Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.5738-20
Intern Med 60: 675-680, 2021
http://internmed.jp

【 ORIGINAL ARTICLE 】

Hypozincemia in Chronic Hepatitis C Is Improved with Viral Clearance by Direct-acting Antiviral Agents

Ryosaku Shirahashi, Toshikuni Suda and Masaya Tamano

Abstract:

Objective Hypozincemia is a decrease in the serum zinc level of patients with hepatitis C and often requires zinc supplementation to improve the hepatic function. Our previous study showed the efficacy of direct-acting antiviral agent (DAA) treatment on serum zinc levels in patients with hepatitis C without zinc supplementation. In this study, we aimed to prospectively examine factors related to the improvement of serum zinc levels of patents with hepatitis C with DAA treatment.

Methods Fifty-three patients with hepatitis C treated with DAAs between March 2018 and February 2019 at a university medical center were divided into two groups based on their initial serum level: the zinc deficiency group (n=43, <80 μg/dL) and the normal zinc group (n=10, ≥80 μg/dL). Their serum zinc levels and clinical parameters were measured before DAA treatment, at the end of treatment and 12 weeks post-treatment.

Results All 53 patients achieved a sustained viral response to DAAs at the end of treatment and at follow-up. There was a significant increase in the serum zinc level from baseline to follow-up in the zinc deficiency group but not in the normal zinc group. The change in serum albumin was the only factor contributing to the observed increase in serum zinc levels by a multiple regression analysis.

Conclusion DAA treatment in patients with hepatitis C improved hypozincemia due to the restored function of serum albumin, which binds to about 60% of serum zinc, upon the amelioration of the hepatitis C infection.

Key words: hypozincemia, hepatitis C, direct-acting antiviral agents

(Intern Med 60: 675-680, 2021)
(DOI: 10.2169/internalmedicine.5738-20)

Introduction

Zinc is an important trace element involved in life-sustaining processes, such as protein synthesis and metabolism, and plays a key role in the growth and development of the human body. Adults have 1.5 to 3 g of zinc in their bodies, and in the blood, 60% of zinc is bound to albumin.

Serum zinc levels decrease in patients with hepatitis C as the disease progresses to chronic hepatitis, compensated cirrhosis and decompensated cirrhosis (1). Zinc supplementation therapy in patients with hepatitis C improves the long-term prognosis by improving the hepatic function and inhibiting hepatocarcinogenesis (2-5). Reports have shown that serum zinc has a decreasing trend during interferon treatment in hepatitis C patients (6) and that zinc supplementation during treatment increases the viral clearance rate (7).

In our previous study, we prospectively showed for the first time that treatment with direct-acting antiviral agents (DAAs) promptly improved hypozincemia in patients with hepatitis C without zinc supplementation (8). Ko et al. reported in their retrospective study of 95 patients that treatment with DAAs improved hypozincemia in patients with hepatitis C for as long as 2 years and showed that factors associated with a lack of improvement in zinc included hyperuricemia and alcohol intake (9).

In the present study, factors related to the improvement of serum zinc levels during treatment with DAAs in patients with hepatitis C were prospectively examined.

Department of Gastroenterology, Dokkyo Medical University Saitama Medical Center, Japan
Received for publication June 30, 2020; Accepted for publication August 10, 2020
Correspondence to Dr. Masaya Tamano, mstamano@dokkyomed.ac.jp

Table 1. Clinical Characteristics in the Zn Normal Group and Zn Deficiency Group at Baseline.

