J Gastroenterol 1994; 29:610–615

Journal of Gastroenterology

Serum trace metals in chronic viral hepatitis and hepatocellular carcinoma in Thailand

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Abstract: This study was conducted to determine and compare serum trace metal levels in viral hepatitis-associated chronic liver disease. Of 98 patients aged 43 (± 13) [mean (± SD)] years, 83 (85%) were seropositive for hepatitis B surface antigen (HBsAg) and 15 (15%) were seropositive for anti-hepatitis C virus (HCV). Twenty-five patients had chronic persistent hepatitis, 32 chronic active hepatitis, 21 post-necrotic cirrhosis, and 20 hepatocellular carcinoma. Determination of fasting serum trace metal levels (zinc, copper, calcium, magnesium, and phosphorus) was performed after the patients had been on a 2-day diet containing 10–12 mg zinc/day. Compared to healthy volunteers (n = 30), serum zinc levels were significantly decreased in patients with chronic active hepatitis, cirrhosis, and hepatocellular carcinoma ($P \leq 0.0001$), and copper levels were significantly elevated only in patients with hepatocellular carcinoma ($P < 0.0001$). The overall serum levels of calcium, magnesium, and phosphorus were within normal ranges, and levels of calcium and magnesium correlated with serum zinc ($P = 0.01–0.03$). Serum zinc levels correlated with bilirubin, albumin, and cholesterol ($P = 0.0004 \leq 0.0001$), but not with daily urinary zinc excretion. Serum copper levels correlated with alkaline phosphatase and gamma-glutamyltransferase ($P = 0.008–0.0001$). These results suggested that changes in liver cell pathology compounded by functional impairment may alter the metabolism of trace metals, in particular, zinc and copper. The possible relationship of these changes to the pathogenesis of chronic liver disease is discussed.

Key words: chronic liver disease, serum zinc, serum copper

Introduction

Trace metals are essential nutrients for normal growth and development. Both zinc and copper are involved in several hepatic enzyme systems and are stored in large quantities in the liver.[1,2] Decreased serum zinc or increased copper levels occur in patients with some acute and chronic liver diseases.[3–6] However, studies comparing serum zinc levels in various types of liver disease have been limited, and it is not clear whether zinc deficiency is directly related to the severity of hepatic dysfunction and/or to liver cell pathology. Zinc supplementation has been suggested to be of therapeutic value in patients with cirrhosis[3,7] and some other chronic liver diseases,[7–9] but the significance of diet as a cause of zinc deficiency in these patients is not known. There are limited data on other essential metals that also participate in the normal physiology of hepatic enzyme systems, i.e., copper, magnesium, calcium, and phosphorus. The aim of this study was to determine and compare serum levels of zinc and other trace metals in patients with chronic viral hepatitis and hepatocellular carcinoma.

Materials and methods

Patients

Patients with histologically proven chronic liver disease admitted to Ramathibodi Hospital, Bangkok, were screened for entry into the study. Entry criteria included: (i) age 15 years or older, (ii) presence of hepatitis B surface antigen (HBsAg) or anti-hepatitis

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(Received for publication on Sept. 7, 1993; accepted on Dec. 17, 1993)

C virus (HCV) for more than 6 months, and (iii) no association with other possible causes of chronic liver disease (long-term drug or alcohol intake, autoimmune disease). Patients who were taking any medication apart from vitamins were excluded from the study. Informed consent was obtained from each subject and the study was approved by the Research Committee of Mahidol University, Thailand. Patients were classified according to liver histology, as described by DeGroote et al.,[10] into four groups; chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), cirrhosis (CIR), and hepatocellular carcinoma (HCC).

Methods

On admission, detailed physical signs and symptoms were recorded in all patients and vital signs were monitored every 6–12 h. Following inclusion in the study, all patients were served with food containing a known quantity of zinc (10–12 mg per day divided into three meals) for 2 days. At the end of each meal, the quantity of zinc consumed was calculated by subtracting the amount of food left uneaten. This procedure was done under supervision of a nutritionist and the average zinc consumption of each patient was calculated by routine methods.[11,12] On the next day, fasting blood samples were taken, via polyethylene syringes, for determining serum levels of trace metals (zinc, copper, magnesium, phosphorus, and calcium). Routine blood tests, i.e., complete blood count, liver function tests, and blood biochemistry were also performed. On days 2 and 3 of the study, 24-h urine specimens were collected in acid-washed polyethylene bottles for measurement of daily zinc excretion.

Fasting serum zinc and copper levels were also determined in 30 healthy adult volunteers (24 males, 6 females) who gave informed consent to the study. These healthy controls did not receive the supervised diet given to the study patients.

