Ind J Clin Biochem (Apr-June 2014) 29(2):232–237
DOI 10.1007/s12291-013-0349-5

ORIGINAL ARTICLE

Alteration of Micronutrient Status in Compensated and Decompensated Liver Cirrhosis

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Received: 19 February 2013 / Accepted: 27 May 2013 / Published online: 14 June 2013

Abstract Decompensation followed by death is the most serious outcome in patients suffering from cirrhosis of the liver. Alteration of trace elements may play a vital role in the process of decompensation. To examine the change in status of trace elements during the decompensation process, we analysed the zinc, copper, iron, magnesium, bilirubin and albumin levels in the serum of compensated ($n = 34$) and decompensated ($n = 31$) liver cirrhosis patients and compared them with healthy control group ($n = 36$) by post hoc ANOVA. We observed significant alteration in the selected micronutrients in the diseased group relative to healthy controls ($P < 0.05$). Moreover, mean serum zinc and iron levels were significantly lower with a higher level of serum copper in decompensated cirrhosis group than in compensated group ($P < 0.05$). However, no significant decrease of serum magnesium was found between the two diseased groups. Our findings imply that the trace elements like zinc, copper and iron might exert important contributory roles in decompensation process in liver cirrhosis and hence, may be utilized as important biomarkers for these patients. Furthermore, we propose that replacements of those micronutrients at an early stage can delay or prevent the severe outcomes like hepatic encephalopathy, gastrointestinal bleeding, severe jaundice or ascites in these patients.

Keywords Liver cirrhosis · Trace elements · Decompensation

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Introduction

Many elements, although present in minute quantities, are essential nutrients in 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 function causing illness. Considering the vital role that trace elements in enzymatic reactions they have been examined critically as potential key factors in varied diseases. Although trace elements are only a part of total picture, they contribute significantly to nutrition and maintenance of health as well as prevention of several diseases [1]. The role of trace elements in the pathogenesis of liver cirrhosis and its sequelae is still not clearly understood [2]. The evaluation of functions that trace elements play in health and disease becomes more difficult when it is recognized that there are not only nutritional but also metabolic interactions among different trace elements [1]. Several authors have pointed out that levels of a few trace elements like iron and magnesium are altered in diseases like cirrhosis of liver but the alteration is not uniform and not always they are significant [2–5]. Similarly, copper and zinc are essential trace elements for several metabolic processes and overload or deficiencies of

these elements have been found to lead to metabolic disorders and some other diseases [6].

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 %. Gifted with an amply generous functional and regenerative reserve, transition from well-compensated cirrhosis to symptomatic decompensation is a clinical and biochemical continuum. 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 micronutrient deficiency [7].

Symptoms of some micronutrient deficiency simulate the features of cirrhosis. Symptoms of acute zinc deficiency like anorexia, dysfunction of smell and taste, mental and cerebellar disturbance as well as symptoms of chronic zinc deficiency like growth retardation, anaemia, testicular atrophy, and impaired wound healing are common in cirrhosis. So, micronutrient deficiency may contribute to the features of cirrhosis or to the development of decompensated cirrhosis as a continuum [8]. Hence, we hypothesized that changes in mineral and micronutrient status may significantly be associated with the pathogenesis of complications found in hepatic cirrhosis and aimed to observe their comparative role in progression of the disease. Accordingly, we designed the present study to assess the comparative role of some common micronutrient deficiencies in deterioration or decompensation of liver cirrhosis.

Materials and Methods

The study was conducted in the Department of Biochemistry, Mamata Medical College and Hospital, Khammam, Andhra Pradesh. A total of 65 patients of liver cirrhosis (compensated-M = 23, F = 11. decompensated-M = 18, F = 13) ranging from 20 to 60 years of age were selected. 36 age matched, healthy subjects (from hospital employees and students) 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 habbit and drinking water quality.

Cases were selected from Outdoor Patient Department and 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.

Hospital admitted patients were studied only after the acute phase of the disease i.e., shock, acidosis, alkalosis, and hypoglycemia were corrected. Liver cirrhosis patients associated with concomitant pathology like diabetes mellitus, hypertension, chronic diarrhoea, renal failure, and infective hepatitis or taking drugs causing alteration of trace mineral status were excluded from the study. Severely ill, unconscious, disabled and non cooperative patients were also excluded from the study.

Normal control subjects were considered as group 1 ($n = 36$). Liver cirrhosis patients with ascites, portal hypertensive gastrointestinal bleeding, and/or, encephalopathy were considered in decompensated group or group 3 ($n = 31$). Liver cirrhosis patients without the above mentioned features were considered in compensated group or group 2 ($n = 34$). 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 tubes (glass tubes free of electrolytes), allowed to clot, centrifuged, and serum removed to another metal free tube for storage at -20° until assayed.

