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Low Serum Zinc and Spontaneous Bacterial Peritonitis in Patients with HCV-Related Liver Cirrhosis

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ABSTRACT

Background: A serious and common consequence in cirrhotic liver patient is spontaneous bacterial peritonitis (SBP), it means infection of ascitic fluid with unknown operationally treatable etiology in the abdomen. The body contains one of the most prevalent trace elements, zinc (Zn), which is important for growth and development.

Objective: To investigate the relationship between low serum Zn levels and SBP development in HCV-related cirrhotic liver patients.

Patients and Methods: The study was done on fifty patients with HCV related cirrhotic liver suffering from ascites. Half of them without SBP, classified as group (A), and the other half with SBP on the basis of ascitic fluid polymorphonuclear (PMN) leukocytic count of ≥250 cells/μL in the absence of subsequent peritonitis, no matter the culture results of the ascitic fluid, classified as group (B).

Results: The levels of C-reactive protein (CRP), platelet count, and white blood cells (WBCs) were significantly different between the two research groups (P<0.001, =0.016, =0.03 respectively). Ascitic fluid PMN count increased significantly in SBP group, which means statistically significant difference between the 2 groups (P<0.001). Serum zinc levels was significantly lower in group B than group A [63.1 μg/dL (46-95) vs 84.79 μg/dL (65-130); P<0.001]. With 0.860 as area under the curve (AUC), serum zinc was significant at a threshold level of ≤70 μg/dL with a sensitivity of 84 percent and 64 percent specificity for predicting SBP.

Conclusion: Serum zinc levels in cirrhotic individuals with HCV who had been diagnosed with SBP were found to be considerably low.

Key words: Cirrhosis, Serum zinc**,** Spontaneous Bacterial Peritonitis**,** Ascites.

INTRODUCTION

Liver cirrhosis is a diffuse process of fibrosis and nodule formation that leads to loss of normal architecture of liver, decrease in hepatocellular mass, more over activation of hepatic stellate cells, increase amount of collagen and forming other components of extracellular matrix that result in fibrosis [(1)].

There are variety of reasons for cirrhosis; the most common are hepatotropic viruses (HCV – HBV) and alcoholism [(2)]. Regardless of the cause, there are many complications to cirrhosis such as hepatic encephalopathy, ascites, SBP, and hepatocellular carcinoma [(3)]. In cirrhotic ascitic patients, SBP is a significant, common consequence in which paracentesis was used for its diagnosis (ascitic fluid PMNL count ≥ 250 cells/mm^3) [(4)]. It occurs in 10-30 percent of hospitalized patients suffering from cirrhotic liver and ranges from 1.5% to 3.5% in outpatients [(5)]. Gram negative bacteria, mainly E. coli, are the causes of SBP, while gram positive bacteria can also play a role in some instances [(6)].

In the body, more than 300 different types of enzymes use zinc as a coenzyme to mediate activities such as synthesis of protein, transcription of RNA, cell development, and cell division. Currently, zinc is regarded as a trace element that is necessary for preserving life [(7)].

Numerous disorders, including chronic liver disease, the malabsorption syndrome, short bowel syndrome, dialysis-required renal illnesses, diabetes mellitus, and inflammatory bowel disease, have been linked to low serum zinc levels [(8)]. Numerous catalytic, regulatory, and protective systems need zinc homeostasis. Patients with chronic liver illness were shown to have disturbances in it [(9)].

Many factors contribute to zinc deficiency in liver cirrhotic patients, including decreased ability to synthesis albumin, malabsorption of Zn from the gut, due to gastric congestion brought on by portal hypertension[(10)] and increased urinary excretion of Zn[(11)].

NADPH oxidase in monocytes and macrophages is decreased by zinc supplementation, which reduces the production of free radicals. Zinc is a useful substance to lessen oxidative stress because superoxide dismutase, which is necessary for controlling free radicals, is dependent on both zinc and copper [(12)]. The activity of the transcription factor A20, which is zinc-dependent and inhibits the production of TNF-**α**, is also increased by zinc supplementation in monocytes and macrophages [(13)].

The aim of this study was investigation of the relationship between low serum Zn levels and SBP development in patients with HCV-related cirrhotic liver.

PATIENTS AND METHODS

This study included fifty patients suffering from HCV-positive cirrhotic liver with ascites who were admitted to the Internal Medicine Unit of the Benha University Hospital. Based on clinical, laboratory, and

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ultrasonographic findings, individuals with cirrhotic liver and ascites who were older than eighteen years old were involved in the study.

