A Case Control Study on Serum Level of Micronutrients in Decompensated Liver Cirrhosis Patients in Mymensingh, Bangladesh

Hossain MS[1], Rahman MM[2], Shahidullah ASM[3], Sen B[4], Hoque MR[5], Paul AR[6], Saha KR[7]

Abstract

Liver cirrhosis is an end stage condition of liver disease and the leading cause of death for both men and women all over the world. It causes death due to acute or chronic gastrointestinal blood loss & multi organ failure. The aim of the study is to evaluate the serum Copper, Zinc and Iron levels in patients with liver cirrhosis. A total 120 subjects were included in this study. Among them, 60 diagnosed decompensated liver cirrhosis patients denoted as case group (n=60) and 60 were normal healthy individuals denoted as control group (n=60), both the case and control groups were male because liver cirrhosis occurs rarely female in Bangladesh. The subjects were briefed and written consents were taken. Under all aseptic precaution 5 ml of venous blood was collected from median cubital vein, analysis was carried out in the Department of Biochemistry, Mymensingh Medical College, Mymensingh, over a period of one year from July 2015 to June 2016. All statistical analysis was performed by SPSS windows package, version 20. Significance of the difference between two groups were evaluated by using student's unpaired 't' test. All the values were expressed as mean ± SD and P value 0.05 was taken as the level of significance. After careful evaluation, in the present study we found significant increase in the serum Copper levels and significant decreases in the serum Zinc and Iron levels in liver cirrhosis patients when compared with that of control group. It can be concluded that to overcome fatal consequences of decompensated liver cirrhosis patients we should avoid high Copper containing food provides Zinc and Iron supplementation as well as blood transfusion may be beneficial.

CBMJ 2019 July: vol. 08 no. 02 P: 10-16

Key words: Liver cirrhosis, Copper, Zinc and Iron.

Introduction

Liver cirrhosis is the end-stage condition of liver disease. Cirrhosis results from persistent and progressive necrosis of hepatocytes, followed by fibrosis and abnormal nodule formation. So, entire liver is involved, loss of architecture, necrosis of hepatocyte, fibrosis, nodules are formed by regeneration of hepatocytes.[1]

The exact prevalence of cirrhosis worldwide is unknown. Cirrhosis prevalence was estimated at 0.15% or 400000 in the USA. This may be an underestimation as we recognize the high prevalence of undiagnosed cirrhosis in both nonalcoholic steatohepatitis (NASH) and hepatitis C. Similar number have been reported from Europe and numbers are even higher in most Asian and African countries when chronic viral hepatitis B or C are frequent. Since compensated cirrhosis often goes undetected for prolonged periods of time, a reasonable estimate is that up to 1% of population may have histological cirrhosis.[2]

1. *Dr. Md. Shahadath Hossain, Assistant Professor (CC), Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*
2. *Professor Dr. Md. Muzibar Rahman, Professor and Head, Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*
3. *Professor Dr. A. S. M. Shahidullah, Professor (CC), Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*
4. *Professor Dr. Bina Sen, Professor (CC), Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*
5. *Dr. Md. Rafiqul Hoque, Associate Professor, Department of Biochemistry, Mymensingh Medical College Mymensingh, Bangladesh.*
6. *Dr. Arup Ratan Paul, Associate Professor, Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*
7. *Dr. Kamona Rani Saha, Associate Professor, Department of Biochemistry, Community Based Medical College Bangladesh, Mymensingh, Bangladesh.*

Address of correspondence:

Email: dr.shahadath.bd@gmail.com
Mobile: 01722117789

In hepatic cirrhosis, the presence of any one or more of jaundice, ascites, portal hypertensive gastrointestinal bleeding, and/or, encephalopathy is considered as decompensation. Decompensation, per se, is a significant risk for mortality. One-year mortality in compensated cirrhosis is 1-3.4%, but in decompensation it is elevated to 20-57%. The above manifestations appear when the disease process overwhelms the compensatory mechanisms, either by disease progression or a superimposed acute insult or some other contributing factors like micronutrients (Cu, Zn, Fe) deficiency.[3]

