Zinc Status and Oral Zinc Tolerance Test in Alcoholic and Non-Alcoholic Cirrhosis

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Summary: *Plasma, erythrocyte (RBC), hair and urine zinc measurements were performed in 25 non-alcoholic and 20 alcoholic cirrhotic patients in order to evaluate the zinc status. Zinc absorption was examined in the same group of patients using the oral zinc tolerance test and comparing results to a healthy control group. Low ($p<0.05$) plasma, RBC and hair zinc levels revealed a state of zinc deficiency in both patient groups independent of chronic alcohol consumption. On the other hand hyperzincuria is more profound in the compensated alcoholic cirrhotics when compared with their nonalcoholic counterparts. With 22.5 mg elementary zinc, the increase in plasma zinc was significantly lower in both cirrhotic patients than in the control group in the 1st($p<0.01$), 2nd($p<0.01$) and 4th($p<0.05$) hour. Small intestinal biopsies showed shortening and prominent distension of villi and intense stromal edema in both patient groups, which can explain the zinc malabsorption. However it is not clear whether these intestinal changes are due to zinc deficiency or to portal hypertension. Thus in addition to hyperzincuria, zinc malabsorption appears to contribute to zinc deficiency in liver cirrhosis and seems to result, in part, from pathological changes in the mucosa.*

Key Words: Zinc absorption, Liver cirrhosis.

The relation between zinc and liver cirrhosis has been studied extensively. Initial studies in the literature were mainly in alcoholic cirrhotic patients. In 1955, Vallee found low plasma zinc and high urinary zinc levels (1) while others have documented low zinc levels in RBC, leukocytes, polymorphonuclear leukocytes (2) and liver tissue (3,4) in alcoholic cirrhotic patients. These findings, taken together, have tended to suggest that alcoholic cirrhosis may be associated with a state of zinc deficiency. It was reported that hepatic alcohol dehydrogenase enzyme activity, which is a zinc metalloenzyme, was low in alcoholic cirrhosis (5), thus rendering the liver more susceptible to damage from continued alcohol consumption.

The cell turn-over rate in the gastrointestinal system is very high. Indeed the epithelial cells in gastrointestinal mucosa are renewed every forty-eight hours. Zinc is essential as a trace element in the activity of metalloenzymes related to protein synthesis, therefore the effects of zinc deficiency are observed mostly in tissues with high mitotic activity. In a genetic zinc deficiency disease, Acrodermatitis Enteropathica, there is severe intestinal mucosal atrophy which can be reversed by effective zinc supplementation. It is therefore reasonable to propose that the existence of chronic zinc deficiency in liver cirrhosis might lead to pathological changes in the intestinal mucosa. On the other hand, malabsorption of zinc by the gut in cirrhotic patients either from chronic ethanol consumption or from other unknown reasons might also aggravate the zinc deficiency, thus creating a vicious cycle.

Zinc absorption in patients with alcoholic liver cirrhosis had been studied by many researc-

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hers, but the results obtained have been conflicting (6,7,8,9). The object of this study was to determine whether there is a decrease in intestinal zinc absorption in patients with the diagnosis of both alcoholic and nonalcoholic cirrhosis. In addition to this by comparing alcoholic and nonalcoholic cirrhotic patients we tried to show whether chronic alcohol consumption have an effect or not on some zinc parameters that were already changed in cirrhotic patients.

MATERIALS and METHODS

Subjects: Our control group consisted of 10 healthy volunteers (6 males and 4 females) between the ages of 22 and 46. The patients were divided into two groups according to the etiology of chronic liver disease. Group 1 consisted of 25 nonalcoholic cirrhotics (19 males and 6 females) between the ages of 17 and 70 while in group 2 there were 20 alcoholic cirrhotics (18 males and 2 females) between the ages of 37 and 59. All patients were hospitalized in the Gastroenterology Department of the Faculty of Medicine, Ankara University.

Both groups were classified as compensated (without any ascites and serum albumin greater than 3g/dl) or as decompensated (with ascites and serum albumin less than 3g/dl) according to the severity of liver disease. In group 1,10 patients were in the compensated state, while 15 patients were decompensated. On the other hand in group 2, 10 patients were in the compensated state and 10 patients were decompensated.

