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EFFECTS OF ORAL ZINC THERAPY ON LIVER FUNCTIONS AND SERUM-LIVER DISTRIBUTION OF ZINC AND COPPER IN PATIENTS WITH BILHARZIAL PERIPORTAL TRACT FIBROSIS

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ABSTRACT

Zinc; copper; total proteins; albumin; globulin, total bilirubin; ASAT and ALAT were determined in serum of 41 bilharzial patients with various degrees of periportal tract fibrosis (PTF). Zinc and copper in liver biopsies were also measured in addition to the intrasplenic portal pressure. 13 patients showed moderate PTF on histopathological examination. Ten of them have agreed to receive zinc sulphate. 7 H_2O orally in gelatinous capsules t.d.s for 15 days. Each patient received 75 mg zinc/day. The same parameters were again determined after zinc therapy.

In all patients (11 male + 30 female), the degree of PTF showed inverse correlation with liver zinc content and direct correlation with serum zinc level. Also PTF showed inverse correlation with both serum and liver copper. However after zinc treatment in patients with moderate PTF, there was a marked increase in liver zinc content amounted to 82% while serum zinc was unchanged. Both serum & liver copper concentrations were significantly decreased by 45% & 38% respectively. Liver functions showed marked improvement following zinc treatment. The data reflected good therapeutic efficacy of zinc sulphate capsules in ameliorating the effects of bilharzial liver fibrosis.

INTRODUCTION

High zinc uptake by the schistosomes has been recorded which can reflect reduced amount of zinc in tissues of the infected host (1). Tissue zinc deficiency can result in serious complications. Growth retardation; gonadal dysfunction; increased blood ammonia and urea and altered enzymatic activities in different metabolic pathways are among the adverse effects of zinc deficiency (2-4). Zinc has proven role in maintaining normal immune functions and in maintaining the integrity of cellular membranes (5, 6).

Zinc and also copper have a role in liver diseases. The liver conserves copper even when the central nervous system; spleen; connective tissue & bone marrow are severly affected by copper deficiency (7). Zinc has been used effectively in the treatment of excessive liver-copper precipitation in Wilson's disease (8). Alterations in serum zinc have been found in patients with schistosomal liver fibrosis (9). Alterations in collagen biosynthesis and cross links have been found in zinc deficiency (10).

The present study deals with the therapeutic efficacy of oral zinc supplementation in patients with schistosomal periportal tract fibrosis. The modulatory effect of zinc therapy on serum and liver concentrations of both copper and zinc has been also investigated.

MATERIALS AND METHODS

Fourty one patients (11 female and 30 male) with intestinohepatic schistosomiasis in the age range of 19-35 years were selected from the inpatient section, Al-Hussein University Hospital, Cairo. They were subjected to full clinical examination and they were free from any other disease. Non of them had received any previous antibilharzial

therapy. Stool examination revealed the presence of S. mansoni eggs. Another group of ten healthy subjects (3 female and 7 male) were also investigated.

Measurements of periportal tract fibrosis (PTF) was done for all patients-after their agreement-using eye piece micrometry on stained sections of liver needle biopsies. The liver biopsies were taken by specialist surgeon using Vim Silverman needle biopsy. The histopathological examination was carried out on parafin sections of the liver tissue stained with hematoxylin-eosin and Manson's trichrome. According to the histopathological examination of liver biopsies, the patients were classified into three groups. The first one consisted of ten patients without evident PTF. The second group involved thirteen patients with moderate PTF (M $\pm$ SE: 26.46 $\pm$ 2.31 u). The third group consisted of the remaining eighteen patients with sever PTF (M $\pm$ SE: 43.75 $\pm$ 1.62 u).

Intrasplenic portal pressure (ISPP) was measured in all patients according to the method of Reynolds et al. (11). Fasting serum samples were obtained from the patients and control subjects for the colourmetric determination of total bilirubin (12); ASAT and ALAT (13); total protiens, albumin and total globulins (14, 15); copper and zinc by atomic absorption (16). The two elements were also determined in the liver biopsy homogenate of each patient after digestion with 10% tetramethylammonium hydroxide.

Ten patients with moderate PTF have agreed to receive oral zinc therapy. The mean value $\pm$ SE of PTF in those ten was 29.7 $\pm$ 5.88 u. Zinc sulphate. 7 H_2O (El-Nasr Company, Abo-Zahbal, Egypt) was capsulated in soft gelatinous capsules (Memphis Company, Egypt) each contained 25 mg zinc. The patients were given zinc capsules t.d.s. one hour before meal (75 mg/day) for continuous 15 days. During that period, no other medication was given. Administration of zinc

capsules before meal was in order to overcome food-phytate interference with zinc absorption. All patients were on satandard hospital diet during the period of treatment. During zinc treatment, patients were asked for any possible gastrointestinal troubles eg. dyspepsia, nausea, vomiting, constipation ... etc.

