

Insulin resistance derived from zinc deficiency in non-diabetic patients with chronic hepatitis C

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Abstract. Chronic hepatitis C virus (HCV) infection frequently evokes metabolic abnormalities including insulin resistance. A decrease in serum zinc (Zn) levels is often observed in association with hepatic fibrosis. Zn also plays important roles in insulin secretion. However, little is known about the relationship between Zn deficiency and insulin resistance in patients with HCV-related chronic liver disease. The main purpose of this study was to examine the contribution of Zn deficiency to insulin resistance in patients with chronic hepatitis C (CH-C). Forty-eight non-diabetic patients with CH-C were enrolled. Serum alanine aminotransferase (ALT), ferritin and Zn levels were examined in the enrolled patients with CH-C. Insulin resistance was determined by the Homeostasis model for assessment of insulin resistance (HOMA-IR). Zn deficiency was defined as serum Zn levels <65 µg/dl. Seven out of the 48 (15%) patients with CH-C fulfilled the criteria for Zn deficiency. Serum Zn levels were inversely correlated with serum ferritin levels ($r=-0.364$, $p=0.0140$). The values of HOMA-IR were positively linked to serum ferritin levels ($r=0.299$, $p=0.0484$). The mean value of HOMA-IR in the Zn deficiency group was significantly higher than that in the normal-range Zn group (3.76 ± 0.66 vs. 2.08 ± 1.35 , $p=0.0019$). Serum ALT levels were also closely associated with serum ferritin levels ($r=0.727$, $p<0.001$). These findings were independent of HCV genotypes or loads of HCV-RNA. Our data suggest that iron

overload in patients with CH-C derives from Zn deficiency and thereby causes insulin resistance.

Introduction

Zinc (Zn) is an essential trace element that serves as the active center of approximately 300 enzymes and participates in physiological functions including immune function, neurosensory function, protein metabolism, wound healing and sexual function (1,2). Zn plays important roles as a growth co-factor, an immunomodulator and a cytoprotectant with antioxidant, antiapoptotic and anti-inflammatory effects (3,4). Therefore, Zn depletion results in numerous types of symptoms including taste disorder, hyperkeratotic skin change, wound healing disturbance and testicular dysfunction.

Zn deficiency is also frequently observed in patients with chronic liver diseases (CLD), including chronic hepatitis and liver cirrhosis (5-8). Serum Zn levels inversely correlate with the severity of hepatic fibrosis (7). Zn depletion in patients with liver cirrhosis is closely related to the progression of hepatic encephalopathy. Zn deficiency usually leads to a decrease in the activity of ornithine transcarbamylase, a key Zn-containing enzyme for the urea cycle. This consequently causes the accumulation of ammonia and seems to account for the pathogenesis of hepatic encephalopathy (9). Zn administration has favorable effects on the inhibition of hepatic fibrosis, activating collagenase, which belongs to a zinc-metallo-enzyme, and/or diminishing prolyl hydroxylase, which contributes to collagen synthesis (10). Zn supplementation also relieves hepatic encephalopathy. In addition, we previously elucidated that oral administration of polaprezinc, a complex of zinc and l-carnosine, markedly improved necroinflammation in the liver of hepatitis C virus (HCV)-related CLD, reducing iron overload in the liver (11).

It has been widely established that persistent HCV infection leads to metabolic abnormalities including insulin resistance, hepatic steatosis and hypertriglycemia (12,13). Impaired signaling of the insulin receptor substrate (IRS) by the HCV core protein is considered to trigger insulin resistance in patients with HCV-related CLD (14). Previous studies revealed that insulin resistance is significantly associated with body mass index (BMI) (15), hepatic steatosis (16), hepatic

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Abbreviations: ALT, alanine aminotransferase; CH-C, chronic hepatitis C; HCV, hepatitis C virus; CLD, chronic liver disease; DM, diabetes mellitus; HOMA, homeostasis model assessment; IR, insulin resistance; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; SOD, superoxide dismutase; Zn, zinc

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fibrosis (17,18) and/or iron overload (19) in patients with chronic hepatitis C (CH-C).