Characteristic	Normal group (n=10)	Deficiency group (n=43)	p value
Age (y)	64.7±10.1	68.6±12.9	0.1663
Sex (Male/Female)	6/4	21/22	0.7354
HCV-RNA (LogIU/mL)	5.9±1.0	5.8±0.9	0.6617
ALT (IU/L)	33.0±16.7	61.0±48.0	0.0596
GGT (mg/dL)	36.4±24.2	49.6±37.1	0.2314
Total bilirubin (mg/dL)	0.7±0.1	0.8±0.3	0.9254
Serum albumin (g/dL)	4.4±0.3	4.1±0.3	0.0017
WBCs ($\times 10^3/mm^3$)	5.91±0.92	4.99±1.56	0.0239
Hb (g/dL)	14.7±1.6	13.6±1.9	0.086
Platelets ($\times 10^4/mm^3$)	23.0±5.0	16.9±7.5	0.0047
Prothrombin activity (%)	103.2±79.7	94.4±13.5	0.0905
AFP (g/dL)	3.2±2.3	6.5±6.0	0.1007
FIB-4 index	1.752±0.974	3.690±2.595	0.0084

AFP: α-fetoprotein, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, Hb: hemoglobin, WBCs: white blood cells

Materials and Methods

Patients

The subjects were 53 consecutive patients diagnosed with hepatitis C in a university medical center who were treated with DAAs between March 2018 and February 2019. Patients taking zinc preparations, patients who consumed ≥20 g of alcohol per day and patients with concurrent hepatocellular carcinoma were excluded. Patients who started a strict diet for diabetes were also excluded.

This prospective study was approved by the Ethics Committee of the university medical center, and written, informed consent was obtained from all participants. This study conformed to the ethical guidelines of the 2008 Declaration of Helsinki.

Serum zinc and other parameters

Serum zinc levels were measured in an early morning fasting state before DAA treatment (Baseline), at the end of treatment (EOT) and 12 weeks after the end of treatment (Follow-up 12), and the changes over time were investigated. The difference between serum zinc levels at Baseline and Follow-up 12 (ΔZn) was also assessed.

The Japanese Society of Clinical Nutrition (JSCN) defines a serum zinc level of <60 μg/dL as zinc deficiency and 60-80 μg/dL as subclinical zinc deficiency. Therefore, in this study, patients with serum zinc levels <80 μg/dL were placed in the zinc deficiency group.

Clinical parameters obtained on the same day that serum zinc levels were measured were compared. These parameters included the following: alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total bilirubin (T-Bil), serum albumin (Alb), white blood cells (WBCs), hemoglobin (Hb), platelets (Plts), prothrombin activity (PT%), and α-fetoprotein (AFP). The FIB-4 index was estimated using the values of serum aspartate aminotransferase (AST), ALT, Plts and age.

The differences in ALT, GGT, serum Alb, Plts, AFP and the FIB-4 index between Baseline and Follow-up 12, represented by ΔALT, ΔGGT, ΔAlb, ΔPlts, ΔAFP and the ΔFIB-4 index, and their correlations with ΔZn were examined.

Statistical analysis

Continuous data for serum zinc levels and other parameters are expressed as the means ± standard deviation (SD). The paired Wilcoxon's test and chi-squared test were used to test for differences in each parameter before and after the start of treatment. Values of $p<0.05$ were considered significant.

Results

The subjects were 53 patients (27 men, 26 women; mean age 67.3 years old, range 40-88 years old) with hepatitis C treated with DAAs. Hepatitis C virus (HCV) genotypes were 1b in 25 patients, 2a in 19 patients, 2b in 8 patients and 3a in 1 patient. The subjects were 49 patients with chronic hepatitis and 3 with compensated cirrhosis. The DAAs used and their treatment durations were as follows: glecaprevir/pibrentasvir (8 weeks) in 47 patients, elbasvir+grazoprevir (12 weeks) in 5 patients and ledipasvir/sofosbuvir (12 weeks) in 1 patient. In all 53 patients, a sustained viral response was achieved at EOT and Follow-up 12.

The mean serum zinc level in all 53 patients was 70.4± 12.0 μg/dL at Baseline. The zinc deficiency group included 43 patients (serum zinc level <60 μg/dL in 10 patients, and 60-80 μg/dL in 33 patients).

