The association between serum zinc levels and subjective symptoms in zinc deficiency patients with chronic liver disease

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This study aimed to analyze the association between serum zinc levels and major subjective symptoms in zinc deficiency patients with chronic liver disease. 578 patients with chronic liver disease were enrolled. The patients, whose serum zinc level of <80 μg/dl, completed a questionnaire to determine whether they had subjective symptoms of the five conditions (taste disorder, aphthous stomatitis, dermatitis, alopecia, and anorexia). Then, the association between these subjective symptoms and serum zinc levels was analyzed. In total, 193 patients (33.4%) experienced any subjective symptoms. The prevalence of each symptom was as follows: 36 patients with taste disorder (6.2%), 46 with aphthous stomatitis (8.0%), 77 with dermatitis (13.3%), 46 with alopecia (8.0%), and 53 with anorexia (9.2%). In total, 70.8%, 34.1%, and 26.1% patients with serum zinc levels of <40, ≥40 to <60, and ≥60 to <80 μg/dl, respectively, had these symptoms. When zinc deficiency was defined as a serum zinc level of <80 μg/dl, approximately one-third of patients displayed symptoms presumably originating from zinc deficiency. As serum zinc levels decreased, the prevalence of these symptoms increased. Dermatitis, especially, was relevant to zinc.

Key Words: chronic liver disease, zinc deficiency, dermatitis, taste disorder, anorexia

Zinc was first reported as an essential element for rat growth in 1933, and in 1963, zinc deficiency was first reported in humans. Subsequent studies have revealed that 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. Furthermore, the SLC39A/ZIP and SLC30A/ZnT families, which are zinc transporter gene families that control the transport of zinc within the cytoplasm and from the outside to the cytoplasm in the gastrointestinal tract, and cytoplasmic metallothionein play an important role in maintaining zinc homeostasis.

In 2002, the World Health Organization (WHO) stated that zinc deficiency is one of the most important risk factors for morbidity and mortality in developing countries. A subsequent survey revealed that zinc deficiency affects 17.3% of the global population and that Japan has the highest prevalence (15–25%) among developed countries. Zinc deficiency is known to cause various symptoms including taste disorders, aphthous stomatitis, dermatitis, alopecia, anorexia, chronic diarrhea, pancytopenia, immune dysfunction, neurosensory disturbance, cognitive dysfunction, growth retardation, and gonadal dysgenesis. 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. In CLD, a decreased capacity to synthesize albumin and the malabsorption of zinc from the intestine cause zinc deficiency. Although zinc deficiency and CLD are closely associated as described above, no previous large-scale studies have investigated the reality of zinc deficiency and its associated symptoms in patients with CLD. With an emphasis placed on five conditions (taste disorder, aphthous stomatitis, dermatitis, alopecia, and anorexia) as symptoms that occur in association with zinc deficiency, the association between the prevalence of these conditions and serum zinc levels were examined.

Materials and Methods

Patients and study design. We measured serum zinc levels and analyzed the association between patient characteristics and blood test values in 578 patients with CLD who admitted to Sapporo Kousei General Hospital between January and December 2017. The plasma concentration of zinc was assessed using an atomic absorption spectrophotometer (Hitachi High-Tech Science Co., Ltd. Tokyo, Japan). Zinc deficiency was defined as a serum zinc level of <80 μg/dl. 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 (ALD) in patients whose daily ethanol consumption was ≥60 g/day. According to the Practice Guideline by the American Association for the Study of Liver Diseases, non-alcoholic fatty liver disease was diagnosed. Liver cirrhosis was diagnosed based on liver histology, transient elastography (liver stiffness of ≥14.5 kPa measured with Fibroscan®), or the presence of gastroesophageal varices. Patients treated with oral polaprezinc® (Zeria Pharmaceutical Co. Ltd., Tokyo, Japan) and nobelzin® (Nobelpharma Co. Ltd., Tokyo, Japan) and those with acute hepatitis were excluded. Furthermore, patients with a serum zinc level of <80 μg/dl completed a questionnaire in an examination room to determine whether they had subjective symptoms of the five conditions (taste disorder, aphthous stomatitis, dermatitis, alopecia, and anorexia). Then, the association between these subjective symptoms and serum zinc levels was analyzed. The study protocol conformed to the 1975 Declaration of Helsinki and was approved by the ethics committees of our institutions.

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Results

Clinical characteristics. The mean age of the study was 68 years, and male gender were 309 patients (53.5%). Cirrhosis and treatment history of hepatocellular carcinoma (HCC) were identified in 326 (56.4%) and 196 (33.9%) patients, respectively. The most common etiology was hepatitis C virus (HCV) infection identified in 326 (56.4%) and 196 (33.9%) patients, respectively (Table 1).