Groups classification: **Group (A):** 25 ascitic individuals with HCV related cirrhotic liver who did not have SBP were included. **Group (B):** 25 HCV-related cirrhotic patients with SBP were included based on a PMN count in the ascitic fluid of 250 cells/L or more with positive ascitic fluid culture or without, moreover an intra-abdominal source of infection or cancer were absent [(14)].

Exclusion criteria were: patients with evidence of secondary bacterial infection, ascites from other causes (malignancy, cardiac disease, renal disease, tuberculosis, or hypothyroidism), sepsis, hepatocellular carcinoma (HCC), and liver cirrhosis from causes other than HCV.

Each patient had a complete medical record, physical examination, and biochemical testing to detect signs of liver cirrhosis, including ascites, and symptoms of spontaneous bacterial peritonitis as altered mental status, fever, diarrhea, increase in ascites, abdominal pain, and new-onset renal failure.

Complete blood count (CBC), C-reactive protein, markers for liver injury (ALT, AST), liver function tests (total, direct, albumin, prothrombin time, serum creatinine and blood urea, international normalized ratio, HCV antibodies by enzyme-linked immunosorbent assay (ELISA), and serum Zn with Zn assay kit by colorimetric end point method . Pelviabdominal ultrasonography was used for detection of radiological signs of LC, ascites, and to rule out HCC. Diagnostic abdominal paracentesis was performed for the examination of ascitic fluid PMN.

Ethical approval:

All subjects provided their informed permission after the Institutional Review Board of the Benha University's Faculty of Medicine gave its approval. The work was held in conformity with the Declaration of Helsinki, which is the World Medical Association's code of ethics for human experimentation.

Statistical analysis

IBM Inc., Chicago, Illinois, USA, SPSS v26 was used to conduct the statistical study. Quantitative data were presented as mean, standard deviation (SD), and range and were compared by the unpaired Student's t-test were used. The qualitative data, which were presented as frequency and percentage (%), were analyzed using the Chi-square test. We used the Pearson correlation. For assessment of test's overall diagnostic performance, ROC curve analysis was used. A two tailed P value of 0.05 is considered statistically significant.

RESULTS

Between the two groups, there was no statistically significant regarding age and sex (Table 1).

Table (1): Demographic data of the studied groups

		Group A (n=25)	Group B (n=25)	P-value
Age (years)	**Mean ± SD**	59.2 ± 4.07	60.2 ± 8.23	0.574
	Range	52 - 67	31 - 70	
Sex	**Male**	16 (64%)	9 (36%)	0.089
	Female	9 (36%)	16 (64%)	

WBCs and CRP were substantially higher in group B than group A in terms of the laboratory investigation's parameters, although platelets were significantly lower in group B than group A. Regarding Hb, ALT, AST, albumin, total bilirubin, creatinine, prothrombin concentration, and INR, there were no statistically significant changes between the two groups under investigation (Table 2).

Table (2): Laboratory findings in both groups

		Group A (n = 25)	Group B (n = 25)	P-value
Hemoglobin (g/dl)	Mean ±SD	8.9 ± 2.21	9.8 ± 1.67	0.095
WBCs (10^3 cell/cmm)	Mean ±SD	6.468 ± 1.41	9.86 ± 2.44	**0.03***
Platelets (10^3 cell/cmm)	Mean ±SD	145.352 ± 34.54	90.44 ± 21.99	**0.016***
ALT (IU/l)	Mean ±SD	47.8 ± 11.47	42 ± 10.13	0.694
AST (IU/l)	Mean ±SD	68.2 ± 6.92	71.4 ± 6.63	0.904
Albumin (g/dl)	Mean ±SD	2.9 ± 0.61	2.7 ± 0.53	0.283
Total bilirubin (mg/dl)	Mean ±SD	2.2 ± 0.54	3.2 ± 0.71	0.461
Creatinine (mg/dl)	Mean ±SD	1.6 ± 0.38	2.1 ± 0.51	0.194
Prothrombin concentration (%)	Mean ±SD	55.2 ± 13.34	58.8 ± 14.61	0.393
INR	Mean ±SD	1.4 ± 0.25	1.4 ± 0.33	0.968
CRP (mg/L)	Mean ±SD	40.7 ± 10.01	66.3 ± 15.81	**<0.001***

*: Significant

Those with SBP (group B) had a substantially higher ascitic fluid PMN count than those without SBP (group A) (Table 3).