Table 1 shows the clinical characteristics of the zinc normal and zinc deficiency groups at Baseline. The serum Alb level, WBCs and Plts were lower and the FIB-4 index higher in the zinc deficiency group than in the normal zinc group. Hemoglobin and PT% tended to be lower in the zinc

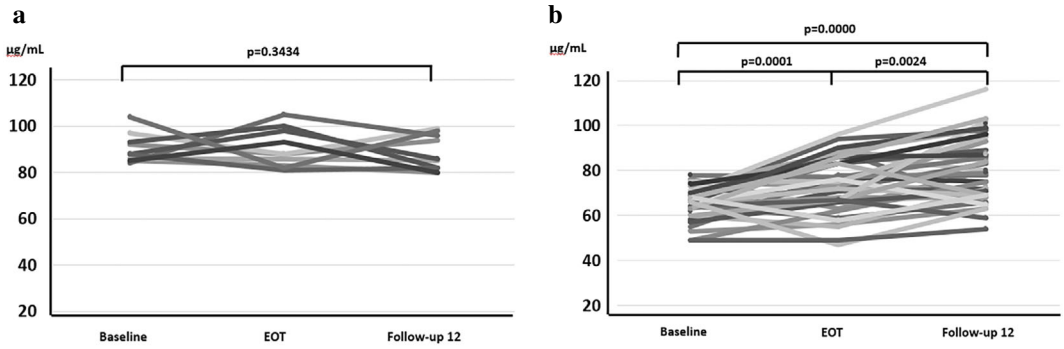

Figure 1. **Changes in serum zinc levels before and after treatment with DAAs. The serum zinc level in 10 subjects in the normal zinc group was 90.2±6.4 μg/dL before treatment, 90.4±8.3 μg/dL at the end of treatment and 88.2±7.7 μg/dL 12 weeks after the end of treatment, showing no significant change during the observation period (p=0.3434). At every point, the level was within the normal range, which was ≥80 μg/dL (a). The serum zinc level in 43 subjects in the zinc deficiency group was 65.8±7.4 μg/dL before treatment, 73.6±12.5 μg/dL at the end of treatment and 80.4±13.9 μg/dL 12 weeks after the end of treatment, showing significant increases from pre-treatment to the end of treatment (p=0.0001) and from the end of treatment to 12 weeks after the end of treatment (p=0.0024) (b).**

Table 2. **Changes in Parameters of All 53 Patients between Baseline and Follow-up 12.**

	Baseline	Follow-up 12	p value
ALT (IU/L)	55.2±44.8	15.6±7.6	<0.0001
GGT (mg/dL)	46.8±35.2	24.7±16.6	<0.0001
Total bilirubin (mg/dL)	0.8±0.3	0.8±0.3	0.13918
Serum albumin (g/dL)	4.1±0.3	4.3±0.3	0.00095
WBCs ($\times 10^3/mm^3$)	5.20±1.50	5.43±1.72	0.11316
Hb (g/dL)	13.9±1.9	13.8±2.0	0.26663
Platelets ($\times 10^4/mm^3$)	18.2±7.5	18.9±7.4	0.03495
Prothrombin activity (%)	97.2±14.4	100.8±15.0	0.06637
AFP (g/dL)	5.8±5.7	4.1±2.9	0.00004
FIB-4 index	3.324±2.487	2.732±1.821	0.00051

AFP: α-fetoprotein, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, Hb: hemoglobin, WBCs: white blood cells

deficiency group than in the normal zinc group, although no significant difference was noted. ALT tended to be higher in the zinc deficiency group than in the normal zinc group, but no significant difference was observed. The HCV viral load was equivalent between the two groups.

Fig. 1 shows the changes in the serum zinc levels from Baseline to EOT and Follow-up 12. In the normal zinc group, the serum zinc level was 90.2±6.4 μg/dL at Baseline, 90.4±8.3 μg/dL at EOT and 88.2±7.7 μg/dL at Follow-up 12, showing no significant change during the observation period (Fig. 1a). In the zinc deficiency group, the serum zinc level was 65.8±7.4 μg/dL at Baseline, 73.6±12.5 μg/dL at EOT and 80.4±13.9 μg/dL at Follow-up 12, showing significant increases from Baseline to EOT (p=0.0001) and from EOT to Follow-up 12 (p=0.0024) (Fig. 1b).

