Plasma copper and zinc levels in chronic viral hepatitis

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ABSTRACT

Objective: The relationship between chronic hepatitis and trace metals has not been understood clearly. Copper (Cu) and zinc (Zn) are essential trace elements for several metabolic processes. Overload or deficiency of these elements can lead to metabolic disorders and some other diseases. In this study, we aimed to examine the relationship between chronic hepatitis and plasma Cu and Zn levels.

Methods: Forty-three patients with chronic viral hepatitis (CVH) and 30 healthy controls were included in this study. The patients were consecutively admitted to the Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Gaziantep University, Turkey, between January 2000 and November 2000. Plasma Cu and Zn levels, and hepatic function test results of the patients and controls were compared. Serologic and virologic markers, and histopathologic assessments were performed for confirmation of CVH. Plasma Cu and Zn concentrations were determined with Bathocuproin, using deproteinization method and 5-Br-PAPS methods.

Results: Patients plasma Cu level was 16.0 ± 2.8 and plasma Zn level was 26.0 ± 7.3. The corresponding values were 12.2 ± 5.4 and 26.6 ± 5.6 in the healthy controls. The patients with CVH had a higher plasma Cu level than the controls ($p<0.05$) while the Zn levels were similar in both groups ($p>0.08$).

Conclusion: Although there is an increased plasma Cu level in CVH, its mechanism is unclear. However, this condition may have clinical importance as Cu is a hepatotoxic element.

Saudi Med J 2004; Vol. 25 (8): 1066-1069

Copper (Cu) is an important trace element, and is associated with a number of metalloproteins.[1-3] The major functions of Cu metalloproteins are oxidation-reduction reactions as Cu-containing enzymes bind and react directly with molecular oxygen. A number of pathological conditions have been attributed to the loss of cuproenzyme activity.[1] During infections or inflammatory stress, serum Cu concentration increases due to acute-phase activity of interleukin-1 (IL-1). Elevated serum Cu concentrations are seen in portal cirrhosis and hepatitis, due to excessive Cu, which is normally excreted in the bile, is probably retained in the circulation.[4-6] The metabolic functions of zinc (Zn) are based largely on its presence as an essential component of many metalloenzymes involved virtually in all aspects of metabolism. Zinc is an integral component of nearly 300 enzymes in different species of all phyla.[7] Zinc plays a major role in protein synthesis, and has an important function in gene expression.[8-10] Zinc has been studied for its antiviral effect in HIV,[11] rhinovirus[12] and herpes virus.[13] In addition to its anti-inflammatory effect, Zn has an antioxidant effect,[14] which has a radical scavenging and immunomodulatory effects. Zinc supplementation enhances the response to interferon therapy in patients with intractable chronic hepatitis C.[15]

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Received 13th January 2004. Accepted for publication in final form 27th March 2004.

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Chronic hepatitis B virus infection (HBV) is a serious condition. One-third to one-forth of the patients who were chronically infected by HBV are expected to have progressive liver disease such as cirrhosis and primary liver cancer.[16] In HBV disease, the immune system of the host attacks HBV infected hepatocytes, thereby cause liver injury.[17] Hepatitis C virus infection (HCV) is a relatively common disease. Almost 3% of the world population are chronically infected with HCV, which accounts for approximately 20% of causes of acute hepatitis and 70% of cases chronic hepatitis. Chronic HCV is a major cause of cirrhosis and hepatocellular carcinoma.[17,18] The humoral response plays a central role in the neutralization of free viral particles, but its protective function is limited against intracellular virus.[19] The severity of the liver injury reflects the strength of the immune response: the strongest response causes the greatest injury and likelihood of viral clearance. Patients with chronic liver disease show impaired trace element metabolism, namely high levels of iron and Cu and low levels of Zn, selenium, phosphorus, calcium and magnesium.[10]

The relationship between chronic hepatitis and trace metals has not been understood clearly. Copper and Zn are essential trace elements required for several metabolic processes. Overload or deficiency of these elements can lead to metabolic disorders and some other diseases. For the purpose of this study, we investigated the relationship between chronic viral hepatitis (CVH) and plasma Cu and Zn levels.

Methods.

