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***Short Communication***

# Zinc deficiency in patients with chronic liver disease in Japan

Itaru Ozeki, Tomohiro Arakawa, Hirokazu Suii, Ryoji Tatsumi, Masakatsu Yamaguchi, Tomoaki Nakajima, Yasuaki Kuwata and Joji Toyota

*Department of Hepatology, Sapporo Kosei General Hospital, Sapporo, Japan*

*Aim:* This study aimed to determine the distributions of serum zinc levels and the prevalence of zinc deficiency in patients with chronic liver disease (CLD) in actual clinical practice, and to analyze the association between serum zinc levels and clinical characteristics.

*Methods:* This study analyzed 1973 patients with CLD, including 749 with liver cirrhosis, who were admitted to Sapporo Kosei General Hospital in 2017.

*Results:* Zinc deficiency, defined as a serum zinc level of <60 μg/dL, was observed in 555 patients overall (28.1%), including 182 (14.9%) patients without liver cirrhosis and 373 (49.8%) with liver cirrhosis. When marginal zinc deficiency was included, zinc deficiency (serum zinc level <80 μg/dL) was observed in 1594 (80.8%) patients overall, including 924 (75.5%) patients without liver cirrhosis and 670 (89.5%) with liver cirrhosis. Serum zinc levels were most strongly correlated with serum albumin levels. Of the 257 CLD patients with an albumin level of <3.5 g/dL, 234 (91.1%) had a serum zinc level of <60 μg/dL.

*Conclusions:* Zinc deficiency is common in patients with CLD. Serum zinc levels should be regularly measured, particularly in patients with liver cirrhosis.

**Key words:** chronic liver diseases, liver cirrhosis, zinc

## INTRODUCTION

ZINC ACTS AS an active center of or coenzyme for >300 types of enzymes to mediate DNA synthesis, RNA transcription, cell growth and division, and other processes including synthesis, regeneration, and protein maintenance in the body. Currently, zinc is considered an essential trace element for maintaining life.[1–4]

In 2002, the World Health Organization stated that zinc deficiency is one of the most important risk factors for morbidity and mortality in developing countries.[5] A subsequent survey showed that zinc deficiency affects 17.3% of the global population, and that Japan has the highest prevalence (15–25%) among developed countries.[6] Diseases that cause zinc deficiency in adults include chronic liver disease (CLD), inflammatory bowel disease, short bowel syndrome, renal diseases including conditions requiring dialysis, and diabetes mellitus.[7] In CLDs, a decreased capacity to synthesize albumin, the malabsorption of zinc from the intestine, and increased zinc excretion in the urine cause zinc deficiency.[8–10] Although zinc deficiency and CLDs are closely associated as described above, no previous large-scale studies have investigated the reality of zinc deficiency. The present study aimed to determine the distributions of serum zinc levels and the prevalence of zinc deficiency in patients with CLD in actual clinical practice, and to analyze the association between serum zinc levels and clinical characteristics.

## METHODS

### Patients

WE MEASURED SERUM zinc levels and analyzed the association between patient characteristics and blood test values in 1973 patients with CLD who were admitted to Sapporo Kousei General Hospital between January and December 2017. Although patients with CLD were defined as those with an identified cause of liver dysfunction who could be regularly followed up for at least 6 months, patients in whom the cause was not identified or was only suspected were classified as those with unknown causes. We concluded there was diagnosable alcoholic liver disease in patients whose daily ethanol consumption was >60g/day. Non-alcoholic fatty liver disease

*Correspondence: Dr Itaru Ozeki, Department of Hepatology, Sapporo Kosei General Hospital, Kita 3 Higashi 8, Chuo-ku, Sapporo 060–0033,* Japan.
*Email:* i.ozeki@ja-hokkaidoukouseiren.or.jp
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was diagnosed according to the Practice Guideline by the American Association for the Study of Liver Diseases.[11] Liver cirrhosis was diagnosed based on liver histology, transient elastography (liver stiffness of ≥14.5 kPa measured with Fibroscan),[12] or the presence of gastroesophageal varices. Patients treated with oral polaprezinc and zinc acetate hydrate, and those with acute hepatitis were excluded. The use of the following concomitant drugs was examined: enteral branched-chain amino acid (BCAA)-enriched nutrient mixtures, BCAA granules, non-absorbable disaccharides, non-absorbable antibiotics, and diuretics. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the ethics committees of our institutions.