Distributions of serum zinc levels. Overall, the serum zinc levels were <40 μg/dl in 48 patients (8.3%), ≥40 to <60 μg/dl in 258 patients (44.6%), and ≥60 to <80 μg/dl in 272 patients (47.1%) (Fig. 1A). In total, 252 patients without cirrhosis were divided into the same categories, which included 0 (0.0%), 77 (30.6%), and 175 patients (69.4%), respectively (Fig. 1B). Furthermore, 326 patients with cirrhosis were divided into the same categories, which included 48 (14.7%), 181 (55.5%), and 97 patients (29.8%) (Fig. 1C). The mean serum zinc levels were 57 μg/dl in overall, 63 μg/dl in patients without cirrhosis, and 52 μg/dl in patients with cirrhosis.

Comparison of serum zinc levels according to clinical characteristics. Table 2 shows a comparison of the mean serum zinc levels according to patient clinical characteristics. Serum zinc levels were lower in patients aged ≥70 years, and those with cirrhosis and a treatment history of HCC.

Questionnaire survey on subjective symptoms. Questionnaire forms were collected from 578 patients with a serum zinc level of <80 μg/dl. The forms were collected from 48 patients with a serum zinc level of <40 μg/dl (55.2%, n = 87), 258 with a level of ≥40 to <60 μg/dl (55.1%, n = 468), and 272 with a level of ≥60 to <80 μg/dl (26.2%, n = 1,039). In total, 193 patients (33.4%) experienced any of the following symptoms: taste disorder, anorexia, portal hypertension, and hepatic echinococcosis. In total, 70.8%, 34.1%, and 26.1% patients with serum zinc levels of <40, ≥40 to <60, and ≥60 to <80 μg/dl, respectively, had these symptoms (Fig. 2). As serum zinc levels decreased, the prevalence of these symptoms increased. The proportions of patients with ≥2 of the symptoms were 9.3%, 14.6%, 10.5%, and 7.4% according to the receiver operating characteristics curve. A p value of <0.05 was considered statistically significant. Statistical analyses were performed with R (http://www.r-project.org/).

Table 1. Patients characteristics

| Characteristics          | Number | age (years) | gender, male | Body weight (kg) | BMI (kg/m²) | Liver cirrhosis | Treatment history of HCC |
|--------------------------|--------|-------------|--------------|------------------|-------------|------------------|--------------------------|
|                          |        | 68 (9)      | 309 (53.5)   | 60.5 (9.9)       | 23.6 (3.0)  | 326 (56.4)      | 196 (33.9)               |

Categorical variables expressed as number (%) and the continuous variables as mean ± SD. Six patients with HBV and HCV coinfection, 3 patients with AIH and PBC overlap syndrome, 3 patients with idiopathic portal hypertension, 1 patient with hepatic echinococcosis, 1 patient with primary sclerosing cholangitis, 1 patient with Wilson disease, 1 patient with extrahepatic portal venous obstruction, and 1 patient with hemochromatosis, γ-GT, γ-glutamyltransferase; AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid and tyrosine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; FIB-4, fibrous-4; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; PBC, primary biliary cholangitis.

Fig. 1. Distributions of serum zinc levels. (A) Overall (n = 578). (B) Patients without liver cirrhosis (n = 252). (C) Patients with liver cirrhosis (n = 326).
Factors contributing to emergency of subjective symptoms. Univariate analysis showed that age ≥79 (OR 1.756), body mass index (BMI) ≥25.2 kg/m² (OR 1.935), hemoglobin <12.9 g/dl (OR 2.292), platelets count <8.0 x 10⁴/μl (OR 2.954), prothrombin <76% (OR 2.601), albumin <4.2 g/dl (OR 4.056), total bilirubin ≥1.3 mg/dl (OR 2.264), alkaline phosphatase (ALP) ≥362 U/L (OR 2.468), branched-chain amino acid and tyrosine ratio (BTR) ≥4.50 (OR 2.406), Mac-2 binding protein glycosylation isomer (M2BPGi) ≥2.3 COI (OR 1.906), serum copper <123 mg/dl (OR 0.571), and serum zinc <61 mg/dl (OR 1.782) were associated with emergency of dermatitis. Multivariate analysis showed that age ≥79 (OR 2.001, \( p = 0.039 \)), platelets count <8.0 x 10⁴/μl (OR 1.071, \( p = 0.029 \)), and serum zinc <61 mg/dl (OR 2.158, \( p = 0.049 \)) were independently associated with emergency of dermatitis (Table 3). Univariate analysis showed that age ≥67 (OR 3.054),

