The Prevalence and Implication of Zinc Deficiency in Patients With Chronic Liver Disease

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Abstract

Background: Patients with liver cirrhosis often exhibit zinc deficiency. Although zinc is involved in many bioactivities, many aspects of clinical implications of zinc deficiency in liver cirrhosis remain unclear. We aimed to reveal the prevalence and implications of zinc deficiency in liver cirrhosis by assessing associations with parameters such as clinical symptoms and laboratory data.

Methods: In 235 cirrhosis patients enrolled at multiple medical institutions in 2009, we assessed how blood zinc levels were associated with their clinical symptoms, patients characteristics, and liver function test results.

Results: Blood zinc levels were most strongly correlated with blood albumin levels among the study parameters (r = 0.587, P < 0.0001). When blood albumin levels were ≤ 3.5 g/dL, blood zinc levels were < 70 μg/dL in 88% of patients. Additionally, significant correlations were observed with age (r = -0.253, P = 0.0014), aspartate aminotransferase levels (r = -0.254, P = 0.0020), total bilirubin levels (r = -0.222, P = 0.0053), prothrombin time (r = -0.255, P = 0.0029), branched-chain amino acid to tyrosine ratio (r = 0.357, P < 0.0001), Child-Pugh score (r = 0.469, P < 0.0001), ammonia levels (r = -0.246, P = 0.0028), and total cholesterol levels (r = 0.314, P < 0.0001). Blood zinc levels were significantly lower in patients with edema/ascites (P < 0.0001), those with hepatic encephalopathy (P = 0.0215), those receiving oral branched-chain amino acid diuretics (P = 0.0045), and those receiving oral branched-chain amino acids (P < 0.0001) than in those without these conditions.

Conclusions: Zinc deficiency is prevalent in cirrhosis patients, whereas nitrogen metabolic disorders, particularly hypoalbuminemia, can be an indicator of zinc deficiency. Thus, cirrhosis patients exhibiting a nitrogen metabolic disorder should be examined for the presence of zinc deficiency.

Keywords: Liver cirrhosis; Nitrogen metabolism; Branched-chain amino acid; Ammonia; Albumin

Introduction

While the liver is the primary organ for metabolism of nutri-
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In the synthesis, storage, and secretion of insulin, zinc deficiency resulted in numerous problems, including growth retardation, apoptosis, and metabolism [12, 20-24]. There were some reports that zinc deficiency occurs as a result of nutritional factors, but also in various disease states (malabsorption, Crohn's disease, alcoholism, liver cirrhosis, chronic renal disease, and other chronically debilitating diseases) [13]. Factors that are potentially responsible for zinc deficiency in liver cirrhosis include disturbed zinc absorption by the digestive tract and increased zinc excretion in the urine [12, 13]. The effects of cytokines, mainly interleukin-6 and endotoxins also contributed zinc deficiency [12]. Furthermore, diuretics aggravate zinc deficiencies in patients with liver cirrhosis by increasing zinc excretion in the urine [29].

Several studies showed a statistically significant inverse relationship between the serum levels of zinc and ammonia, and that zinc deficiency is related to the pathogenesis of hepatic encephalopathy [30-32]. On the basis of these findings, there were some reports which examined the effects of zinc supplementation in liver cirrhosis with hyperammonemia [30, 33-36]. Marchesini et al. showed that 3-month supplementation of zinc in patients with hepatic encephalopathy reduced serum ammonia levels, but this was not a randomized-controlled trial [14]. Two randomized-controlled trials of rather short period (8 and 10 days) have been performed to examine these effects, and the results were controversial [33, 37]. Recently, we performed a longer period randomized, placebo-controlled double-blind trial, and indicated that zinc supplementation for 3 months seems effective and safe for treating hyperammonemia in liver cirrhosis [38].

The main effect of zinc supplementation on reducing serum levels of ammonia proposed thus far is increased activity of ornithine transcarbamylase, a key enzyme of the urea cycle in the liver [13]. Riggio O et al. showed that liver ornithine transcarbamylase activity decreased and plasma ammonia level increased in zinc-deficient rats, while the activity of the enzyme significantly increased in zinc-supplemented cirrhotic rats [34].