### Measurement of the plasma concentration of zinc and definitions of zinc deficiency

The plasma concentration of zinc was assessed using an atomic absorption spectrophotometer (Hitachi High-Tech Science, Tokyo, Japan). According to the 2016 Practice Guidelines for Zinc Deficiency issued by the Japanese Society of Clinical Nutrition,[13] zinc deficiency was defined as a zinc level of <60 μg/dL; marginal zinc deficiency was defined as a zinc level of ≥60 and <80 μg/dL.

### Statistical analysis

Descriptive statistics are reported as a proportion (%) for categorical variables, and as the mean ± SD for continuous variables. Frequency comparisons between groups were analyzed using the $\chi^2$-test. The Mann–Whitney *U*-test was used to analyze continuous data. Spearman's rank correlation coefficient analysis was used to evaluate relationships between serum zinc levels and blood test values and patients' clinical characteristics. A *P*-value of <0.05 was considered statistically significant. Statistical analyses were carried out with R (http://www.r-project.org/).

## RESULTS

### Patients' clinical characteristics

THE MEAN PATIENT age was 66 years, and there were 1017 men (51.5%). The present study included 477 patients with a treatment history of hepatocellular carcinoma (HCC; 24.2%), 270 with a treatment history of gastroesophageal varices (13.7%), 57 with a treatment history of hepatic encephalopathy (2.9%), and 121 with ascites (6.1%). Compared with patients without cirrhosis, the patients with cirrhosis were older, predominately male, and had a higher body mass index. The patients with cirrhosis were more likely to have diabetes; a treatment history of HCC, gastroesophageal varices, or hepatic encephalopathy; and ascites. A total of 682 (34.6%) patients with chronic hepatitis C, 681 (34.5%) with chronic hepatitis B, 194 (9.8%) with alcoholic liver disease, 193 (9.8%) with non-alcoholic fatty liver disease, 51 (2.6%) with autoimmune hepatitis, 43 (2.2%) with primary biliary cholangitis, and 100 (5.1%) with unknown causes had underlying etiology. The prevalence of chronic hepatitis B was significantly higher in patients without cirrhosis than in those with cirrhosis. By contrast, compared with patients without cirrhosis, patients with cirrhosis were significantly more likely to have alcoholic liver disease, non-alcoholic fatty liver disease, others, and unknown etiology. All concomitant drugs were more frequently used in patients with cirrhosis. Blood test results showed significant differences between patients with and without cirrhosis for all parameters, except for glycated hemoglobin and serum iron (Table 1).

### Distributions of serum zinc levels

Overall, the serum zinc levels were <40 μg/dL in 87 patients (4.4%), ≥40 to <60 μg/dL in 468 patients (23.7%), ≥60 to <80 μg/dL in 1039 patients (52.7%), and ≥80 μg/dL in 379 patients (19.2%). In total, 1224 patients without cirrhosis were divided into the same categories, which included three (0.2%), 179 (14.6%), 742 (60.6%), and 300 (24.5%) patients, respectively. Furthermore, 749 patients with cirrhosis were divided into the same categories, which included 84 (11.2%), 289 (38.6%), 297 (39.7%), and 79 (10.5%) patients, respectively. Zinc deficiency was observed in 555 patients overall (28.1%), including 182 (14.9%) patients without cirrhosis and 373 (49.8%) with cirrhosis. When marginal zinc deficiency was included, zinc deficiency (serum zinc level <80 μg/dL) was observed in 1594 (80.8%) patients overall, including 924 (75.5%) patients without cirrhosis and 670 (89.5%) with cirrhosis (Fig. 1). The mean serum zinc levels were 66.9 μg/dL (SD 11.5 μg/dL) overall, 71.6 μg/dL (SD 9.4 μg/dL) in patients without cirrhosis, and 59.3 μg/dL (SD 12.7 μg/dL) in patients with cirrhosis.

