver cirrhosis. It is defined as an infection of ascitic fluid that cannot be attributed to any abdominal inflammation or surgically correctable condition where the polymorph nuclear leukocytic count in ascetic fluid is more than or equal to 250 cells/mm³ [4].

SBP needs prompt recognition and treatment. SBP has an estimated prevalence of 1.5–3.5% in outpatients and 10–30% in hospitalized cirrhotic patients [5].

Typically, Gram-negative bacteria are the main causative organisms of SBP in patients with cirrhotic ascites; however, there is a constant rising number of SBP cases due to Gram-positive bacteria [6].

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Zinc as an essential micronutrient is needed for the development and integrity of monocytes and macrophages as well as regulation of their functions such as phagocytosis and proinflammatory cytokine production. Zinc deficiency was reported in many disorders like chronic liver disease, chronic alcoholism, malabsorption syndrome, chronic renal disease, and other chronic diseases including malignancy [7].

Zinc homeostasis is mandatory for many catalytic, regulatory and defensive mechanisms. It was noticed to be disturbed in patients with chronic liver disease [8].

Zinc plays a vital role in the early response and recovery after acute illnesses or major surgical procedures. During acute stress, zinc is transferred from the circulation to specific tissues such as the liver and the spleen with increased turnover in those organs, and increased disposal in the urine and injured tissues. Severe zinc deficiency is manifested by delayed recovery and increased mortality following major surgery, peritonitis, and sepsis [9].

SBP is a known predictor of mortality in patients with liver cirrhosis and ascites. Zinc deficiency has also been linked, in few studies, to an increased risk of developing SBP [10].

Patients and methods

The current study included 306 patients with liver cirrhosis and ascites who underwent ascitic fluid sampling for the exclusion of SBP during admission to the Gastroenterology and Hepatology Unit, Ain Shams University Hospital, Egypt, from March 2017 to March 2018. Of these, 79 patients were diagnosed with SBP by means of ascitic fluid polymorph nuclear leukocytic count more than or equal to 250 cells/mm³ and were assigned to group 1. The remaining 227 patients with cirrhotic ascites were assigned to group 2.

The inclusion criteria were liver cirrhosis and age over 18 years. Patients with less than 18 years, secondary bacterial peritonitis, sepsis, hepatocellular carcinoma, as well as all patients with local causes of ascites such as tuberculosis or malignancy were excluded from the start.

The following information was gathered from the patients' medical records on the date of the diagnosis: age, sex, alcohol intake, history of diabetes, hypertension, cardiovascular disorders, viral hepatitis, cirrhosis, hepatic encephalopathy, hematemesis, SBP, chronic kidney disease, nonalcoholic steatohepatitis, primary biliary cirrhosis, hepatic focal lesions, as well as a full review

of all past medical records including previous admissions and diagnoses and previous laboratory and imaging studies.

Laboratory investigations included liver function tests (serum aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, total protein, albumin, alkaline phosphatase, and alpha-fetoprotein), renal function tests (serum sodium, potassium, creatinine, and blood urea nitrogen), coagulation profile (prothrombin time, partial thromboplastin time, and international normalized ratio), complete blood count, and inflammatory markers (C-reactive protein) by standard laboratory tests. Serum zinc concentration was measured using the colorimetric end point method using zinc assay kit.

Diagnostic paracentesis was performed to obtain 15 ml of ascitic fluid under complete aseptic conditions; 10 ml were immediately inoculated in blood culture bottles. The rest was sent for chemical and cytological examination in tubes containing EDTA and was analyzed within 3 h of aspiration. Ascitic fluid protein was measured by spectrophotometry. Ascitic neutrophil count was detected by microscopic examination after centrifugation.

All patients signed a written informed consent prior to inclusion into this study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a prior approval by the institution's ethics committee.

Statistical analysis

Statistical analysis of the present study was conducted using statistical package for the social sciences, version 23 (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp, USA). The normality of the distribution was checked by Shapiro–Wilk test. Numerical variables were expressed as mean, SD, median, and interquartile range. For comparison between two groups, *t* test was used for parametric data, while Mann–Whitney test was used for nonparametric data. The cutoff point with the highest sensitivity and specificity rates was chosen using the receiver operating characteristic (ROC) curve. A *P* value less than 0.05 was considered statistically significant.