On the other hand, Zn also plays crucial roles in the secretion and activation of insulin (20). Therefore, abnormalities of Zn metabolism seem to be associated with the pathogenesis of type 2 diabetes mellitus (DM) (21). A recent report revealed that oral administration of a Zn complex improved glucose tolerance in an experimental model of type 2 DM (22).

However, little is known about the relationship between insulin resistance and Zn deficiency in patients with HCV-related CLD. The primary purpose of this study was to investigate whether or not Zn deficiency contributes to insulin resistance in patients with HCV-related CLD.

Materials and methods

Study population. Forty-eight non-diabetic patients with CH-C, who had HCV-RNA detectable in the sera by polymerase chain reaction (PCR) and showed histological characteristics consistent with chronic hepatitis, were randomly selected for this study. Informed consent was obtained from the enrolled patients.

Laboratory assessments. The severity of obesity was evaluated using BMI. Insulin resistance was determined by the Homeostasis model for assessment of insulin resistance (HOMA-IR) method using the following equation: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mg/dl)} / 405$ (23). Serum alanine aminotransferase (ALT) levels were assessed as a serological parameter of necroinflammation in the liver. Serum ferritin levels were examined as a serological hallmark of iron storage in the liver. Blood samples were drawn in the morning with fasting on the basis of circadian serum Zn levels (24). Zn deficiency was defined as a serum Zn level $<65 \mu\text{g/dl}$, which corresponds to the normal lower limit of normal serum Zn. Quantitative detection of serum HCV-RNA was performed by Amplicor-HCV monitor assay (Roche Molecular Diagnostics, Tokyo, Japan) (25). The HCV genotype was determined by the HCV-RNA Genotyping assay system (Home Brew SRL Inc., Tokyo, Japan) (26).

Evaluation of hepatic fibrosis and steatosis. The grades of hepatic fibrosis were determined by the New Inuyama classification system, which is a standard criterion for the histological assessment of chronic hepatitis in Japan (27). The stages in chronic hepatitis were classified from F_0 through F_3 . F_0 was defined as no fibrosis in the liver, while F_4 was defined as liver cirrhosis. The enrolled patients were divided into two groups on the basis of hepatic fibrosis: early-stage (F_0 and F_1) and late-stage (F_2 and F_3).

The severity of hepatic steatosis was evaluated in accordance with the classification proposed by Brunt and colleagues (28): grade 0, no steatosis; grade 1, $<33\%$ of hepatocytes with steatosis; grade 2, 33–66% of hepatocytes affected.

Statistical analyses. Data values are represented as means \pm standard deviation (SD). The Mann-Whitney U test was applied for the comparison of continuous variables. The linear regression analysis was used to analyze the relation of the serum ferritin levels with serum Zn and ALT levels, or

Table I. Demographic, viral and histological characteristics of 48 patients with chronic hepatitis C.

| Characteristics | Data |
|-------------------------|----------------------------|
| Age (range) | 59 \pm 11 (23–76) |
| Gender (male/female) | 34/14 |
| Body mass index (range) | 23.7 \pm 2.6 (17.9–30.6) |
| Genotype | |
| 1b | 32 (67%) |
| 2a | 11 (23%) |
| 2b | 5 (10%) |
| Hepatic fibrosis | |
| F_0 | 1 (2%) |
| F_1 | 20 (42%) |
| F_2 | 6 (12%) |
| F_3 | 21 (44%) |
| Hepatic steatosis | |
| Grade 0 | 27 (57%) |
| Grade 1 | 16 (33%) |
| Grade 2 | 5 (10%) |
| Zinc deficiency | 7 (15%) |

the values of HOMA-IR. p-values <0.05 were considered to indicate a significant difference between groups.

Results

Demographic features of the enrolled patients with CH-C. Demographic characteristics are shown in Table I. Of the enrolled patients, 32 (67%) had a HCV genotype of 1b, 11 (23%) had a genotype of 2a and 5 (10%), 2b, respectively. There were no significant differences in the serum Zn levels among the enrolled patients with the three HCV genotypes (genotype 1b, 79.5 ± 15.1 ; 2a, 71.9 ± 9.8 ; 2b, 70.4 ± 7.0). Serum Zn levels were not associated with the load of HCV-RNA ($r=0.0074$, $p=0.6230$).