Serologic test for HBsAg and anti-HCV

HBsAg was determined by enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, North Chicago, ILL.). The presence of anti-HCV was determined by enzyme immunoassay (EIA) with purified C100-3 antigen, using an HCV EIA 1.1 test kit (Abbott).

Determination of serum trace metals

Serum and urine zinc were determined by flame atomic absorption spectrophotometry, using a Varian Techtron model AA6 instrument (Melbourne, Australia), as described by Hackley et al.[13] All equipment involved in the measurement of zinc content were zinc-free (disposable polyethylene or acid-washed). Serum copper, magnesium, and calcium were determined by graphite furnace atomic absorption spectrophotometry, using an SP 9 Pye Unicam instrument (Cambridge, England), as described by Trudeau and Freier.[14] Inorganic phosphate was determined with a Beckman Synchron CX5 (BREA, Calif.).

Statistical analysis

The data for the patient groups concerning serum metals, daily urine zinc excretion, and laboratory findings (Table 1) were analyzed by one-way analysis of variance. Differences in serum trace metal levels between patient groups as well as comparisons of the combined data of all groups and their age distributions with values for the healthy controls were determined by the Wilcoxon rank-sum test. Correlations between serum trace metals and liver function tests were assessed by the method of Pearson.[15]

Results

We examined 98 patients (83 males, 15 females) with chronic liver disease whose sex and age distributions were comparable to those of the healthy controls [mean (± SD) age, 43 (± 13) vs 40 (± 11) years; $P = 0.12$]. Of these 98 patients, 83 (85%) showed persistent HBsAg and 15 (15%) were seropositive for anti-HCV. The daily zinc consumption of these patients during the first 2 hospital days ranged from 3.6–10.8 [mean (± SD), 6.5 (±2.0) mg/day. Admission laboratory data are shown in Table 1.

Serum trace metal levels

The overall serum zinc levels of all patients were significantly lower than those in the control group [mean (± SD); 96.1 (± 27.8) vs 118.9 (± 21.8) μg/dl; $P < 0.0001$]. This was attributed to the significantly decreased serum zinc levels in patients with CAH, CIR, and HCC ($P \leq 0.0001$). Of the four study groups, patients with CIR had the lowest serum zinc levels, while patients with CPH had normal levels ($P = 0.23$) (Fig. 1). The average daily urine zinc excretion in the CPH, CAH, and CIR groups was normal and did not differ significantly among groups ($P = 0.28$) (Table 1). As data on urine zinc levels in patients with HCC were incomplete, this group was excluded from the urine zinc analysis.

Compared to the control group, serum copper was significantly elevated only in patients with HCC ($P < 0.0001$; Fig. 1). Serum levels of albumin-corrected calcium in all patients were generally within normal ranges;

Table 1. Admission laboratory findings [mean (± SD)] in patients with chronic liver diseases

Parameters (normal values)	Patient groups				
	CPH (n = 25)	CAH (n = 32)	CIR (n = 21)	HCC (n = 20)	Total (n = 98)
Age in years	36 (8)	37 (12)	58 (7)	47 (12)	43 (13)
Calcium (8.8–10 mg/dl)	9.4 (0.6)	9.3 (0.7)	8.9 (1.1)	8.8 (1.1)	9.2 (0.9)
Magnesium (1.8–3.0 mg/dl)	2.0 (0.1)	2.0 (0.2)	1.8 (0.4)	2.0 (0.5)	2.0 (0.3)
Phosphorus (3.3–4.6 mg/dl)	3.6 (0.4)	3.5 (0.4)	3.7 (1.3)	3.1 (0.9)	3.5 (0.8)
Total bilirubin (0.2–1.2 mg/dl)	1.0 (0.3)	1.2 (0.5)	2.7 (2.4)	4.5 (6.8)	2.1 (3.5)
SGOT (5–40 units/l)	67 (47)	195 (185)	98 (65)	196 (311)	144 (190)
SGPT (5–40 units/l)	110 (87)	240 (220)	57 (25)	76 (101)	135 (161)
Alkaline phosphatase (40–105 units/l)	60 (20)	85 (40)	94 (39)	247 (146)	114 (99)
Albumin (42–52 gm/l)	45 (4)	42 (5)	34 (9)	34 (8)	40 (8)
Cholesterol (140–270 mg/dl)	206 (37)	198 (49)	141 (48)	163 (57)	181 (54)
Gamma-GT (5–35 units/l)	31 (16)	39 (22)	109 (103)	187 (171)	82 (108)
Urine zinc (300–600 µg/24 h)	436 (336)	482 (263)	599 (414)	—	497 (336)

