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Clinical Significance of Serum Zinc Levels on the Development of Sarcopenia in Cirrhotic Patients

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Abstract. *Background/Aim: Sarcopenia increases the mortality in patients with cirrhosis. Approximately 60% of zinc is accumulated in skeletal muscle. We aimed to determine the role of subclinical zinc deficiency on sarcopenia development in patients with cirrhosis. Patients and Methods: We enrolled 151 patients with cirrhosis and divided them into the group with normal serum zinc levels (Group N: 80-130 μg/dl; n=38) and group with subclinical zinc deficiency (Group D: <80 μg/dl; n=113). The risk factors for sarcopenia were then investigated. Results: Group D had more sarcopenia cases than Group N (31.0% vs. 13.2%). In group D, HGS exhibited a weakly positive but significant correlation with serum zinc levels (R=0.287, p=0.00212), serum zinc levels negatively correlated with both ammonia and myostatin levels (R=−0.254, p=0.0078; R=−0.33, p<0.01), and low zinc levels were independently associated with sarcopenia development. Conclusion: Patients with cirrhosis showing subclinical zinc deficiency have a significantly higher risk of developing sarcopenia.*

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The nutritional status of patients with cirrhosis is often poor. Sarcopenia is a form of malnutrition caused by deficiencies of macronutrients (protein) and micronutrients [trace elements such as calcium, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc (1), and vitamins] and results in increased mortality for patients with cirrhosis (2). The factors that induce sarcopenia in patients with chronic liver disease include hyperammonemia, lower levels of branched-chain amino acids (BCAA), and lower testosterone levels (3). Recently, the liver–muscle axis has been identified as a cause of sarcopenia in cirrhosis, and hyperammonemia has been recognized as a potential mediator. The upregulation of myostatin is one of the mechanisms responsible for the deterioration of protein synthesis and increased autophagy, both of which are associated with the development of sarcopenia in patients with cirrhosis. Higher serum myostatin levels are correlated with lower albumin (Alb) and lower BCAA and tyrosine ratio (BTR), signifying the development of hyperammonemia and loss of skeletal muscle mass (4). BCAAs, especially leucine, are key regulators of the target of rapamycin complex 1 signaling, which is associated with the insulin/insulin-like growth factor 1 regulatory pathway (5). Supplementation with BCAA increases serum Alb levels and improves the Fischer's ratio (the ratio of BCAA to aromatic amino acids) in the blood of cirrhotic patients (6). Recent data suggest that BCAA supplements will be useful for preventing and treating sarcopenia (7). The roles of testosterone in developing and maintaining muscle mass and function are known to be important (8). Testosterone supplementation has been demonstrated to exert beneficial effects on skeletal muscle mass and function, although the results remain inconsistent. However, the etiologies of sarcopenia are not fully understood. Therefore, an improved understanding of the mechanisms involved in sarcopenia is important to identify possible

molecular targets for pharmacological treatment. The Japan Society of Hepatology proposed guidelines according to which the loss of skeletal muscle mass and strength would indicate sarcopenia (9). One recognized parameter of muscle function is handgrip strength (HGS). In cirrhosis, HGS decline is associated with numerous adverse clinical outcomes, including malnutrition, low physical activity, and disease development and progression (10). Skeletal muscle mass is determined by the skeletal muscle index (SMI) (11). In addition, other tools such as computed tomography (CT), bioelectrical impedance analysis, and dual-energy X-ray absorptiometry should be mentioned as they are regarded as the gold standard methods to detect sarcopenia and can also assess skeletal muscle mass. Generally, HGS measurement is simple, rapid, inexpensive, plausible, and suitable for use at the bedside. HGS measurement can also be conducted reciprocally for patients with cirrhosis. The influence of skeletal muscle mass on sarcopenia has already been extensively investigated (12, 13). However, in patients with cirrhosis it is the reduced HGS, rather than the loss of skeletal muscle, that is associated with increased mortality risk (12).

Zinc is an important trace element and is ubiquitously distributed in all tissues, but the highest levels are found in liver and skeletal muscle (13). The liver maintains systemic zinc homeostasis that is predominantly regulated by its intestinal absorption. Zinc contributes to diverse cellular and metabolic processes and is a prerequisite for protein synthesis (11). Zinc deficiency has been attributed to endotoxins and cytokines (14). Patients with cirrhosis usually have elevated endotoxin levels, and frequently also exhibit reduced rates of muscle protein synthesis (15). According to the Japanese practical guidelines for zinc deficiency developed by the Japanese Society of Clinical Nutrition, a serum zinc level <60 μg/dl is defined as zinc deficiency, while 60 μg/dl ≤ serum zinc level <80 μg/dl is regarded as subclinical zinc deficiency, and 80 μg/dl ≤ serum zinc level <130 μg/dl is considered to be the normal zinc range (16). Nishikawa *et al*. demonstrated that zinc deficiency (<60 μg/dl) is an independent predictor of sarcopenia in patients with chronic liver diseases. Given the relative lack of awareness of zinc deficiency disorder, zinc deficiency is frequently overlooked in the clinical setting (14). Recent evidence suggests that zinc levels are correlated with HGS (17). In this study we aimed to determine the clinical significance of subclinical zinc deficiency on the development of sarcopenia in patients with cirrhosis.

