SERUM TRACE METAL LEVELS IN PATIENTS WITH ACUTE HEPATITIS B

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Abstract. This study was conducted to determine serum levels of trace metals in young adult patients in the early icteric phase of acute hepatitis B virus infection. There were 15 patients (10 males, 5 females) and 15 healthy volunteers (11 males, 4 females). The age distribution of both groups ranged from 15-40 years and were comparable [mean (SD) = 28(6) *vs* 31(7) years; p = 0.12]. Compared to the healthy controls, the patients had significantly decreased serum zinc but elevated serum copper levels [means (SD) of zinc = 118(22) *vs* 97(20) μg/dl, p = 0.012; and of copper = 82(15) *vs* 135(40) μg/dl, p < 0.001]. The overall serum levels of calcium, magnesium and phosphorus in the studied patients were within normal ranges. Serum zinc concentrations of these patients correlated with albumin (r = 0.69, p = 0.005) and their serum calcium correlated with alkaline phosphatase (r = 0.61, p = 0.015). These results demonstrate that alterations of zinc and copper metabolism occur early during the acute icteric phase of uncomplicated hepatitis. These changes may be of pathophysiological significance in acute hepatitis, in particular in patients with pre-existing zinc deficiency.

INTRODUCTION

Trace metals are essential nutrients for normal growth and maintenance of cellular functions. Zinc and copper are involved in several hepatic enzyme systems and are stored in large quantities in the liver (Aggett and Harries, 1979; Becking, 1976; Evans, 1973). As metabolic disturbance of trace metals may contribute to the pathogenesis of liver disease, effective management should be based on a better understanding of these alterations. Abnormalities of zinc and copper metabolism have been well documented in various type of chronic liver disease (Pramoolsinsap *et al,* 1994; Karayalcin *et al,* 1988; Versieck *et al,* 1974; Brewer *et al,* 1983) but reports in acute hepatitis have been inconsistent. Interpretation of these results can be complicated by variations in factors influencing trace metal metabolism in the patients studied *ie* age groups, severity of clinical feature and any underlying cause of liver cell injury. The present study for determination of serum levels of zinc and other trace metals was confined to young adults with acute hepatitis B viral infection during the early icteric phase.

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MATERIALS AND METHODS

Patients

Patients aged 15-40 years were included in the study if they had been jaundiced for 5 days or less, were sero-positive for anti-HBc IgM and had more than 10-fold increase in serum transaminase. Patients were excluded from the study if they had other systemic complications (fulminant hepatitis, septicemic or disseminated intravascular coagulation), were known to have any underlying systemic disease, were concomitantly sero-positive for anti-HCV, or were taking any medication apart from vitamins.

Methods

All studied patients were admitted to the hospital. Detailed physical signs and symptoms were recorded and vital signs were monitorred every 6-12 hours. On the next hospital day, fasting blood samples were taken, via polyethylene syringes, for serum concentrations of trace metals (zinc, copper, magnesium, phosphorus and calcium). A 24 hour urine zinc was collected for measurement of zinc concentrations. Routine blood tests *ie* complete blood count, liver function tests and blood biochemistry were performed. Fasting serum zinc and copper levels were determined in 15 adult healthy volunteers (11 males and 4 females) aged 15-40 years who gave informed consent to the study.

Determination of serum trace metals

All utensils involved in the procedure for measurement of serum zinc content were zinc-free (disposable polyethylene or acid-washed) (Pramoolsinsap *et al,* 1994). Serum zinc was determined by flame atomic absorption spectophotometry using a Varian Techtron model AA6 instrument (Melbourne, Australia), as described by Hackley *et al* (1968). Serum copper, magnesium and calcium were determined by graphite furnace atomic absorption spectophotometry using SP 9 Pye Unicam instrument (Cambridge, England), as described by Trudeau and Ereier (1967). Inorganic phosphate was determined with a Beckman Synchron CX5 (BREA, CAL, USA).

Serological tests for HBsAg and anti-HCV

HBsAg and anti-HBc IgM were determined by enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, North Chicago, ILL, USA). 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).

Statistical analysis

Differences in serum zinc, copper and age distribution between studied patients and healthy volunteers were analyzed by Student's unpaired *t* test. Correlations between serum trace metals and liver function tests were assessed by the method of Pearson.

RESULTS

Patient data

Fifteen patients (10 males, 5 females) with acute hepatitis B virus infection and 15 healthy volunteers (11 males, 4 females) were studied. The age distributions of patients [mean (SD) = 27.9 (5.9) years] were comparable to those of the healthy controls [30.6 (6.7) years; p = 0.12]. These patients had a history of acute jaundice ranging from 1-5 days [mean (SD) = 3 (2) days] duration and their liver function tests on admission are shown in Table 1.