### Table 2. Comparison of serum zinc levels according to patient clinical characteristics

| Frequency comparisons between groups were analyzed using chi-square test. Mean differences were evaluated by the Mann-Whitney U test. BMI, body mass index; HCC, hepatocellular carcinoma. |
| Gender | N | Mean (SD) | \( p \) value |
|--------|---|-----------|----------------|
| Male   | 309 | 56 (9)   | 0.132          |
| Female | 269 | 58 (9)   | 0.012          |
| Age (years) | | | |
| <70 | 494 | 59 (9) | 0.012 |
| ≥70 | 84 | 56 (8) | 0.012 |
| BMI (kg/m²) | | | |
| <25.0 | 401 | 57 (9) | 0.754 |
| ≥25.0 | 177 | 57 (10) | 0.001 |
| Background liver disease | | | |
| Non-cirrhosis | 252 | 63 (6) | 0.001 |
| Cirrhosis | 326 | 52 (9) | 0.001 |
| Treatment history of HCC | | | |
| No | 382 | 59 (9) | 0.001 |
| Yes | 196 | 54 (10) | 0.001 |

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Fig. 2. Association between serum zinc levels and the prevalence of any symptoms. (A) Overall (\( n = 578 \)). (B) Serum zinc levels <40 μg/dl (\( n = 48 \)). (C) Serum zinc levels ≥40 μg/dl to <60 μg/dl (\( n = 258 \)). (D) Serum zinc levels ≥60 μg/dl to <80 μg/dl (\( n = 272 \)). *<40 μg/dl vs ≥40 μg/dl to <60 μg/dl, and ≥40 μg/dl to <60 μg/dl vs ≥60 μg/dl to <80 μg/dl, all \( p < 0.001 \). \( \star \) patients with any symptoms, \( \star \) patients without symptoms.

Fig. 3. The prevalence of each symptom. Overall (\( n = 578 \)); <40 μg/dl (\( n = 48 \)); ≥40, <60 μg/dl (\( n = 258 \)); ≥60, <80 μg/dl (\( n = 272 \)). *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \).
Multivariate analysis showed that female was independently associated with emergency of anorexia (Table 5). Multivariate analysis showed that age (OR 4.200), albumin <4.0 g/dl (OR 2.928), total bilirubin (OR 2.092), cirrhosis (OR 2.032), and serum zinc <53 μg/dl (OR 2.262) were associated with emergency of anorexia (Table 5). Multivariate analysis showed that female was independently associated with emergency of dermatitis and alopecia (Table 6 and 7).

Discussion

Although CLD have been regarded as diseases associated with a high risk of zinc deficiency, this relationship has not been elucidated. In the present study, which included 578 patients with CLD who admitted to our hospital, we comprehensively measured serum zinc levels and examined whether serum zinc levels were associated with patient clinical characteristics, blood test values, and subjective symptoms. Age was shown to be associated with serum zinc levels, with lower levels reported in older patients. The reasons for this include the onset and exacerbation of hypalbuminemia due to decreases in the capacity of the gastrointestinal tract to absorb albumin, the oral intake of protein, and the capacity to synthesize albumin with increasing age. Another reason is the decreased bioavailability of zinc due to the enhanced expression of metallothionein. Next, serum zinc levels were significantly lower in patients with cirrhosis. This is consistent with previous reports and has been attributed to the decreased capacity of the liver to synthesize albumin and the impaired intestinal absorption of albumin. Additionally, serum zinc levels were lower in patients with concomitant HCC. Such patients might often concomitantly have cirrhosis. No consistent views have been reached on whether zinc deficiency increase the risk of developing HCC or not. A recent paper revealed hypozincemia has been associated with developing HCC in HCV-related cirrhosis. This study concluded that HCV induces hypozincemia due to a reduction in copper-zinc superoxide dismutase and antioxidative activity, which results in the development HCC. Meanwhile, studies are being vigorously conducted to analyze the association between zinc transporters and HCC, and their results are awaited.