CPH, Chronic persistent hepatitis; *CAH*, chronic active hepatitis; *CIR*, cirrhosis; *HCC*, hepatocellular carcinoma; *Gamma-GT*, gamma-glutamyltransferase; *SGOT*, serum glutamic-oxaloacetic transaminase; *SGPT*, serum glutamic-pyruvic transaminase

however, CIR and HCC patients had lower levels than CPH and CAH patients (P = 0.008; Table 1). Serum magnesium and phosphorus levels in all patient groups were within normal ranges and did not differ significantly among groups ($P \geq 0.16$; Table 1).

Correlation between serum trace metals and liver function test

The overall serum levels of zinc correlated with those of calcium (Fig. 2; P = 0.013) and magnesium (r = 0.23, P = 0.03), but not with daily urinary zinc excretion (r = 0.003, P = 0.91). Serum copper and phosphorus levels were not related to levels of any other serum trace metals determined in this study.

Except for aminotransferase [serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT)], all liver function test parameters showed some relationship with the serum trace metals. Serum zinc correlated inversely with bilirubin (Fig. 2; P = 0.0004), and directly with albumin and cholesterol levels (Fig. 3; $P < 0.0001$), but weakly with gamma-glutamyltransferase levels (r = 0.21, P = 0.06). Serum calcium correlated directly with albumin (r = 0.53, $P < 0.0001$) and cholesterol levels (r = 0.52, $P < 0.0001$). Serum copper correlated directly with alkaline phosphatase and gamma-glutamyltransferase levels (Fig. 4; $P < 0.0001$ and P = 0.008, respectively). Serum levels of phosphorus and alkaline phosphatase were correlated (r = −0.24, P = 0.021), as were magnesium and cholesterol levels (r = 0.20, P = 0.056).

Discussion

Zinc plays an essential role in many biochemical and physiological functions, including metabolism, enzyme function, protein synthesis, sexual maturation, and host defense reactions.[16,17] Common causes of zinc deficiency include dietary deficiency, malabsorption,[18] increased urine excretion, and liver cell damage.[19] In our patients, the average daily zinc consumed in the hospital was equivalent to the normal intake in the Thai population, which intake ranges from 4.5 mg in females to 6.5 mg in males.[20] Despite this apparently normal zinc intake, variable degrees of decreased serum zinc were detected in some of the study groups. There was no evidence of increased renal zinc excretion either in patients with (CIR and CAH) or without

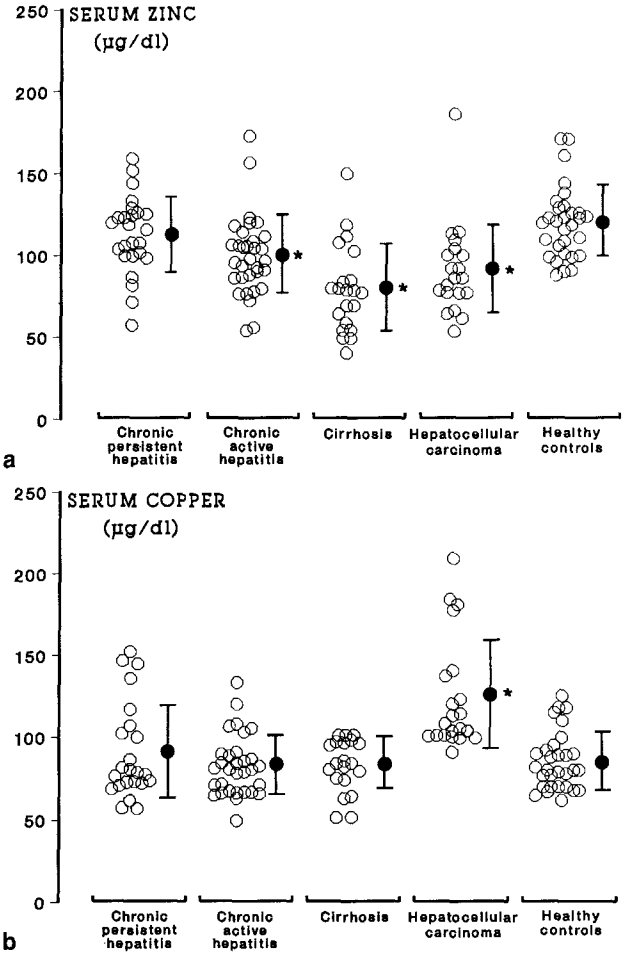

Fig. 1a,b. Serum levels of **a** zinc and **b** copper in patients with chronic liver disease and in healthy controls. Means (*black circles*) and 95% confidence intervals are shown. *($P < 0.05$), significantly different from values for healthy volunteers by the Wilcoxon rank-sum test