Patients and Methods

A single-center cohort of outpatients with cirrhosis was conducted from January 2015 to December 2019 at the Nara Medical University Hospital. A total of 151 consecutive cirrhotic patients were studied. Liver cirrhosis was diagnosed according to the clinical data including laboratory tests (*e.g*., Alb, bilirubin, and prothrombin time), medical imaging features, liver histology, and clinical complications (*e.g*., hepatic encephalopathy and ascites). Skeletal muscle mass and HGS were measured at admission, and clinical parameters and serum assayed for endotoxin activity (EA) were evaluated in 151 patients with cirrhosis. All patients enrolled in this study received dietary advice from dietitians. No differences in dietary protein intake were observed in individuals. Patients with hepatocellular carcinoma or extrahepatic cancers, infectious diseases, and concomitant liver disease (*i.e*., chronic hepatitis B infection, hepatitis C infection) were excluded. Zinc levels were measured in patients with cirrhosis using a colorimetric assay kit (Abcam, ab102507) as per the manufacturer's instructions; absorbance was measured at 560 nm (18). In the present study, serum zinc levels of less than 80 μg/dl were defined as low serum zinc levels according to the definition proposed by the Japanese Society of Clinical Nutrition. A total of 151 patients were divided into two groups, patients with cirrhosis whose serum zinc level of 80-130 μg/dl (Group N) (n=38) and those whose serum zinc level of <80 μg/dl (Group D) (n=113). The study protocol was approved by the Medical Ethics Committee of Nara Medical University (Nara-med, 0152-12-5). All 151 patients enrolled provided written informed consent for blood samples before enrollment in the study.

Diagnosis of sarcopenia. The Japanese Society of Hepatology established the original criteria for liver disease-related sarcopenia based on the Asian criteria for sarcopenia in 2016. In this study, sarcopenia was diagnosed using the Assessment Criteria for Sarcopenia in Liver Disease (1st edition) based on the Sarcopenia Assessment Criteria of the Japan Society of Hepatology (9).

A decline in handgrip strength was measured using hand dynamometers (19). The amount of skeletal muscle mass was retrospectively defined using the SMI, which was calculated by carrying out skeletal muscle measurements at the level of the third lumbar (L3) vertebra on CT images (SMI-CT). CT scan was perfumed at the time of blood examination. The cutoff values for HGS were <26 kg in men and <18 kg in women. The cutoff values for SMI-CT were ≤42 cm^2/m^2 in men and ≤38 cm^2/m^2 in women. Presarcopenia was defined as patients with normal HGS and decreased SMI and dynapenia is defined as patients with normal SMI and decreased HGS (20).

EA measurements. Endotoxin was measured using the Endotoxin Activity Assay (EAA) according to the assay manufacturers' protocols (Toxicolor LS-50-M Set; Seikagaku Corp., Tokyo, Japan) (21, 22). In brief, the EAA is based on the principle that endotoxin interacts with antiendotoxin antibodies and is transported to neutrophils by complement receptors. Neutrophils undergo a respiratory burst accompanied by light emission, in the presence of zymosan and luminol. A chemiluminometer is used to quantify the light produced, and its intensity is proportional to the concentration of endotoxin in the sample (23). EA is expressed in relative units derived from the integral of the basal level and the facilitated chemiluminescent response (on a scale from 0 to 1). For fasting venous ammonia levels, blood samples were obtained in the morning after the patients had fasted overnight or for at least 6 hours and placed on ice immediately after collection. An enzymatic method was used for ammonia measurement (24). Serum myostatin concentrations were measured in duplicate using commercially available kits (DGDF80, R&D Systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation were less than 10% (25).

Statistical analysis. All statistical analyses were performed using R Ver.4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria). The data were expressed as median±standard deviation. The baseline characteristics between groups were compared using the

Table I. *Clinical and demographic characteristics of cirrhotic patients*.