Between January 2000 and November 2000, 43 patients with CVH consecutively admitted to the Department of Infectious Disease and Clinical Microbiology, University Hospital, Turkey were included in this study. Thirty age and sex matched healthy subjects were also included, and comprised of the control group. The diagnosis of CVH was based on serologic and virologic markers, and histopathologic assessments were performed for confirmation of CVH. Plasma Cu, Zn, alanine aminotransferase (ALT), aspartate transaminase (AST) and bilirubin levels were assessed in both groups.

Sample collection. After 12 hours of fasting, venous blood samples were collected using standard venipuncture technique between 9:30-11:00 a.m. Sera was separated immediately by centrifugation at 3000 G for 10 minutes, and stored at -20°C until analysis was performed in the same run. Hemolyzed specimens were excluded. Plastic disposable syringes with stainless steel needles were used for blood collection. Glassware was cleared of surface trace metal contamination by soaking overnight in nitric acid-hydrochloric acid (1:3 V/V) solution, followed by rinsing with deionized water. Water met specifications for type. One water with ≥14 μg/cm^2 resistance and iron, Zn and Cu content of <10 μg/l .[1]

Measurement of metal content. Plasma Cu and Zn concentrations were determined with Bathocuproin using deproteinization method (Boehringer Mannheim GmBh. Mannheim, Germany) and 5-Bromo-adenosine 3'-phosphate-5'phosphosulfate (5-Br-PAPS) method (Elitech Diagnostics, Sees, France) according to manufacturer's instructions.

Statistical analysis. Plasma Cu and Zn levels, and hepatic function test results were compared between the groups as well as in the patients with CVH were treated with interferon and to those who did not take any interferon treatment. Statistical Package for Social Sciences 9.0 (Chicago, Illinois. USA) and Med calc (Medcale Software, Mariakerke, Belgium) programs were used for statistical analyses. Comparison of the groups was performed using analysis of variance (ANOVA) and x^2 test. Data were presented as mean ± SEM.

Results.

The groups were age and gender matched (p>0.05). The ages ranged from 18-70 years (mean 41.2 ± 12.7 years) in CVH group and from 18-76 years (mean 38.7 ± 15.0 years) in the control group. Of 43 patients, 20 (46.5%) were taking interferon treatment, and 23 (53.5%) were new cases without treatment. Plasma Cu, ALT, AST, and total and direct bilirubin levels of the patients with CVH were significantly higher than the controls (p=0.042, p<0.001, and p<0.003). Plasma Cu concentration was 16.0 ± 2.8 mmol/L (11.9-19.7) in the patients and 12.2 ± 5.4 mmol/L (1.2-22. 8) in the control group (**Table 1**) (**Figure 1**). Plasma Zn concentrations of CVH [26.0 ± 7.3 mmol/L (9.8-45)] and control [26.6 ± 5.6 mmol/L (13.3-37.8)] groups were similar (p=0.53). Plasma AST level was significantly lower in the patients treated with interferon than in the patients without treatment (p=0.041). Plasma Cu and Zn levels of the patients with and without interferon treatment were similar (p>0.05) (**Table 2**).

Discussion.

Hepatic Cu overload has been studied in Wilson's disease, primary cirrhosis and alcoholic liver disease as well as chronic hepatitis. Copper accumulation in fibrotic livers caused by chronic hepatitis C may contribute to hepatic injury. Although the real mechanism is still unclear, excessive amounts of Cu may damage liver by oxidative stress.[18] It was found that cirrhotic subjects had significantly decreased blood Zn and selenium (Se) levels, which were independent from the nutritional status, whereas plasma iron (Fe) levels significantly decreased only in malnourished cirrhotic patients.[20] Our patients were not cirrhotic,

Table 1 - Plasma copper and zinc levels in patients and healthy controls.

Parameter measured	Control N=30	Patients N=43	*p* value
Alanine aminotransferase(U/l)	24.1±12.5 (12-78)	42.1±39.3 (10-186)	<0.01
Aspartate transaminase (U/l)	21.4±5.7 (12-34)	49.3±43.1 (10-205)	<0.01
Total bilirubin (mg/dl)	0.53±0.22 (0.2-1.4)	0.79±0.61 (0.25-3.96)	0.036
Direct bilirubin (mg/dl)	0.18±0.3 (0.1-0.5)	0.27±0.35 (0.08-2.09)	0.038
Copper (mmol/l)	12.2±5.4 (1.2-22.8)	16.0±2.8 (11.9-19.7)	0.042
Zinc (mmol/l)	26.6±5.6 (13.3-37.8)	26.0±7.3 (9.8-45)	0.536