Although various symptoms are reported to appear in zinc deficiency, no large-scale studies have evaluated the incidence rates of actual subjective symptoms. Based on the questionnaire responses collected from 578 patients in the present study, 193 (33.4%) had any of the following symptoms: taste disorder, aphthous stomatitis, dermatitis, alopecia, and anorexia. When these five symptoms were separately analyzed, dermatitis showed the highest prevalence at 13.3%. In the present study, a multivariate analysis showed that older age, lower platelets count, and low serum zinc levels were independently associated with emergency of dermatitis. As skin ages, increased transdermal

| Table 3. Factors contributing to emergency of dermatitis |
|----------------------------------|-----------------|--------|-----------------|-----------------|
| Age (years; ≥79≤79)             | 1.756           | 1.011–3.049 | 0.044           | 2.001           | 1.034–3.873 | 0.039 |
| Gender (male:female)            | 1.348           | 0.827–2.196 | 0.229           |                 |           |       |
| BMI (kg/m²; ≥25.2≤25.2)         | 1.935           | 1.162–3.222 | 0.010           |                 |           |       |
| Liver cirrhosis (yes:no)        | 1.618           | 0.977–2.678 | 0.060           |                 |           |       |
| Treatment history of HCC (yes:no)| 1.216           | 0.739–1.998 | 0.441           |                 |           |       |
| ALD (yes:no)                    | 1.388           | 0.737–2.613 | 0.308           |                 |           |       |
| Diabetes (yes:no)               | 1.220           | 0.689–2.161 | 0.494           |                 |           |       |
| Hemoglobin (g/dl; <12.9≤12.9)    | 2.292           | 1.284–4.093 | 0.001           |                 |           |       |
| Platelets count (>10.5≤10.5)     | 2.945           | 1.806–4.802 | <0.001          |                 |           |       |
| Prothrombin (%; <76≤76)         | 2.601           | 1.561–4.336 | <0.001          |                 |           |       |
| Albumin (g/dl; <4.0≤4.0)         | 4.056           | 1.821–9.034 | <0.001          |                 |           |       |
| Total bilirubin (mg/dl; ≥13≤13)  | 2.264           | 1.324–3.873 | 0.002           |                 |           |       |
| AST (U/L; ≥35≤35)               | 1.558           | 0.962–2.522 | 0.070           |                 |           |       |
| ALT (U/L; ≥34≤34)               | 0.646           | 0.350–1.192 | 0.159           |                 |           |       |
| ALP (U/L; ≥36≤36)               | 2.468           | 1.518–4.012 | <0.001          |                 |           |       |
| eGFR (ml/min/1.73 m²; <47.6≤47.6) | 1.782          | 0.975–3.255 | 0.058           |                 |           |       |
| BTR (≥4.50≤4.50)                | 2.406           | 1.453–3.985 | 0.001           |                 |           |       |
| M2BPGi (COI; ≥2.3≤2.3)          | 1.906           | 1.112–3.267 | 0.018           |                 |           |       |
| Serum copper (μg/dl; ≥123≤123)  | 0.571           | 0.337–0.966 | 0.035           |                 |           |       |
| Serum iron (μg/dl; ≥132≤132)    | 0.647           | 0.344–1.218 | 0.175           |                 |           |       |
| Serum zinc (μg/dl; ≥61≤61)      | 1.782           | 1.485–4.725 | 0.001           | 2.158           | 1.001–4.721 | 0.049 |

ALD, alcoholic liver disease; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acids and tyrosine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; OR, odds ratio.
Table 4. Factors contributing to emergency of taste disorder