Table 2 shows the changes in other parameters from Baseline to Follow-up 12 in all 53 subjects. The ALT, GGT, AFP and the FIB-4 index were significantly decreased, whereas the serum Alb level and Plts were significantly increased. The PT% tended to increase, although it did not show any significant difference.

Fig. 2 shows the correlations between the ΔZn and ΔALT, ΔGGT, ΔAlb, ΔPlt, ΔAFP and ΔFIB-4 index. The ΔZn showed a strong positive correlation with ΔAlb (r=0.4666, p=0.00043) and weak positive correlations with the ΔPlt (r=0.2880, p=0.03650) and ΔFIB-4 index (r=0.2289, p=0.09922). No correlations were observed with ΔALT, ΔGGT or ΔAFP.

Table 3 shows the results of a multiple regression analysis using the ΔZn as the explanatory variable and the ΔALT, ΔGGT, ΔAlb, ΔPlts, ΔAFP and ΔFIB-4 index as the objective variables. Only the ΔAlb was identified as a factor contributing to the ΔZn.

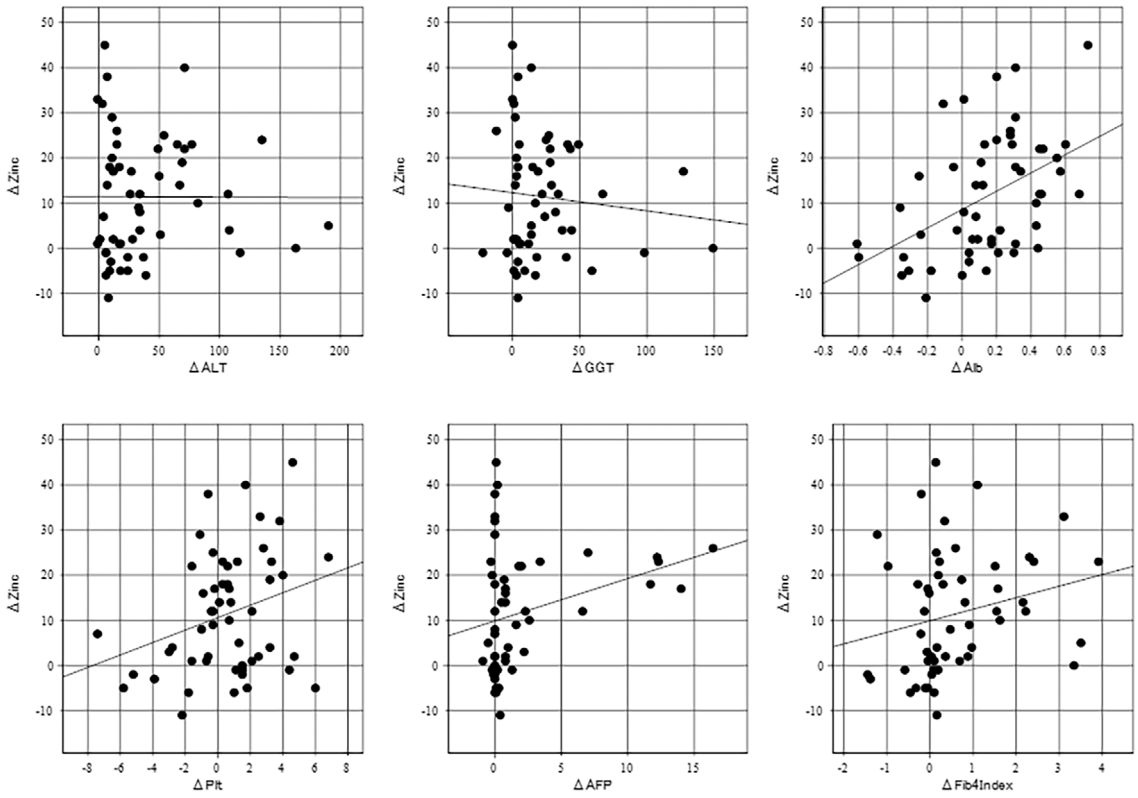

Figure 2. **Correlations between ΔZn and various parameters. The differences in serum zinc levels, ALT, GGT, serum albumin levels, Plts, AFP and the FIB-4 index between Baseline and Follow-up 12 are represented by the ΔZn, ΔALT, ΔGGT, ΔAlb, ΔPlt, ΔAFP and ΔFIB-4 index, respectively. An examination of the correlations between ΔZn and other parameters showed a strong positive correlation between the ΔZn and the ΔAlb (r=0.4666, p=0.00043) and weak positive correlations between the ΔZn and the ΔPlt (r=0.2880, p=0.03650) and between the ΔZn and the ΔFIB-4 index (r=0.2289, p=0.09922). No correlation was observed between the ΔZn and the ΔALT, ΔGGT or ΔAFP. The ΔALT, ΔGGT, Δalbumin, ΔPlt, ΔAFP and ΔFIB4-index were calculated with differences between Baseline and Follow-up 12. AFP: α-fetoprotein, Alb: albumin, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, Plts: platelets, WBCs: white blood cells**

Table 3. **Multiple Regression Analysis That Assumed ΔZn as the Objective Variable.**

	β	SE(β)	stdβ	t-value	p value