### Correlation of serum zinc levels with blood test values and patients' clinical characteristics

Serum zinc levels were moderately correlated with serum albumin, cholinesterase, Mac-2 binding protein glycosylation isomer (M2BPGi), FIB-4 index, and hemoglobin levels; they were weakly correlated with total cholesterol, prothrombin, ammonia, platelet, serum alkaline phosphatase (ALP), serum alanine aminotransferase levels; BCAA/tyrosine ratio; and age. When only the patients with cirrhosis were considered, the serum zinc levels were strongly correlated with albumin and cholinesterase levels,

**Table 1** Patients' characteristics

| | Overall ($n = 1973$) | Non-cirrhosis ($n = 1224$) | Cirrhosis ($n = 749$) | *P*-value |
|---|---|---|---|---|
| Age (years) | 66 (9) | 64 (9) | 70 (10) | <0.001 |
| Sex (male) | 1017 (51.5) | 565 (46.2) | 452 (60.3) | <0.001 |
| Height (cm) | 160.0 (7.8) | 160.3 (7.7) | 159.6 (8.0) | 0.416 |
| Body weight (kg) | 61.1 (10.2) | 60.8 (10.3) | 61.5 (10.1) | 0.191 |
| BMI ($kg/m^2$) | 23.8 (3.1) | 23.6 (3.1) | 24.0 (3.2) | 0.021 |
| Diabetes mellitus | 245 (12.4) | 100 (8.2) | 145 (19.4) | <0.001 |
| Treatment history of HCC | 477 (24.2) | 155 (12.7) | 322 (43.0) | <0.001 |
| Treatment history of gastroesophageal varices | 270 (13.7) | 11 (0.9) | 259 (34.6) | <0.001 |
| Treatment history of hepatic encephalopathy | 57 (2.9) | 2 (0.2) | 55 (7.3) | <0.001 |
| Present of ascites | 121 (6.1) | 0 (0) | 121 (16.2) | <0.001 |
| Etiology | | | | |
| HCV | 682 (34.6) | 435 (35.5) | 247 (33.0) | 0.246 |
| HBV | 681 (34.5) | 517 (42.2) | 164 (21.9) | <0.001 |
| Alcoholic liver disease | 194 (9.8) | 52 (4.2) | 142 (19.0) | <0.001 |
| Non-alcoholic fatty liver disease | 193 (9.8) | 107 (8.7) | 86 (11.5) | 0.047 |
| AIH | 51 (2.6) | 36 (2.9) | 15 (2.0) | 0.202 |
| PBC | 43 (2.2) | 27 (2.2) | 16 (2.1) | 0.918 |
| Others† | 29 (1.5) | 9 (0.7) | 20 (2.7) | 0.001 |
| Unknown | 100 (5.1) | 41 (3.3) | 59 (7.9) | <0.001 |
| Dosing | | | | |
| Diuretics | 269 (13.6) | 24 (2.0) | 245 (32.7) | <0.001 |
| Non-absorbable disaccharides | 131 (6.6) | 5 (0.4) | 126 (16.8) | <0.001 |
| Non-absorbable antibiotics | 92 (4.7) | 1 (0.1) | 91 (12.1) | <0.001 |
| BCAA-enriched nutrient | 147 (7.5) | 5 (0.4) | 142 (19.0) | <0.001 |
| BCAA granules | 222 (11.2) | 24 (2.0) | 198 (26.4) | <0.001 |
| WBC ($\times 10^3/\mu L$) | 4.7 (1.2) | 5.0 (1.2) | 4.3 (1.2) | <0.001 |
| Hemoglobin (g/dL) | 12.8 (1.4) | 13.2 (1.1) | 12.1 (1.7) | <0.001 |
| Platelets count ($\times 10^4/\mu L$) | 13.7 (4.7) | 16.2 (4.3) | 9.6 (3.2) | <0.001 |
| Prothrombin (%) | 86 (14) | 94 (11) | 73 (13) | <0.001 |
| Albumin (g/dL) | 4.1 (0.4) | 4.3 (0.3) | 3.7 (0.5) | <0.001 |
| Total bilirubin (mg/dL) | 0.9 (0.4) | 0.7 (0.2) | 1.2 (0.7) | <0.001 |
| AST (U/L) | 35 (16) | 31 (13) | 42 (18) | <0.001 |
| ALT (U/L) | 29 (17) | 29 (17) | 30 (15) | <0.001 |
| γ-GT (U/L) | 59 (52) | 46 (38) | 80 (70) | <0.001 |
| ALP (U/L) | 304 (108) | 261 (77) | 370 (137) | <0.001 |
| ChE (U/L) | 266 (76) | 305 (61) | 206 (71) | <0.001 |
| eGFR ($mL/min/1.73\ m^2$) | 69.5 (15.2) | 71.4 (13.3) | 66.5 (17.7) | <0.001 |
| Total cholesterol (mg/dL) | 180 (30) | 189 (28) | 163 (28) | <0.001 |
| Ammonia (μg/dL) | 46 (21) | 35 (11) | 61 (28) | <0.001 |
| BTR | 5.8 (1.5) | 6.3 (1.2) | 4.9 (1.8) | <0.001 |
| HbA1c (%) | 5.9 (0.6) | 5.9 (0.5) | 6.0 (0.7) | 0.121 |
| FIB-4 | 4.2 (2.4) | 2.7 (1.2) | 6.8 (3.1) | <0.001 |
| M2BPGi (COI) | 2.6 (2.1) | 1.2 (0.6) | 4.5 (3.2) | <0.001 |
| Serum zinc levels (μg/dL) | 67 (12) | 72 (9) | 59 (13) | <0.001 |
| Serum copper levels (μg/dL) | 109 (19) | 117 (19) | 91 (12) | <0.001 |
| Serum iron levels (μg/dL) | 106 (36) | 106 (32) | 105 (41) | 0.090 |
| Ferritin (ng/mL) | 136 (112) | 136 (105) | 136 (120) | 0.024 |

