Hypozincemia in chronic hepatitis C is improved with viral clearance by direct-acting antiviral agents

Running title: Improving hypozincemia in hepatitis C

Ryosaku Shirahashi, Toshikuni Suda, Masaya Tamano

Department of Gastroenterology, Dokkyo Medical University Saitama Medical Center, 2-1-50 Minami-Koshigaya, Koshigaya-shi, Saitama 343-8555, Japan

Correspondence to: Masaya Tamano

Professor, Department of Gastroenterology

Dokkyo Medical University Saitama Medical Center

2-1-50 Minami-Koshigaya, Koshigaya-shi, Saitama 343-8555, Japan

Tel: +81-48-965-1111; Fax: +81-965-1169

E-mail: mstamano@dokkyomed.ac.jp

Abstract

Objective Hypozincemia is a decrease in serum zinc level of patients with hepatitis C and often requires zinc supplementation to improve 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 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 (Alb) was the only factor contributing to the observed increase in serum zinc levels by multiple regression analysis.

Conclusions: DAA treatment in patients with hepatitis C improved hypozincemia due to the restored ability of Alb which binds about 60% of serum zinc, upon the amelioration of the hepatitis C infection.

Key words: Hypozincemia, Hepatitis C, Direct-acting antiviral agents

Introduction

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 hepatic function and inhibiting hepatocarcinogenesis (2-5).

Reports have shown that serum zinc shows a decreasing trend during interferon treatment in hepatitis C patients(6), and that zinc supplementation during treatment raises the viral clearance rate(7).

In our previous study, we prospectively showed for the first time in the world 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 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.

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 less than 60 μg/dL as zinc deficiency and 60-80 μg/dL as subclinical zinc deficiency. Therefore, in this study, patients with serum zinc levels less than 80 μg/dL were placed in the zinc deficiency group.

Clinical parameters, which were obtained on the same day that serum zinc levels were measured, were compared. Clinical 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 means ± standard deviation (SD). The paired Wilcoxon 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, range 40-88 years) with hepatitis C treated with DAAs. The DAAs used and their treatment durations were: 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/mL 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 group and the zinc deficiency group at Baseline.

Serum Alb level, WBCs, and Plts were lower, and the FIB-4 index was higher in the zinc deficiency group than in the normal zinc group. Hemoglobin and PT% tended to be lower in the zinc deficiency 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.

Figure 1 shows changes in 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/mL at Baseline, 90.4 ± 8.3 μg/mL at EOT, and 88.2 ± 7.7 μg/mL 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/mL at Baseline, 73.6 ± 12.5 μg/mL at EOT, and 80.4 ± 13.9 μg/mL 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 changes in other parameters from Baseline to Follow-up 12 in all 53 subjects. ALT, GGT, AFP, and the FIB-4 index were significantly decreased, whereas the serum Alb level and Plts were significantly increased. PT% tended to increase, although it did not show any significant

difference.

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

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

Discussion

Zinc, one of the elements 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, playing a key role in 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 of quality of life has been reported (10).

Adults have 1.5 to 3 g of zinc, which is widely distributed throughout the body including 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 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 impaired absorption associated with changes in 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 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 this 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 1973 Japanese patients with chronic liver disease (18), which is consistent with the present data. In the zinc deficiency group, 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 progression of hepatic fibrosis.

Serum zinc in the zinc deficiency group improved promptly with DAA

treatment for 8 to 12 weeks. Moreover, Plts were significantly increased and ALT, GGT, AFP, and the FIB-4 index were decreased by DAA treatment. Histological fibrosis in hepatitis C has been reported not to improve early after DAA treatment, though histological improvement of inflammation has been reported early after treatment (19). Therefore, amelioration of hepatitis may contribute to the increase in zinc. 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 ΔZn and these factors. In contrast, a strong correlation was observed between ΔZn and ΔAlb ($r = 0.4666$, $p = 0.00043$).

When multiple regression analysis was performed using ΔZn as the objective variable and ΔALT, ΔGGT, ΔAlb, ΔPlts, ΔAFP, and ΔFib-4 index as explanatory variables, only 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 ΔAlb, ΔZn also showed a positive correlation with 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, it is possible that zinc from the cellular component, although it may be a small amount, was

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 factors improving serum zinc levels other than the increase in Alb. Hepatitis C virus itself, however, has been reported not to directly affect the serum zinc level(22). It is presumed that the mechanism for 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.

Conclusions

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.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors do not have any

commercial or financial involvement in connection with this study that represents or appears to represent any conflicts of interest.

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. 2006 Nov;51: 1967-77.

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. 2009 Nov;45: 292-303.

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. 2012 Nov;51: 178-84.

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. 2015 Mar;11: 2159-66.

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. 2018 Dec 10;10.

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. 1999 Dec;11: 1076-80.

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. 2001 Sep;8: 367-71.

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. 2019 Nov;49: 1353-6.

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. 2018 Nov 2;10.

10. Prasad AS. Clinical, endocrinological and biochemical effects of zinc deficiency. Clin Endocrinol Metab. 1985 Aug;14: 567-89.

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. 2019 Jun 17;8.

12. Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol. 1986 Aug;251: R398-408.

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

14. Prasad AS, Oberleas D. Binding of zinc to amino acids and serum proteins in vitro. J Lab Clin Med. 1970 Sep;76: 416-25.

15. Giroux EL, Durieux M, Schechter PJ. A study of zinc distribution in human serum. Bioinorg Chem. 1976;5: 211-8.

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. 2018 May;10: 437-44.

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. 2020 Feb 28;9.

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

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. 2018 Nov;6: 1391-400.

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. 1985 Aug;115: 1073-8.

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. 1992 Sep;68: 515-27.

22. Himoto T, Masaki T. Associations between Zinc Deficiency and Metabolic Abnormalities in Patients with Chronic Liver Disease. Nutrients. 2018 Jan 14;10.

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. 1996 Oct 18;87: 331-42.

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

1997 Apr;71: 2881-6.

25. Tellinghuisen TL, Marcotrigiano J, Gorbalenya AE, Rice CM. The NS5A protein of hepatitis C virus is a zinc metalloprotein. J Biol Chem. 2004 Nov 19;279: 48576-87.

Figure Legends

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 is 90.2 ± 6.4 μg/mL before treatment, 90.4 ± 8.3 μg/mL at the end of treatment, and 88.2 ± 7.7 μg/mL 12 weeks after the end of treatment, showing no significant change during the observation period ($p = 0.3434$). At every point, the level is within the normal range, which is 80 μg/mL or higher (a). The serum zinc level in 43 subjects in the zinc deficiency group is 65.8 ± 7.4 μg/mL before treatment, 73.6 ± 12.5 μg/mL at the end of treatment, and 80.4 ± 13.9 μg/mL 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).

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 ΔZn, ΔALT, ΔGGT, ΔAlb, ΔPlt, ΔAFP, and ΔFIB-4 index. Examination of correlations between ΔZn and other parameters shows a strong positive correlation between ΔZn and ΔAlb ($r = 0.4666$, $p = 0.00043$) and weak positive correlations between ΔZn and ΔPlt ($r = 0.2880$, $p = 0.03650$) and between ΔZn and ΔFIB-4 index ($r = 0.2289$, $p = 0.09922$). No correlation is observed between ΔZn and ΔALT, ΔGGT, or ΔAFP. 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