Because zinc has been revealed to play an important role in the synthesis, storage, and secretion of insulin, zinc deficiency affects not only insulin secretion but also insulin resistance [39-41]. In diabetes mellitus, zinc deficiency has been found to be associated with glucose tolerance and the development of complications of diabetes mellitus, whereas there are some reports that zinc supplementation improves glucose tolerance and reduces the incidence of complications [42, 43]. Although glucose intolerance is also prevalent in liver cirrhosis, the association between glucose intolerance and zinc in liver cirrhosis has been little studied.

These previous studies have increasingly shown that zinc deficiency is associated with nitrogen metabolic disorders, mainly affecting ammonia in liver cirrhosis, and that zinc supplementation is effective for patients with these conditions. However, not all patients with liver cirrhosis exhibit zinc deficiency. In addition to nitrogen metabolic disorders, other metabolic disorders, such as glucose intolerance, often coexist with liver cirrhosis. A large-scale study on the prevalence of zinc deficiency in liver cirrhosis and the associations between zinc deficiency and various pathological conditions of liver cirrhosis may provide answers to the questions of which cirrhosis patients should be suspected of having zinc deficiency and for which cirrhosis patients should zinc supplementation be considered. We previously conducted a prospective study enrolling 299 cirrhosis patients to assess the effects of BCAA therapy. Using data obtained in this previous study, we investigated the prevalence of zinc deficiency in liver cirrhosis and the association between zinc deficiency and clinical symptoms in the present study.

Patients and Methods

Patients

In 2009, 299 cirrhosis patients without hepatocellular carcinoma were enrolled from 14 medical institutes in Japan. The diagnosis of liver cirrhosis was based on an aspartate aminotransferase (AST)-to-platelet ratio index > 1.5; morphologic changes of the liver such as hypertrophy of the left lateral and caudate lobes or atrophy of the right posterior hepatic lobe on ultrasonography, computed tomography, and/or magnetic resonance imaging; or a pseudo-lobule formation finding on histopathologic examination.

Of these 299 patients, 235 who had not received any oral zinc preparations were included in the present study. In addition to age and sex, the following clinical data were collected: cause of liver diseases; the presence or absence of current or prior history of hepatic encephalopathy, ascites, and edema; the presence or absence of the use of diuretics and BCAAs; and the presence or absence of treatment for diabetes mellitus (Table 1).

Methods

Correlations of blood zinc concentrations with patients’ characteristics and liver function test results

After overnight fasting, blood samples were obtained from
the antecubital vein in the morning. The association between serum zinc and patients’ characteristics or liver function tests was analysed by the statistical method described in the following section.

Statistical analysis

The correlation between blood zinc levels and continuous variables was analysed using linear regression. An absolute $r$ value of 0.2 to less than 0.4 was considered to indicate a weak correlation, that of 0.4 to less than 0.7 a correlation, and that of less than 0.2 no correlation. Regarding the correlation between blood zinc levels and categorical variables, intergroup significant differences in blood zinc levels were tested using the Mann-Whitney test for two-level categorical variables and by the Kruskal-Wallis test for more than three-level categorical variables. A $P$ value of less than 0.05 was considered to indicate a significant difference, in other words, an association with blood zinc levels.

Ethics

This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the prior approval given by the institutional review board of each institution.

Results

Association of blood zinc concentrations with patient characteristics and laboratory data

Blood zinc concentrations were correlated with blood albumin levels and Child-Pugh scores, whereas they were weakly correlated with age, AST, prothrombin time, BCAA-to-tyrosine ratio (BTR), blood ammonia levels, and total cholesterol levels (Table 2) (Fig. 1). Among the categorical variables, blood zinc levels showed significant intergroup differences in cause of liver disease, the presence or absence of edema/ascites and hepatic encephalopathy, and the use of diuretics and BCAAs. These variables were found to be associated with blood zinc levels (Table 3).

Blood albumin levels and the probability of zinc deficiency

Because the strongest correlation was observed between blood albumin levels and Child-Pugh scores, whereas they were weakly correlated with age, AST, prothrombin time, BCAA-to-tyrosine ratio (BTR), blood ammonia levels, and total cholesterol levels (Table 2) (Fig. 1). Among the categorical variables, blood zinc levels showed significant intergroup differences in cause of liver disease, the presence or absence of edema/ascites and hepatic encephalopathy, and the use of diuretics and BCAAs. These variables were found to be associated with blood zinc levels (Table 3).