Many elements, although present in minute quantities, are essential nutrients for humans. Their presence was long overlooked and it has only been found in recent years that these elements perform functions indispensable for maintenance of life, growth and reproduction. Inadequate levels of some elements may impair cellular and physiological functions causing illness. Considering the vital role, those trace elements in enzymatic reactions they have been examined critically as potential key factors in varied diseases like liver cirrhosis. Although micronutrients are only a part of total picture, they contribute significantly to nutrition and maintenance of health as well as prevention of several diseases like liver cirrhosis.[4]

Copper (Cu) is an important trace element which is widely distributed in all human tissues, with high concentration in liver, heart, brain and kidney. The majority (96%) of serum Cu is firmly bound with $\alpha 2$ globulin that is called ceruloplasmin. Remaining 4% is loosely bound to albumin that is metabolically active. Some study reported that the fraction of copper loosely bound to protein (nonceruloplasmin fraction) was increased in cirrhosis. They suggested that hypercupremia might be produced by decreased capacity of liver to inactivate the circulating oestrogen which leads to excessive circulating oestrogen resulting in hypercupremia.[5] Cu is highly toxic in excess and results in cellular damage and hepatocellular carcinoma.[6]

Zinc (Zn) is an essential trace element. It is an essential component of more than 300 enzymes, which participate in metabolism of carbohydrate, protein, fat and nucleic acid. Protein metabolism impairment in cirrhotic patients would appear to affect the plasma transport of Zn rather than its overall availability in the organ.[7] Zn is transported in plasma mostly by albumin (60-70%) and by a_2 macroglobulin (30-40%). Low serum Zn level in liver cirrhosis patients might be the result of decreased liver albumin content, decreased a_2 macroglobulin synthesis, poor dietary intake, or protein restriction.[8]

Iron (Fe) is one of the very important trace elements widely distributed in body. Iron is distributed as hemoglobin (65-70%), ferritin and hemosiderin (25-30%), myoglobin and transferin (5%). Regarding iron homeostasis iron level significantly lower in decompensated liver cirrhosis. Many studies observed that iron and total iron binding capacity levels were lowered than control in all child Pugh groups of cirrhosis.[8] Acute gastrointestinal hemorrhage is common and potentially a serious complication of portal hypertension. Rupture of oesophageal varices may cause hypovolemia and secondary iron deficiency anaemia. Chronic loss of blood in the GIT causes chronic hemorrhage and iron deficiency anaemia. Acute and chronic hepatocellular disease is also associated with defective blood coagulation. Decreased synthesis of factors II, VII, IX, X may cause severe blood loss and anaemia. Thrombocytopenia also partially contributes to anaemia in cirrhosis.[9]

Table 1: Child-Pugh classification of prognosis in cirrhosis

Score	1	2	3
Encephalopathy	None	Mild	Marked
Bilirubin (µmol/L)	<34	34-50	>50
Albumin (G/L)	>35	28-35	<28
Prothrombin time Prolonged in sec.	<4	4-6	>6
Ascites	None	Mild	Marked

Add the individual score <7=child's A, 7-9= child's B, >9= child's C. To convert bilirubin in µmole/L to mg/dl, divide by 17.1. [10]

Table 2: Survival in Cirrhosis

Child Puge Grade	Survival (%)			Hepatic Death (%)
	1 Year	5 Year	10 Year	
A	82	45	25	43
B	62	20	7	72
C	42	20	0	85

There has been a growing awareness of possible alteration of serum Zn level in patients with liver cirrhosis. Various studies of serum Zn level in patients with liver cirrhosis were undertaken and significant decreases in serum zinc levels were found in these patients. Symptoms of acute Zn deficiency like anorexia, dysfunction of smell and taste, mental and cerebellar disturbance as well as symptoms of chronic Zn deficiency like growth retardation, anaemia, testicular atrophy, and impaired wound healing are common in cirrhotic patients. So, micronutrient deficiency may contribute to the features of cirrhosis or to the development of decompensated cirrhosis as a continuum. Despite this controversy persists regarding the importance of serum Cu, Zn and Fe level in the patients of liver cirrhosis, the merits of routine screening of these elements continue to be debated.