	Total (n=151)	Group N (n=38)	Group D (n=113)	*p*-Value
Gender (Male/Female)	95/56	22/16	73/40	0.58
Age[a]	70±10	70±9	73±10	0.12
Child–pugh classification (A/B/C)	117/28/6	37/1/0	80/27/6	0.01
Etiology (HBV/HCV/alcohol/NASH/Others)	24/64/37/10/16	8/15/8/2/5	16/49/29/8/11	0.79
BMI (kg/m^2)[a]	24.3±4.1	24.5±3.6	24.1±4.0	0.23
Skeletal mass index[a]	41.6±8.1	41.1±8.5	41.8±8.0	0.65
Grip strength (kg)[a]	26.0±9.8	29.6±9.4	24.8±9.2	<0.01
Sarcopenia	40/151	5/38	35/113	0.03
PT (%)[a]	77±15	78±14	75±16	0.28
Alb (g/dl)[a]	4.1±0.6	4.4±0.4	3.8±0.6	<0.01
Ch-E (U/l)[a]	214±94	260±90	196±88	<0.01
BTR (μmol/l)[a]	4.8±1.8	5.5±1.5	4.6±1.8	<0.01
Ammonia (μg/dl)	38±27	31±18	48±29	<0.01
Myostatin (pg/ml)	3158±1383	2642±1200	3331±1401	<0.01
Zinc (μg/dl)[a]	72±17	89±14	63±12	<0.01
EA levels[a]	0.28±0.12	0.27±0.08	0.29±0.13	0.40

[a]Mean±standard error of mean. BMI: Body mass index; PT: prothrombin time; Alb: albumin; Ch-E: cholinesterase; BTR: branched chain amino acid and tyrosine ratio; Zn: zinc; EA: endotoxin activity; Group N: normal zinc level; Group D: deficiency of zinc.

unpaired *t*-test or the Mann–Whitney *U*-test. We used parametric tests for continuous data with normal distribution, and nonparametric tests for continuous data without normal distribution. Categorical data were analyzed by the Fisher's exact test. Correlations were calculated by Spearman's rank test. In addition, the association between zinc levels and sarcopenia severity was assessed using the Steel–Dwass test. A two-sided *p*-value of less than 0.05 was considered to be statistically significant. Baseline parameters significantly correlated with serum zinc levels in the univariate analysis were subjected to multivariate logistic regression analysis to select candidate valuables.

Ethics approval and consent to participate. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Nara Medical University (protocol code 0152-12-5 and date of approval: October 14, 2014). Informed consent was obtained from all subjects involved in the study.

Results

Clinical characteristics of participants. The cause of liver disease was hepatitis B virus infection in 24 patients, hepatitis C virus infection in 64 patients, alcohol abuse in 37, nonalcoholic steatohepatitis in 10 and other disorders in 16, including 6 with primary biliary cholangitis, 5 with autoimmune hepatitis, and 5 with idiopathic causes (Table I). Group D had significantly lower liver function reserve (Child–Pugh class A/B/C; 37/1/0 *vs*. 82/27/6: $p<0.01$) and grip strength than Group N (29.6±9.4 kg *vs*. 24.8±9.2 kg: $p<0.01$). Group N had significantly higher serum levels of albumin (Alb), cholinesterase (Ch-E), BCAA and tyrosine ratio (BTR), and zinc than group D [4.4±0.4 (g/dl) *vs*. 3.8±0.6 (g/dl), $p<0.01$, 260±90 (U/l) *vs*. 196±88 (U/l), $p<0.01$, 5.5±1.5 (μmol/l) *vs*. 4.6±1.8 (μmol/l), $p<0.01$, 89±14 (μg/dl) *vs*. 63±12 (μg/dl), $p<0.01$]. Serum levels of ammonia (NH3) and myostatin were higher in Group D than Group N [31±18 (μg/dl) *vs*. 48±29 (μg/dl), $p<0.01$, 2,642±1,200 (pg/ml) *vs*. 3,331±1,401 (pg/ml), $p<0.01$]. The prevalence of sarcopenia was higher in Group D than in Group N [(13.2% (5/38) *vs*. 31.0% (35/113), $p=0.031$]. No significant differences in SMI-CT or EA levels were found between Group D and Group N (41.1±8.5 *vs*. 41.8±8.0, $p=0.65$, 0.27±0.08 *vs*. 0.29±0.13, $p=0.40$).

Correlations between handgrip strength and clinical parameters. HGS had significant positive correlations with SMI-CT in both Group N and D (R=0.65, $p<0.001$; R=0.451, $p<0.01$) (Table II). HGS showed only a tendency toward positive correlation with Alb in Group N and had significant correlation with Alb in Group D (R=0.319, $p<0.0508$; R=0.203, $p=0.0319$). HGS was significantly correlated with BMI in Group N (R=0.566, $p<0.01$), but not in group D (R=0.0993, $p=0.306$). HGS had significant negative correlation with age in both Groups of N and D (R=–0.373, $p=0.021$; R=–0.446, $p<0.01$). No significant correlation was found between grip strength and EAA in either group (R=–0.106, $p=0.557$; R=–0.0752, $p=0.479$). HGS exhibited positive weak but significant correlation with serum zinc levels in group D (R=0.287, $p<0.01$), whereas no significant correlation was observed between HSG and zinc levels in group N (R=–0.0698, $p=0.677$). HGS exhibited significant positive correlation with Ch-E levels and BTR in Group N (R=0.616, $p<0.01$; R=0.39, $p=0.0156$). HGS inversely correlated with serum levels of ammonia (R=–0.226, $p=0.0186$), but not those of myostatin (R=–0.185, $p=0.0517$) in Group D.