Serum (S) iron in µg/dl was measured by Ferrozine method, spectrophotometrically [9], (S) magnesium in mg/dl was measured by Calmagite method, spectrophotometrically [10], (S) copper and (S) zinc in µg/dl were measured spectrophotometrically [11]. All the kits were supplied by Crest Biosystems. (S) bilirubin (mg/dl) was estimated by modified Jendrassik and Grof's method (Crest Biosystems) [12]. (S) albumin (g/dl) was estimated by bromocresol green dye binding method (Crest Biosystems) [13].

Statistical analysis was done by one way and post hoc analysis of variance (ANOVA) with Bonferroni correction; P value was considered significant at the confidence level of ≤ 0.05. All statistical analyses were performed using SPSS software version 16.0 for Windows.

The whole process strictly followed the guidelines and regulations set by the Helsinki Declaration of 1975, as revised in 2000. Ethical clearance was taken from the properly constituted institutional ethical committee before initiating the work.

Result Analysis

Results of one way ANOVA was shown in Table 1. All parameters were altered significantly in the disease groups in comparison to control group. For more extensive analysis between individual groups ANOVA with Post hoc analysis (Bonferroni) was performed and the result was shown in Table 2.

Analysis of the data shown in Table 2 revealed that mean (S) zinc and mean (S) iron levels were significantly

Table 1 ANOVA to show the significance of difference between mean values of the control group, compensated group and decompensated group of liver cirrhosis patients

	Control group ($n = 36$)	Compensated group ($n = 34$)	Decompensated group ($n = 31$)	[a]Level of significance between group
Serum zinc in μg/dl (mean ± SD)	80.5 ± 8.1	51.4 ± 1.3	61.8 ± 1.6	F = 281.2 $P < 0.001$
Serum magnesium (mg/dl) (mean ± SD)	2.5 ± 0.6	1.4 ± 0.5	1.4 ± 0.3	F = 57.6 $P < 0.001$
Serum copper (μg/dl) (mean ± SD)	106.4 ± 1.3	199.4 ± 14.2	165.7 ± 1.2	F = 1158.3 $P < 0.001$
Serum iron (μg/dl) (mean ± SD)	80.3 ± 1.1	58.5 ± 1.4	75.1 ± 1.1	F = 2642.3 $P < 0.001$
Serum albumin (g/dl) (mean ± SD)	3.7 ± 0.8	2.8 ± 0.3	3.2 ± 0.7	F = 11.4 $P < 0.001$
Serum bilirubin (mg/dl) (mean ± SD)	0.86 ± 0.02	8.9 ± 0.7	4.8 ± 0.8	F = 1455.6 $P < 0.001$

[a] ANOVA performed with SPSS version 16.0 for Windows to show the significance between different groups at 95 % confidence interval

decreased in the cirrhosis patients ($P < 0.05$). Furthermore, we found group 3 patients (decompensated cirrhosis) showed a significant reduction in (S) zinc and iron values in comparison to their group 2 (compensated cirrhosis) counterparts and control subjects ($P < 0.05$). Opposite trends were observed for mean (S) copper and mean (S) bilirubin levels in the decompensated group (all increased, $P < 0.05$). Although a significant decrease in (S) magnesium levels was found in cirrhosis patients overall (both group I and 2) in comparison to the control subjects ($P < 0.05$), no such difference was observed between the compensated and decompensated groups within the case population ($P > 0.05$). Same trend was observed for the mean (S) albumin level ($P > 0.05$), an important marker of the basal cellular function of the hepatocytes.

Discussion

In the present study our aim was to assess any alteration in the micronutrient homeostasis in compensated and decompensated liver cirrhosis. We observed significant decrease in mean (S) zinc and iron levels ($P < 0.05$) along with a significant increase in mean (S) copper and bilirubin levels ($P < 0.05$) in all cirrhotic patients (both group I and group 2) in comparison to the healthy subjects (group 3). Furthermore, the levels of zinc and iron were found to be significantly reduced in the decompensate group (group 3) when compared with their compensated counterparts (group 2). The two disease groups i.e., group I and 2, didn't show significant difference in mean (S) magnesium and albumin levels between them as evident from the post hoc ANOVA results in Table 2 ($P = 1.000$ between group 2 and 3). Although mean (S) bilirubin level was high in compensated liver cirrhosis patients in comparison to healthy controls, but other features of decompensations like ascites, encephalopathy and portal hypertensive gastrointestinal bleeding were absent in this group.