Discussion

In the present study, many cirrhosis patients exhibited hypoalbuminemia, whereas blood zinc levels were associated with indicators of nitrogen metabolism, mainly blood albumin levels, as well as several blood test parameters, clinical symptoms, and the presence or absence of the use of oral medication. Particularly, blood albumin levels were strongly associated with blood zinc levels. Thus, hypoalbuminemia detected in cirrhosis patients can be a useful indicator of zinc deficiency.

It cannot be concluded from the present study alone

Table 1. Patients’ Characteristics

| N       | 235  |
|---------|------|
| Age (y/o) | 67.4 ± 11.1 |
| Gender (male/female) | 109/126 |
| Etiology (HCV/HBV/alcohol/NASH/others) | 148/27/25/15/20 |
| BMI (kg/m²) | 23.8 ± 4.1 |
| Daily alcohol intake (none/ < 60 g/ ≥ 60 g) | 179/25/23 |
| Platelets count (× 10³/mm³) | 102.9 ± 64.4 |
| AST (IU/L) | 52.0 ± 32.1 |
| ALT (IU/L) | 41.8 ± 29.4 |
| Albumin (g/dL) | 3.67 ± 0.53 |
| Total bilirubin (mg/dL) | 1.10 ± 0.53 |
| Prothrombin time (international normalized ratio) | 1.13 ± 0.18 |
| Creatinine (mg/dL) | 0.90 ± 1.30 |
| BUN (mg/dL) | 16.4 ± 10.8 |
| BCAA-to-tyrosine ratio (BTR) | 3.97 ± 1.77 |
| Child-Pugh score | 6.03 ± 1.15 |
| Ammonia (µg/dL) | 48.7 ± 33.5 |
| Total cholesterol (mg/dL) | 157.9 ± 33.5 |
| Triglycerides (mg/dL) | 93.4 ± 49.6 |
| Fasting blood glucose (mg/dL) | 114.8 ± 31.7 |
| HbA1c (%) | 5.50 ± 1.03 |
| HOMA-IR | 5.71 ± 8.86 |
| Zinc (µg/dL) | 64.1 ± 15.8 |
| Ferritin (ng/mL) | 168.2 ± 522.0 |
| AFP (ng/mL) | 18.1 ± 37.4 |
| Edema, ascites (+/-) | 46/187 |
| Administration of diuretics (+/-) | 78/157 |
| Administration of BCAA (+/-) | 78/157 |
| Administration of oral DM agents (+/-) | 56/179 |
| Administration of insulin (+/-) | 22/213 |

Values are expressed as mean ± SD. y/o: years old; HCV: hepatitis C virus; HBV: hepatitis B virus; NASH: non-alcoholic steatohepatitis; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; BCAA: branched-chain amino acid; HOMA-IR: homeostasis model assessment-insulin resistance; AFP: alpha-fetoprotein; DM: diabetes mellitus.
whether these variables correlated with blood zinc levels are a cause or outcome of zinc deficiency. This issue is discussed along with previously reported studies in the following section (Fig. 3).

Blood albumin levels, blood ammonia levels, BTR, and prothrombin time, which were correlated with blood zinc levels, are all blood test parameters reflecting protein metabolism. As described above in the Introduction, zinc is involved in the function of the hepatic urea cycle, and zinc deficiency causes hyperammonemia through impaired ammonia metabolic capacity due to hypofunction of the urea cycle [6, 14]. When ammonia that is not metabolised in the urea cycle is metabolised by glutamine synthetase present in skeletal muscles and other organs, BCAAs are consumed and become deficient, which decreases the BTR. BCAA deficiency, which reduces the protein synthetic capacity, ultimately leads to decreased blood albumin levels [6, 44]. Moreover, the present study revealed that blood zinc levels were significantly lower in patients with a current or prior history of hepatic encephalopathy. These findings, on the whole, support that zinc deficiency may be involved in the development of hepatic encephalopathy because zinc deficiency impairs ammonia metabolism, subsequently causing hyperammonemia. Among the parameters reflecting nitrogen metabolism, blood albumin levels were most strongly correlated with blood zinc concentrations. As described above, while zinc deficiency may contribute to decreased blood albumin levels, hypoalbuminemia leads to decreased blood zinc concentrations because zinc binds mainly to albumin and is transported in blood [13]. In other words, zinc deficiency is a cause as well as an outcome of hypoalbuminemia. Thus, because of the strong association between blood zinc and blood albumin levels, a strong correlation can be assumed to exist between them. Because several studies have revealed that such nitrogen metabolic disorders and hepatic encephalopathy are

