

Selenium deficiency is associated with insulin resistance in patients with hepatitis C virus–related chronic liver disease

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Abstract

The relationship between selenium (Se) deficiency and insulin resistance has not much been established in persistent hepatitis C virus (HCV) infection, although Se deficiency is often observed in patients with liver cirrhosis. We hypothesized that the decreased serum Se levels were associated with the severity of hepatic fibrosis or insulin resistance in patients with HCV-related chronic liver disease (CLD). To test the hypothesis, 52 patients with HCV-related CLD including chronic hepatitis and liver cirrhosis were enrolled in this study. The severity of hepatic fibrosis was divided into 4 categories (F₁ through F₄) according to the new Inuyama classification. Insulin resistance was defined by the homeostasis model for assessment of insulin resistance value. Serum Se levels significantly declined in proportion to the severity of hepatic fibrosis and were positively correlated with serum albumin ($r = 0.372$, $P = .0065$) and zinc ($r = 0.403$, $P = .0081$) concentrations. Serum Se levels were also linked to glutathione peroxidase activities in the sera of the enrolled patients ($r = 0.374$, $P = .0148$). By contrast, serum Se levels were inversely correlated with the homeostasis model for assessment of insulin resistance values ($r = -0.304$, $P = .0338$). However, serum Se levels were independent of HCV genotype and loads of HCV-RNA. These findings suggest that Se deficiency was associated with the severity of hepatic fibrosis in patients with HCV-related CLD and that Se deficiency was likely to be one of the factors contributing to insulin resistance in those patients.

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Keywords:

Chronic liver disease; Glutathione peroxidase; Hepatitis C virus; Hepatic fibrosis; Insulin resistance; Selenium

Abbreviations:

Alb, albumin; ALT, alanine aminotransferase; BMI, body mass index; CLD, chronic liver disease; DM, diabetes mellitus; GPx, glutathione peroxidase; HCV, hepatitis C virus; HOMA-IR, homeostasis model for assessment of insulin resistance; NHC, normal healthy controls; Se, selenium; Zn, zinc.

1. Introduction

Selenium (Se), one of the essential trace elements for human beings, has a major metabolic significance. It has been well recognized that Se is incorporated as the amino

acid selenocystein during translation of primary structures [1]. Se also plays an important role as an essential constituent of selenoproteins [2]. Approximately 100 kinds of selenoproteins are speculated to exist in mammalian systems [3]. To date, glutathione peroxidases (GPxs), thioredoxin reductases, iodothyronine deiodinases, and selenoprotein P have been identified as major selenoproteins [4,5].

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It has been well known that severe Se deficiency often causes a fatal cardiomyopathy like Keshan disease [6] and degenerative osteoarticular disorders such as Kashin-Beck disease [7]. In liver disease, Se deficiency has been frequently observed in patients with alcoholic liver cirrhosis [8–14] and primary biliary cirrhosis [9,12,15]. According to cited reports, plasma Se levels in patients with liver cirrhosis declined in proportion to the severity of the impaired hepatic reserve [12–14].

On the other hand, the relationships between insulin resistance and abnormalities in metabolism of trace elements including Se [16,17], zinc (Zn) [18], copper [19], and chromium [20] have been widely discussed in experimental animal models of type 2 diabetes mellitus (DM) and/or patients with type 2 DM. Serum Se levels frequently decline in patients with type 2 DM [16,17].

Recently, a decline in serum Se concentration was shown in patients with hepatitis C virus (HCV)–related chronic liver disease (CLD) [21–23]. It has been well established that persistent HCV infection often evokes metabolic abnormalities, including hepatic steatosis [24], insulin resistance [25], dyslipidemia [26], and iron overload [27]. However, the association of Se deficiency with insulin resistance has not been well established in patients with HCV-related CLD. Our hypothesis is that serum Se levels decline in proportion to the severity of hepatic fibrosis in patients with HCV-related CLD, and the decrease in Se levels contribute to insulin resistance in those patients. To test our hypothesis, we examined the correlation between the serum Se levels and the severity of hepatic fibrosis and insulin resistance in patients with HCV-related CLD.