Although, liver plays an important role in metabolism of trace minerals [14] there are significant differences regarding the roles of different trace elements in cirrhosis of liver. Role of magnesium has been described diversely in different studies. Paik et al. [15] showed significant anti fibrotic effect of magnesium salts like magnesium lithospermate in cirrhotic rats. However, our findings suggest that (S) magnesium levels are not uniformly decreased in the cirrhotic patients depending on the degree of severity of symptoms or decompensation. These observations are endorsed by the studies of Rahelic et al. [2] that observed no significant changes in (S) magnesium levels between liver cirrhosis patients and healthy population. It has been suggested in several studies that documented reduction of mean (S) magnesium in liver cirrhosis patients rather may be the consequence of direct and indirect effects of alcohol, secondary hyperaldosteronism, use of diuretics and hypoalbuminemia [3]. Kalbfleisch et al. [16, 17] explained the low (S) magnesium in liver cirrhosis due to decreased nutritional intake of the metal, poor absorption of magnesium in distal jejunum, decreased plasma level of albumin as well as increased excretion of magnesium due to indirect effect of alcohol on renal tubules and administration of magnesuric diuretics (furosemide).

Protein metabolism impairment in cirrhotic patients would appear to affect the plasma transport of zinc rather than its overall availability in the organism [18]. Zinc is transported in plasma mostly by albumin (60–70 %) and by α_2 macroglobulin (30–40 %). Low serum zinc level in liver cirrhosis patients might be the result of decreased liver albumin content, decreased α_2 macroglobulin synthesis, poor dietary intake, or protein restriction [19]. Keeping in track with these findings serum zinc level was found to be

Table 2 Multiple comparisons between control group, compensated group and decompensated group of liver cirrhosis patients

Post hoc analysis with Bonferroni correction							
Dependent variable	(I) group	(J) group	Mean difference (I−J)	SE	Sig.	95 % confidence interval	
						Lower bound	Upper bound
Serum zinc	1	2	18.68777*	1.22036	0.000	15.7136	21.6619
		3	29.03405*	1.25137	0.000	25.9843	32.0838
	2	1	−18.68777*	1.22036	0.000	−21.6619	−15.7136
		3	10.34627*	1.26875	0.000	7.2542	13.4384
	3	1	−29.03405*	1.25137	0.000	−32.0838	−25.9843
		2	−10.34627*	1.26875	0.000	−13.4384	−7.2542
Serum magnesium	1	2	1.08268*	0.11832	0.000	0.7943	1.3710
		3	1.12505*	0.12133	0.000	0.8294	1.4207
	2	1	−1.08268*	0.11832	0.000	−1.3710	−0.7943
		3	0.04236	0.12301	1.000	-0.2574	0.3422
	3	1	−1.12505*	0.12133	0.000	−1.4207	−0.8294
		2	−0.04236	0.12301	1.000	−0.3422	0.2574
Serum copper	1	2	−59.30358*	1.92313	0.000	−63.9905	−54.6167
		3	−92.97586*	1.97200	0.000	−97.7818	−88.1699
	2	1	59.30358*	1.92313	0.000	54.6167	63.9905
		3	−33.67227*	1.99939	0.000	−38.5450	−28.7995
	3	1	92.97586*	1.97200	0.000	88.1699	97.7818
		2	33.67227*	1.99939	0.000	28.7995	38.5450
Serum iron	1	2	5.19892*	0.30327	0.000	4.4598	5.9380
		3	21.87586*	0.31098	0.000	21.1180	22.6337
	2	1	−5.19892*	0.30327	0.000	−5.9380	−4.4598
		3	16.67694*	0.31530	0.000	15.9085	17.4454
	3	1	−21.87586*	0.31098	0.000	−22.6337	−21.1180
		2	−16.67694*	0.31530	0.000	−17.4454	−15.9085
Serum albumin	1	2	0.47657*	0.16921	0.018	0.0642	0.8889
		3	0.82224*	0.17351	0.000	0.3994	1.2451
	2	1	−0.47657*	0.16921	0.018	−0.8889	−0.0642
		3	0.34567	0.17592	0.157	−0.0831	0.7744
	3	1	−0.82224*	0.17351	0.000	−1.2451	−0.3994
		2	−0.34567	0.17592	0.157	−0.7744	0.0831
Serum bilirubin	1	2	−3.93946*	0.14542	0.000	−4.2939	−3.5851
		3	−8.04119*	0.14912	0.000	−8.4046	−7.6778
	2	1	3.93946*	0.14542	0.000	3.5851	4.2939
		3	−4.10173*	0.15119	0.000	−4.4702	−3.7333
	3	1	8.04119*	0.14912	0.000	7.6778	8.4046
		2	4.10173*	0.15119	0.000	3.7333	4.4702