Table 2. Relationship Between Serum Zinc and Patients’ Characteristics (Continuous Variables)

|                      | r    | P value |
|----------------------|------|---------|
| Age (y/o)            | 0.253| 0.0014  |
| BMI (kg/m²)          | 0.158| 0.067   |
| Platelets count (× 10³/mm³) | 0.189| 0.0177  |
| AST (IU/L)           | 0.245| 0.002   |
| ALT (IU/L)           | 0.077| 0.3384  |
| Albumin (g/dL)       | 0.587| < 0.0001|
| Total bilirubin (mg/dL) | 0.222| 0.0053  |
| Prothrombin time (international normalized ratio) | 0.255| 0.0029  |
| Creatinine (mg/dL)   | 0.149| 0.0688  |
| BUN (mg/dL)          | 0.128| 0.1231  |
| BCAA-to-tyrosine ratio (BTR) | 0.357| < 0.0001|
| Child-Pugh score     | 0.469| < 0.0001|
| Ammonia (µg/dL)      | 0.246| 0.0028  |
| Total cholesterol (mg/dL) | 0.314| < 0.0001|
| Triglycerides (mg/dL)| 0.079| 0.3493  |
| Fasting blood glucose (mg/dL) | 0.076| 0.3527  |
| HbA1c (%)            | 0.115| 0.1623  |
| HOMA-IR              | 0.072| 0.4165  |
| Ferritin (ng/mL)     | 0.069| 0.4146  |
| AFP (ng/mL)          | 0.17 | 0.0344  |

y/o: years old; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; BCAA: branched-chain amino acid; HOMA-IR: homeostasis model assessment-insulin resistance; AFP: alpha-fetoprotein; DM: diabetes mellitus.

Figure 1. Correlations between blood zinc concentrations (vertical axis) and blood test results (absolute r ≥ 2.0). (a) Albumin. (b) Aspartate aminotransferase (AST). (c) Ammonia. (d) Prothrombin time. (e) Total bilirubin. (f) Total cholesterol. (g) BCAA-to-tyrosine ratio (BTR).
relieved by zinc supplementation [13, 14, 38, 44], zinc deficiency appears to be the cause of such nitrogen metabolic disorders observed in liver cirrhosis.

Total bilirubin levels also showed a weak negative correlation with blood zinc concentrations. Regarding the association between bilirubin metabolism and zinc, there is a report indicating a negative correlation as observed in the present study [45]; however, the detailed mechanism remains unknown. Because zinc has been reported to suppress the enterohepatic circulation [46], it is possible that impairment of this function due to zinc deficiency contributes to increased blood bilirubin levels. However, given the small sample size, this issue needs to be investigated in the future.

AST levels also showed a weak negative correlation with blood zinc concentrations. Because zinc has been reported to have antioxidant and anti-inflammatory activities [13], zinc deficiency may be involved in aggravation of hepatocellular injury. However, the correlation coefficient was low, although there is also a report indicating that zinc levels were not correlated with either alanine aminotransferase or AST levels [45]. Thus, the association between hepatocellular injury and zinc also needs to be investigated in the future.

Blood cholesterol levels also showed a weak positive correlation with blood zinc levels. Regarding cholesterol metabolism, because zinc is involved in insulin secretion and insulin resistance, zinc deficiency has been reported to be a possible cause of increased cholesterol levels [47]. This finding, which differs from the results of the present study, indicates that zinc may play different roles in dyslipidaemia in people with and without liver cirrhosis.

In patients receiving diuretics or BCAAs, blood zinc concentrations were significantly lower. Whether the use of these drugs is a cause or outcome of zinc deficiency is discussed as follows. In Japan, because the use of BCAAs is recommended for patients with liver cirrhosis accompanied by hypoalbuminemia, BCAAs are administered to patients exhibiting hypoalbuminemia. Meanwhile, the use of diuretics is recommended for patients with liver cirrhosis accompanied by ascites or edema. However, because one of the causes of ascites and edema is generally hypoalbuminemia, diuretics are also highly likely to be administered in consequence of the hypoalbuminemia. However, because other studies have shown that the use of diuretics may increase urinary excretion of zinc [29, 48], the drugs may enhance zinc deficiency.