Table II. *Correlation of hand grip strength with clinical parameters in Group N and Group D.*

	Group N Cirrhotic patients whose serum zinc level is 80-130 μg/dl (n=38)		Group D Cirrhotic patients whose serum zinc level is <80 μg/dl (n=113)	
	R	*p*-Value	R	*p*-Value
BMI	0.566	<0.001	0.0993	0.31
SMI (CT)	0.65	<0.001	0.451	<0.01
Age	–0.373	0.021	–0.446	<0.01
Alb	0.319	0.0508	0.203	0.03
PT	0.367	0.0236	–0.118	0.22
Zn	–0.0698	0.677	0.287	<0.01
ChE	0.616	<0.001	0.179	0.06
BTR	0.39	0.0156	0.166	0.08
Ammonia	–0.0436	0.795	–0.226	0.02
Myostatin	0.058	0.728	–0.185	0.05
EA levels	–0.106	0.557	–0.0752	0.48

BMI: Body mass index; SMI: skeletal mass index; BIA: bioelectrical impedance analysis; PT: prothrombin time; Alb: albumin; TG: triglyceride; CT: Computed tomography; T-Cho: total cholesterol; Ch-E: cholinesterase; BTR: branched chain amino acid and tyrosine ratio; EA: endotoxin activity; Group N: normal zinc level; Group D: deficiency of zinc.

Correlations of zinc levels with clinical parameters in cirrhotic patients with zinc deficiency. Zinc levels had significant positive correlations with Alb and BTR (R=0.81, $p<0.01$; R=0.45, $p<0.01$) (Figure 1A and B) and significant negative correlations with NH3 (R=–0.254, $p<0.01$) in Group D (Figure 1C). No significant correlation was found between zinc and EA levels (R=–0.03, $p=0.798$) (Figure 1D). Myostatin levels exhibited a significant positive correlation with ammonia levels (R=0.69, $p<0.01$) and a significant inverse correlation with zinc levels in Group D (R=–0.33, $p<0.01$) (Figure 2A and B).

Association between zinc levels and sarcopenia severity in Group D. Zinc levels in patients with no sarcopenia (normal SMI and HGS), dynapenia (normal SMI and reduced HGS), presarcopenia (reduced SMI and normal HGS), and sarcopenia (reduced SMI and reduced HGS) were 67.9±11.9 μg/dl, 60.8±13.8 μg/dl, 65.1±12.2 μg/dl, and 58.5±10.5 ug/dl, respectively (Figure 3). We examined the association between zinc levels and sarcopenia severity in Group D (n=113). Zinc levels were significantly lower in the presarcopenia group than in the no sarcopenia group ($p<0.01$). Further, zinc levels were significantly lower in the presarcopenia group than in the no sarcopenia group ($p<0.05$).

Comparison of parameters between patients with sarcopenia and those without sarcopenia. Patients with sarcopenia had significantly lower zinc levels than those without sarcopenia in group D [58 (μg/dl) *vs*. 66 (μg/dl), $p<0.01$], whereas no significant differences in zinc levels have been found between patients with sarcopenia and those without sarcopenia in group N [91 (μg/dl) *vs*. 94 (μg/dl), $p=0.786$] (Table III). Liver function reserve was not significantly different between patients with sarcopenia and those without sarcopenia (Child–Pugh class A/B/C; 26/7/2 *vs*. 54/20/4: $p=0.81$). In group D, cirrhotic patients with sarcopenia showed significantly lower HGS and SMI-CT than those without sarcopenia (17.0±5.3 *vs*. 29.3±9.1, $p<0.01$, 36.4±4.1 *vs*. 44.3±8.1, $p<0.01$) (Table IV). The number of male patients with sarcopenia was significantly lower than those without sarcopenia ($p<0.01$). Patients with sarcopenia were significantly older than those without sarcopenia (75.3±8.7 years *vs*. 69.6±8.7 years, $p<0.01$). Serum Ch-E levels were significantly higher in cirrhotic patients with sarcopenia than in those without sarcopenia (3.7±0.5 g/dl *vs*. 3.9±0.6 g/dl, $p<0.01$, 172±67 U/l *vs*. 207±95 U/l, $p=0.027$).