Data analysed by SPSS 16.0 version for Windows

* The mean difference is significant at the 0.05 level

significantly reduced in the diseased group in our study. Furthermore, a significant decrease was observed in decompensated state in comparison to the compensated cirrhosis patients. These observations strongly suggest that zinc plays a crucial role in progression of pathogenesis of hepatic cirrhosis. Low zinc concentrations have been reported in patients with cirrhosis of the liver, particularly those with hepatic encephalopathy (HE) [14, 20]. Zinc deficiency was found to alter neurotransmitters like gamma aminobutyric acid and norepinephrine and in animal experiments zinc supplementation was reported to lead to a reduction in blood ammonia [20]. Possible role of zinc deficiency in the pathogenesis of HE has been also suggested by inducing alterations in urea metabolism. In carbon tetrachloride (CCl_4) induced cirrhotic rats oral zinc supplementation reduces ammonia levels and increases

ornithine carbamoyl transferase activity in their liver [21]. In addition epidemiological studies suggest that zinc deficiency might be associated with increased cancer risks in cirrhotic patients, and zinc levels are possible prognostic factors for determining the chance of a patient reaching remission. So zinc supplementation in patients having liver cancer cannot be overemphasized [19].

Numerous studies have shown that (S) copper: zinc ratio is a sensitive indicator for the diagnosis of various cancers [22, 23]. In our study elevated copper and reduced zinc levels in decompensated cirrhosis patients are found in comparison to control and compensated groups (Table 1). This finding might serve as a more significant prognostic index than other parameters examined in this study. Not only we found serum copper to be significantly raised in cirrhotic patients but furthermore, we observed this increase to be significantly higher in the decompensated group in comparison to the compensated patients (Table 1, 2). In a previous study [24] reported that the fraction of copper loosely bound to protein (non ceruloplasmin fraction) was increased in cirrhosis. They suggested that hypercupremia might be produced by decreased capacity of liver to inactivate the circulating oestrogen leading to excessive circulating oestrogen resulting in hypercupremia [24]. Billiary copper excretion capacity has been also reported to correlate with hepatic mass that may also explain our findings in part [25]. Copper is highly toxic in excess and results in cellular damage and hepatocellular carcinoma [26]. Copper functions as a cofactor in various redox enzymes. Copper ions can bind to proteins and nucleic acids and cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by the presence of copper ions [27]. Several studies have also shown that plasma copper concentrations are increased in various cancers [28]. Zowczak et al. [22] demonstrated significant increase in mean serum total copper levels and (S) copper: zinc ratio in all cancer patients groups relative to a control group. In agreement with most of the previous studies, we found that higher copper concentrations in blood were associated with the degree of severity of the disease in hepatic cirrhosis. Hence, we suggest that copper might play an important contributory role in progression of these patients towards the decompensated state. Our present findings in conjunction with the oxidative and malignant potentials initiated by copper suggest 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 of malignancy.

Regarding the iron homoeostasis in these patients, our result indicated that (S) iron levels were significantly lower in decompensated cirrhosis patients than that of controls and compensated groups. Aberrent (S) iron values in hepatic cirrhosis are characteristically documented by different studies [4, 5]. However similar findings like ours are also observed by Buyukasik et al. [4], who suggested that (S) iron and total iron binding capacity levels were lowered than controls in all child pugh groups of cirrhosis. Along with these findings our results indicate that iron deficiency anaemia might be common in this group. Ching Chiang Lin has suggested that iron deficiency anaemia may be common at the onset of liver cancer [19]. 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 chronic iron deficiency anaemia. Acute or 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 [29]. Studies of 20 cirrhotic patients with anaemia not caused by blood loss indicated that an extracorpuscular hemolytic process was present which was proportional in severity to the degree of anaemia [30]. Other than these common causes a reduced iron level is also attributable to a decreased haem oxygenase expression in kidney of cirrhotic rats [31]. Considering all these facts the results of our present study indicate a decrease in overall total body iron status in the cirrhosis of liver in our study population that aggravates with progression of the disease.

Concerning the changes in albumin level in these patients our observations indicate that although the serum albumin was significantly decreased in the cirrhotic patients overall, there was a lack of statistically significant difference in mean (S) albumin concentrations between the two diseased groups (Group 2 and 3). Iwata Ozaki noticed that although, the main regulatory factor for (S) albumin concentration is albumin mRNA expression in the liver, in severe cases there is a discrepancy between albumin mRNA level and severity of the diseases [32]. In agreement with these studies we suggest that albumin itself may not be a good marker for assessing the progression of severity of the disease (decompensation).

Finally we conclude that in our study population there was a definite and significant alteration of (S) zinc, (S) copper and (S) iron concentrations with the progression of decompensation in liver cirrhosis, whereas, alteration in (S) magnesium and (S) albumin were not reflected to a significant extent. Hence, we propose that estimation of these micronutrients can predict the decompensation in liver cirrhosis and so recommend their analysis at the time of diagnosis of liver cirrhosis. If the micronutrient levels are altered, replacements may delay or prevent the decompensation as well as the most severe outcome of liver cirrhosis like hepatocellular carcinoma or liver transplantation.

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