Fasting serum samples and liver biopsies were taken from the ten patients at the end of 15-days zinc treatment. The samples were subjected for determination of copper & zinc in serum and liver and for determination of serum total bilirubin, ASAT, ALAT, total proteins, albumin and total globulins by the methods mentioned before. The degree of PTF after therapy was evaluated.

RESULTS

Histopathological examination of liver biopsies showed that in patients without evident fibrosis (Group A: 3 female and 7 male), bilharzial ova was present in 4 cases only and bilharzial pigment was present in 6 cases. In patients with moderate PTF (Group B: 3 female and 10 male), bilharzial ova was present in 9 cases and large PTF was detected in 11 cases with granulomatus reaction and chronic inflammation in 6 cases and interstitial fibrosis in 4 cases. In patients with sever PTF (Group C: 5 female and 13 male), bilharzial ova was present in 9 cases and bilharzial pigment was present in 14 cases and large PTF was present in all cases with granulomatus reaction and chronic inflammation in 13 cases. The intrasplenic portal pressure (ISPP) in the three groups showed marked differences and it was positively correlated with the degree of PTF (Table 1).

Evaluation of liver functions in bilharzial patients reflected that patients with moderate PTF (Group B) and sever PTF (Group C) have decreased serum albumin level associated with increased serum globu-

TABLE (I)
Effect of Periportal Tract Fibrosis (PTF) on the Intrasplenic Portal Pressure (ISPP) and Liver Functions in 3 Groups of Bilharzial Patients (M ± SE) and liver functions in normal subjects.

	Group (A) n=10 3F + 7M	Group (B) n=13 3F + 10M	Group (C) n=18 5F + 13M	Group (D) n=10 3F + 7M
PTF (microns)	b,c 2.38 ± 1.21	a,c 26.46 ± 2.31	a,b 43.73 ± 1.62	-
ISPP (mm H_2O)	b,c 189 ± 13	a,c 238 ± 13	a,b 304 ± 18	-
Serum Total Proteins (gm%)	d 6.8 ± 0.2	d 6.9 ± 0.2	d 7.1 ± 0.1	a,b,c 7.8 ± 0.2
Serum Albumin (gm%)	b,c,d 4.0 ± 0.1	a,d 3.1 ± 0.2	a,d 3.3 ± 0.1	a,b,c 5.1 ± 0.1
Serum Globulins (gm%)	b,c 2.8 ± 0.2	a,d 3.8 ± 0.2	a,d 3.8 ± 0.1	b,c 2.7 ± 0.1
Serum ASAT (u/ml)	c,d 27.5 ± 1.9	d 32.2 ± 5.1	a,d 39.2 ± 2.9	a,b,c 15.0 ± 3.8
Serum ALAT (u/ml)	d 31.7 ± 3.2	d 42.7 ± 5.6	d 34.6 ± 3.2	a,b,c 19.5 ± 5.2
S. Total Bilirubin(mg%)	1.06 ± 0.02	1.1 ± 0.02	1.04 ± 0.1	0.95 ± 0.05

n: Number of patients in each group. F & M female and male.
a,b,c,d: Significantly different from groups A, B, C and D respectively at P 0.02

lins compared with those without evident fibrosis (group A). Patients with sever PTF showed marked increase in serum ASAT. Serum total bilirubin was normal in the three groups. The three gorups of bilharzial patients showed disturbed liver function when compared with healthy controls.

Serum zinc level showed positive correlation (P 0.05) with the degree of PTF. Serum zinc in patients with sever PTF was significantly (P 0.02) higher than in those without or with moderate fibrosis (Table 2). On the contrary, serum copper showed negative correlation (P 0.05)

TABLE (2)
Effect of Periportal Tract Fibrosis (PTF) on Serum and Liver Concentrations of zinc and Copper (M±SE) in addition to Serum Levels of Control Subjects.