Univariate and multivariate analysis of variables associated with development of sarcopenia in patients with cirrhosis with subclinical zinc deficiency. Univariate and multivariate logistic regression analyses showed that low zinc levels and old age were independent factors associated with the development of sarcopenia in patients with cirrhosis whose serum zinc level of <80 μg/dl [odds ratio (OR)=0.916, 95% CI=0.858-0.977, $p<0.01$; OR=1.09, 95% CI=1.020-1.150, $p<0.01$] (Table V and Table VI). Univariate and multivariate analysis of variables associated with decreased hand grip strength in patients with cirrhosis with subclinical zinc deficiency. Univariate logistic regression analyses showed that old age, female, low SMI and low zinc levels were independent factors associated with decreased HGS in patients with cirrhosis whose serum zinc level of <80 μg/dl (OR=1.110, 95%

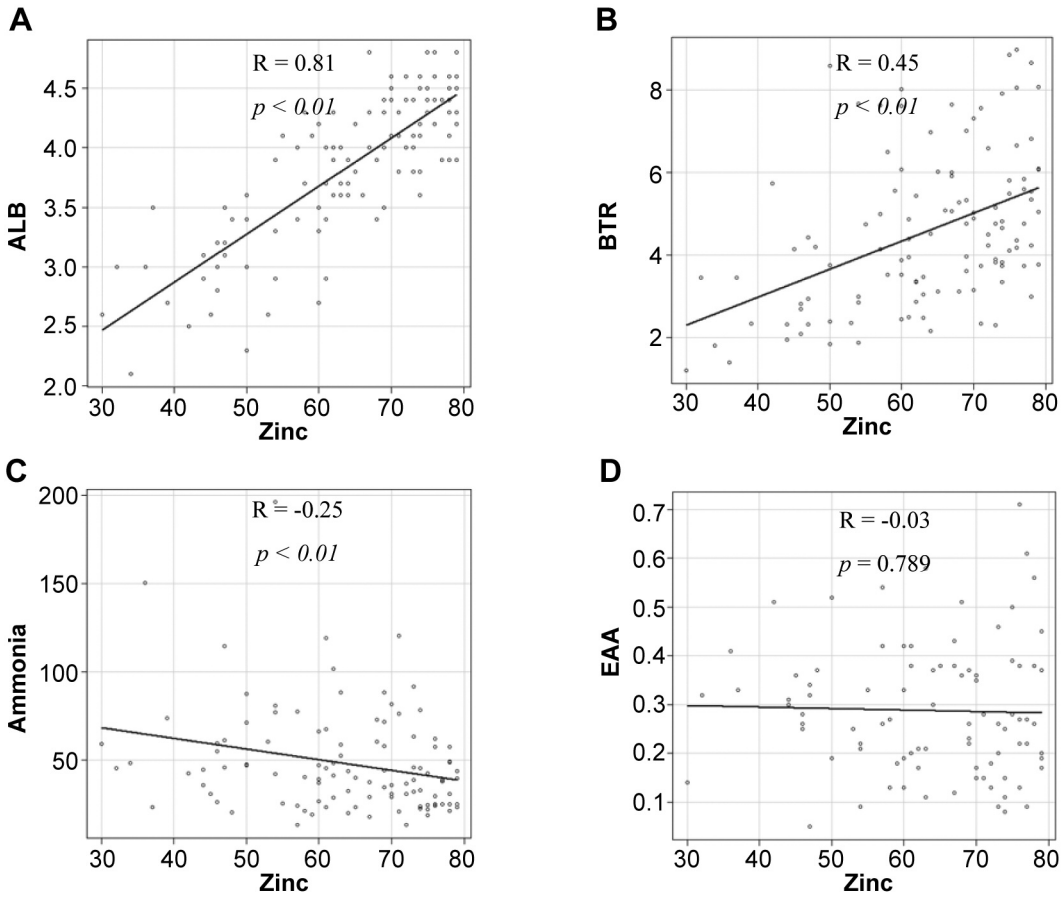

Figure 1. *Correlation between zinc levels (horizontal axis) and laboratory results (A) albumin, (B) branched-chain amino acids and tyrosine ratio (BTR), (C) ammonia, (D) endotoxin activity.*

CI=1.040-1.190, p<0.01; OR=4.280, 95% CI=1.490-12.30, p<0.01; OR=0.916, 95% CI=0.848-0.989, p=0.02; OR=0.943, 95% CI=0.905-0.984, p<0.01) (Table VII and Table VIII).