ΔALT (IU/L)	0.00124	0.00188	0.1164	0.6585	0.51364
ΔGGT (mg/dL)	0.00262	0.00216	0.1853	1.215	0.23086
Δalbumin (g/dL)	-0.57	0.2143	-0.3719	-2.6595	0.01087
ΔPlts ($\times 10^4$/mm^3)	0.02218	0.02315	0.137	0.958	0.34332
ΔAFP (g/dL)	-0.01753	0.01494	-0.1587	-1.1732	0.24703
ΔFIB4-index	-0.1134	0.6394	-0.3053	-1.7732	0.08312

AFP: α-fetoprotein, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, Plts: platelets

ΔALT, ΔGGT, Δalbumin, ΔPlts, ΔAFP, and ΔFIB4-index were calculated with differences between Baseline and Follow-up 12.

Discussion

Zinc, an element contained in various food items, including meats, grains, legumes and dairy products, is an important trace element involved in life-sustaining processes, such as protein synthesis and metabolism, and plays a key role in the growth and development of the human body. Zinc deficiency is reportedly manifested in various ways, including growth and developmental disorders, dysgeusia, glossalgia, anemia, loss of appetite and diarrhea, and a possible decline in the quality of life has been reported (10).

Adult bodies contain 1.5 to 3 g of zinc, which is widely distributed throughout the body, including in the skeletal muscles (60%), bones (20% to 30%), skin and hair (8%), liver (4% to 6%), gastrointestinal tract and pancreas (2.8%) and spleen (1.6%) (11-13). In the blood, 60% of zinc is bound to Alb and 30% to macroglobulin (14, 15). In patients with chronic liver disease, the serum zinc level is thought to be decreased by abnormal nitrogen metabolism, specifically hypoalbuminemia (16). Other factors related to the decrease in serum zinc levels in patients with chronic liver disease are an impaired absorption associated with changes in the small intestinal mucosa, decreased zinc content of the liver associated with reduction in the functional liver cell count and an imbalanced diet. In patients with hepatic cirrhosis, increased urinary zinc excretion associated with portal-systemic shunting is thought to be a background factor (11).