2. Methods and materials

2.1. Study population

Fifty-two nondiabetic patients with HCV-related CLD, who had HCV-RNA detectable in their sera by polymerase chain reaction and showed histological characteristics consistent with chronic hepatitis or liver cirrhosis, were randomly selected for this study. This clinical study was approved by the ethics committee of Kagawa University School of Medicine. Fully informed consent was obtained from each participant. Ages at entry, sex, and body mass index (BMI) were examined in the enrolled patients with HCV-related CLD (Table 1). As a comparison group, 11

cases of normal healthy controls (NHC) were also assigned to this study. None of the NHC cases took Se supplements.

2.2. Laboratory assessments

Liver function tests including serum alanine aminotransferase (ALT) and albumin (Alb) were assessed in the enrolled patients. Serum Se and Zn levels were assessed at fasting in the morning because Zn has a circadian rhythm [28]. Glutathione peroxidase levels in sera were analyzed using a commercially available enzyme-linked immunosorbent assay kit (Chyman Chemical Company, Ann Arbor, USA). Insulin resistance was determined by the homeostasis model for assessment of insulin resistance (HOMA-IR) method using the following equation: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mg/dL)} / 405$ [29]. Quantitative detection of serum HCV-RNA was performed by Amplicor-HCV monitor assay (Roche Molecular Diagnostics, Tokyo, Japan) [30]. The HCV genotype was determined by the HCV-RNA genotyping assay system (Home Brew SRL Inc, Tokyo, Japan) [31].

2.3. Histological assessments

Liver tissue specimens were obtained by liver biopsy using 16-gauge needles under the guidance of ultrasound. The tissue samples were fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin. Histological staging was performed using the new Inuyama classification system, which is the standard criterion for the histological assessment of chronic hepatitis in Japan [32]. The stages in chronic hepatitis were classified from F₀ through F₃. F₀ was defined as no fibrosis in the liver, whereas F₄ was defined as liver cirrhosis. Fibrous portal expansion was observed in F₁. Bridging fibrosis and bridging fibrosis with lobular destruction were present in F₂ and F₃, respectively.

2.4. Statistical analyses

Data are represented as means \pm standard deviations and were analyzed using the commercially available software, SPSS version 11.0 software (SPSS Inc, Chicago, IL). The Bonferroni/Dunn method was applied for comparison of more than 2 groups. Linear regression analysis was used to analyze the correlations among variables. *P* values less than .05 were considered to indicate significance.

3. Results

3.1. Demographic features of patients enrolled in this study

Clinical characteristics of the enrolled patients are shown in Table 1. Of the enrolled patients with HCV-related CLD, 30 were men and the rest were women. The patients' ages ranged from 30 to 76 years. Hepatic fibrosis in these patients was evaluated as follows: 19 patients in F₁, 13 in F₂, 10 in F₃, and 10 in F₄. On the other hand, 30 patients were infected

Table 1
Clinical characteristics of the enrolled patients with HCV-related CLD

Age (y)	57.7 \pm 10.47 (30–76)
Sex (male/female)	30/22
Staging (F ₁ /F ₂ /F ₃ /F ₄)	19/13/10/10
HCV genotype (1b/2a/2b)	30/15/7
Loads of HCV-RNA (KIU/mL)	1024 \pm 933 (54–3800)
BMI	23.7 \pm 2.8 (17.2–31.9)
HOMA-IR values	1.96 \pm 1.00 (0.51–5.13)

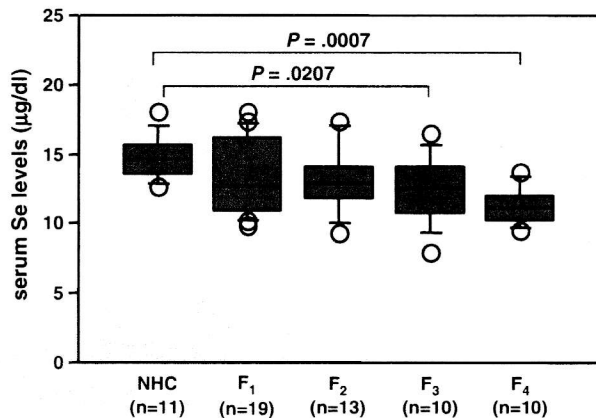


Fig. 1. Relationship between serum Se levels and degrees of hepatic fibrosis in patients with HCV-related CLD.

with genotype 1b, whereas 15 and 7 patients were infected with genotype 2a and 2b, respectively. Overall, BMI and HOMA-IR values in these patients were 23.7 ± 2.8 and 1.96 ± 1.00 , respectively.