Table (3): Ascitic fluid PMNs of the studied groups

		Group A (n=25)	Group B (n=25)	P-value
PMNs in ascitic fluid (cells/mm^3)	**Mean ± SD**	108.2 ± 26.82	481.4 ± 117.70	**<0.001***

PMNs: Polymorphonuclear leukocytes**,** *: Significant

Concerning serum zinc, it was significantly lower in group B (SBP group) than in group A (Non-SBP group) (Table 4).

Table (4): Serum zinc of the studied groups

		Group A (n=25)	Group B (n=25)	P-value
Serum zinc (µg/dL)	**Mean ± SD**	84.8 ± 19.12	63.1 ± 11.64	**<0.001***

*: Significant

On correlating serum zinc with ascitic fluid PMNs, there was negative correlation between serum zinc and ascitic fluid PMNs (Table 5).

Table (5): Correlation between serum zinc and ascitic fluid PMNs of the studied groups

		PMNs in ascitic fluid (cells/mm^3)
Serum zinc (µg/dL)	**r**	- 0.341
	P value	**0.015***

*: Significant, r: Pearson correlation coefficient

Serum zinc was significant at cut-off ≤70 µg/dL for predicting SBP (Table 6 & Figure 1).

Table (6): Validity of serum zinc in predicting SBP in patients with HCV related cirrhotic ascites

Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P-value
≤70 µg/dL	84%	64%	70%	80%	0.860	**<0.001***

PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve, *: Significant

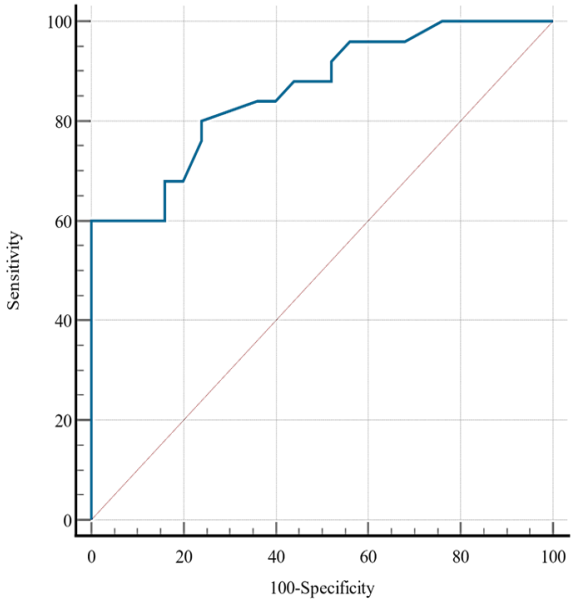

Figure (1): ROC curve of serum zinc in predicting SBP in patient with HCV related cirrhotic ascites.

DISCUSSION

Spontaneous bacterial peritonitis is a specific and frequent complication in those suffering from cirrhosis. At the time of hospital admission, the proportion of cirrhotic ascitic patients ranges from 10 to 30%, and around 50% develop while receiving treatment there. Various variables affect the mortality rate, which can vary from 20 to 30 percent. The main causes of SBP in ascitic fluid include local and systemic immunological dysfunction, diminished opsonic activity and bacterial translocation [(15)].

Zinc is micronutrient which lowers oxidative stress and inflammation. It is necessary as an enzyme cofactor for immunity needed in several cellular and metabolic functions [(10)].

The main goal of this research was to investigate the relationship between low zinc levels in serum and SBP in individuals with HCV-related cirrhotic liver. Zinc supplementation in persons with ascites and cirrhotic liver could change the probability of infection, which may therefore significantly decrease morbidity and mortality in such a high-risk people if zinc deficiency is proved to be a significant for prediction of SBP.

According to the findings of our study, group B (the SBP group) had considerably greater WBCs (9.866±6.445) than group A (the Non-SBP group) (6.468±4.014) in terms of the parameters of laboratory studies. **Cholongitas *et al.*** [(16)] found a similar result, stating that leukocytosis was more in spontaneous bacterial peritonitis people as a component of the body's responsiveness to infection. Similar to **Elsadek *et al.*** [(17)] study, who stated significantly higher ESR and CRP in the SBP group (P 0.001, for both parameters), which implement the presence of inflammation.