The classification of zinc deficiency by the JSCN is considered to be useful for the prediction of hepatic events, including carcinogenesis, ascites, encephalopathy and variceal rupture in patients with hepatitis C (17). For this reason, subjects were divided into two groups-the normal zinc group and the zinc deficiency group-using the JSCN classification for the analysis in the present study. Zinc deficiency was observed in 43 of 53 patients (81.1%) with hepatitis C prior to treatment. Ozeki et al. reported zinc deficiency in 80.8% of 1,973 Japanese patients with chronic liver disease (18), which is consistent with the present data. In the zinc deficiency group, the serum Alb levels and Plts were lower and the FIB-4 index was higher than in the normal zinc group. Based on these findings, zinc deficiency in patients with hepatitis C appears to be caused by reduced Alb synthesis associated with the progression of hepatic fibrosis.

Serum zinc levels in the zinc deficiency group improved promptly with DAA treatment for 8 to 12 weeks. Furthermore, Plts were significantly increased, and the ALT, GGT, AFP and FIB-4 index were decreased by DAA treatment. Histological fibrosis in hepatitis C has been reported not to improve early after DAA treatment, although histological improvement of inflammation has been reported early after treatment (19). Therefore, amelioration of hepatitis may contribute to the increase in zinc levels. However, no correlations were noted between the improvement in zinc and ΔALT, ΔGGT and ΔAFP. If consumption due to inflammation is a cause of the decrease in serum zinc levels, there should be certain correlations between the ΔZn and these factors. In contrast, a moderate correlation was observed between the ΔZn and ΔAlb (r=0.4666, p=0.00043).

When a multiple regression analysis was performed using the ΔZn as the objective variable and the ΔALT, ΔGGT, ΔAlb, ΔPlts, ΔAFP and ΔFib-4 index as the explanatory variables, only the ΔAlb was a significant factor contributing to the increase in zinc. As described earlier, 60% of serum zinc is bound to Alb. DAA treatment appears to have ameliorated hepatitis, restoring the ability of the liver to synthesize Alb, a transport protein, and thereby increasing Alb, which in turn improved serum zinc levels.

In addition to the correlation with the ΔAlb, the ΔZn also showed a positive correlation with the ΔPlts. The clear reason for this correlation is unknown. In the present study, serum zinc levels were measured by separating the sera as promptly as possible after blood collection. However, zinc from the cellular component, while a small amount, may have been included in the measurement. Since Plts contain 0.48 ng/10^6 cells of zinc (20), and 3% of zinc is considered to be contained in white blood cells and Plts (21), zinc from platelets may have also been measured.

Nevertheless, our previous study showed an increase in serum zinc levels prior to the increase in serum Alb (8), suggesting that there are other factors improving the serum zinc levels besides an increase in Alb. Of note, the hepatitis C virus itself has been reported not to directly affect the serum zinc level (22). It is presumed that the mechanism underlying the decreased zinc levels involves the non-structural proteins NS3 and NS5A of the hepatitis C virus. NS3 is a zinc-containing enzyme (23, 24), and NS5A is a zinc metalloprotein (25). DAAs inhibit viral growth by suppressing the functions of these non-structural proteins of hepatitis C virus. This mechanism may affect serum zinc levels after treatment.

Conclusion

Treatment with DAAs improved hypozincemia in patients with hepatitis C in a short period without zinc supplementation. The increase in serum zinc levels was thought to be caused by an increase in Alb, a transport protein.

The authors state that they have no Conflict of Interest (COI).

References

1. Moriyama M, Matsumura H, Fukushima A, et al. Clinical significance of evaluation of serum zinc concentrations in C-viral chronic liver disease. Dig Dis Sci **51**: 1967-1977, 2006.
2. Matsuoka S, Matsumura H, Nakamura H, et al. Zinc supplementation improves the outcome of chronic hepatitis C and liver cirrhosis. J Clin Biochem Nutr **45**: 292-303, 2009.
3. Matsumura H, Nirei K, Nakamura H, et al. Zinc supplementation therapy improves the outcome of patients with chronic hepatitis C. J Clin Biochem Nutr **51**: 178-184, 2012.
4. Kawaguchi T, Nagao Y, Abe K, et al. Effects of branched-chain amino acids and zinc-enriched nutrients on prognosticators in HCV-infected patients: a multicenter randomized controlled trial. Mol Med Rep **11**: 2159-2166, 2015.
5. Hosui A, Kimura E, Abe S, et al. Long-term zinc supplementation improves liver function and decreases the risk of developing hepatocellular carcinoma. Nutrients **10**: 1955, 2018.
6. Grungreiff K, Reinhold D, Ansorge S. Serum concentrations of sIL-2R, IL-6, TGF-beta1, neopterin, and zinc in chronic hepatitis C patients treated with interferon-alpha. Cytokine **11**: 1076-1080, 1999.
7. Takagi H, Nagamine T, Abe T, et al. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. J Viral Hepat **8**: 367-371, 2001.