3.2. Correlation between serum Se levels and hepatic fibrosis in patients with HCV-related CLD

Fig. 1 shows the mean serum Se level in each stage of hepatic fibrosis. Serum Se levels declined in proportion to the severity of hepatic fibrosis. Overall serum Se levels in F₃ (12.5 ± 2.4 µg/dL) and F₄ (11.3 ± 1.3 µg/dL) were significantly lower than that in NHC (14.8 ± 1.6 µg/dL).

3.3. Correlation between serum Se levels and GPx activities or ALT levels in patients with HCV-related CLD

We examined the correlation between serum Se concentrations and GPx activities in their sera (Fig. 2A). Glutathione peroxidase activities were elevated in proportion to serum Se levels in patients with HCV-related CLD ($r = 0.374$, $P = .0148$). However, no significant correlation between serum Se and ALT levels was found in patients with HCV-related CLD (Fig. 2B).

3.4. Correlation between serum Se and Alb or Zn levels in patients with HCV-related CLD

As shown in Fig. 3A, serum Se levels positively correlated with serum Alb levels ($r = 0.372$, $P = .0065$), suggesting that the synthesis of Alb might depend on the serum Se concentration. Linear regression analysis revealed a positive correlation between serum Se and Zn levels in the enrolled patients with HCV-related CLD ($r = 0.403$, $P = .0081$; Fig. 3B).

3.5. Correlation between serum Se or Zn levels and insulin resistance in patients with HCV-related CLD

The relationship between serum Se levels and HOMA-IR values was investigated in the enrolled patients with HCV-related CLD. Serum Se levels inversely correlated with

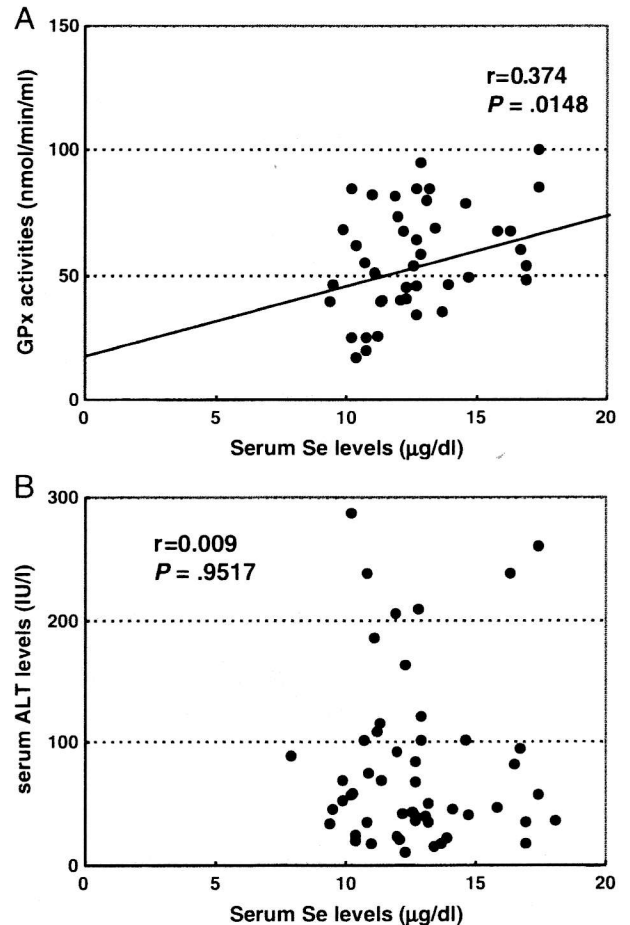


Fig. 2. Relationship between serum Se levels and GPx activities (A) or ALT levels (B) in patients with HCV-related CLD.

HOMA-IR values ($r = -0.304$, $P = .0338$; Fig. 4A). A significant inverse correlation between serum Zn concentrations and HOMA-IR values was also found in these patients ($r = -0.514$, $P = .0008$; Fig. 4B). These results indicated that insulin resistance was associated with the degree of Se and Zn deficiency in patients with HCV-related CLD.