	Group (A) n=10 No PTF	Group (B) n=13 Moderate PTF	Group (C) n=18 Sever PTF	Group (D) n=10 Normal
Serum zinc (ug/dL)	d 122 ± 7.5	a,c,d 308 ± 19.5	a,b,d 420 ± 14.6	a,b 93 ± 4.5
Serum Copper (ug/dL)	b,c 105 ± 4.2	a,c,d 90 ± 4.0	a,b,d 70 ± 2.5	b,c 111 ± 5.2
Liver zinc (ug/gm wet tissue)	b,c 186 ± 14.3	a 117 ± 7.4	a 110 ± 8.5	-
Liver Copper (ug/gm wet tissue)	b,c 96 ± 3.2	a,c 76 ± 3.3	a,b 57 ± 3.2	-

n: Number of patients.
a,b,c,d: Significantly different from groups A, B, C and D respectively at P 0.02

with the degree of PTF. The two groups with moderate and sever PTF showed markedly high serum zinc level and low copper level compared with normal subjects. Both zinc and copper in liver biopsies showed negative correlation (P 0.05) with the degree of PTF (Fig. 1). However the group with sever PTF showed statistically non significant decrease in liver zinc content compared with the groups having moderate fibrosis.

Administration of zinc sulphate (75 mg/day for 15 days) to patients with moderate PTF (29.7 ± 5.88 u) caused significant (P 0.05) increase in serum albumin together with marked decrease in serum globulins. Also, serum ALAT showed significant (P 0.05) decrease after zinc therapy. ASAT showed a decrease which was statistically non significant (Table 3). Although the investigated liver function indexes showed improvement after zinc treatment, yet they were still different from the healthy control values (Table 1) which might be related to the relatively short duration of treatment.

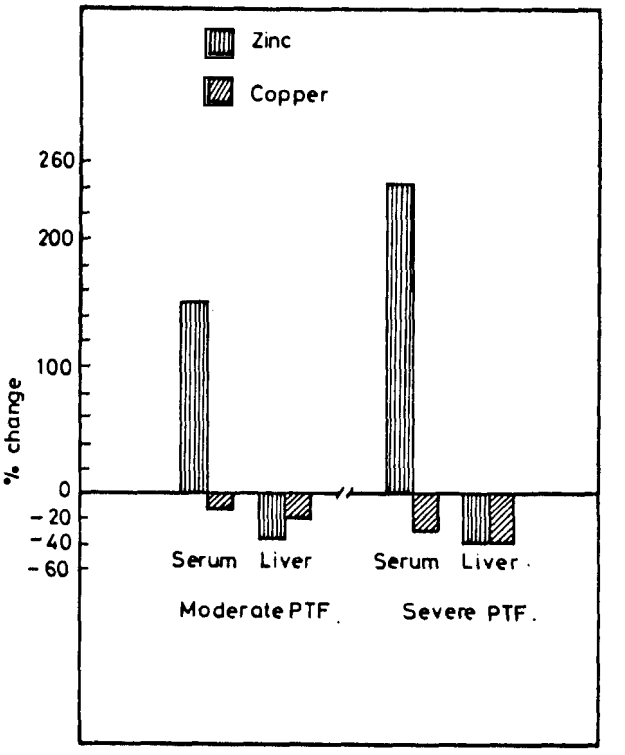

FIGURE (1)
Percent Change in Zinc and Copper in Patients with PTF Compared with Those Without Evident Fibrosis.

Both serum and liver copper concentrations showed marked (P 0.05) decrease after zinc therapy (Table 4). On the other hand, liver zinc content showed significant (P 0.05) increase but serum zinc concentration was unchanged (Fig. 2).

Administration of zinc sulphate in such small divided doses did not result in any remarkable gastrointestinal disorders in all patients.

TABLE (3)

Effect of Oral zinc Sulphate Administration (25 mg $ZnSO_4.7\,H_2O$ t.d.s. for 15 days) on Liver Functions in Bilharzial Patients With Moderate PTF (29.7 ± 5.88 u)

	Mean of 10 patients ± SE	
	Before zinc	After zinc
Serum Total Proteins (gm%)	6.9 ± 0.15	7.2 ± 0.1
Serum Albumin (gm%)	3.3 ± 0.1	3.9* ± 0.1
Serum Globulins (gm%)	3.6 ± 0.1	3.2* ± 0.1
Serum ASAT (u/ml)	42.5 ± 5.5	31.8 ± 1.8
Serum ALAT (u/ml)	40.6 ± 4.1	27.5* ± 1.9
Serum Total Bilirubin (mg%)	1.07 ± 0.02	1.09 ± 0.03

*: Significantly different from the value before treatment at P 0.05.