Materials & Methods

This case control study was conducted in the Department of Biochemistry, Mymensingh Medical College, Mymensingh from over a period of one year from July 2015 to June 2016. A total of 60 patients of decompensated liver cirrhosis (all are child's Pugh B & C) ranging from 20-60 years were selected. Sixty ages matched, healthy subjects (from students and hospital employees) were selected in the control group. All participants were recruited after obtaining their written consents. All study subjects were from the same geographical area with no significant difference in their food habits and drinking water quality. Cases were selected from indoor ward of the same hospital. Liver cirrhosis patients were diagnosed provisionally from detailed history, positive findings on clinical examination. Final diagnostic criteria were fulfilled by ultrasonographic examination of the abdomen and relevant biochemical tests. Liver cirrhosis patients associated with concomitant pathology like diabetes mellitus, hyper tension, chronic diarrhoea, renal failure or taking drugs causing alteration of trace elements status were excluded from this study. Blood was obtained from patients and controls after an overnight fast. The blood was drawn into plastic syringes fitted with stainless steel needles, immediately transferred to metal free tube, allowed to clot, centrifuged and serum removed to another metal free tube for storage -20 degree centigrade until assayed.

Serum Iron is in μgm/dl was measured by Ferrozine method, by spectrophotometrically.[11] Serum Copper and Zinc in μgm/dl were measured spectrophotometrically. All data were recorded systematically in a preformed data collection sheet. The collected data were processed and analyzed by computer software SPSS (Statistical package for social science) version 20. Students unpaired 't' test was used to analyze the data between groups. For analytical test, 95% confidence limit $P<0.05$ was taken as the level of significance.

Result Analysis

In the present study, a total number of 120 subjects were participated. Among them, 60 were decompensated liver cirrhosis patients denoted as case group and 60 were normal healthy individuals denoted as control group. Different variables of the subjects were being analyzed in this section. Serum Copper, Zinc and Iron levels were estimated from blood samples collected from 120 human subjects. Data were expressed in Mean ± SD and statistical significance of difference among the groups were calculated by unpaired student's 't' test. If p value is less than 5% ($p <0.05$) it indicates statistically significant result. If p value is less than 1% ($p<0.01$) it indicates statistically highly significant result. If p value is more than 5% ($p>0.05$) it indicates statistically not significant result. The results were expressed as serum copper, zinc and

iron is in µgm/dl. The study revealed that mean serum copper level was higher in case group as compared to control group. The mean values of copper were 104.69 ± 9.15 and 149.17 ± 5.72 in control and case group respectively. (Table 3)

The analysis showed that, the difference in mean serum copper levels between two groups was highly significant. Analysis of mean serum copper levels of study population were presented in figure 1. The study revealed that mean serum zinc level was lower in case group as compared to control group. The mean values of zinc were 91.08 ± 6.16 and 45.89 ± 14.10 in control and case group respectively. The analysis showed that, the difference in mean serum zinc levels between two groups was highly significant. Analysis of mean serum zinc levels of study population were presented in figure 2. The study revealed that mean serum iron level was lower in case group as compared to control group. The mean values of iron were 118.83 ± 11.93 and 35.26 ± 7.74 in control and case group respectively in figure 3. The analysis showed that, the difference in mean serum iron levels between two groups was highly significant.

Table 3: Serum Copper, Zinc and Iron levels in the study population.