In the current study, Group B (66.3±15.81) showed significantly higher CRP when compared to group A (40.7±10.01) (P 0.001). In terms of the investigated patients' platelet counts, both the SBP and non-SBP groups had lower platelet counts, but the SBP group was significantly lower. These results are similar to **Lata *et al.*** [18] study, who detected that SBP patients' platelet counts showed statistically significant decrease compared to non-SBP patients' platelet counts and hypothesized that this difference was due to the increase in portal pressure. The amount of protein in ascites, which influences the incidence of SBP even through facilitating bacterial translocation, is a key component. May be affected by portal hypertension. Additionally, **Kuckleburg *et al.*** [19] hypothesized that platelets perform a variety of immunologic tasks as a result of their participation in triggering neutrophil granulocyte activation in bacterial infections. Thus, in cirrhotic individuals, a low platelet count may lead to inadequate neutrophil activation and an increased risk of infections.

Regarding other laboratory measurements and demographic variables, there were no appreciable variations between the two study groups. This is consistent with research by **Mohammad *et al.*** [20]**,** who found no statistically significant variations in Hb, ALT, albumin, bilirubin, INR, or creatinine levels between individuals with and without SBP. Our results were different from those of **Hanna and colleagues** [21], who claimed that patients without SBP had blood albumin levels that were statistically considerably higher. Additionally, the SBP group's AST and serum creatinine were statistically significantly greater than those of the other group. Additionally, **Abo Galala and colleagues** [22] reported data that were distinct from our findings because ALT and AST levels between SBP positive and negative patients differed statistically substantially and were greater in SBP patients. Albumin, however, showed no statistically significant difference among studied groups.

According to our research, the ascitic fluid polymorphonuclear count was significantly higher in the SBP group when compared to non-SBP group (P<0.001). These findings were consistent with **Hanna *et al.*** [21] finding that SBP patients had a higher ascitic PMN count than non-SBP patients. They also reported similar findings.

Concerning serum zinc level, our study showed that serum zinc was significantly lower in group B (SBP group) compared to group A (non-SBP group) (63.1 ±11.64 vs. 84.79 ±19.12 µg/dl; P< 0.001), and at a cutoff level ≤70 µg/dL, it had a sensitivity of 84%, 64% specificity, 70% PPV and 80% NPV for predicting SBP with an area under the curve (AUC) = 0.860). These findings were corroborated by **Hanna *et al.*** [21] study, which found that patients with SBP had lower serum zinc levels than those with no complicated ascites (49.11±11.8 vs. 79.27±9.58 g/dl; P<0.001) and that SBP could be accurately predicted in cirrhotic ascitic diseased people at a cutoff level below 70.5µ g/dl with a sensitivity of 94. This was inconsistent with the results of **Soomro *et al.*** [23]**,** who showed that zinc levels were statistically significantly lower in individuals with liver cirrhosis associated with SBP. This also concurs with a prior study by **Mohammad and colleagues** [20], which observed by using multivariate analysis that SBP patients had significantly lower zinc concentrations in serum, and that SBP was independently predicted by zinc insufficiency (serum zinc concentration 60 µg/dl).

Many different pathways may be responsible for the zinc shortage seen in people with liver cirrhosis. Early satiety and malnutrition are results of the mechanical compression on the stomach that ascites creates. In SBP, an infection that causes anorexia and decreased appetite speeds up this process. Additionally, reduced zinc extraction from the portal veins and intestinal absorption are linked to liver cirrhosis [24]. Last but not least, elevated portosystemic shunting is linked to greater zinc excretion and hence elevated urine loss [25]. Previous research examined zinc's potential impact on immune deficiencies such T-cell abnormalities. The functions of neutrophils and lymphocytes appear to be influenced by zinc. As a result, zinc deficiency is still a leading suspect in SBP patients [26].

According to our research, blood zinc levels and PMN levels in ascitic fluid correlated negatively (r = -0.341). **Hanna *et al.*** [21]**,** who discovered a correlation between a zinc shortage and an increase in ascitic fluid PMNs, reported similar findings. **Sengupta and colleagues**[10] came to the conclusion that ascites and SBP were negatively linked with serum zinc concentrations. Low blood zinc levels were associated with worsening of clinical consequences and a shorter transplant-free survival time. SBP in individuals with cirrhotic ascites was thought to be independently predicted by zinc in these patients. Additionally, they claimed that in these individuals, zinc might be a helpful diagnostic and prognostic marker.

Limitation of the study: The study is single centered, and variations may occur among other settings and study sample size was small.

CONCLUSION

In cirrhotic individuals with HCV who had been diagnosed with spontaneous bacterial peritonitis, serum zinc was found to be considerably low. The emergence of SBP in some patients may be linked to zinc deficiency. Therefore, zinc supplementation may be crucial in the prevention of SBP.

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Competing interests: Nil.

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