Results

This cross-sectional study was conducted between March 2017 and March 2018 in the Gastroenterology and Hepatology Unit, Internal Medicine Department, Ain Shams University,

Egypt. The study included 306 patients with liver cirrhosis who were selected from the Ain Shams University Hospital and were assigned to two groups. Seventy-nine patients, who were diagnosed with SBP, were assigned to group 1. The remaining 227 patients, who were diagnosed with cirrhotic ascites, were assigned to group 2.

Group 1 included 52 (65.8%) male patients and 27 (34.2%) female patients, while group 2 included 149 (65.6%) male patients and 78 (34.4%) female patients. There was no statistically significant sex difference between the two groups ($P=0.546$). The mean age for group 1 patients was 55.89 ± 6.7 , while the mean age for group 2 patients was 55.5 ± 7.5 with no statistically significant age difference between the two groups ($P=0.174$).

Table 1 shows univariate analysis of data from patients with SBP (group 1) and patients with cirrhotic ascites (group 2), as well as a comparison between laboratory parameters and ascitic fluid parameters between the two groups.

In group 1, cultures of ascetic fluid samples were positive for *Escherichia coli* in nine patients (11.4%), *Staphylococcus* spp. in nine (11.4%) patients, and *Klebsiella* spp. in six (7.6%) patients. The rest of group 1 patients were culture negative (69.6%).

Patients with SBP showed lower serum zinc in comparison to patients with uncomplicated ascites

(49.11 ± 11.8 vs. 79.27 ± 9.58 $\mu\text{g/dl}$). The difference was highly significant ($P<0.001$). They also showed lower serum albumin in comparison to patients with uncomplicated ascites (1.87 ± 0.31 vs. 2.91 ± 0.23 g/dl). The difference was also highly significant ($P<0.001$). Total leukocytic count was significantly higher among patients with SBP than patients with uncomplicated ascites (11.34 ± 4.16 vs. 8.65 ± 2.02 with $P=0.001$). Additionally, C-reactive protein was significantly higher among patients with SBP than patients with uncomplicated ascites (22.84 ± 4.33 vs. 8.15 ± 2.87 mg/l with $P<0.001$).

Patients with SBP showed lower ascitic fluid protein in comparison to patients with uncomplicated ascites (0.64 ± 0.23 vs. 1.69 ± 0.14 g/dl). The difference was highly significant ($P<0.001$). Ascitic fluid neutrophil count was significantly higher among patients with SBP than patients with uncomplicated ascites (935.08 ± 223.75 vs. 222.95 ± 15.03 with $P<0.001$).

Patients with SBP showed significantly lower levels of ascitic fluid albumin than patients with cirrhotic ascites (0.3 ± 0.2 vs. 0.8 ± 0.2 with $P<0.001$).

Patients with SBP showed significantly lower serum ascites albumin gradient than patients with cirrhotic ascites (1.5 ± 0.3 vs. 2.1 ± 0.3 with $P<0.001$).

Patients with SBP showed insignificantly lower zinc/serum ascites albumin gradient ratio than patients with cirrhotic ascites (36.8 ± 30.9 vs. 38.9 ± 8 with $P=0.55$).

Table 1 Univariate analysis comparing the two studied groups regarding blood tests and ascitic fluid composition

Compared variables	Group 1 (SBP) ($N=79$) (mean/median)	SD/IQR	Group 2 (ascites) ($N=227$) (mean/median)	SD/IQR	Significance (t test, MW test) P value
Age	55.886	6.7	55.507	7.544	0.692
S.Alb (g/dl)	1.868	0.307	2.905	0.2261	<0.001
ALP (U/l)	99.911	11.006	91.899	12.158	<0.001
T.Bil (mg/dl)	3.59	3.1–3.9	1.8	1.6–2.1	<0.001
AST (IU/l)	33	23–44	27	22–33	0.002
ALT (IU/l)	27	20–39	28	21–34	0.368
TLC ($\times 10^3/\text{m}^3$)	11.338	4.161	8.646	2.019	<0.001
PLT ($\times 10^3/\text{m}^3$)	66.829	28.149	138.933	27.055	<0.001
BUN (mg/dl)	33	22–47	26	15–43	0.005
Cr mg/dl	2.132	0.912	1.877	0.936	0.036
PT (s)	24.729	2.141	18.904	1.593	<0.001
AFP (ng/ml)	4	3–4	4	3–4	0.238
S. Zn $\mu\text{g/dl}$	49.11	11.841	79.27	9.58	<0.001
S. Na (mEq/l)	123	121–127	139	137–141	<0.001
CRP (mg/l)	22.835	4.331	8.155	2.867	<0.001
AFNC (>250)	935.076	223.754	222.947	15.033	<0.001
As fl Pr (g/dl)	0.6363	0.233	1.688	0.143	<0.001