Patients with any other coexisting diseases and/or alcohol consumption were excluded from group 1 while in group 2 all patients were HBV seronegative. Routine laboratory tests were performed using standard methods and HBsAg was determined by radioimmunoassay (RIA) (Abbots). Ultrasonography (Toshiba Model Sal 30) was used in all patients for the evaluation of hepatic parenchyma, portal hypertension and ascites. Liver cirrhosis was diagnosed by histological examination of percutaneous liver biopsy specimens in 40 patients. In the remaining 5 patients in whom biopsy was unsuccessful or not done for reasons of safety, the diagnosis of cirrhosis was based on typical clinical manifestations of portal hypertension and compatible ultrasonographic appearance of the liver with ancillary signs of portal hypertension.

Oral Zinc Tolerance test (OZTT) and other zinc measurements: After and 8 hour overnight fast, blood was taken from subjects to determine fasting plasma zinc. Then 100mg $ZnSO_4$. $7H_2O$ (22.5 mg elementary zinc) was given. All the subjects were allowed only water during the test. After the oral zinc test dose, blood was taken in the first, second and fourth hours to measure plasma zinc levels. Using the data obtained, the increase in plasma zinc concentration above fasting levels (plasma zinc level for a specific hour - fasting plasma zinc) was plotted (Fig 1,2) (10). The control group was tested similarly. Plasma, RBC, hair and urine zinc levels were assessed by atomic absorption spectrophotometer (Perkin Elmer Model 2380) (11) and zinc-free plastic containers and tubes were used to collect the various specimens stated above.

Small Intestinal Biopsy: After the OZTT, the Crosby capsule was passed under fluoroscopic control to the ligament Treitz and small intestinal biopsy was performed in 29 patients. Three control subjects underwent the same operation and the tissues were fixed with formalin and stained with hematoxylineosin for histopathological examination.

Statistical Analysis

Means (x) and standard errors (SE) were calculated for all blood and urine values. To

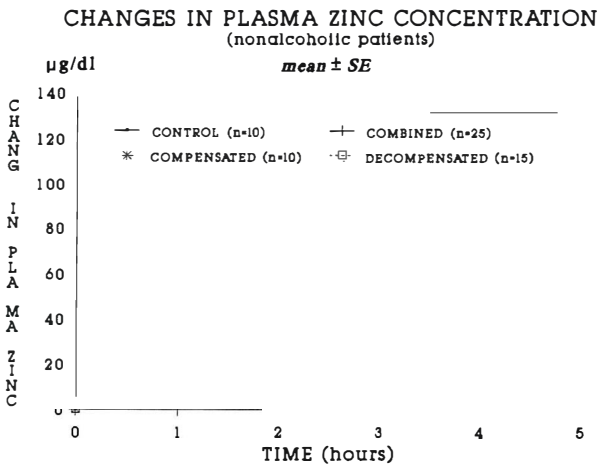

Figure 1: Changes in plasma zinc concentration (mean±SE) following administration of 22.5 mg. elementary zinc in 10 healthy subjects and in 25 nonalcoholic cirrhotic patients. When all patients (combined) and the decompensated subgroup were examined, the changes in plasma zinc were significantly lower in the first ($p<0.01$), second ($p<0.01$) and fourth ($p<0.05$) hour when compared with the control group. In the compensated subgroup they were significantly lower only in the second hour ($p<0.05$).

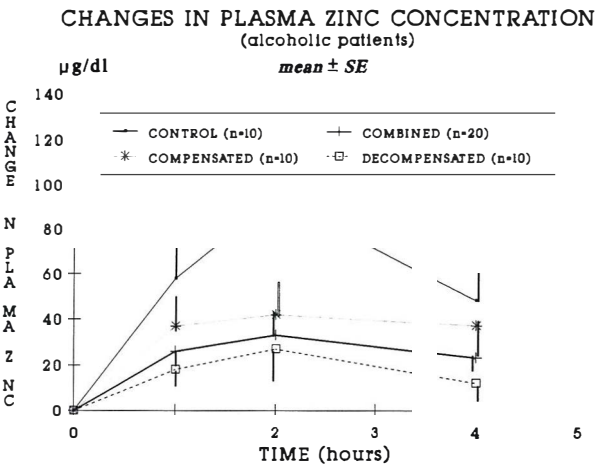

Figure 2: Changes in plasma zinc concentration (mean±SE) following administration of 22.5 mg. elementary zinc in 10 healthy subjects and in 20 alcoholic cirrhotic patients. The results were similar to nonalcoholic patients, compare to Fig 1.

compare the mean values of the patient group with those of the control group, student's test was employed. P values less than 0.05 were considered significant.