Regarding alcohol drinking, because previous studies have indicated that patients with alcoholic liver cirrhosis exhibit zinc deficiency, it has been suggested that alcohol drinking may cause zinc deficiency [13]. However, the present study did not show any correlation between alcohol consumption and blood zinc concentrations. In alcoholic liver cirrhosis, nitrogen metabolic disorders, such as hypoalbuminemia, are observed, for example, because of disease progression or malnutrition due to alcohol drinking. Thus, patients with alcoholic liver cirrhosis may have exhibited zinc deficiency. Further studies are needed to determine the causal relationship between alcohol drinking and zinc deficiency. Although blood zinc levels showed significant intergroup differences in cause of liver disease (Table 3), blood albumin levels also showed significant intergroup differences in cause of liver disease (HCV, 3.6 ± 0.5 g/dL; HBV, 4.0 ± 0.6; alcohol, 3.6 ± 0.7; non-alcoholic steatohepatitis (NASH), 3.8 ± 0.7; P = 0.0203 by Mann-Whitney). Therefore, it is likely that the intergroup difference in blood zinc levels was associated with the stage (function) of liver disease.

### Table 3. Relationship Between Serum Zinc and Patients’ Characteristics (Categorized Variables)

| Serum level of zinc (µg/dL) | Gender (male/female) | Etiology (C/B/Al/N/O) | Daily alcohol intake (none/< 60g/≥ 60 g) | Edema or ascites (+/-) | Hepatic encephalopathy (+/-) | Administration of diuretics (+/-) | Administration of BCAA (+/-) | Administration of oral DM agents (+/-) | Administration of insulin (+/-) | P value |
|-----------------------------|----------------------|----------------------|----------------------------------------|-----------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------|--------|
| 65.7 ± 16.3/62.6 ± 15.3     | 0.2423               | 62.2 ± 14.9/72.9 ± 14.3/66.1 ± 20.5/60.8 ± 13.7/60.9 ± 14.8 | 62.3 ± 15.0/73.0 ± 19.3/63.4±16.1 | 56.0 ± 19.6/65.9 ± 14.3 | 51.3 ± 18.3/64.8 ± 15.4 | 58.6 ± 18.9/65.6 ± 14.6 | 55.0 ± 13.1/70.5 ± 14.4 | 65.0 ± 16.4/63.8 ± 15.7 | 61.9 ± 19.3/64.3 ± 15.5 | 0.04   |

Values are expressed as mean ± SD. C: hepatitis C virus; B: hepatitis B virus; Al: alcohol; N: non-alcoholic steatohepatitis; O: others; BCAA: branched-chain amino acid; DM: diabetes mellitus.

Blood cholesterol levels also showed a weak positive correlation with blood zinc levels. Regarding cholesterol metabolism, because zinc is involved in insulin secretion and insulin resistance, zinc deficiency has been reported to be a possible cause of increased cholesterol levels [47]. This finding, which differs from the results of the present study, indicates that zinc may play different roles in dyslipidaemia in people with and without liver cirrhosis.

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![Figure 2. Blood albumin concentrations and the probability of zinc deficiency (blood zinc concentrations < 70 µg/dL).](image-url)
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In addition, no significant correlations were observed with glucose and lipid metabolism-related parameters, such as the presence or absence of abnormal glucose tolerance, the presence or absence of treatment of diabetes mellitus, and body mass index. Although several reports suggest that zinc is involved in insulin secretion and glucose tolerance [39-43], the present study does not prove any significant association between zinc and abnormal glucose tolerance due to liver cirrhosis.

The present study showed that blood zinc concentrations were correlated with many test parameters and clinical symptoms characteristic of liver cirrhosis. Particularly, zinc deficiency is involved in the pathological conditions of nitrogen metabolic disorders and hepatic encephalopathy. This supports the importance of zinc supplementation. As for the other parameters associated with blood zinc levels, the mechanisms of their associations need to be elucidated in the future. Moreover, because patients with hepatocellular carcinoma were excluded from the cirrhosis patients included in the present study, we did not assess the association between zinc and hepatocarcinogenesis. There is a report of a study suggesting the presence of this association [13], which is an important future issue to be addressed.

In conclusion, zinc deficiency is prevalent in liver cirrhosis, while many metabolic disorders, particularly hypoalbuminemia and other nitrogen metabolic disorders, are good indicators of zinc deficiency. Thus, the presence or absence of zinc deficiency should be evaluated in cirrhosis patients exhibiting nitrogen metabolic disorders.

Conflict of Interest

The authors declare that they have no conflict of interest.

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