

# Serum Copper And Zinc Levels In Addicts With Hepatitis C Virus Infection: A Case-Control Study In Hamadan, Iran

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## Abstract:

**Background:** The study aimed to investigate the serum levels of copper and zinc in addicts with Hepatitis C virus (HCV) infection and compare them with those of non-infected addicts.

**Materials and Methods:** The current case-control study was conducted on 74 addicts with HCV infection and 74 non-infected addicts matched by age and gender referring to Imam Khomeini Clinic in Hamadan, Iran. Copper and zinc serum levels were measured by the enzyme-linked immunosorbent assay. Data were analyzed with STATA software and the level of significance was 95%.

**Results:** The mean serum level of copper in the case and control groups was  $154.29 \pm 62.75$  and  $132.85 \pm 35.42$   $\mu\text{g/dL}$ , respectively ( $P = 0.011$ ), and the mean zinc level was  $70.34 \pm 19.9$  and  $37.07 \pm 19.9 \pm 29.88$   $\mu\text{g/dL}$ , respectively ( $P < 0.001$ ). There was no significant correlation between age, gender, and viral load with copper and zinc serum levels in the case group. However, in both the case and control groups, there was a positive and significant correlation between AST and ALT with copper serum levels, and a significant and reverse correlation between zinc and AST serum levels ( $P < 0.05$ ).

**Conclusion:** Therefore, the study concludes that in addicts with HCV infection, copper serum level increases and zinc level decreases. There is a correlation between serum copper changes and ALT and AST levels as well as serum zinc changes and AST levels.

**Key Word:** Copper; Zinc; Hepatitis C; Hamadan

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## I. Introduction

Hepatitis C is a single-stranded RNA virus with six different variants; according to the World Health Organization report, about 3% of the world's population is infected with HCV [1] of which at least 21.3 million live in East Mediterranean region [2]. The prevalence of this infection in the general population of Iran is less than 1% [3]. Hepatitis C virus (HCV) infection is epidemic throughout the world as a disease transmitted through blood or blood products [4]. In developed countries, the main route to transmit the disease is through the use of infected needles among injection drug users. However, in developing countries it is more likely to be transmitted through blood donations, especially in those who are in need of a blood supply [5]. Due to the strong tendency of the disease to be developed into chronic form, the liver is the main organ affected by HCV infection. Since the metabolism of many substances and elements, including copper and zinc, carries out in liver, the metabolism of these elements may also be impaired in liver diseases, and consequently, the concentration of these elements may also change. Minerals play different roles in immunological, physiological, and biochemical reactions [6]. Since these elements are metabolized in the liver, the concentration of each of these elements can be affected by various liver diseases [7]. Zinc is a micronutrient, which its primary metabolism occurs in the liver cells. This element affects the growth, development, and function of the immune system [6]. It is also a structural component of a wide range of enzymes, proteins, neuropeptides, hormone receptors, and polynucleotides [8]. Studies showed that Zinc has an antiviral effect on HIV, rhinoviruses, and herpes simplex virus. In addition, it has anti-inflammatory and anti-oxidant properties [9]. Metallothionein is the main and core protein in the regulation of zinc concentration, which its serum level changes with the production of various cytokines; however, the production of cytokines is affected by infections [10]. Since zinc deficiency

is one of the causes of defective regeneration and may play an important role as a negative regulator in HCV proliferation, zinc supplements can be a new approach to treat HCV infection [11]. The serum and tissue levels of zinc and copper are affected by stress, trauma, and infections, which under such circumstances the serum level of zinc decreases and that of copper increases; it is attributed to IL-1, which increases in the acute phase of infection [10]. Copper is essential for a variety of biochemical processes as well as the nervous system activity [12]. Copper acts as an anti-hepatofibrosis cofactor in chronic liver diseases. When hepatitis progresses toward cirrhosis, the serum level of copper increases [7]. Some evidence suggests that reduced iron and copper serum levels can improve the response to chronic hepatitis treatment, and even zinc ions in serum can affect the HCV load [6]. Possibly, by measuring parameters such as copper and zinc in injection drug users with HCV infection and being aware of the serum level changes of these elements, a relationship can be found between HCV infection, and copper and zinc serum levels, and even a new approach be introduced to diagnose, treat, and determine the degree of disease progression. Considering the limited research in this field in Iran and other countries, the current study aimed at comparing the serum levels of copper and zinc in injection drug users with chronic HCV infection with those of their non-infected healthy counterparts.