3.6. Correlation between serum Se levels and loads of HCV-RNA or HCV genotypes in patients with HCV-related CLD

Fig. 5A shows that there was no significant relationship between serum Se concentrations and loads of HCV-RNA in the enrolled patients. Serum Se levels were also independent of HCV genotypes in the enrolled patients (Fig. 5B).

4. Discussion

In the present study, we observed that a decrease in serum Se levels was significantly associated with the severity of hepatic fibrosis in patients with HCV-related CLD, indicating that our hypothesis was correct. The Se content in the average Japanese diet is estimated to be approximately 100 µg/d [33]. Thus, there is little possibility that the Se

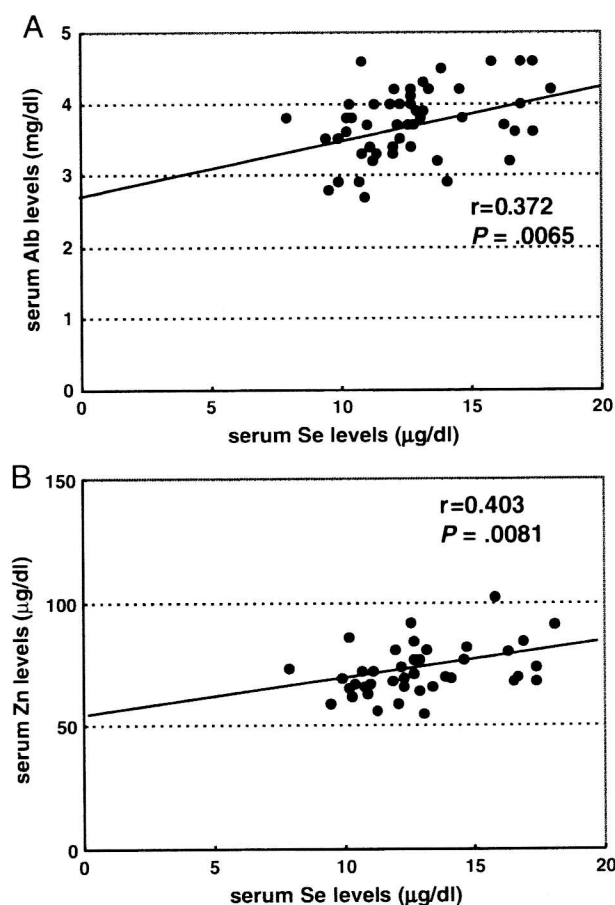


Fig. 3. Relationship between serum Se levels and Alb (A) or Zn (B) levels in patients with HCV-related CLD.

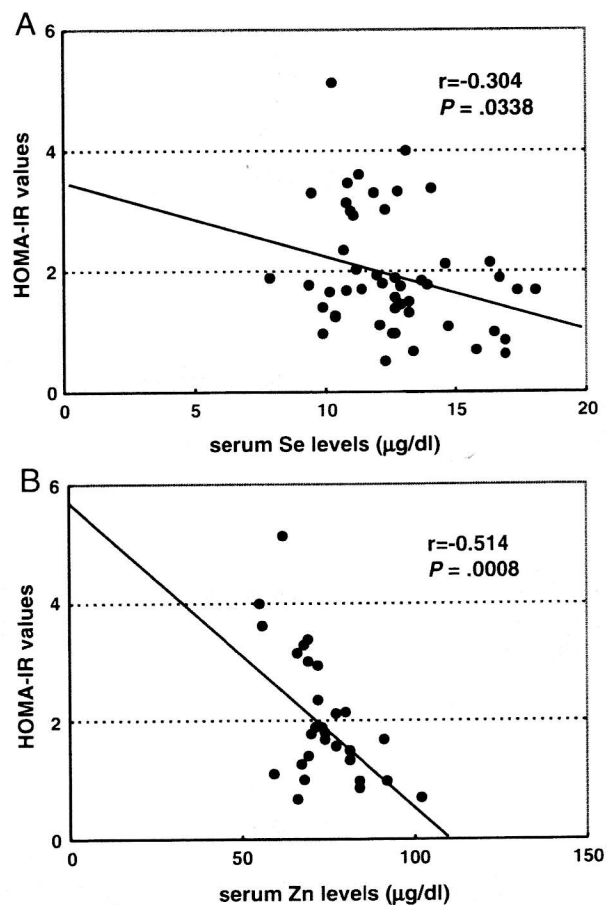


Fig. 4. Relationship between insulin resistance and Se deficiency (A) or Zn deficiency (B) in patients with HCV-related CLD.