|                        | Univariate analysis |                      | Multivariate analysis |                      |
|------------------------|---------------------|----------------------|-----------------------|----------------------|
|                        | OR                  | 95% CI               | p value               | OR                  | 95% CI               | p value               |
| Age (years; ≥67:<67)   | 3.054               | 1.315–7.094          | 0.007                 | 3.063               | 1.139–8.239          | 0.027                 |
| Gender (male:female)   | 0.680               | 0.345–1.340          | 0.263                 |                      |                      |                      |
| BMI (kg/m²; ≥25.7:<25.7) | 1.166               | 0.542–2.507          | 0.694                 |                      |                      |                      |
| Liver cirrhosis (yes:no) | 2.438               | 1.125–5.282          | 0.020                 |                      |                      |                      |
| Treatment history of HCC (yes:no) | 1.609               | 0.814–3.180          | 0.168                 |                      |                      |                      |
| ALD (yes:no)           | 1.779               | 0.781–4.050          | 0.165                 |                      |                      |                      |
| Diabetes (yes:no)      | 0.612               | 0.233–1.611          | 0.316                 |                      |                      |                      |
| Hemoglobin (g/dl; <12.7:<12.7) | 5.802               | 2.023–16.636         | <0.001                |                      |                      |                      |
| Platelets count (>10¹⁵/μl; <10.0:<10.0) | 1.566               | 0.785–3.124          | 0.200                 |                      |                      |                      |
| Prothrombin (%) (<80:<80) | 2.491               | 1.177–5.271          | 0.014                 |                      |                      |                      |
| Albumin (g/dl; <3.0:<3.0) | 4.702               | 2.222–9.950          | 0.001                 |                      |                      |                      |
| Total bilirubin (mg/dl; ≥1.4:<1.4) | 4.429               | 2.186–8.972          | <0.001                | 3.548               | 1.500–8.393          | 0.004                 |
| AST (U/L; ≥35:<35)     | 2.832               | 2.222–9.950          | 0.003                 | 2.862               | 1.194–6.859          | 0.018                 |
| ALT (U/L; ≥22:<22)     | 1.619               | 0.813–2.320          | 0.169                 |                      |                      |                      |
| ALP (U/L; ≥348:<348)   | 3.377               | 1.672–6.819          | 0.001                 |                      |                      |                      |
| eGFR (ml/min/1.73 m²; ≥50.6:<50.6) | 2.840               | 1.403–5.750          | 0.003                 | 3.136               | 1.357–7.249          | 0.007                 |
| BTR (≥3.2:<3.20)       | 2.307               | 1.107–4.809          | 0.022                 |                      |                      |                      |
| M2BPGi (COI; ≥3.6:<3.6) | 4.844               | 2.180–10.760         | <0.001                |                      |                      |                      |
| Serum copper (μg/dl; ≥124:<124) | 0.616               | 0.294–1.292          | 0.196                 |                      |                      |                      |
| Serum iron (μg/dl; ≥136:<136) | 1.429               | 0.669–3.054          | 0.355                 |                      |                      |                      |
| Serum zinc (μg/dl; ≥50:<50) | 3.659               | 1.844–7.261          | <0.001                |                      |                      |                      |

ALD, alcoholic liver disease; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acids and tyrosine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; OR, odds ratio.

Table 5. Factors contributing to emergency of anorexia

|                        | Univariate analysis |                      | Multivariate analysis |                      |
|------------------------|---------------------|----------------------|-----------------------|----------------------|
|                        | OR                  | 95% CI               | p value               | OR                  | 95% CI               | p value               |
| Age (years; ≥79:<79)   | 1.449               | 0.746–2.814          | 0.271                 |                      |                      |                      |
| Gender (male:female)   | 0.760               | 0.432–1.339          | 0.346                 |                      |                      |                      |
| BMI (kg/m²; ≥26.9:<26.9) | 0.375               | 0.131–1.071          | 0.057                 |                      |                      |                      |
| Liver cirrhosis (yes:no) | 2.092               | 1.123–3.896          | 0.018                 |                      |                      |                      |
| Treatment history of HCC (yes:no) | 0.680               | 0.360–1.286          | 0.233                 |                      |                      |                      |
| ALD (yes:no)           | 2.108               | 1.074–4.138          | 0.027                 |                      |                      |                      |
| Diabetes (yes:no)      | 0.669               | 0.306–1.461          | 0.310                 |                      |                      |                      |
| Hemoglobin (g/dl; <11.9:<11.9) | 1.761               | 0.919–3.375          | 0.085                 |                      |                      |                      |
| Platelets count (>10¹⁵/μl; <10.5:<10.5) | 2.148               | 1.154–4.001          | 0.014                 |                      |                      |                      |
| Prothrombin (%) (<80:<80) | 4.200               | 2.058–8.570          | <0.001                |                      |                      |                      |
| Albumin (g/dl; <4.0:<4.0) | 2.928               | 1.530–5.601          | 0.001                 |                      |                      |                      |
| Total bilirubin (mg/dl; ≥1.3:<1.3) | 3.327               | 1.831–6.044          | <0.001                |                      |                      |                      |
| AST (U/L; ≥40:<40)     | 2.576               | 1.455–4.560          | 0.001                 |                      |                      |                      |
| ALT (U/L; ≥52:<52)     | 0.473               | 0.143–1.564          | 0.210                 |                      |                      |                      |
| ALP (U/L; ≥400:<400)   | 2.447               | 1.367–4.382          | 0.002                 |                      |                      |                      |
| eGFR (ml/min/1.73 m²; ≥41.3:<41.3) | 3.529               | 1.681–7.412          | <0.001                | 2.542               | 1.007–6.416          | 0.048                 |
| BTR (≥3.2:<3.2)        | 2.262               | 1.193–4.289          | 0.011                 |                      |                      |                      |
| M2BPGi (COI; ≥4.4:<4.4) | 4.685               | 2.467–8.897          | <0.001                | 3.466               | 1.652–7.272          | 0.001                 |
| Serum copper (μg/dl; ≥127:<127) | 0.631               | 0.329–1.210          | 0.163                 |                      |                      |                      |
| Serum iron (μg/dl; ≥136:<136) | 2.032               | 1.067–3.871          | 0.028                 |                      |                      |                      |
| Serum zinc (μg/dl; ≥53:<53) | 3.173               | 1.777–5.667          | <0.001                |                      |                      |                      |