Of the 48 patients with CH-C, 1 had no fibrosis (F_0) in the liver and 20 patients had stage F_1 fibrosis. Six and 21 patients with CH-C fulfilled stage F_2 and F_3 fibrosis, respectively. Therefore, the enrolled patients were divided into 2 groups: 21 patients with CH-C in the early stage of hepatic fibrosis and 27 patients with CH-C in the late stage of hepatic fibrosis.

Severity of hepatic steatosis in the enrolled patients was evaluated as follows: 27 (57%) out of the 48 patients with CH-C had no hepatic steatosis (grade 0). Sixteen (33%) and 5 (10%) patients had hepatic steatosis of grade 1 and grade 2, respectively.

Seven out of the 48 (15%) patients with CH-C fulfilled the criteria for Zn deficiency. These 7 patients with CH-C had hepatic fibrosis of stage F_2 or F_3 .

Correlation between serum ferritin levels and serum Zn levels, HOMA-IR values or serum ALT levels. The relationship between serum ferritin and Zn levels in the enrolled patients with CH-C was examined. As shown in Fig. 1A, the serum ferritin levels were inversely correlated with the serum Zn

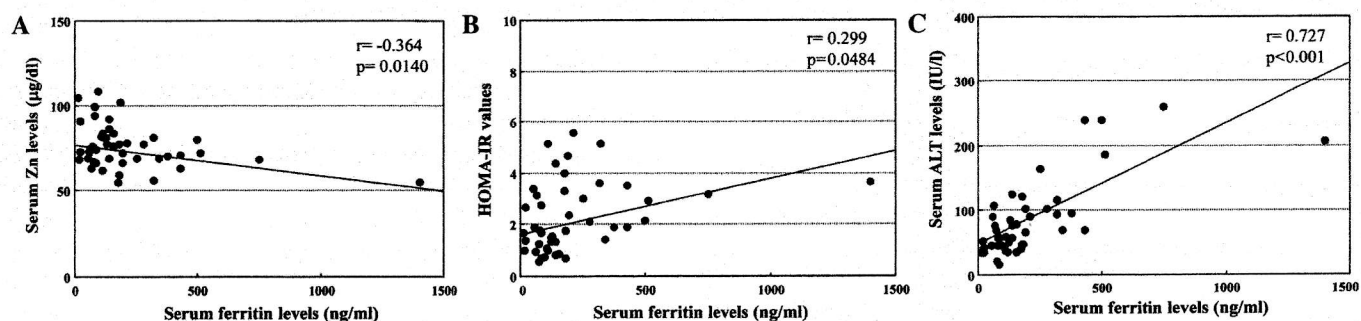


Figure 1. Correlation between serum ferritin levels and serum Zn levels (A), HOMA-IR values (B) or serum ALT levels (C) in patients with CH-C.

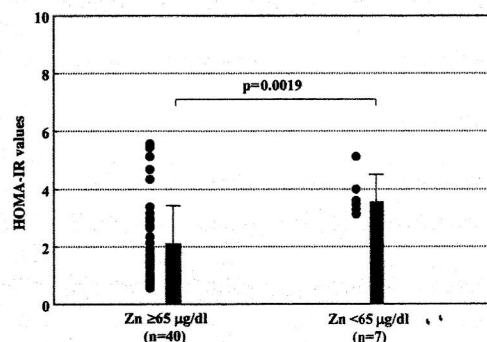


Figure 2. Comparison of HOMA-IR values between the Zn-deficient group and the group with a normal range of Zn values. Columns and bars represent the mean values \pm SDs of the enrolled patients.

levels ($r = -0.364$, $p = 0.0140$). The correlation was independent of HCV genotype or load of HCV-RNA (data not shown).

Moreover, serum ferritin levels were significantly associated with serum HOMA-IR values in the enrolled patients ($r = 0.299$, $p = 0.0484$, Fig. 1B), regardless of HCV genotype or load of HCV-RNA. On the other hand, the linear regression analysis showed a close relationship between serum ferritin and ALT concentrations in patients with CH-C ($r = 0.727$, $p < 0.001$, Fig. 1C).