TABLE (4)

Effect of Oral zinc Sulphate Administration (25 mg t.d.s. for 15 days) on Serum Level and Liver content of zinc and Copper in Bilharzial Patients With Moderate PTF (29.7 u ± 5.88 SE)

	Mean of 10 patients ± SE	
	Before zinc	After zinc
Serum zinc (ug%)	304 ± 23.5	299 ± 26.2
Liver zinc (ug/gm wet tissue)	131 ± 6.7	236* ± 12.4
Serum Copper (ug%)	95 ± 5.4	59* ± 4.3
Liver Copper (ug/gm wet tissue)	77 ± 6.3	43* ± 3.5

*: Significantly different from the value before treatment of P 0.05

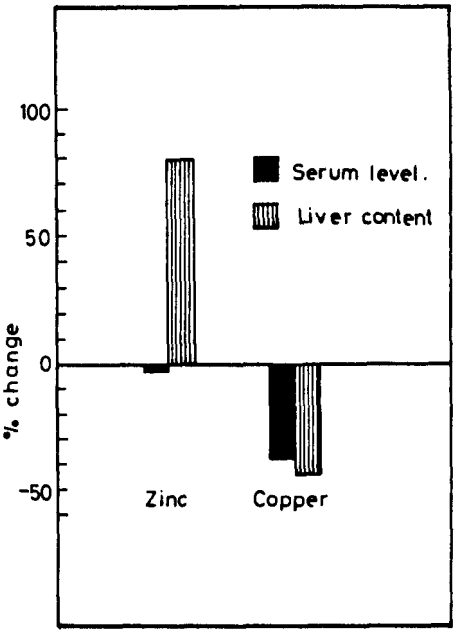

FIGURE (2)
Percent Change in Zinc and Copper in Patients with Moderate PTF After 15 days Oral Administration of $ZnSO_4$ (75 mg/day).

DISCUSSION

Liver zinc content in bilharzial patient showed negative correlation with the degree of PTF. Such decrease in liver zinc could not be attributed to body deficiency of this element since serum zinc concentration was markedly high compared with its level in normal subjects. The cause might be the rise in intrasplenic portal pressure with consequent reduction in the amount of circulating zinc that reached the liver. In addition, affected hepatocytes might contribute in the release of hepatic zinc into circulation. This concept could find support in the data of serum transaminases which showed values more or less at the upper limit of normal figures (Table 1). Similar liver involvement in alcoholic cirrhosis has been accused for an increase in urinary zinc excretion and decrease in hepatic zinc content (17).

The increase in serum zinc level and serum globulins in bilharzial patients (Tables 1, 2) seemed consistent with the findings of Prasad (18). The author has found increased serum level of alpha$_2$-macroglobulin in bilharzial liver fibrosis. Alpha$_2$-macroglobulin as well as ceruloplasmin exhibite specific binding sites to zinc (19). Similar increase of serum zinc in bilharzial patients has been reported by Soliman et al. (9).

Oral administration of zinc sulphate (75 mg/day) resulted in significant increase of liver zinc content of patients with moderate PTF (Table 4). However serum zinc did not show any marked change. This could be explained on the basis that protein binding sites of zinc in serum of patients with bilharzial fibrosis might be saturated with zinc ions. So, the exogenously administered zinc might be more readily taken by the liver tissue and thus provide an essential protective mechanism for the diseased organ. The protective effect of zinc is evident from its role in maintaining the integrity of cell membranes (5).

Both serum level and liver content of copper showed negative correlations with the degree of PTF in bilharzial patients (Table 2). Moreover, oral zinc administration caused further significant reduction in copper concentration in serum as well as liver (Table 4). The increase in intrasplenic portal pressure might contribute in the reduction of liver copper content in the same way as in case of zinc. Affected hepatocytes might also share in this effect. However a state of body deficiency of copper might more satisfactory explain the reduction in both liver & serum copper before as well as after zinc administration. Both copper & zinc have been found to affect the bioavailability of each other through competition for protein binding sites in the intestinal wall (20). Further, zinc has specific property to bind with serum ceruloplasmin (19). Thus in view of the increased serum zinc level in bilharzial patients both before & after exogenous zinc administration, released liver copper into circulation

and basal serum copper content might be more readily excreted. Decreased intestinal copper absorption after zinc administration might be responsible for further reduction in both serum and liver copper content. The effect of zinc therapy to reduce serum and liver copper in our study is analogous to that found in patients with Wilson's disease following zinc supplementation (8).

Zinc therapy caused improvement in serum proteins and transaminases (Table 3). This might be related to the role of zinc in different metabolic processes (18). Also, it might be related to the role of zinc in maintaining the integrity of cellular membranes (5).

In conclusion, this study points to the importance of zinc therapy as a protective tool in liver disease. Zinc treatment may result in more promising benificial effects if used for longer periods than in our study. Further, gastrointestinal troubles can be overcomed by giving zinc in divided small doses -as in the present study- allover the day.

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