ALD, alcoholic liver disease; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acids and tyrosine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; OR, odds ratio.
Table 6. Factors contributing to emergency of alopecia

|                      | Univariate analysis |                  |                  | Multivariate analysis |                  |                  |
|----------------------|---------------------|------------------|------------------|-----------------------|------------------|------------------|
|                      | OR                  | 95% CI           | p value          | OR                    | 95% CI           | p value          |
| Age (years; ≥75:<75) | 1.866               | 1.012–3.442      | 0.043            | 0.040                 | 0.009–0.166      | <0.001           |
| Gender (male:female) | 0.052               | 0.016–0.169      | <0.001           |                       |                  |                  |
| BMI (kg/m²; ≥23.7:<23.7) | 1.610               | 0.858–3.020      | 0.135            |                       |                  |                  |
| Liver cirrhosis (yes:no) | 0.758               | 0.415–1.386      | 0.357            |                       |                  |                  |
| Treatment history of HCC (yes:no) | 0.387               | 0.177–0.847      | 0.014            |                       |                  |                  |
| ALD (yes:no)        | 0.253               | 0.060–1.062      | 0.043            |                       |                  |                  |
| Diabetes (yes:no)   | 1.088               | 0.523–2.262      | 0.821            |                       |                  |                  |
| Hemoglobin (g/dl; <13.0:≥13.0) | 4.290               | 1.665–11.056     | 0.001            |                       |                  |                  |
| Platelets count (×10⁴/µl; <12.5:≥12.5) | 0.539               | 0.290–1.002      | 0.048            |                       |                  |                  |
| Prothrombin (%) <70:≥70 | 0.544               | 0.256–1.159      | 0.110            |                       |                  |                  |
| Albumin (g/dl; <3.5:≥3.5) | 0.580               | 0.273–1.232      | 0.152            |                       |                  |                  |
| Total bilirubin (mg/dl; ≥0.7:<0.7) | 0.326               | 0.066–1.053      | 0.058            |                       |                  |                  |
| AST (U/L; ≥27:<27)  | 2.832               | 2.222–9.950      | 0.003            |                       |                  |                  |
| ALT (U/L; ≥60:<60)  | 1.029               | 0.353–2.999      | 0.959            |                       |                  |                  |
| ALP (U/L; ≥400:<400) | 1.255               | 0.650–2.422      | 0.498            |                       |                  |                  |
| eGFR (ml/min/1.73 m²; <57.0:≥57.0) | 0.607               | 0.290–1.271      | 0.182            |                       |                  |                  |
| BTR (≥4.00:<4.00)   | 0.623               | 0.277–1.404      | 0.250            |                       |                  |                  |
| M2BPGi (COI; ≥5.2:<5.2) | 1.256               | 0.623–2.534      | 0.524            |                       |                  |                  |
| Serum copper (µg/dl; <100:≥100) | 0.607               | 0.306–1.205      | 0.150            |                       |                  |                  |
| Serum iron (µg/dl; ≥230:<230) | 2.500               | 0.691–9.047      | 0.149            |                       |                  |                  |
| Serum zinc (µg/dl; ≥53:<53) | 0.426               | 0.201–0.901      | 0.022            |                       |                  |                  |

ALD, alcoholic liver disease; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acids and tyrosine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; OR, odds ratio.