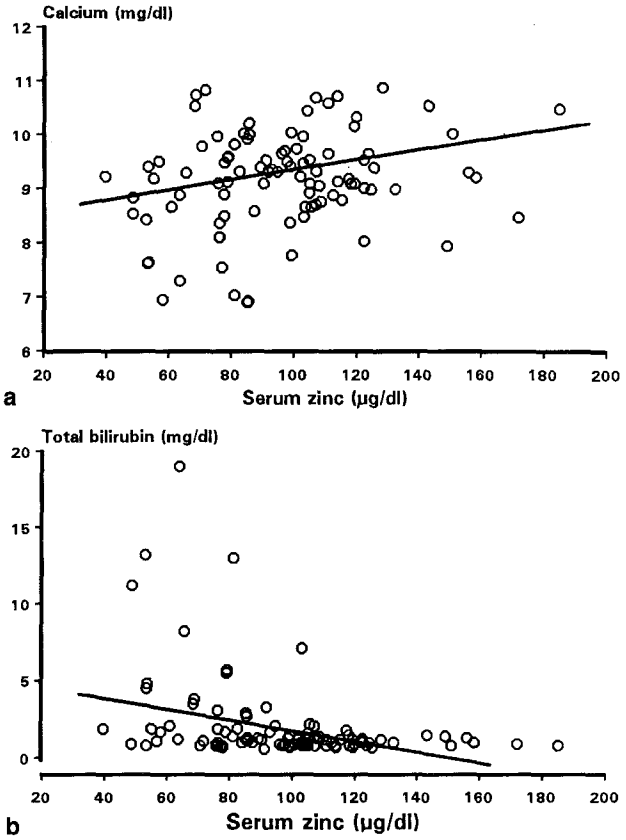

Fig. 2a,b. Correlations of serum zinc levels, **a** with serum calcium, **b** with total bilirubin in patients with chronic liver disease

serum zinc deficiency (CPH) and there was no correlation between urine and serum zinc levels. These findings suggested that dietary deficiency and increased renal excretion were not the major causes of zinc deficiency in these patients. In this study, the decreased serum zinc appeared to be related to the severity of liver cell pathology, although malabsorption, as a contributing factor to zinc deficiency, cannot be ruled out. The mean serum zinc level was normal in patients with CPH, and zinc deficiency was less marked in patients with CAH than in those with a more severe form of liver pathology, i.e., CIR or HCC. The relationship between serum zinc levels and some liver function test parameters, in particular bilirubin, found in these patients may further support the hypothesis that liver impairment is a contributing factor in zinc deficiency. Whether these associations are causal or incidental remains to be determined, as abnormal liver function tests are also usually related to pathological hepatic changes.

There have been attempts to relate zinc deficiency to the pathogenesis of carcinoma. Many investigators have found significantly decreased zinc levels in tumorous liver, with increased levels in normal surrounding tissue, both in humans[21–23] and in animal models.[23] Zinc supplementation has been shown to inhibit or to prevent carcinogenesis[24] and to prevent the spread of transplanted tumors in animal models.[25,26] Whether an abnormal zinc metabolism contributes to malignant cell transformation in humans remains to be clarified. In a study of 301 Thai patients with cancer of various organs other than the liver, the mean level of serum zinc in cancer patients was found to be significantly lower than that in the control group.[27] In this present study, we found zinc deficiency in HCC patients and also in patients with CIR and CAH, both of which conditions are classified as high risk for HCC development.[28]

One of the most important enzyme systems involved in the metabolism of foreign compounds is the hepatic microsomal cytochrome P450 mixed-function oxidase