RESULTS

The results of several liver tests for both patient groups are shown in Table 1. The values given under the term combined represents the mean values for each group. In order to compare the values in different stages of liver disease in both groups, values were given separately for compensated and decompensated subgroups. In statistical analysis the patient values in both groups were first compared with the control group and then group 1 and group 2 patients were compared with each other.

The decreases in serum albumin levels were especially prominent in the decompensated subgroups of both alcoholic and nonalcoholic patients. The SGOT and serum bilirubin levels were slightly elevated in both subgroups when compared with the control values. The SGOT levels were found to be significantly high in group 2 when compared with group 1 as a result of chronic alcohol consumption. HBsAg determinations were positive in 17 patients in group 1, while as group 2 patients were by definition must be seronegative for hepatitis B serology so that they represent a homogeneous group of alcoholic patients, none of them were HBsAg seropositive. Examination of the percutaneous liver biopsy specimens showed liver cirrhosis in 40 patients. In the remaining 5 patients liver biopsy was technically unsuccessful because of liver atrophy with massive ascites and/or not attempted because of a prolonged prothrombin time (>25 sec). Ultrasonographic examinations of the liver showed various changes (heterogeneous increase in the echogenicity of liver parenchyma 100%, increase in attenuation of the ultrasonic beam 88%, loss of detail of vascular echoes 62% and indentation of liver contour 77%). Ancillary signs of portal hypertension were as follows: portal veins were greater than 13 mm and

Table I: Liver Profile in Control and Cirrhotic Patients (Mean±SE)

	n	Albumin (g/dl)	SGOT (IU/L)	Bilirubin (mg/dl)	HBsAg +	Ultrasound Examination	Liver Biopsy (+)
Control	10	4.5±0.2	15±4	0.5±0.2	0	Normal	Not done
GROUP 1 (Nonalcoholic Cirrhosis)							
Compensated	10	3.8±0.7	30±12	1.7±0.6	7	LPD/PH	10
Decompensated	15	2.9±0.5	44±30	1.2±1.1	10	LPD/PH/A	10
Combined	25	3.2±0.7	41±27	1.3±0.1	17	LPD/PH/A	20
GROUP 2 (Alcoholic Cirrhosis)							10
Compensated	10	3.7±0.5	65±10	1.2±0.5	0	LPD/PH	10
Decompensated	10	2.7±0.4	72±15	1.6±0.5	0	LPD/PH/A	20
Combined	20	3.3±0.5	68±13	1.4±0.5	0	LPD/PH/A	

LPD: Liver Parenchymal Disease, PH: Portal Hyertension, A: Ascites

splenic veins were greater than 10 mm in all patients, collateral veins (dilated coronary vein 5±0.3 mm, umblical vein 3±0.2 mm, short gastric veins) were also demonstrated in 44% of the patients. Ascites was demonstrated only in the decompensated subgroup, while splenomegaly was demonstrated in all patients.

As shown in Table 2 the mean (±SE) plasma, RBC and hair zinc levels in both patient groups were significantly lower than those in the control group ($p<0.05$). When the combined plasma zinc values of group 1 and group 2 patients were compared the difference was not significant ($p>0.05$) but plasma zinc values of the compensated subgroup of alcoholic patients were found to be significantly ($p<0.05$) low when compared with the compensated subgroup of nonalcoholic patients. RBC zinc values were also low in both patient groups when compared with the control values but the difference was insignificant when group 1 and group 2 RBC zinc values were compared with each other. Similarly combined hair zinc values of group 2 patients were also lower ($p<0.05$) than the combined group 1 values but within the same group there was not any significant difference between the subgroups. Daily urinary zinc excretion on the other hand was significantly higher in the both patient groups when compared with the control group ($p<0.01$). Hyperzincuria was more prominent in the decompensated subgroup of nonalcoholic patients when compared with the compensated nonalcoholics and daily urine zinc excretion in the compensated alcoholic patients was significantly higher then the compensated nonalcoholic patients.