Table 7. Factors contributing to emergency of aphthous stomatitis

|                      | Univariate analysis |                  |                  | Multivariate analysis |                  |                  |
|----------------------|---------------------|------------------|------------------|-----------------------|------------------|------------------|
|                      | OR                  | 95% CI           | p value          | OR                    | 95% CI           | p value          |
| Age (years; ≥75:<75) | 1.248               | 0.662–2.354      | 0.493            | 0.458                 | 0.243–0.862      | 0.016            |
| Gender (male:female) | 0.437               | 0.232–0.820      | 0.008            |                       |                  |                  |
| BMI (kg/m²; ≥25.0:<25.0) | 0.375               | 0.617–1.194      | 0.601            |                       |                  |                  |
| Liver cirrhosis (yes:no) | 0.834               | 0.271–1.524      | 0.479            |                       |                  |                  |
| Treatment history of HCC (yes:no) | 0.846               | 0.440–1.626      | 0.615            |                       |                  |                  |
| ALD (yes:no)        | 0.708               | 0.271–1.848      | 0.479            |                       |                  |                  |
| Diabetes (yes:no)   | 1.088               | 0.523–2.262      | 0.821            |                       |                  |                  |
| Hemoglobin (g/dl; <13.1:≥13.1) | 2.661               | 1.166–6.072      | 0.016            |                       |                  |                  |
| Platelets count (×10⁴/µl; <11.7:≥11.7) | 0.953               | 0.511–1.780      | 0.881            |                       |                  |                  |
| Prothrombin (%) <89:≥89 | 0.903               | 0.459–1.776      | 0.768            |                       |                  |                  |
| Albumin (g/dl; <3.4:≥3.4) | 1.333               | 0.690–2.574      | 0.391            |                       |                  |                  |
| Total bilirubin (mg/dl; ≥2.0:<2.0) | 1.280               | 0.482–1.280      | 0.619            |                       |                  |                  |
| AST (U/L; ≥33:<33)  | 1.205               | 0.659–2.203      | 0.545            |                       |                  |                  |
| ALT (U/L; ≥28:<28)  | 0.440               | 0.208–0.932      | 0.028            |                       |                  |                  |
| ALP (U/L; ≥373:<373) | 0.505               | 0.238–1.070      | 0.070            |                       |                  |                  |
| eGFR (ml/min/1.73 m²; <60.0:≥60.0) | 0.676               | 0.338–1.351      | 0.265            |                       |                  |                  |
| BTR (≥3.50:<3.50)   | 1.629               | 0.801–3.312      | 0.174            |                       |                  |                  |
| M2BPGi (COI; ≥4.3:<4.3) | 1.087               | 0.559–2.111      | 0.808            |                       |                  |                  |
| Serum copper (µg/dl; <138:≥138) | 0.762               | 0.308–1.855      | 0.555            |                       |                  |                  |
| Serum iron (µg/dl; ≥207:<207) | 2.395               | 0.870–6.595      | 0.082            |                       |                  |                  |
| Serum zinc (µg/dl; ≥68:<68) | 0.525               | 0.261–1.056      | 0.067            |                       |                  |                  |

ALD, alcoholic liver disease; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BTR, branched-chain amino acids and tyrosine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; OR, odds ratio.
water loss leads to dry skin. Dry skin is often itchy and prone to dermatitis. Meanwhile, skin manifestations associated with liver diseases are also diverse and nonspecific. In addition, dermatitis is correlated with the severity of liver function. Lower platelets count is associated with the severity of liver function. A particularly strong association is reported between zinc deficiency and dermatitis. Although the characteristic symptoms in patients with disease conditions such as erythematous rash and scale plaques, skin disorders such as dandruff, acne, and diaper rash are also relieved by zinc administration. Regarding the pathogenesis of dermatitis, zinc deficiency is considered to reduce epidermal Langerhans cells, which inhibit adenosine triphosphate derived from chemically damaged keratin-producing cells. Thus, it is difficult for gastroenterologists to precisely differentiate skin manifestations caused by zinc deficiency from those caused by older age and liver diseases. Whether zinc supplementation results in symptomatic improvement might be helpful information for differentiating these skin manifestations. Next, the prevalence of anorexia and taste disorder was 9.2%, 6.2%, respectively. Zinc deficiency is reported to cause anorexia leading to weight loss and to cause gastrointestinal mucosal atrophy, leading to the decreased secretion of gastric juice or impaired motility of the gastrointestinal tract. In the present study, a multivariate analysis showed that lower eGFR and higher M2BPGi were independently associated with emergency of anorexia. The pathogenesis of anorexia in chronic kidney disease is not fully understood but it has been reported that the progressive decline of glomerular filtration rate in chronic kidney disease patients are associated with a significant reduction in food intake. Moreover, many patients with higher M2BPGi had cirrhosis. One of the pathogenesis of malnutrition in cirrhosis was shown poor dietary intake. Potential reasons for low energy intake include reduced appetite possibly associated with increased brain tryptophan availability, satiety due to ascites, poor palatability of low-sodium diets, and hepatic encephalopathy. gastrointestinal symptoms, and gut dysfunction. A univariate analysis showed that lower serum zinc level was associated with emergency of anorexia. Although zinc is assumed to be directly and indirectly involved, the mechanism of anorexia associated with zinc deficiency remains unclear. Because taste disorder and aphthous stomatitis are the possible main causes of anorexia, they are mutually associated symptoms. Taste disorder is a taste disturbance associated with the distorted perception of taste or a persistent sense of taste in the absence of objective assessment of their symptoms. In addition, the study did not define a fixed duration of symptoms. 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. Third, this study only used a simple questionnaire to collect information about subjective symptoms. Therefore, the results might have been biased to overrepresent the patients’ subjective assessment of their symptoms. In addition, the study did not include a placebo group with a serum zinc level of ≥280 μg/dL. Consequently, multi-center larger studies will be needed to confirm these results.