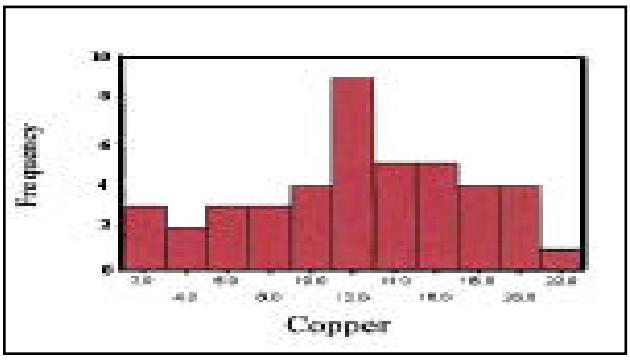

Figure 1 - Plasma copper concentration in patients with chronic viral hepatitis. SD=5.42, Mean=12.2, N=43

Table 2 - Plasma copper and zinc levels versus interferon treatment.

Parameter measured	Without treatment	With treatment	*p* value
Alanine aminotransferase(U/l)	45.2±35.6	39.4±42.9	0.97
Aspartate transaminase (U/l)	59.9±51.2	40.0±32.9	0.041
Total bilirubin (mg/dl)	0.88±0.81	0.70±0.37	0.134
Double bilirubin (mg/dl)	0.31 ±0.43	0.23±0.27	0.271
Copper (mmol/l)	12.52±5.38	11.97±5.59	0.89
Zinc (mmol/l)	26.28 ±6.66	27.11±4.27	0.082

and had a normal nutritional status and almost normal plasma Zn level. In this study, increased Cu levels were encountered in CVH. Copper levels were not changed with interferon treatment. Changes in the content of microelements may depend on the severity of the underlying disease.[21] Some of the trace elements such as Cu function like a co-factor against hepatic fibrosis in chronic liver disease, particularly in the biosynthesis of collagen. As the disease progresses from chronic hepatitis to liver cirrhosis, serum calcium, magnesium, phosphorus and Zn concentrations decrease while the Cu concentration increases.[22]

Copper, zinc superoxide dismutase is produced in the rough endoplasmic reticulum hepatocytes and protects the cells from cellular injury caused by superoxide anion radical in various disorders of the liver.[23] A reduction of Zn and increase of Cu was found in patients with chronic hepatitis.[21] In chronic persisting hepatitis, Kryska et al[24] showed that hepatitis B surface antigen (HbsAg) positive cases had decreased magnesium and increased Cu levels in the serum. Pramoolsinsap et al[25] suggested that serum Zn levels decreased significantly in patients with chronic active hepatitis, cirrhosis, and hepatocellular carcinoma and Cu levels increased significantly only in patients with hepatocellular carcinoma. Many studies have proved Zn deficiency in alcoholic liver disease, chronic hepatitis and liver failure.[25-27] It seems that Cu and Zn are involved in chronic liver diseases though the results of different studies in the literature are conflicting. This condition might have resulted from the assessment of these trace elements in different chronic liver disorders. Our results showed that plasma Zn and Cu levels did not change with interferon treatment in CVH while plasma level of the latter increased in CVH.

Zinc has an antioxidant and anti-inflammatory activities, and induces metallothionein, which has radical scavenging and immunomodulatory effects.[14,28] Nagame et al[29] proposed that Zn increased antiviral effect and in turns response to interferon therapy. In the same study, the patients responded completely to interferon therapy were found to have a higher serum Zn/Cu ratio than the patients who did not respond to interferon. Therefore, Nagame et al,[29] advised Zn administration as an adjunct to interferon therapy in chronic HCV infections. However, our results are not parallel with these contentions as serum Zn levels were similar between the patients and controls, and did not related to interferon treatment in CVH.

In conclusion, increased plasma Cu, which can cause damage in the liver, may be important in CVH. However, its role in the etiology or pathogenesis in CVH is unclear. Plasma Zn level is not associated with CVH.

Acknowledgment. This study was supported by Gaziantep University Research Foundation and it was presented as an abstract in the 2nd International Meeting on Free Radicals in Health and Disease (May 2002). We thank Dr. Yildirim A. Bayazit for editing the manuscript.

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