Variables	Mean± SD Control group	Mean± SD Case group	t value	p value
Serum Copper (µgm/dl)	104.69±9.15	149.17±5.72	32	P< 0.001
Serum Zinc (µgm/dl)	91.08±6.16	45.89±14.10	22.82	P<0.001
Serum Iron (µgm/dl)	118.83 ± 11.93	35.26 ± 7.74	45.59	P<0.001

Unpaired student's 't' test, Significant

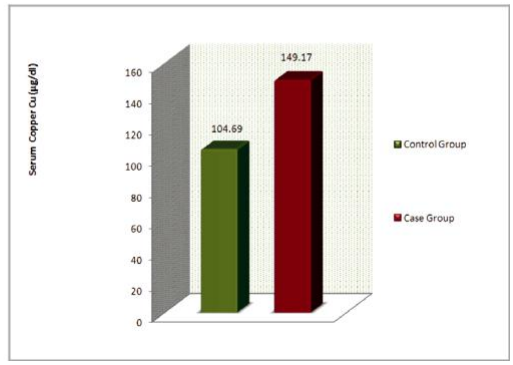

Fig.1 Mean serum copper levels in study group

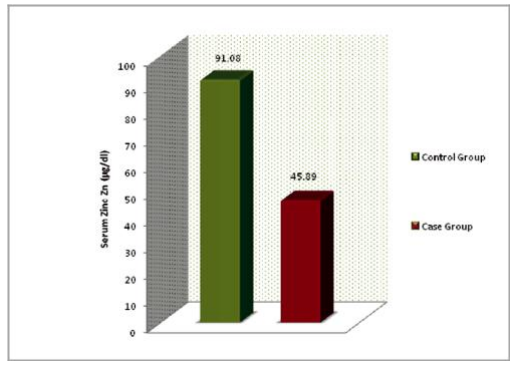

Fig.2 Mean serum zinc levels in study group

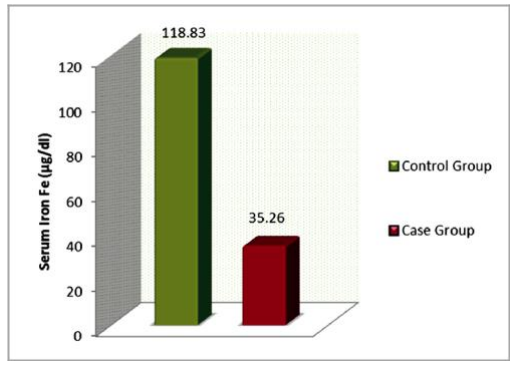

Fig.3 Comparison of mean serum iron levels in the study population.

Discussion

This present work was carried out to evaluate the serum copper, zinc and iron status in liver cirrhosis patients. In statistical analysis mean of the different characteristics for each group were compared and student's unpaired 't' test were done. The results were calculated and analyzed by using SPSS windows package, version 20 (statistical package for social science). In the present study, the results showed a significant ($p<0.001$) increase in serum Copper and significant ($p<0.001$) decreases in serum zinc and iron levels in liver cirrhosis patients when compared with that of control group.

Copper (Cu) is an essential trace element that is involved in the function of several cuproenzymes. It also shows anti-oxidant activity. As a cofactor it is involved in metabolic reactions angiogenesis, oxygen transport, and anti-oxidant protection.[12] Cu and Zn are involved in several reactions in the protection from free radical damage. They are component of several metallo enzyme with redox capacity. This redox capacity converse then an antioxidizing and anti-reactive oxygen radical action.[13] In the present study, the mean values of serum Cu levels were 104.69 ± 9.15 and 149.17 ± 5.72 in control and case group respectively. The analysis showed the significant increase level of serum Cu in liver cirrhosis patient when compared with that of control group. This finding is in agreement with the result of Gubbler et al.[5], kar et al.[14] and Lin et al.[8]. The possible role of estrogens in the pathogenesis of the hypercupremia associated cirrhosis of the liver is worthy of comment.

Gubbler et al.[5] suggested that hypercupremia may be produced in human subjects by estrogen administration and is present in pregnant women in whom there is a high level of estrogen in subject with cirrhosis of the liver, there are many manifestations of excessive estrogenic stimulation because of the diminished capacity of the liver to "inactivate" the circulating estrogens. Several studies have also shown that plasma copper concentrations are increased in various cancers.[15] Zowczak et al.[16] demonstrated significant increase in mean serum total copper levels and serum copper: zinc ratio in all cancer patients group relative to a control group. Pramoolsinsap et al.[17] suggested that serum Zn levels decreased significantly in patients with chronic active hepatitis, cirrhosis and hepatocellular carcinoma and Cu levels increased significantly only in patients with hepatocellular carcinoma. In agreement with most of the previous studies, we found that higher copper concentration in blood were associated with the degree of severity of the disease in liver cirrhosis. Hence, we observed and suggested that high copper levels in decompensated cirrhosis might lead to a more severe outcome and low copper diet might be necessary for decompensated patients to prevent the fatal outcome.