The present study revealed that many patients with cirrhosis suffer zinc deficiency and that various concomitant symptoms presumably associated with zinc deficiency are observed at a certain frequency in patients with CLD. Historically, there were no zinc preparations approved for the treatment of hypozincemia in Japan. However, in March 2017, zinc acetate hydrate, which had been demonstrated to be safe and effective for the long-term treatment of Wilson disease, was approved for the additional indication of hypozincemia. Because the long-term administration of zinc to patients with CLD with zinc deficiency is reported to prevent worsening of liver function and progression of hepatic fibrosis and to reduce the risk of developing HCC, 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 CLD.

In conclusion, when zinc deficiency was defined as a serum factors, such as nutrient deficiencies (e.g., iron, folic acid, and vitamin B), infection (e.g., viruses and candida), drugs, stress, and autoimmunity. The role of nutritional deficiency as a cause of aphthous stomatitis has been highlighted by the association of a subset of 5% to 10% of aphthous stomatitis patients with low serum levels of iron, folate, zinc, or vitamins B1, B2, B6 and B12. Because zinc is deeply involved with the stability of the structure function of the exocytotic membrane, aphthous stomatitis might not be caused by a single factor but rather by multiple factors, such as prolonged inflammation and delayed wound healing due to zinc deficiency and an immune-compromised state caused due to the imbalance of T helper (Th) 1/Th2 cytokines. Exacerbations of the condition have been observed mainly in the luteal phase of the menstrual cycle and during the menopause. Although the mean patient age was 68 years in the present study, the proportion of female might be high. However, the pathogenesis of aphthous stomatitis has not yet been clearly elucidated. Finally, the prevalence of alopecia was 8.0%. Because zinc plays an important role in the regeneration and repair of hair follicles, the association between serum zinc levels and alopecia has been described in many reports. Alopecia is caused by the combined effects of aging, nutritional status, and gut inflammation associated with androgen. The majority of men start to lose hairs in the twenties, while women begin to lose their hair in forties or fifties. Also, female hair loss will not end up with complete baldness; whereas male hair loss can end up with complete baldness. Androgenetic alopecia is a common disorder affecting 50% of men and 15% of women, especially postmenopausal women. Although we enrolled patients whose mean age was 68, the percent of the patients with alopecia might be higher in female. In the present study, the prevalence of all symptoms except for aphthous stomatitis and alopecia individually increased as serum zinc levels decreased. This may have occurred because the involvement of factors other than zinc could not be excluded in aphthous stomatitis and alopecia.

The limitations of the present study were as follows. First, the study was a retrospective single-center observational study. Second, the serum zinc levels show circadian variations, are high in the early morning, and decrease toward the afternoon. Therefore, blood sample collection should preferably be done 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. Third, this study only used a simple questionnaire to collect information about subjective symptoms. Therefore, the results might have been biased to overrepresent the patients’ subjective assessment of their symptoms. In addition, the study did not include a placebo group with a serum zinc level of ≥280 μg/dL. Consequently, multi-center larger studies will be needed to confirm these results.
zinc level of <80 μg/dl, approximately one-third of patients with zinc deficiency displayed symptoms presumably originating from zinc deficiency. As serum zinc levels decreased, the prevalence of these symptoms increased. In such patients, detailed history taking is necessary in daily clinical practice.

**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| ALD          | alcoholic liver disease |
| ALP          | alkaline phosphatase |
| AST          | aspartate aminotransferase |
| BMI          | body mass index |
| BTR          | branched-chain amino acid and tyrosine ratio |
| CLD          | chronic liver disease |
| eGFR         | estimated glomerular filtration rate |
| HCC          | hepatocellular carcinoma |
| HCV          | hepatitis C virus |
| M2BPGi       | Mac-2 binding protein glycosylation isomer OR |
| OR           | odds ratio |

**Conflict of Interest**

No potential conflicts of interest were disclosed.

**Ethical Approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee (registration no. 499) and with the 1964 Helsinki declaration.

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