Categorical variables are expressed as number (%), and the continuous variables as the mean ± SD. Frequency comparisons between groups were analyzed using the $\chi^2$-test or Fisher's exact test. Mean differences were evaluated by Mann–Whitney *U*-test.

†Eight patients with hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection, six patients with autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) overlap syndrome, five patients with idiopathic portal hypertension, three patients with hepatic echinococcosis, two patients with primary sclerosing cholangitis, two patients with Wilson's disease, two patients with extrahepatic portal venous obstruction, and one patient with hemochromatosis.

γ-GT, γ-glutamyltransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BCAA, branched-chain amino acids; BTR, branched-chain amino acid and tyrosine ratio; BMI, body mass index; ChE, cholinesterase; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; WBC, white blood cell.

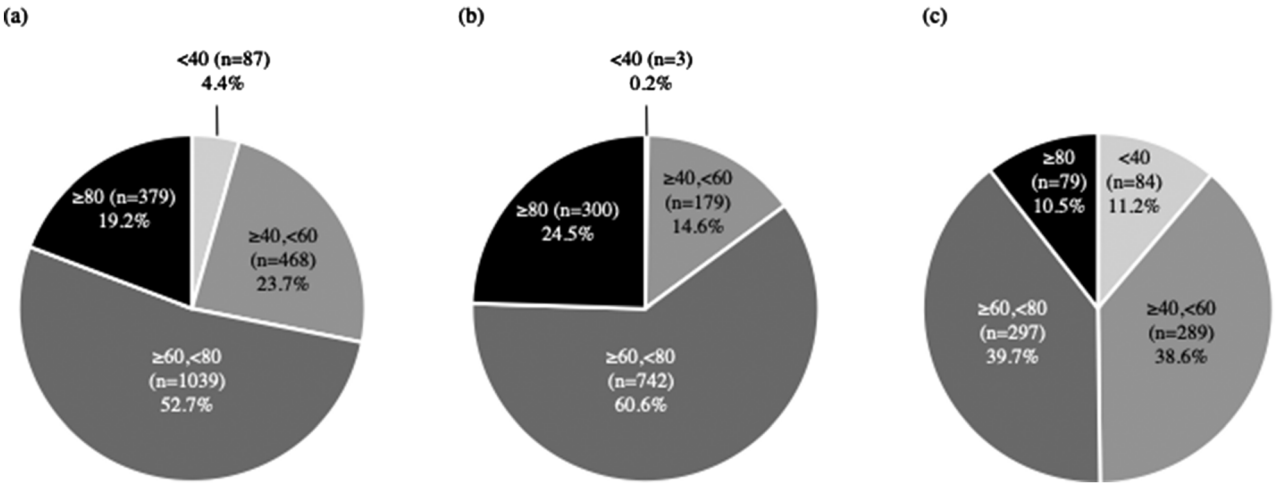


**Figure 1** Distributions of serum zinc levels.

and were moderately correlated with M2BPGi, FIB-4 index, and hemoglobin, total cholesterol, prothrombin, and ammonia levels (Table 2).