AFNC, ascetic fluid neutrophil count; AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cr, creatinine; CRP, C-reactive protein; IQR, interquartile range; MW, Mann–Whitney; Na, sodium; PLT, platelet count; Pr, protein; PT, prothrombin time; S.Alb, serum albumin; SBP, spontaneous bacterial peritonitis; T.Bil, total bilirubin; TLC, total leukocytic count; Zn, zinc.

Eta-squared value was calculated using serum zinc as an independent variable for comparing patients with cirrhotic ascites to those with SBP aiming to estimate the effect size of serum zinc concentration on the development of SBP. The Eta-squared value was 0.80 which indicates that serum zinc concentration strongly correlates to SBP. Serum zinc concentration accounts for 80% of variance between patients with cirrhotic ascites and patients with SBP.

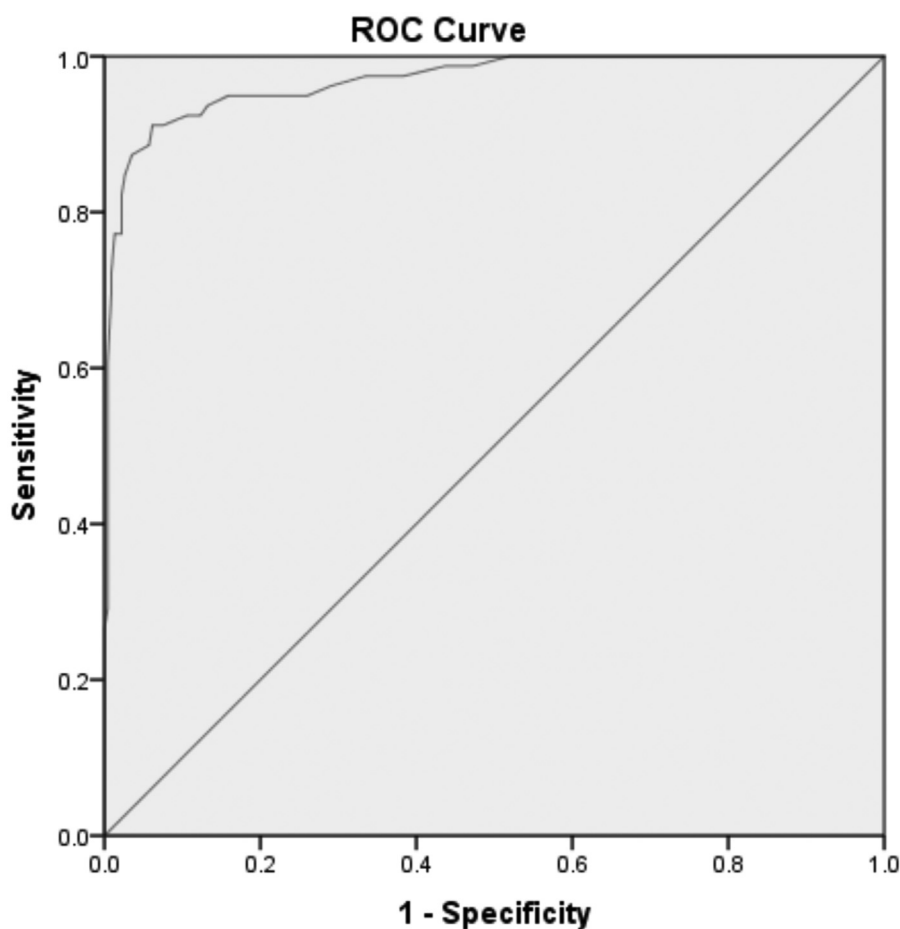
The ROC curve was obtained to determine the sensitivity and specificity of serum zinc concentration in differentiating SBP patients from those with cirrhotic ascites, as well as in determining the best cutoff value at which serum zinc levels could be used for such a purpose. Serum zinc concentration below 70.5 µg/dl predicted SBP in patients with cirrhotic ascites with a sensitivity of 94.9% and specificity of 84.1% (Fig. 1).