deficiency in Japanese patients with HCV-related CLD could be attributed to inadequate Se intake. On the other hand, a previous study demonstrated that the amount of urinary Se excretion in patients with CLDs was almost equivalent to that in NHC [12]. Therefore, malabsorption from the small intestine may account for Se deficiency in patients with HCV-related CLD. Indeed, mucosal abnormalities of the small intestine were revealed in patients with liver cirrhosis [34]. It can be speculated that the mucosal abnormalities of the small intestine resulted in the malabsorption of Se and consequently led to Se deficiency in patients with HCV-related CLD.

It has been conceivable that the administration of Se attenuated hepatic fibrosis in an experimental animal model of liver fibrosis [35]. Selenium supplementation is likely to diminish the degree of oxidative stress, which causes reduced collagen formation and increased collagen degradation, rather than directly inhibiting hepatic fibrosis [35].

We ascertained that activities of GPx were decreased in relation to the severity of Se deficiency in patients with HCV-related CLD because GPx is one of the major selenoproteins. Glutathione peroxidase primarily serves as an oxidative stress scavenger [5]. Therefore, Se deficiency

eventually facilitates oxidative stress in the liver of patients with HCV-related CLD [22]. However, we could not reveal the relationship between Se deficiency and the elevation of serum ALT levels in this study, which was consistent with a previous study [36]. On the other hand, the decline of selenoprotein P activity is also speculated to be in proportion to the decrease in serum Se concentration [37] because selenoprotein P serves as a carrier protein of Se. Unfortunately, we did not examine that in this study. It is noteworthy that selenoprotein P has been shown to prevent lipid peroxidation and subsequent liver injury by supplementation of Se in Se-deficient rats [38].

Serum Se levels were also associated with serum Alb levels in patients with HCV-related CLD, as shown in previous studies involving patients with liver cirrhosis [10,11,13]. We presumed that Se deficiency was associated with the deterioration of the hepatic reserve [8,13]. Välimäki et al [8] documented that the serum Se concentration in patients with hypoalbuminemia of the renal organ was within reference range. On the other hand, Se is likely to be mainly transported in globulin from the small intestine to the liver [39]. Hence, Se deficiency did not result from the depletion of the Se-binding protein in patients with HCV-related CLD.

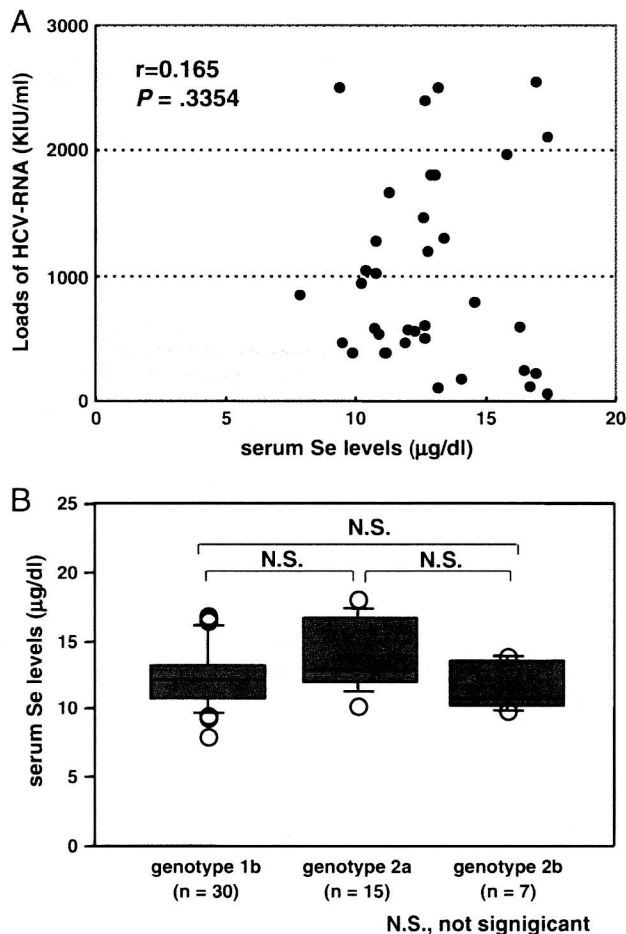


Fig. 5. Relationship between serum Se levels and loads of HCV-RNA (A) or HCV genotypes (B).