When the combined values of patients in both groups were examined together after the zinc test dose was given, the increase in plasma zinc was significantly less in the first ($p<0.01$), second ($p<0.01$) and fourth hours ($p<0.05$) when compared with the control group (Table 2 and Fig 1,2). When analyzed separately in the subgroups, the increase in plasma zinc in the decompensated patients was significantly lower for every hour when compared with the control group ($p<0.01$), while in the compensated subgroups the increase in plasma zinc was significantly lower only in the second hour ($p<0.05$). On the other hand a clear cut difference was not observed between the alcoholic and nonalcoholic patients ($p>0.05$).

Small intestinal biopsy was performed in 6 compensated and 9 decompensated patients in group 1, and in 8 compensated and 7 decompensated patients in group 2. 5 healthy

Table I I: Zinc Profile in Control and Cirrhotic Patients (Mean±SE)

	n	Plasma Zn (mg/dl)	RBC Zn (mg/ml)	Urine Zn (mg/day)	Hair Zn (mg/g)	Oral Zn tolerance test (Plasma Zn mg/dl) 1.hr	2.hr	4.hr
Control	10	91.8±5	13±5	335±65	190±12	150±7	186±7	140±6
GROUP 1 (Nonalcoholic Cirrhosis)								
Compensated	10	77.4±7	11±0.8	980±146	156±7	111±10	136±10	117±9
Decompensated	15	58.4±4	11±0.6	1793±448	152±9	75±4	85±6	69±4
Combined	25	63.8±4	11±0.4	1468±285	154±9	89±5	105±6	86±5
GROUP 2 (Alcoholic Cirrhosis)								
Compensated	10	62.3±6*	11±0.5	1640±315*	135±7	99±6	104±7	99±6
Decompensated	10	55.4±5	11±0.6	1850±320	129±8	73±5	82±6	67±7
Combined	20	59.6±6	11±0.6	1745±318	132±9*	86±6	93±6	83±7

Mean values of plasma Zn, RBC Zn, urine Zn and Hair Zn in both patient groups are significantly different than the control values ($p<0.05$).
*: Mean value significantly different from group 1 ($p<0.05$)

subjects served as a control (Fig. 3). A typical example of a pathological specimen from a cirrhotic patient was shown in Fig. 4. There was shortening and distension of the villi to various degrees in all patients. Stromal edema was one of the most prominent findings. Edema frequently began at the tip of the villus, where it may sometimes cause a saccular ectasia, or in other instances it gradually extended down the length of the villus. There was also overt inflammatory cell infiltration mainly in deeper portions of the lamina propria. These findings were most evident in the decompensated patients. The histopathological findings observed in the mucosa were similar in both nonalcoholic and alcoholic patients.

DISCUSSION

In this study, plasma zinc levels in cirrhotic patients were found to be significantly low

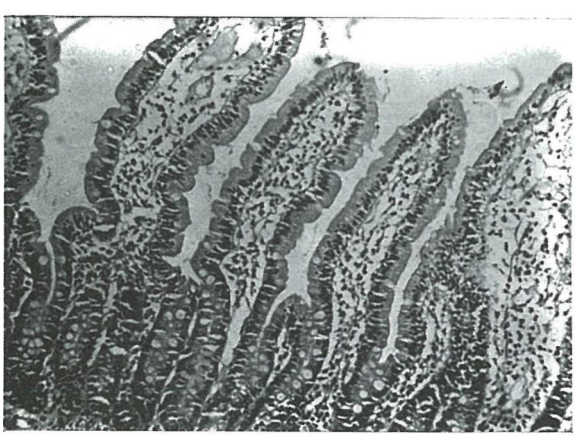

Figure 3: Small intestinal biopsy of a healthy control subject. Tall slender villi occupying most of the total mucosal height while the generative epithelium of the crypts account for a much smaller proportion. (hematoxylin-eosin, x100).