Serum trace metal levels

Compared to the control group, the overall serum zinc levels of all patients were significantly lower [mean (SD); 118.5 (21.7) *vs.* 97.3 (20.4) μg/dl; p = 0.012] but serum copper were significantly elevated [82.1 (15.4) *vs* 134.9 (40.2) μg/dl; p < 0.001] (Fig 1). The average daily urine zinc excretion was variable (Table 1), 2 patients had significant hyperzincuria (urine zinc > 1,000 μg/24 hours). The remaining patients had either low (n = 4) or normal (n = 9) zinc excretion. There was no correlation between serum zinc and urinary zinc excretions (r = 0.05, p > 0.05).

The average levels of serum calcium, magnesium and phosphorus in the studied patients are shown in Table 1. Serum calcium and phosphorus were within normal ranges. Except for one patient with hypomagnesemia (<1.8 mg/dl), all patients had normal levels of serum magnesium.

Correlation between serum trace metals and liver function test

The overall levels of serum zinc correlated with serum albumin (r = 0.68, p = 0.005) and serum calcium correlated with alkaline phosphatase (r = 0.61, p = 0.015) (Fig 2). There was no relationship among serum concentrations of different trace metal and none was correlated with any other parameter of liver function.

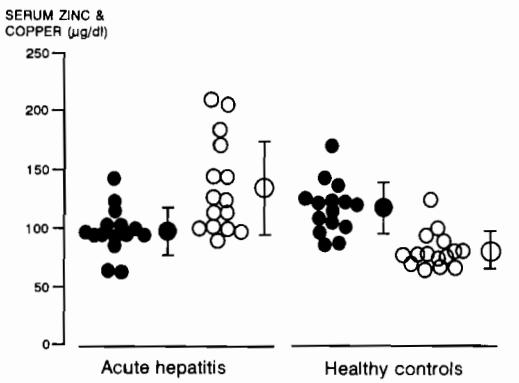

Fig 1– Serum levels of zinc (•) and copper (◦) in patients with acute viral hepatitis and in healthy controls. Means and SD are shown.

DISCUSSION

The maintenance of trace metal concentrations within a physiological range is vital to the integrity of a variety of cellular metabolic processes and is

Table 1

Serum trace metals and liver function tests in patients with acute viral hepatitis.

Parameters (normal values)	Means (SD)
Calcium (8.8 - 10 mg/dl)	9.9 (0.4)
Magnesium (1.8 - 3.0 mg/dl)	1.9 (0.3)
Phosphorus (3.3-4.6 mg/dl)	3.6 (0.3)
Urine zinc (300-600 µg/24 hrs)	477 (53)
Total bilirubin (0.2-1.2 mg/dl)	8.9 (7.2)
SGOT (5-40 U/l)	1,493 (1,022)
SGPT (5-40 U/l)	1,718 (851)
Alkaline phosphatase (40-105 U/l)	121 (63)
Albumin (42-52 gm/l)	42 (2.1)
Chlolesterol (140-270 mg/dl)	182 (76)
Gamma-GT (5-35 U/l)	171 (112)

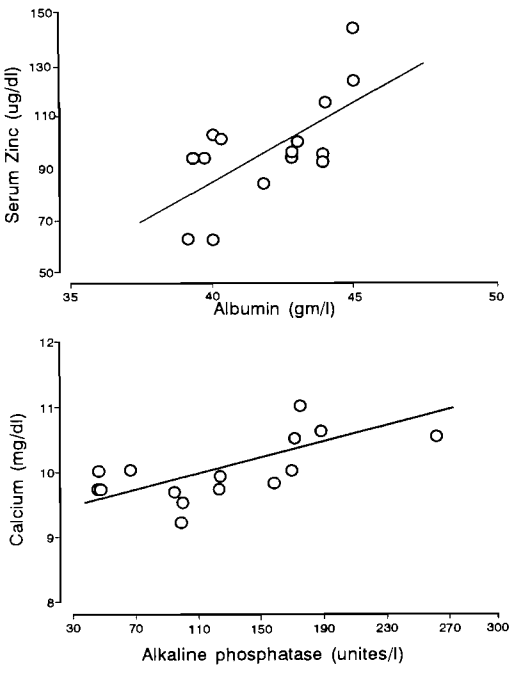

Fig 2- Correlations of serum zinc levels with albumin and of serum calcium with alkaline phosphatase in patients with acute viral hepatitis.