Discussion

Between 60 and 80% of patients with cirrhosis have been found to be malnourished (26). Sarcopenia is common in cirrhotic patients, and has been reported to be an independent risk factor of mortality in such cases, with a reported incidence of 23-60%. An imbalance between the synthesis and degradation of skeletal muscle protein may lead to development of sarcopenia or to various mechanisms that may be involved in its pathogenesis. Skeletal muscle atrophy and weakness have been attributed to both intrinsic factors within skeletal muscles, such as apoptosis, autophagy, calcium metabolism, inflammation, mitochondrial metabolism, and neuromuscular junctions, as well as extrinsic factors in systemic environments, such as endocrine factors, nutritional status, and immobility (27-29). It is, therefore, crucial to identify the pathogenesis and clinical characteristics of sarcopenia and develop preventive and therapeutic strategies against sarcopenia in patients with cirrhosis (30). Zinc contributes to essential physiological processes regulated by the enzymatic activities and maintenance of protein synthesis (31). We found that zinc deficiency is an independent risk factor for sarcopenia in cirrhotic patients with subclinical zinc deficiency. This is the first study to show that cirrhotic patients with subclinical zinc deficiency are at significantly higher risk of developing sarcopenia. Zinc levels were inversely correlated with myostatin levels in cirrhotic patients with low serum levels of zinc. Elevated levels of ammonia may lead to a reduction in skeletal muscle mass, or sarcopenia, and this has been shown to be linked to increased levels of myostatin, which negatively regulates muscle growth (3). We have shown that the incidence of sarcopenia is significantly increased in patients with cirrhosis and subclinical zinc deficiency compared to those with normal serum zinc levels. A strong correlation has been shown between serum levels of albumin and zinc (r=0.81, p<0.01) and multivariate analysis has revealed that serum zinc level is an independent predictor of sarcopenia and reduced HGS. Because

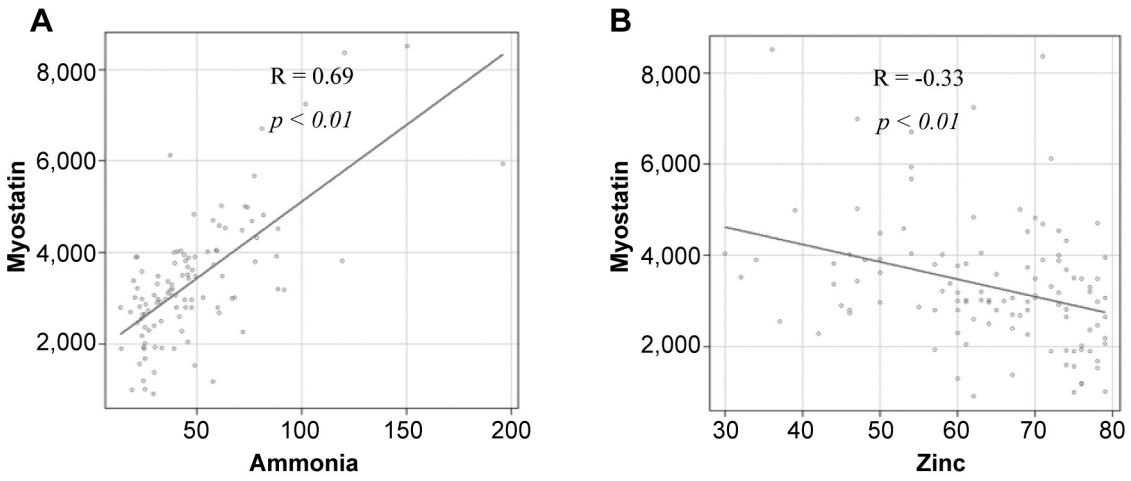

Figure 2. *Correlation between myostatin levels (vertical axis) and laboratory results (A) ammonia and (B) zinc.*

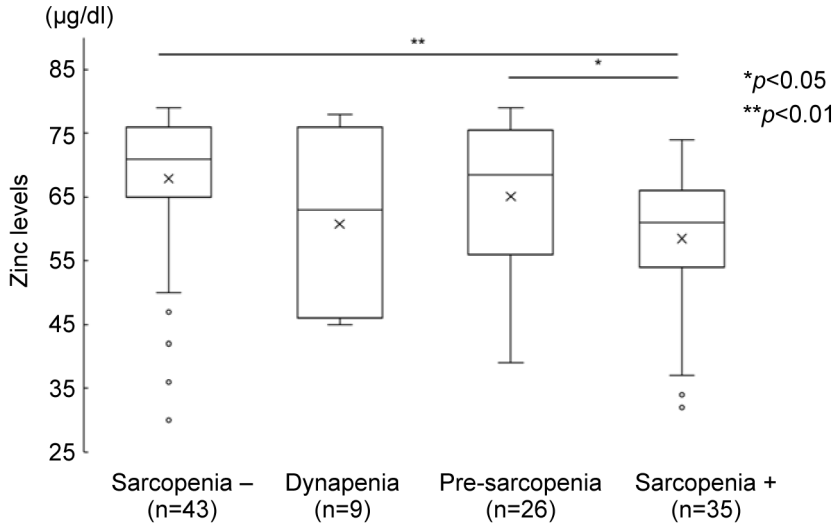

Figure 3. *Association between zinc concentration and sarcopenia severity in group D.*

Table III. *Serum zinc concentration in cirrhotic patients with and without sarcopenia in Group N and Group D.*