8. Suda T, Okawa O, Shirahashi R, Tokutomi N, Tamano M. Changes in serum zinc levels in hepatitis C patients before and after treatment with direct-acting antiviral agents. Hepatol Res **49**: 1353-1356, 2019.

9. Ko YL, Morihara D, Shibata K, et al. Factors attenuating zinc deficiency improvement in direct-acting antiviral agent-treated chronic hepatitis C virus infection. Nutrients **10**: 1620, 2018.

10. Prasad AS. Clinical, endocrinological and biochemical effects of zinc deficiency. Clin Endocrinol Metab **14**: 567-589, 1985.

11. Gupta S, Read SA, Shackel NA, Hebbard L, George J, Ahlenstiel G. The Role of micronutrients in the infection and subsequent response to hepatitis C virus. Cells **8**: 603, 2019.

12. Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol **251**: R398-R408, 1986.

13. Aggett PJ. Aspects of neonatal metabolism of trace metals. Acta Paediatr Suppl **402**: 75-82, 1994.

14. Prasad AS, Oberleas D. Binding of zinc to amino acids and serum proteins in vitro. J Lab Clin Med **76**: 416-425, 1970.

15. Giroux EL, Durieux M, Schechter PJ. A study of zinc distribution in human serum. Bioinorg Chem **5**: 211-218, 1976.

16. Katayama K, Kawaguchi T, Shiraishi K, et al. The prevalence and implication of zinc deficiency in patients with chronic liver disease. J Clin Med Res **10**: 437-444, 2018.

17. Nishikawa H, Enomoto H, Yoh K, et al. Serum zinc level grading system: a useful model for composite hepatic events in hepatitis C virus-associated liver cirrhosis. J Clin Med **9**: 643, 2020.

18. Ozeki I, Arakawa T, Suii H, et al. Zinc deficiency in patients with chronic liver disease in Japan. Hepatol Res **50**: 396-401, 2020.

19. Enomoto M, Ikura Y, Tamori A, et al. Short-term histological evaluations after achieving a sustained virologic response to direct-acting antiviral treatment for chronic hepatitis C. United European Gastroenterol J **6**: 1391-1400, 2018.

20. Milne DB, Ralston NV, Wallwork JC. Zinc content of blood cellular components and lymph node and spleen lymphocytes in severely zinc-deficient rats. J Nutr **115**: 1073-1078, 1985.

21. Ruz M, Cavan KR, Bettger WJ, Gibson RS. Erythrocytes, erythrocyte membranes, neutrophils and platelets as biopsy materials for the assessment of zinc status in humans. Br J Nutr **68**: 515-527, 1992.

22. Himoto T, Masaki T. Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. Nutrients **10**: 88, 2018.

23. Love RA, Parge HE, Wickersham JA, et al. The crystal structure of hepatitis C virus NS3 proteinase reveals a trypsin-like fold and a structural zinc binding site. Cell **87**: 331-342, 1996.

24. Stempniak M, Hostomska Z, Nodes BR, Hostomsky Z. The NS3 proteinase domain of hepatitis C virus is a zinc-containing enzyme. J Virol **71**: 2881-2886, 1997.

25. Tellinghuisen TL, Marcotrigiano J, Gorbalenya AE, Rice CM. The NS5A protein of hepatitis C virus is a zinc metalloprotein. J Biol Chem **279**: 48576-48587, 2004.