We provided the evidence that serum Se levels were closely related to serum Zn levels in patients with HCV-related CLD, which was similar to that from a previous report [12]. Se deficiency is not likely to directly affect Zn status in patients with HCV-related CLD. Se deficiency could result in hypoalbuminemia and subsequently lead to malabsorption of Zn from the small intestine of patients with HCV-related CLD [40] because Alb largely exerts as a carrier protein of Zn [41].

Our findings reveal an inverse correlation between serum Se concentration and HOMA-IR values obtained from patients with HCV-related CLD, supporting our hypothesis that Se deficiency was associated with insulin resistance in those patients. Previous studies revealed that insulin resistance was also associated with hepatic steatosis [42] and iron overload [43] in patients with HCV-related CLD. We confirmed that all of these factors contributed to insulin resistance in these patients. We recently revealed a relationship between Zn deficiency and insulin resistance in patients with chronic hepatitis C [44]. To the best of our knowledge, this is the first report on the relationship between insulin resistance and Se deficiency in patients with HCV-

related CLD, although the relationship between a depressed serum Se concentration and insulin resistance was already shown in patients with type 2 DM [16,17].

It has been well recognized that Se seems to have insulin-mimetic effects [45,46]. Selenate plays crucial roles in the translocation of glucose transporters and the activation of mitogen-activated protein kinases. We postulated the mechanism of insulin resistance deriving from Se deficiency as follows: Se deficiency may result in higher amount of insulin secretion from B cells in the pancreas as a complementary effect and subsequently evoke insulin resistance in patients with HCV-related CLD.

Recent studies demonstrated that administration of sodium selenate attenuated insulin resistance in an experimental animal model of type 2 DM [47,48]. However, previous reports showed that oral Se intake exacerbated glucose tolerance in patients with type 2 DM [49,50]. Unfortunately, serum Se concentrations were not examined before the treatment with selenate in those studies. Those controversial results may be responsible for the narrow therapeutic range of Se. If serum Se levels before the treatment with selenate are below the lower limit of reference range, the administration of selenate will provide a beneficial effect. However, when the serum Se concentrations before the treatment with selenate are within reference range, the additional Se supplementation resulted in overall Se levels that exceeded the therapeutic range and consequently exacerbated insulin resistance in patients with type 2 DM [49]. In the present study, the efficacy of Se supplementation was not confirmed, although the decline of serum Se concentration resulted in insulin resistance in patients with HCV-related CLD. This is the limitation of this study. Therefore, we have to determine an optimal dose of Se supplementation in patients with HCV-related CLD to attenuate insulin resistance. Further examinations will be required to clarify that.

Selenium deficiency is also associated with carcinogenesis, including prostate cancer, hepatocellular carcinoma, and colorectal cancer [51]. Selenium appears to act through the inhibition of androgen receptor signaling in the prostate cancer cells [52]. Thus, Se deficiency results in the elevation of serum prostate-specific antigen levels in patients with prostate cancer. Recently, a large-scale trial (termed the *Nutritional prevention of cancer (NPC) trial*) on the efficacy of Se supplementation (200 μg/d) in patients with skin cancer was carried out [53]. The administration of Se did not affect the recurrence rate of skin cancer, although Se supplementation reduced the incidence of prostate, lung, and colorectal cancer development. Another previous trial revealed that Se supplementation provided a protective effect only for patients who had lower levels of baseline serum Se [54]. Surprisingly, the NPC trial raised a possibility that the administration of Se increased the risk for development of type 2 DM [53].

We found a lack of association between Se deficiency and loads of HCV-RNA or HCV genotypes in patients with HCV-related CLD, although Ko et al [22] revealed an

inverse correlation between serum Se levels and loads of HCV-RNA in those patients. These results may imply that host factors rather than viral factors affect Se deficiency in those patients.

In summary, insulin resistance was associated with the severity of Se deficiency in patients with HCV-related CLD. We should take Se deficiency into consideration as one of the factors affecting insulin resistance in patients with HCV-related CLD.

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