Zinc levels (µg/dl)	Sarcopenia (+) (n=40)	Sarcopenia (–) (n=111)	*p*-Value
Group N (n=38)	91 (µg/dl; n=5)	94 (µg/dl; n=33)	0.79
Group D (N=113)	58 (µg/dl; n=35)	66 (µg/dl; n=78)	<0.01

zinc levels can represent protein synthesis ability, these findings are not very surprising. Moreover, HGS is inversely correlated with age in patients with normal zinc levels and in those with zinc deficiency. Age was a significant risk factor for both sarcopenia and reduced HGS in cirrhotic patients. During aging, many parameters have shown an association with inflammation and oxidative stress, and disrupted zinc homeostasis is a common characteristic of aging (32-34). The high prevalence of

Table IV. *Clinical and demographic characteristics of cirrhotic patients with sarcopenia and without sarcopenia in Group D.*

	Sarcopenia (+) (n=35)	Sarcopenia (–) (n=78)	*p*-Value
Gender (Male/Female)	15/20	58/20	<0.01
Age[a]	75.3±8.7	69.6±8.7	<0.01
Child–pugh classification (A/B/C)	26/7/2	54/20/4	0.81
Etiology (HBV/HCV/Alcohol/NASH/Others)	3/22/5/3/2	13/27/24/5/9	0.058
BMI (kg/m^2)[a]	23.5±3.4	24.5±4.0	0.187
SMI (CT)[a]	36.4±4.1	44.3±8.1	<0.01
Grip strength (kg)[a]	17.0±5.3	29.3±9.1	<0.01
PT (%)[a]	74.3±16.5	75.1±16.6	0.808
Alb (g/dl)[a]	3.7±0.5	3.9±0.6	0.057
Ch-E (U/l)[a]	172±67	207±95	0.027
BTR (μmol/l)[a]	4.24±1.55	4.75±1.97	0.147
Ammonia (μg/dl)[a]	43.9±20.2	50.1±32.0	0.227
Myostatin (pg/ml)[a]	3,294±1,297	3,348±1,454	0.844
EA levels[a]	0.32±0.12	0.28±0.14	0.177

[a]Mean±standard error of mean. BMI: Body mass index; SMI: skeletal mass index; PT: prothrombin time; Alb: albumin; Ch-E: cholinesterase; BTR: branched chain amino acid and tyrosine ratio; Zn: zinc; EA: endotoxin activity; BIA: bioelectrical impedance analysis; Group N: normal zinc level; Group D: deficiency of zinc.

Table V. *Univariate analyses of factors linked to the development of sarcopenia for cirrhotic patients developing zinc deficiency.*

	Univariate analyses		
	Odds ratio	95% CI	*p*-Value
Gender	1.590	0.699-3.610	0.268
Age	1.090	1.030-1.140	<0.01
Alb	0.558	0.291-1.070	0.080
BMI	0.932	0.834-1.040	0.214
ChE	0.995	0.990-1.000	0.053
Ammonia	0.992	0.976-1.010	0.310
Zn	0.950	0.918-0.983	0.026
BTR	0.857	0.683-1.080	0.183
PT	0.997	0.973-1.020	0.806
EA levels	8.980	0.297-272.0	0.207
Myostatin	1.000	1.000-1.000	0.849

Table VI. *Multivariate analyses of factors linked to the development of sarcopenia for cirrhotic patients developing zinc deficiency.*

	Multivariate analyses		
	Hazard ratio	95% CI	*p*-Value
Age	1.090	1.020-1.150	<0.01
Alb	2.410	0.619-9.370	0.204
ChE	1.000	0.993-1.010	0.849
Zn	0.916	0.858-0.977	<0.01

hypozincemia in our cirrhotic patients (74.8%) could be partly due to their older age (average age=70.0 years) and limited protein synthesis (35, 36). On the other hand, it is important to bear in mind that a substantial number of patients without cirrhosis have been shown to be hypozincemic (38.4%) (17). Additionally, the Practical Guideline recommend zinc supplementation even for patients with subclinical zinc deficiency (16, 37). Tomita *et al*. proposed a lower cutoff level of 80 μg/dl for zinc deficiency (38). These findings support the hypothesis that maintenance of serum zinc levels above 80 μg/dl has the potential to reduce both development and progression of sarcopenia in cirrhotic patients, and indicate that patients with subclinical zinc deficiency may develop medical complications including sarcopenia. Patients with advanced liver disease often have low serum zinc levels, and in patients with cirrhosis, decreased serum zinc has been shown to be a surrogate marker for nutritional status (39). Zinc supplementation would be of great benefit to patients with cirrhosis and hyperammonemia (40). Serum zinc levels are inversely correlated with serum ammonia levels in cirrhotic patients who exhibit reduced zinc levels. Zinc supplementation boosts ammonia detoxification in the liver (41), and consequently leads to alleviation of ammonia disposal in skeletal muscle (42). Hyperammonemia promotes transcriptional regulation of myostatin *via* an NF-κB-mediated mechanism in patients with cirrhosis (43). However, our study showed that myostatin had no correlation with HGS in Group D. Myostatin acts by negatively regulating skeletal muscle mass (44) and reducing protein synthesis in muscle by inhibiting the insulin-like growth factor-1 (IGF-1)/AKT pathway as well as the mammalian target of rapamycin (mTOR) pathway (45). IGF-I promotes skeletal muscle growth by inhibiting expression of