essential for many biochemical and physiological functions in man (Aggett and Harries, 1979; Evans, 1973; Aurbach *et al,* 1985). Zinc and copper concentrations affect many hepatic anzymes systems, including the microsomal cytochrome P450 mixed-function oxidase system which is involved in the metabolism of foreign compounds (Becking 1976). Zinc deficiency is a common and persistent finding in patients with chronic liver disease (Pramoolsinsap *et al,* 1994; Karayalcin *et al,* 1988; Versieck *et al,* 1974). Copper levels are elevated in patients with hepatocellular carcinoma and Wilson's disease but are usually unchanged in other types of chronic liver disease (Pramoolsinsap *et al,* 1994; Brewer *et al,* 1983; Hill *et al,* 1978). The majority of reports in patients with acute hepatitis have found variable degrees of zinc deficiency (Halstead *et al,* 1968; Halstead and Smith, 1970; Henkin and Smith, 1972) while others found either unchanged (Versieck *et al,* 1974; Davies *et al,* 1968) or increased serum zinc levels (Kahn *et al,* 1965). These varying results could be attributed to differences in factors influencing trace metal metabolism in the studied patients. In adult Thais, normal levels of serum zinc are higher in age groups below 41 years compared to older age groups (Lekhakul *et al,* 1987). According to some studies, alterations of serum zinc and copper levels in acute hepatitis are more marked in the severe fulminant variety (Versieck *et al,* 1974). In the present study, zinc deficiency and excess copper occurred early even in young adults with uncomplicated acute hepatitis. This abnormality of zinc and copper metabolism occurred in the absence of any significant changes of other serum trace metals (calcium, magnesium and phosphorus).

The common causes of zinc deficiency include dietary deficiency, malabsorption (Karayalcin *et al,* 1988), increased urine excretion and liver cell damage (Aggett and Harries, 1979). In acute hepatitis, the causes of alteration in zinc and copper metabolism are not fully known. Increased serum copper level in these patients is suggested to be through the release of copper-containing enzymes from damaged hepatic cells, or is the result of increases in serum ceruloplasmin (Henkin and Smith, 1972). Some studies in acute hepatitis have suggested that increased urinary zinc excretion, through an increase in diffusible zinc, is a contributing factor in zinc deficiency (Henkin and Smith, 1972; Kahn *et al,* 1965). In the present study, there was no correlation between serum and urine zinc concentrations and hyperzincurea occurred in only 2 patients. Exchangeable zinc is mainly bound to albumin (Giroux and Henkin, 1972) and albumin deficiency has been suggested as a cause of zinc

deficiency in cirrhotic patients (Kahn *et al,* 1965). In the present study, the correlation between serum zinc and albumin occurred despite normal serum albumin. The relationship between zinc and albumin seen in this and in other studies (Pramoolsinsap *et al,* 1994; Henkin and Smith, 1972; Kahn *et al,* 1965) may be simply because they occur in circulation as macromolecular complexes.

In some liver disease, an unknown interaction between zinc and copper have been suggested as a cause of excesses of one and deficiency of others (Festa *et al,* 1985). Zinc deficiency with copper elevation has been found in Wilson's disease, hepatocellular carcinoma and acute hepatitis (Henkin and Smith, 1972). The use of zinc sulphate as an "anticopper" agent has been advocated for treatment of Wilson's disease and apparent clinical improvement has been reported (Brewer *et al,* 1983; Hill *et al,* 1987). The cause of zinc deficiency with copper elevation in acute hepatitis as seen in the present study may be through such suggested interaction.

The significance of alteration of zinc and copper metabolism on the pathophysiology of acute hepatitis is not fully known. It has been shown both in Wilson's disease and in experimental animals that an excess of copper and zinc-superoxide results in hepatic cell damage, including acute hepatitis (Suzuki *et al,* 1993; Gill *et al,* 1994). As seen in the present study, the abnormalities of zinc and copper metabolism occurred even in a young age group without other underlying liver pathology. All of our patients recovered without zinc supplementation and their zinc deficiency was probably self-limited as shown in a previous study (Evans, 1973). However, in patients with pre-existing or borderline zinc deficiency such as that found in old age, chronic liver disease and alcoholic cirrhosis, an attack of acute hepatitis may aggravate the pathology of zinc deficiency and clinical severity. Further studies are needed to determine the therapeutic value of zinc supplementation in acute hepatitis in these high risk groups.

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