Zinc (Zn) is an essential trace element. It is second to iron as the most abundant trace element in the body and it is the only metal which appears in all energy classes, which participate in metabolism of carbohydrate, Protein, fat and nucleic acid.[18] Variation in trace element can occur in several pathological conditions. In the present study, the mean values of serum Zn levels were 45.89 ± 14.10 and 91.08± 6.16 in case and control group respectively. The analysis showed a significant lower level of serum Zn in liver cirrhosis patients when compared with that of control group. This finding is in agreement with the study of Soomro et al.[19], Triwikatmani et al.[20] and Atia et al.[21]. Prasad and Pandey[22] reviewed association between Zn and liver cirrhosis patients. The protein synthesis is reduced in the liver cirrhosis patients.

The important element in the Zn binding to protein is "metallothionein". This protein is involved in Zn metabolism, homeostasis and its release in number of oxidants. The released Zn then inhibits the activity of the enzymes involved in fibro genesis in the liver. Impaired synthesis of this protein may decrease the availability of Zn. However, Kar et al.[14] have been

suggested that Zn is transported in plasma mostly by albumin (60-70%) and by α_2 macroglobulin. Low serum Zn level in liver cirrhosis patient might be the result of decreased liver albumin content, decreased α_2 macroglobulin synthesis, poor dietary intake or protein restriction.

In the present study, the mean value of serum iron levels was 118.83 ± 11.93 and 35.26 ± 7.74 in control and case group respectively. The analysis showed significant lower of serum iron in liver cirrhosis patients when compared with that of control group. Kar et al.[14] suggested that acute gastrointestinal hemorrhage is common and potentially a complication of portal hypertension. Rupture of esophageal varices may cause hypovolemia and secondary iron deficiency anaemia. Chronic losses of blood in the GIT causes chronic hemorrhage and iron deficiency anaemia.

The liver plays a central role in blood coagulation. Acute and chronic hepatocellular diseases are usually associated with defective blood coagulation due to a variety of causes. These include: decreased hepatic synthesis of factors II, VII, IX and X; the presence of inhibitors of these factors; decreased clearance of activated coagulation factors; thrombocytopenia; impaired platelet function; hyper fibrinolysis; and disseminated intravascular coagulation. Coagulation defects complicating liver disease predispose to an increased bleeding tendency, which increase both morbidity and mortality.[9] This finding is in agreement with result of Gonzalez-casas et al. [9], Kar et al.[14] and Buyukasik et al.[23].

Conclusion

The findings of this study revealed significant changes in the studied parameters in liver cirrhosis patients. Here serum copper levels increased and serum zinc and iron levels decreased in significant.

Financial support and Sponsor: No specific support was provided for this study.

Acknowledgements

The authors are profoundly grateful to Department of Biochemistry, Mymensingh Medical College Mymensingh for approval of this study. The authors also express their gratitude to the respondents (liver cirrhosis patients admitted in hepatology unit) for giving consent for collection of data and other information that were greatly instrumental in the successful completion of the study.