## Proportions of patients with serum zinc level <60 μg/dL according to serum albumin levels

When patients with a serum zinc level of <60 μg/dL were divided according to serum albumin levels, 102 patients had an albumin level of <3.0 g/dL (94.4%, $n = 108$), 132 had an albumin level of ≥3.0 to <3.5 g/dL (88.6%, $n = 149$), 165 had an albumin level of ≥3.5 to <4.0 g/dL (47.0%, $n = 351$), and 155 had an albumin level of ≥4.0 g/dL (11.4%, $n = 1365$). Of the 257 patients with an albumin level of <3.5 g/dL, 234 (91.1%) had a serum zinc level of <60 μg/dL (Fig. 2).

## DISCUSSION

ALTHOUGH CLDS HAVE been regarded as diseases associated with a high risk of zinc deficiency, this relationship has not been elucidated.[7] In the present study, zinc deficiency was detected in approximately 15% of patients without cirrhosis and half of patients with cirrhosis. Serum zinc levels should be regularly measured, particularly in patients with cirrhosis. Even in patients without cirrhosis, the similar measurement might be necessary when serum albumin levels are <3.5 g/dL.

Among blood test parameters, serum zinc levels were moderately correlated with albumin, cholinesterase, M2BPGi, FIB-4 index, and hemoglobin levels. When only patients with cirrhosis were considered, these parameters showed even higher correlation coefficients. In particular, serum albumin levels are strongly correlated with serum

**Table 2** Correlation of serum zinc levels with blood test values and patients' clinical characteristics

| | Overall | | Non-cirrhosis | | Cirrhosis | |
|---|---|---|---|---|---|---|
| | *r* | *P*-value | *r* | *P*-value | *r* | *P*-value |
| Albumin | 0.641 | <0.001 | 0.432 | <0.001 | 0.767 | <0.001 |
| ChE | 0.587 | <0.001 | 0.354 | <0.001 | 0.712 | <0.001 |
| M2BPGi | −0.490 | <0.001 | −0.158 | <0.001 | −0.647 | <0.001 |
| FIB-4 | −0.430 | <0.001 | −0.153 | <0.001 | −0.436 | <0.001 |
| Hemoglobin | 0.414 | <0.001 | 0.244 | <0.001 | 0.465 | <0.001 |
| Total cholesterol | 0.399 | <0.001 | 0.273 | <0.001 | 0.420 | <0.001 |
| Prothrombin | 0.389 | <0.001 | 0.073 | 0.019 | 0.451 | <0.001 |
| Ammonia | −0.386 | <0.001 | −0.099 | 0.006 | −0.406 | <0.001 |
| BTR | 0.376 | <0.001 | 0.205 | <0.001 | 0.377 | <0.001 |
| Platelet | 0.331 | <0.001 | 0.055 | 0.055 | 0.252 | <0.001 |
| ALP | −0.328 | <0.001 | −0.132 | <0.001 | −0.392 | <0.001 |
| AST | −0.242 | <0.001 | −0.044 | 0.127 | −0.278 | <0.001 |
| Age | −0.200 | <0.001 | −0.144 | <0.001 | −0.097 | 0.008 |
| WBC | 0.164 | <0.001 | 0.004 | 0.878 | 0.154 | <0.001 |
| eGFR | 0.154 | <0.001 | 0.097 | 0.004 | 0.113 | 0.006 |
| Fe | 0.134 | <0.001 | 0.167 | <0.001 | 0.065 | 0.115 |
| Ferritin | 0.114 | <0.001 | 0.144 | <0.001 | 0.041 | 0.342 |
| Total bilirubin | −0.105 | <0.001 | 0.186 | <0.001 | −0.279 | <0.001 |
| BMI | 0.087 | 0.001 | 0.132 | <0.001 | 0.121 | 0.002 |
| γ-GT | −0.068 | 0.003 | 0.042 | 0.147 | 0.030 | 0.408 |
| HbA1c | 0.020 | 0.510 | 0.005 | 0.899 | 0.091 | 0.080 |
| ALT | −0.015 | 0.498 | 0.092 | 0.001 | −0.035 | 0.340 |
| Cu | −0.010 | 0.712 | −0.012 | 0.695 | 0.015 | 0.725 |

Spearman's rank correlation coefficient analysis was used to evaluate relationships between serum zinc levels and blood test values and patients' clinical characteristics. γ-GT, γ-glutamyltransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acid and tyrosine ratio; ChE, cholinesterase; Cu, copper; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; M2BPGi, Mac-2 binding protein glycosylation isomer; WBC, white blood cell.