## **II. Material And Methods**

The current case-control study was conducted on the subjects selected from injection drug users infected with HCV referring to Imam Khomeini Clinic in Hamadan Province that their disease diagnosis was confirmed by HCV RNA PCR. The case and control group subjects, matched by age and gender, were selected from injection drug users referring to a behavioral disease counseling center, but the controls were not afflicted with any type of hepatitis. Based on the results of the study by Cesur et al., [13] at 95% confidence level and 90% test power, the sample size was 148, which included 74 injection drug users infected with HCV as the case group and 74 non-HCV infected injection drug users as the control group. After obtaining a written consent from the participants in both groups, their information was collected using a checklist including demographic data such as age and gender, and some other data recorded in their profiles including virus genotype, the liver enzymes and bilirubin serum levels. A 5-mL of venous blood sample was taken from each subject in laboratory and stored at -20°C. Then, the serum levels of copper and zinc were measured by the enzyme-linked immunosorbent assay (ELISA) technique (Randox kit; UK) and the results were recorded on the checklist. The inclusion criteria were the absence of comorbid liver diseases, lack of taking vitamin supplements during the past six months, lack of receiving any treatment for both groups, and the HCV-RNA positivity for at least six months in the case group. The data were analyzed with STATA software version 11. In order to compare the mean serum levels of copper and zinc between the two groups and in terms of two-component qualitative (nominal) variables, the Mann-Whitney test, in terms of the multi-component qualitative (ranked) variables, the Kruskal-Wallis test, and to compare the serum levels of zinc and copper in terms of quantitative variables (age and serum liver enzymes levels), Spearman correlation coefficient were used. All statistical analyzes were performed at 95% confidence level.

## **III. Result**

In the current study, a total of 148 eligible patients who signed the written consent form were enrolled. There were no dropouts in the sample size.

Of the total 148 subjects, 70 (94.6%) were male and 4 (5.4%) females. Both the two groups were individually matched by gender. The mean age of the patients in the case and control groups was  $42.74 \pm 11.93$  and  $42.47 \pm 12.29$ , respectively; the two groups were matched by mean age too. Based on the non-parametric Mann-Whitney test results, the serum level of copper was significantly higher in the HCV-infected drug users than the controls ( $P = 0.012$ ), while their serum level of zinc was significantly lower than that of the controls ( $P < 0.001$ ). Also, according to the results of the student t-test, copper: zinc ratio in the HCV-infected addicts was significantly higher than that of the controls ( $P < 0.001$ ).

Based on the results of the non-parametric Mann-Whitney test, no significant difference was observed between the male and female subjects in the case group in terms of the mean serum levels of copper and zinc ( $P = 0.232$ ). According to the results of non-parametric Kruskal-Wallis test, no significant difference was observed among the different genotypes of HCV in the case group in terms of mean serum levels of copper and zinc.

Based on the results of the non-parametric Kruskal-Wallis test, there was no significant relationship between serum levels of zinc and copper and viral load in the case group ( $P = 0.273$ ). In the case and control groups, there was a significant and positive correlation between serum level of copper and AST and ALT levels; while the correlation was significant and reverse between serum levels of zinc and ASL ( $P < 0.05$ ).

## **IV. Discussion**

In the present study, the serum level of copper and copper: zinc ratio were significantly higher in the case group than the controls; while the serum level of zinc was lower in the case group than the controls. There

was no significant difference between gender and viral load in the case group with the mean serum levels of copper and zinc. There was a significant and positive correlation between AST-ALT and copper serum levels; while the correlation was significant and reversed between zinc and AST serum levels; however, no significant correlation was observed between age and serum bilirubin level with serum levels of copper and zinc.