References

1. *Khaleque KA & Mamun KZ. Practical Pathology and Microbiology. Dhaka. 2011.*
2. *Schuppan D & Afdhal NH. Liver Cirrhosis' Lancet. 2009; 371(9651): 838-851.*
3. *Mukerji AN, Patel V & Jain A. Improving survival in decompensated cirrhosis. Int J Hepatol. 2012; 2012: 1-14.*
4. *Sullivan JF, Blotcky AJ, Jetton MM, Hahn HK & Burch RE.Serum levels of selenium, calcium, copper magnesium, manganese & zinc in various human diseases. J Nutr. 1979; 109(8): 1432-1437.*
5. *Gubler CJ, Brown H, Markowitz H, Cartwright GE & Wintrobe MM. Studies on copper metabolism. XXIII. Portal (Laennec's) cirrhosis of the liver. M J Clin Investig. 1957; 36(8): 1208–1216.*
6. *Eagon PK, Teepe AG, Elm MS, Tadic SD, Epley MJ, Beiler BE et al. Hepatic hyperplasia and cancer in rats: alterations in copper metabolism. Carcinogenesis. 1999; 20(6): 1091-6.*
7. *Rocchi E, Borella P, Borghi A, Paolillo F, Pradelli M, Farina F et al. Zinc & magnesium in liver cirrhosis. Eur J Clin Invest. 1994; 24(3): 149–155.*
8. *Lin CC, Huang JF, Tsai LY & Huang YL. Selenium, iron, copper, & zinc levels & copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem. 2006; 109(1): 15–24.*
9. *Gonzalez-Casas R, Jones EA & Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. World J Gastroenterol. 2009; 15(37): 4653–4658.*

10. *Walker BR, Colledge NR, Ralston SH & Penman ID. Davidson's Principles and Practice of Medicine. 2014; 22nd edn, Elsevier, London.*

11. *Iron JF. In: Cook JD, editor. Methods in hematology. New York: Churchill Livingstone. 1980;15-43.*

12. *Akinloye O, Oyewale OJ & Oguntibeju OO. Evaluation of trace elements in pregnant women with pre-eclampsia. Afr J Biotech. 2010; 9: 5196-5202.*

13. *Gang X. Serum magnesium, zinc, copper and selenium concentration in myocardial infarction or receiving digoxin, Thesis (MSc), The Chinese University of Hong Kong. 1998; viewed 12 march 2016.*

14. *Kar K, Dasgupta A, Bhaskar MV & Sudhakar K. Alteration of micronutrient status in compensated and decompensated liver cirrhosis. Ind J Clin Biochem. 2014; 29(2): 232-237.*

15. *Wu T, Sempos CT, Freudenheim JL, Muti P, Smit E. Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. Ann Epidemiol. 2004; 14(3): 195-201.*

16. *Zowczak M, Iskra M, Torlinski L, Cofta S. Analysis of serum copper and zinc concentration in cancer patients. Biol Trace Elem Res. 2001; 82(1-3): 1-8.*

17. *Pramoolsinsap C, Promvanit N, Komindr S & Lerdverasiriku P. Serum trace metals in chronic viral hepatitis & hepatocellular carcinoma. Gastroenterol. 1994; 29: 610-615.*

18. *Broadly MR, White PJ, Hammond JP, Zelko I, & Lox A. Zinc in Plant. New Physiologist. 2007;173: 677.*

19. *Soomro AA, Devrajani BR, Shaikh K, Shah SZA, Devrajani T & Bibi I. Serum zinc level in patients with liver cirrhosis. Pak J Med Sci.2009; 25(6): 986-991.*

20. *Triwikatmani C, Bayupurnama P & Maduseno S. Serum zinc level & urinary zinc excretion in liver cirrhotic patient. Indonesian Journal of Gastroenterology, Hepatology, & Digestive Endoscopy. 2009; 10(1); 2-6.*

21. *Atia F, Sultana N, Ahmed S, Ferdousi S, Sultana R, Atiquzzaman M. A study of serum Zinc level in cirrhosis of liver. Bangladesh J Med Biochem.2012; 5(2): 44-47.*

22. *Prasad CK, Panday JC. Monitoring of Zinc in Liver Cirrhosis Patients. Asian Journal of Biomedical & Pharmaceutical Science. 2016; 6(53): 32-34.*

23. *Buyukasik NS, Nadir I, Akin FE, Cakal B, Kav T, Erosy O, et al. Serum iron parameters in cirrhosis and chronic hepatitis: detailed description. Turk j Gastroenterol. 2011;22(6):606-11.*