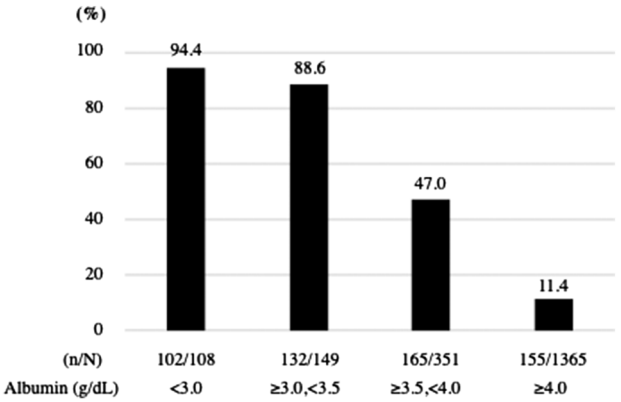


**Figure 2** Prevalence of a serum zinc level of <60 μg/dL according to serum albumin levels.

zinc levels. A similar study of 235 cirrhosis patients carried out in Japan also showed exactly the same result. The study proposed that hypoalbuminemia detected in cirrhotic patients could be a useful indicator of zinc deficiency.[14] Zinc existing in the blood is mainly bound to albumin. As serum albumin levels decrease, more zinc binds to other amino acids, such as histidine and cystine. Because such non-albumin-bound zinc is excreted in the urine, serum zinc levels are considered correlated with serum albumin levels.[8,9] In other words, zinc deficiency caused by liver diseases is a series of the following conditions: progression of hepatic fibrosis (e.g. liver cirrhosis) exacerbate hepatic reserve, which decreases the capacity to synthesize albumin and consequently causes hypoalbuminemia. Because of hypoalbuminemia, zinc is excreted from the body. Thus, hepatic synthesis markers (e.g. albumin, cholinesterase, total cholesterol, and prothrombin) and hepatic fibrosis markers (e.g., M2BPGi and FIB-4 index) are moderately correlated with serum zinc levels, particularly in patients with cirrhosis. However, it is possible that the BCAA/tyrosine ratio and ammonia levels, which showed low correlation coefficients, might have been modified, because many patients had already been treated with BCAA-enriched nutrient mixtures, BCAA granules, non-absorbable disaccharides, non-absorbable antibiotics, and so on. Despite the weak correlation between ALP and serum zinc levels, ALP, which is a zinc-requiring enzyme, is considered useful for diagnosing zinc deficiency and determining the degree of adherence to treatment with zinc preparations, because ALP is rendered less active by zinc deficiency.[15,16] However, when only patients with CLD are considered, ALP levels might be increased by primary diseases; therefore, ALP appears less useful as a diagnostic marker.

The limitations of the present study were that the serum zinc levels show circadian variations, are high in the early morning, and decrease toward the afternoon.[17,18] Therefore, blood sample collection should preferably be carried out in the early morning when patients have fasted. However, because of the difficulty in collecting samples at the same time from all patients in a clinical setting, this study did not define a fixed sampling time.

Historically, there were no zinc preparations approved for the treatment of hypozincemia in Japan. However, in March 2017, zinc acetate hydrate was approved for the additional indication of hypozincemia.[19] Recently, it was reported that hypozincemia was associated with developing HCC in hepatitis C virus-related cirrhosis.[20] Because the long-term administration of zinc to patients with CLD with zinc deficiency was reported to prevent worsening of liver function and progression of hepatic fibrosis, and to reduce the risk of developing HCC,[21,22] zinc supplementation with zinc acetate hydrate might not only relieve various symptoms associated with zinc deficiency, but can also improve the quality of life and prognosis in patients with CLD. We hope that the present study will be helpful for better understanding zinc deficiency associated with CLDs.

## ACKNOWLEDGMENTS

NONE.

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