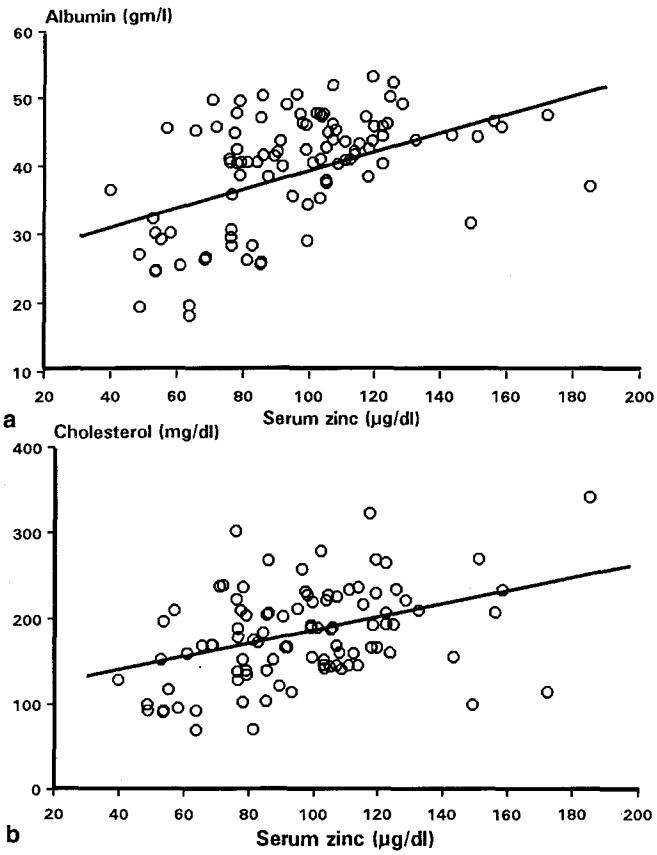

Fig. 3a,b. Correlations of serum zinc levels, **a** with albumin, **b** with cholesterol in patients with chronic liver disease

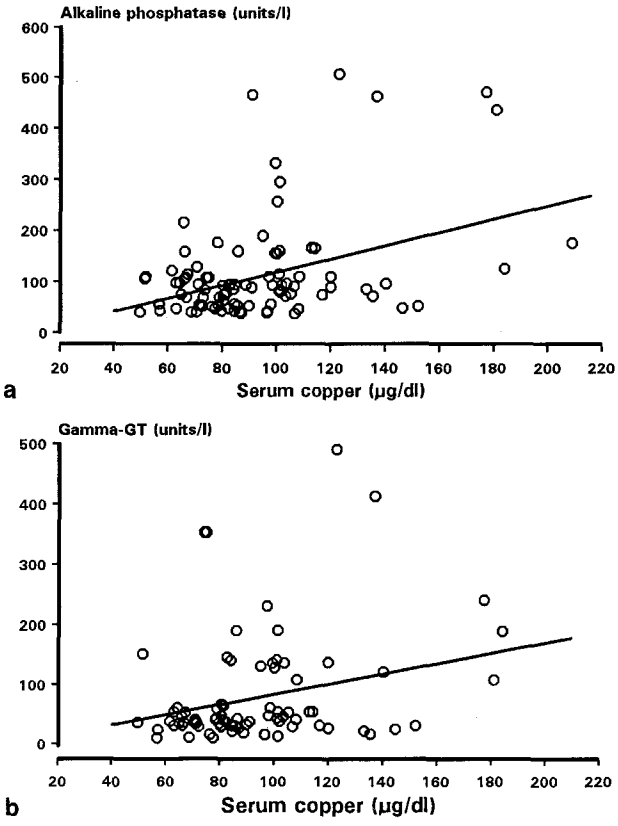

Fig. 4a,b. Correlations of serum copper, **a** with alkaline phosphatase and **b** with gamma-glutamyltransferase in patients with chronic liver disease

system in the endoplasmic reticulum. A number of trace metals have been shown to affect this system, although their interactions are not yet fully established.[29] In the present study, significantly increased serum copper was detected only in HCC patients. Although there was no significant change in the other trace metals studied, serum calcium and magnesium both correlated with serum zinc. Alteration in some trace metals may lead to interactions between trace minerals such as zinc and copper, and excesses of one may lead to deficiency of others.[30] Zinc sulfate has been employed as an "anticopper" agent for the treatment of Wilson's disease, with apparent clinical improvement.[8,9] The elevated serum copper levels seen in our HCC patients may be directly related to, or secondary to zinc deficiency. As the liver is the principal site of copper storage and excretion, as well as the organ in which the plasma copper protein, ceruloplasmin, is synthesized, it may be expected that abnormalities of copper metabolism would be observed in diseases in which liver function is compromised.[31] In the present study, serum copper levels in all patients were correlated with alkaline phosphatase and gamma-glutamyltransferase levels, suggesting that liver impairment is associated with alterations in copper metabolism.

The results of this study demonstrated variable degrees of alteration in serum zinc and copper levels in patients with chronic liver disease. As these serum trace metals are essential for the hepatic microsomal cytochrome P450 mixed-function oxidase system, alterations in their levels may affect normal hepatic metabolism,[29] and this may also contribute to the pathogenesis of liver disease.

Acknowledgment. This study was supported by the research fund of Mahidol University.

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