Table VII. *Univariate analyses of factors linked to low grip strength for cirrhotic patients developing zinc deficiency.*

	Univariate analyses		
	Hazard ratio	95% CI	*p*-Value
Gender	3.500	1.530-7.990	<0.01
Age	1.110	1.050-1.170	<0.01
Alb	0.453	0.230-0.890	0.02
BMI	0.959	0.864-1.060	0.43
SMI	0.886	0.828-0.948	<0.01
ChE	0.994	0.989-0.999	0.02
NH3	0.981	0.963-0.999	0.04
Zn	0.950	0.918-0.983	<0.01
BTR	0.755	0.596-0.957	0.02
PT	1.000	0.980-1.030	0.73
EA levels	8.240	0.335-203.0	0.20
Myostatin	1.000	1.000-1.000	0.58

Table VIII. *Multivariate analyses of factors linked to the low grip strength for cirrhotic patients developing zinc deficiency.*

	Multivariate analyses		
	Hazard ratio	95% CI	*p*-Value
Age	1.110	1.040-1.190	<0.01
Gender	4.280	1.490-12.30	<0.01
SMI	0.916	0.848-0.989	0.02
Zn	0.943	0.905-0.984	<0.01

atrophy-related ubiquitin ligases, atrogin-1, and muscle RING-finger protein-1 and blocking protein breakdown (46). During myogenic differentiation, IGF-1 has been shown to inhibit the myostatin signaling pathway (47). Further, testosterone, a positive regulator of muscle growth, reduces muscular myostatin content, indicating that myostatin levels are influenced by several factors, including ammonia levels (43). These findings explain why myostatin was not significantly associated with HGS. Nevertheless, loss of HGS is attributed to zinc deficiency-induced laboratory parameters including hyperammonemia and increased myostatin levels.

We must acknowledge several limitations of the study. First, this was a single-center prospective study, which including only a small number of cases of cirrhosis. Second, risk factors associated with a decline in grip strength were not evaluated for the development of sarcopenia, although SMI-CT did correlate with HGS in both study groups. Third, we did not analyze patients in Group N because the number of patients with sarcopenia was inadequate for the statistical analysis. Fourth, the number of the patients in Group D was too small to analyze the risk factors of sarcopenia and HGS in different etiologies of liver cirrhosis. Fifth, serum zinc levels constitute only 1% of the total amount of zinc in an organism. Zinc levels should therefore be measured in erythrocytes as well. Taken together, our findings show that cirrhotic patients with subclinical zinc deficiency are at significantly higher risk of developing sarcopenia. Zinc supplementation is an effective means of maintaining serum zinc levels at 80 μg/dl or above, and is potent in preventing the development of sarcopenia in patients with cirrhosis. Zinc deficiency is common in cirrhosis patients and hypoalbuminemia could indicate zinc deficiency. Zinc supplementation may prevent worsening of malnutrition and sarcopenia in cirrhotic patients with subclinical zinc deficiency. We urgently need to conduct studies on whether oral zinc supplementation reduces the prevalence of sarcopenia and alleviates hypoalbuminemia in cirrhotic patients. Moreover, sarcopenia is also an essential feature of liver cirrhosis, representing a negative prognostic factor and influencing mortality. Increased awareness and understanding of the pathophysiology of sarcopenia could allow the development of novel therapeutic approaches that could improve the prognosis of cirrhotic patients. Nevertheless, larger studies will be necessary to clarify the relationship between the development and progression of sarcopenia and serum zinc levels in cirrhotic patients.

Conclusion

Zinc deficiency is an independent risk factor of sarcopenia in cirrhotic patients who exhibited reduced zinc levels (<80 μg/dl). Close monitoring for serum zinc levels is helpful for the management of patients with cirrhosis.

Availability of Data and Materials

Raw data were generated at Nara medical university hospital. Derived data supporting the findings of this study are available from the corresponding author [T.N] on request.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, T.N. and H.Y.; methodology, Y.Fujim.; software, S.T.; validation, M.E., H.Takay., K.I., H.O., K.Moriy., M.T., M.K., S.A., A.K., N.Y., T.M. and Y.T.; formal analysis, A.S., A.K., J.S., T.K., S.I., F.T., K.A. and T.I.; investigation, T.O.; resources, H.Takag.; data curation, Y.Fujin.; writing—original draft preparation, K.M.; writing—review and editing, T.N.; visualization, M.F. and H.K.; supervision, K.Kaji., T.A., and A.M.; project administration, Y.S. and K.Kita.; funding acquisition, N.N. and S.S. All Authors have read and agreed to the published version of the manuscript.

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