Discussion

Zinc plays an important role in the immune system serving as an enzyme cofactor in a number of cellular and metabolic processes. This micronutrient has a role in oxidative stress and in anti-inflammatory effects [11]. SBP prophylaxis has become an important issue in the era of the increasing resistance to antibiotics. If zinc deficiency proves to be an important predictor of SBP, zinc supplementation in patients with cirrhotic ascites might alter the risk of infection and decrease the need for unnecessary antibiotic administration. This might, then, significantly reduce morbidity and mortality in such a risky population.

The hallmark of the current study is that low serum zinc concentration is linked to the development of SBP in patients with cirrhotic ascites. The prevalence of SBP in the current study was 25.82%. According to the

Figure 1



ROC curve to determine the sensitivity and specificity of serum zinc in predicting SBP in patients with liver cirrhotic ascites. AUC, area under the curve; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; ROC, receiver operating characteristic.

best cutoff value from the ROC curve and for the purpose of data analysis in the current study, low serum zinc was defined as serum zinc concentration of less than 70.5 µg/dl. 67.6% of patients with cirrhotic ascites and low serum zinc ($n=75/111$) had SBP, while 94.9% of patients with SBP ($n=75/79$) had low serum zinc. Univariate analysis (Table 1) showed that there is a significant difference between patients with cirrhotic ascites and patients with SBP in terms of serum zinc concentrations ($P<0.001$). The effect size of serum zinc concentration on the presence of SBP was strong (Eta-squared=0.8). At a cutoff value of 70.5 µg/dl, serum zinc concentration was able to differentiate between patients with cirrhotic ascites and patients with SBP with 94.9% sensitivity, 84.1% specificity, and 86.93% accuracy. The positive predictive value of low serum zinc was 67.57%, while the negative predictive value was 97.95% (Fig. 1).

This stands in agreement with a previous study by Mohammad and colleagues where the prevalence of SBP among patients with cirrhotic ascites was 31%. In that study, 35 (64.8%) of the 54 SBP-positive patients had a serum zinc level less than 60 µg/dl, whereas only 45 (36.9%) of 122 SBP-negative patients were found to have low serum zinc ($P=0.001$). The study concluded that serum zinc concentration was significantly lower in patients with SBP, and multivariate analysis showed that zinc deficiency (serum zinc concentration <60 µg/dl) was an independent predictor of SBP [10].

Sengupta and colleagues concluded that serum zinc concentrations were inversely correlated with ascites and SBP. Low serum zinc leads to poorer clinical outcomes and shorter transplant-free survival. Zinc was considered to be an independent predictor of SBP in patients with cirrhotic ascites. They also stated that zinc might serve as a useful diagnostic and prognostic marker in such patients [11]. Zinc deficiency in patients with liver cirrhosis could be attributed to various mechanisms. Ascites causes mechanical compression on the stomach resulting in early satiety and malnutrition. In SBP, this process is enhanced by infection that leads to anorexia and decreased appetite. In addition, liver cirrhosis is associated with limited intestinal absorption and limited portal venous extraction of zinc [12]. Finally, increased portosystemic shunting is associated with higher zinc excretion and thus increased urinary loss [13]. Previous studies

addressed the possible effect of zinc on immunodeficiency states such as T-cell defects. Zinc appears to influence both lymphocyte and neutrophil function. Thus, zinc deficiency remains an important suspect in cases of SBP [14].

Conclusion

Zinc deficiency might be strongly correlated to the development of SBP in patients with cirrhotic ascites. Zinc supplementation might play an important role in prophylaxis against SBP.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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