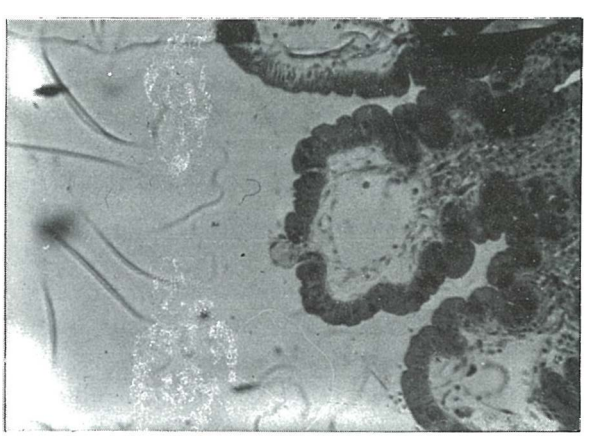

Figure 4: Small intestinal biopsy of a decompensated cirrhotic patient. Histology shows partial shortening and prominent distension of villi. Both villi show stromal edema, one with a typical saccular ectasia. There is marked inflammatory cell infiltration in submucosa (hematoxylin-eosin, x100).

when compared with the control group. In controlled experiments the plasma zinc concentration is a sensitive index of zinc depletion but in diseases it is affected by many factors, particularly leukocyte endogenous mediators (LEM) and serum albumin values, and is unreliable as the sole estimate of zinc status. In liver cirrhosis as a result of continues endotoxemia, LEM values are generally high and zinc is apparently mobilized from the serum and enters the liver under the influence of LEM (12). On the other hand 60-70% of plasma zinc is loosely bound to serum albumin and albumin levels are low as a result of decreased hepatic synthesis. For this reason in addition to plasma zinc we measured zinc levels in RBC and hair where it's level is less affected by the factors stated above. In both patient groups RBC and hair zinc levels were found to be low when compared with the controls reflecting a state of zinc deficiency in our patient groups.

Studies conducted during experimental dietary zinc restriction in healthy humans have shown that urinary excretion of zinc decreases in response to depletion, indicating the presence of homeostatic mechanisms to conserve zinc operating at the level of the kidney (13). Paradoxically the urinary zinc loss was significantly increased in both patient groups, even though they were in a state of zinc depletion, a finding consistent with previous studies (1,3,4,10,14). Most of the previous studies however were on patients with alcoholic cirrhosis. In order to eliminate the possible effects of alcohol itself on zinc metabolism (15) we conducted our study in two different groups of patients namely nonalcoholic cirrhotics (group 1) and alcoholic cirrhotics (group 2). The only difference between the groups was related to the severity of liver disease, hyperzincuria was even more profound in the compensated group 2 patients than in compensated group 1 patients. This finding suggests that hyperzincuria in liver cirrhosis is not related to the etiology of liver disease but chronic alcohol consumption amplifies hyperzincuria even in the initial stages of liver disease. Unfortunately the mechanism of hyperzincuria in liver disease is unknown. In the advanced stages of the disease when pressure in the portal system becomes very high or after portocaval operations (16), hyperzincuria becomes even more significant but decreases after the development of hepatorenal syndrome. Indeed in our decompensated patients urinary zinc excretion was higher than in compensated patients (Table 2). It was shown that the mechanism underlying hyperzincuria in sickle cell anemia was impaired renal tubular handling of zinc (17). In addition to this it was demonstrated that by oral histidine supplementation to a patient with liver disease it is possible to induce hyperzincuria (18). It is likely that zinc in the ultrafiltrate binds to some ligandins, such as amino acids like histidine, and this complex interferes with renal tubular reabsorption of zinc.

Studies of zinc absorption in cirrhotics are contradictory. Using an oral zinc test dose Sullivan et al (6) established that the rise in plasma zinc levels was lower in decompensated alcoholic cirrhotics than controls. On the other hand Mills (7) and Milman et al (8) showed that after an oral dose of radioactive zinc, absorption of zinc increased in compensated alcoholic cirrhotics, indicating a homeostatic mechanism to conserve zinc operating at the level of small intestinal mucosa. Unequivocally, intestinal zinc absorption was found to be increased in zinc depleted rats (19). Finally, Valberg et al (9) found in their experiments with oral ^{65}Zn that zinc absorption decreased in alcoholic cirrhotics while absorption of zinc was normal in the decompensated nonalcoholics. They concluded that chronic alcohol consumption contributed to malabsorption of zinc. This conflicting results in the literature may be the result of problems

related to patient selection in different stages of liver disease with different etiologies. To overcome this problem we selected cirrhotic patients with different etiology (those who used alcohol and those who don't) and divided them into compensated and decompensated subgroups.