In a study by Kalkan et al., (2000) on 52 patients infected with different types of hepatitis virus (A, B, C, D and E), the serum copper level was significantly higher and the serum zinc level was significantly lower in patients compared to those of 52 healthy controls [14]. The results of the current study were consistent with those of the study by Kalkan et al. However, the sample size of the present study was larger than that of their study, and the present study only recruited patients with chronic HCV infection.

In a study by Nakayama et al., In Japan on 147 healthy controls, patients with HCV infection, liver cirrhosis, and hepatocellular carcinoma, the serum level of copper in the control group was at its minimum permissible level, while it was slightly higher in patients with chronic HCV infection compared to the controls, and the increasing trend was even higher in patients with HCC and cirrhosis, respectively. On the contrary, the highest zinc serum level was found in the control group, while it decreased respectively in the liver cirrhosis group, followed by miscellaneous and chronic hepatitis groups; the lowest serum level of zinc was found in patients with HCC. The lowest copper: zinc ratio was observed in the control group; while it was significantly higher in other groups [15]. The results of the current study in terms of serum level of copper, zinc, and copper: zinc ratio was consistent with those of the study by Nakayama et al. In the current study, cirrhosis and HCC cases were not examined; in addition, viral load and the liver enzymes levels were used in the present study to determine the severity of the disease, in which viral load showed no significant correlation with serum levels of copper and zinc, but the correlation between liver enzymes (AST, ALT) and serum copper levels as well as zinc and AST levels were significant.

In a study by Meram et al., in Turkey on 43 patients with chronic hepatitis and 30 healthy controls, serum levels of copper, zinc, ALT, AST and bilirubin were compared between the both groups and the results showed that the serum levels of AST, ALT, total and direct bilirubin, and copper were significantly higher in patients than in controls, but the difference was not statistically significant between the two groups in terms of serum level of zinc [9]. The results of the current study were in line with those of the study by Meram et al., in terms of the high level of copper and liver enzymes (AST and ALT) in the injection drug users with chronic hepatitis. But in terms of serum bilirubin and zinc levels, the results were inconsistent; the difference may be due to the effect of interferon treatment, or different sample sizes. In the study by Meram et al., a number of patients were treated with interferon, and the sample size of their study was smaller than that of the present study.

Other studies comparing serum copper and zinc levels are as follows:

The study by Ko et al., in China compared 33 patients with HCV infection with 31 healthy subjects [6]; the study by Grungreiff et al., in Germany compared 50 patients with HCV infection with 30 healthy controls [10]; Afridi et al., studied 253 female patients with different types of hepatitis [16]; and studies by Saod et al., in Iraq [17] and Kolachi et al., compared 438 patients with different types of hepatitis with 177 healthy subjects not infected with hepatitis [18], consistent with the results of the present study, serum copper levels significantly increased and serum zinc level decreased in the injection drug addicts with HCV infection. In studies comparing only serum zinc levels between patients with and without hepatitis, including the study by Nazir et al., on 40 patients with HCV infection and 15 healthy individuals [19], and the study by Reda in Egypt on 50 healthy subjects and 70 patients with chronic HCV [11], serum zinc level significantly decreased in patients compared to controls, which was consistent with the results of the current study. But in terms of other diseases, including benign and malignant tumors of the ovary [20], liver cirrhosis following HBV and HCV infections, and bladder cancer [21], the alterations in serum levels of copper and zinc were also studied, and the results were similar to those observed in HCV-infected patients. In contrast to the results of the current study, Cesur et al., compared 17 patients with HCV infection with 17 healthy subjects [13], Ali et al., studied 60 patients with chronic HCV and 50 healthy subjects and reported no significant difference between the case and control groups in terms of serum levels of copper and zinc [22]; the contradictory between the results can be attributed to the small sample size of both studies compared to that of the present study.

There was no significant difference in the serum zinc level in the study by Nazir et al., between male and female patients [19] that was consistent with the results of the current study. It should be noted that the number of female subjects in both case and control groups was very low in the present study, which is probably due to the lower incidence of HCV in women than men.

## **V. Conclusion**

In patients with chronic HCV, serum levels of copper increases and serum level of zinc decreases. There is a significant correlation between serum copper changes and ALT-AST levels as well as serum zinc changes and AST level.

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