Comparison of insulin resistance between Zn-deficient and normal Zn groups. The values of HOMA-IR were compared between the group with Zn-deficiency and the group whose Zn levels were within a normal range. As shown in Fig. 2, the mean value of HOMA-IR was significantly higher in the Zn-deficient than that in the normal Zn group (3.76 ± 0.66 vs. 2.08 ± 1.35 , $p = 0.0019$).

Relationship between hepatic fibrosis and serum Zn levels or HOMA-IR values. The relationship between the severity of hepatic fibrosis and serum Zn levels or insulin resistance in the enrolled patients with CH-C was investigated. CH-C patients at late stages of hepatic fibrosis (F_2 and F_3) had significantly lower serum Zn levels than those at early stages of hepatic fibrosis (F_0 and F_1) (73 ± 13 vs. 81 ± 13 $\mu\text{g/dl}$, $p = 0.0366$, Fig. 3A). However, there were no significant differences in the HOMA-IR values between early- and late-stage hepatic fibrosis patients (2.19 ± 1.55 vs. 2.44 ± 1.30 , $p = 0.5527$, Fig. 3B).

Other factors contributing to insulin resistance in patients with CH-C. Recent reports revealed that BMI (15) and hepatic

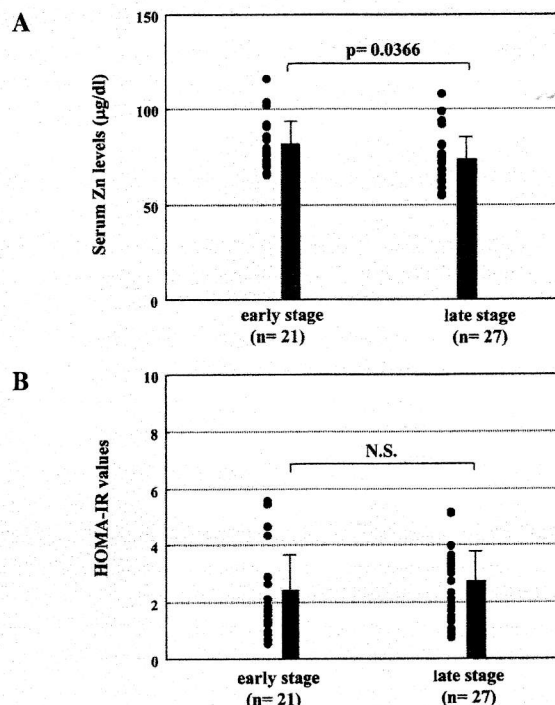


Figure 3. Comparison of serum Zn levels (A) and HOMA-IR values (B) between early- and late-stage hepatic fibrosis in patients with CH-C. Columns and bars represent the mean values \pm SDs of the enrolled patients. NS, not significant.

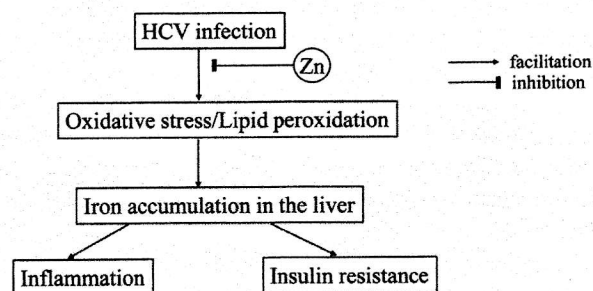


Figure 4. Postulated mechanism of insulin resistance caused by Zn deficiency in patients with CH-C.

steatosis (16) also affect insulin resistance in patients with CH-C. We confirmed that the mean HOMA-IR value in CH-C patients with hepatic steatosis of grade 2 was significantly higher than that in those without hepatic steatosis (3.88 ± 1.08 vs. 1.90 ± 1.20 , $p = 0.0044$). However, there was no significant

correlation between the HOMA-IR value and BMI ($r=0.015$, $p=0.9203$).