The oral plasma zınc tolerance test (OZTT), which measures the increase in plasma zinc concentration above fasting levels following the oral zinc test dose was selected to evaluate zinc absorption as it is a safe, inexpensive and a reliable method (20,21). Under standard conditions this test gives valuable information about zinc absorption by the small intestine. Parallel results were achieved by radioactive zinc absorption tests to the OZTT (22). Other tests which measure small intestinal absorptive functions such as D-xylose absorption and fecal fat analysis were also compared with OZTT and OZTT was found to be a very sensitive test (23). In most trace metal deficiency states the absorption of the metal is increased as an avidence of homeostasis (19,24). Paradoxically in our study there was a significant depression of plasma zinc curves in both patient groups when compared with the controls (Fig 1,2).

The orally administered zinc enters the vascular pool and can be rapidly cleared by the zinc deficient liver tissue which might explain the low plasma zinc levels after an OZTT in our cirrhotic patients. However, it was shown in patients with liver cirrhosis that intravenous radioactive zinc accumulation was low in the zinc deficient liver tissue (7). This observation can be explained hypothetically by the decreased synthesis of zinc binding protein, metallothionein, by the hepatocytes in liver cirrhosis (25).

Alternatively rapid excretion of zinc by the kidneys might lower zinc plasma concentrations following ingestion. Sullivan et al showed that it was not possible for further increments in urine zinc excretion in the already hyperzincuric patients by giving oral zinc indicating the possibility of zinc malabsorption (20). For this reason we performed small intestinal biopsies in the hope of finding a defect in the mucosa which might explain the depressed plasma zinc curves. Microscopic analysis of the specimens showed distension of the villi with various degrees of shortening, prominent stromal edema and inflammatory round cell infiltration in both the alcoholic and nonalcoholic patients, particularly in our decompensated subgroups. Similar findings in the small intestine of cirrhotics have been reported by others (26,27), but no one correlated the pathological changes in intestinal mucosa with zinc malabsorption. Experiments with rats demonstrated that chronic ethanol ingestion significantly impairs net zinc absorption (28). On the other hand it was reported that villous atrophy showed regression after discontinuation of alcohol in man (29). It is likely that ethanol have an adverse effect on intestinal mucosa but it is not the sole etiologic factor which causes the pathological changes in the mucosa of cirrhotics as we found similar changes in nonalcoholic cirrhotic patients who never used alcohol. It is possible that the mucosal changes and zinc malabsorption are a result of either portal hypertension or zinc deficiency itself. In Acrodermatitis Enteropathica there is severe mucosal atrophy and this can only be reversed through an effective zinc supplementation (30). If effective zinc replacement therapy improved the oral plasma zinc tolerance test and reverted the small intestinal mucosa to a normal state, we could then say that zinc deficiency is responsible for these changes. On the other hand the finding of intense mucosal distension and stromal edema, which is unusual in Acrodermatitis Enteropathica, leads one to think that mucosal changes and zinc malabsorption are a result of portal hypertension.

The findings in our study suggest that;

(1) there is a state of zinc depletion (low plasma, RBC and hair zinc values) in liver cirrhosis and chronic alcohol consumption is not the principal factor in the etiology but it augments this state.

(2) one of the main reasons for chronic zinc deficiency in liver cirrhosis is hyperzincuria, which results from unknown factors related to cirrhosis but is again amplified by chronic alcohol consumption.

(3) the other reason for chronic zinc deficiency in liver cirrhosis is zinc malabsorption by the gut. It is likely that pathological changes in the small intestinal mucosa are responsible from zinc malabsorption. These changes in liver cirrhosis may result from chronic portal hypertension or may be caused by zinc deficiency but not related mainly to chronic alcohol consumption.

(4) in general the clinical stages of liver failure significantly alters the results. Decompensated cirrhotic patients are affected more then their compensated counterparts.

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