Discussion

In this study, we revealed that insulin resistance in patients with CH-C is closely associated with Zn deficiency (Fig. 2). Zn deficiency was found in 15% of the patients with chronic hepatitis. To our knowledge, this is the first report that describes the relationship between insulin resistance and Zn deficiency in patients with CH-C. Previously, Furutani and colleagues revealed that insulin resistance was characterized by iron accumulation in the liver (19). We confirmed the correlation between insulin resistance and iron overload in patients with CH-C and postulated that iron overload may derive from Zn deficiency. The data obtained in the present study suggest the possible mechanism of insulin resistance in patients with CH-C (Fig. 4).

It is well recognized that chronic HCV infection eventually leads to reactive oxygen species in the liver (29). The oxidative stress induced by HCV infection consequently facilitates iron storage in the liver. On the other hand, Zn plays crucial roles in antioxidative actions and confers protective effects on hepatocytes (3,4). Zn deficiency has been shown to result in attenuation of antioxidative properties and thereby a significant elevation in the serum ferritin level, which corresponds to iron accumulation in the liver. Iron overload in the liver eventually evokes insulin resistance (19), as well as necroinflammation (30) in the liver.

To date, several types of oxidative stress markers, including 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), 4-hydroxy-2'-nonenal (4-HNE) and thioredoxin, have been identified. On the other hand, superoxide dismutase (SOD) and glutathione peroxidase are well recognized as useful hallmarks for scavengers of oxidative stress. Patients with CH-C usually show increased serum and liver levels of these oxidant products as well as attenuation of antioxidant defenses. Oxidative stress sequentially leads to lipid peroxidation, hepatic steatosis and finally hepatocarcinogenesis in patients with HCV-related CLD (31).

The hypothesis that oxidative stress by persistent HCV infection enhances excessive iron accumulation in the liver is supported by a recent study (32). Fujita and colleagues revealed that hepatic 8-OHdG counts in patients with CH-C were significantly correlated with serum ferritin levels, indicating that oxidative stress caused by HCV infection is strongly associated with iron overload.

In the present study, we determined that hyperferritinemia, which reflects iron storage in the liver, in the patients with CH-C may be responsible for the Zn deficiency (Fig. 1). The decrease in the activity of copper/zinc-SOD due to Zn deficiency may account for iron overload in patients with CH-C.

An inverse correlation between serum ferritin and Zn levels is also observed in patients with β -thalassemia (33), which fulfills the criteria for secondary iron overload syndrome. Notably, iron overload and Zn deficiency in these patients may play a part in the pathogenesis of impaired glucose tolerance (34).

Hyperferritinemia primarily seems to be responsible for the down-regulation of hepcidin, which has been recognized as a peptide hormone modulating iron uptake from the small

intestine (35) and/or up-regulation of transferrin receptors (36). Miura and colleagues (35) recently revealed that a decrease in the activity of hepcidin caused by HCV-induced oxidative stress was primarily derived from i) the stabilization of the negative hepcidin regulators, hypoxia inducible factor (HIF) 1 α and HIF2 α , and ii) the hypoacetylation of histone and subsequent inhibition of the binding of two positive regulators, CCAT/enhancer-binding protein α (C/EBP α) and the signal transducer and activator of transcription 3 (STAT3), to the hepcidin promoter.

A close relationship between insulin resistance and iron overload has been observed in patients with type 2 DM (37). We also revealed that iron overload participated in insulin resistance in patients with CH-C (Fig. 1). Moreover, we confirmed that iron overload in patients with CH-C eventually caused liver damage (Fig. 1).

It has been well established that the severity of hepatic fibrosis is inversely related to serum Zn levels (11). Previous reports affirm that the degree of hepatic fibrosis is associated with insulin resistance in patients with HCV-related CLD (12,17,18). However, we did not confirm the relationship between hepatic fibrosis and insulin resistance in patients with CH-C. Other factors including iron overload may contribute to insulin resistance in patients with CH-C.

The data described above allowed us to predict that Zn supplementation may facilitate protection against oxidative stress caused by HCV infection, subsequently reduce hepatic iron accumulation and thereby improve insulin resistance. Further clinical trials are required to investigate this prediction.

In conclusion, Zn deficiency is likely to contribute to iron overload in patients with CH-C. Therefore, Zn deficiency seems to be an important factor contributing to insulin resistance in patients with CH-C.

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