

Serum Zinc and Copper Levels in Amebic Dysentery

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Objective: It is suggested that zinc alters the functionality of *Entamoeba histolytica* in vitro as reflected by decrease in replication and adhesion and in vivo as manifested by inhibition of amebic pathogenicity. Suggesting a possible role in amebic intestinal disease copper significantly inhibits *Entamoeba histolytica* acid phosphatase activity.

Methods: Thirty-one patients with *Entamoeba histolytica* rectocolitis and 26 age/gender matched healthy subjects were recruited for the study. Plasma zinc and copper levels were determined with 5-Br-PAPS and bathocuproin with deproteinization methods respectively.

Results: Serum zinc concentration was (median; min-max) significantly lower in patients with amebic dysentery (9.9; 4.7-19.6 mmol/L) than the control group (12.4; 7.9-20.0 mmol/L), ($p < 0.05$). Serum copper concentrations of the patients (19.7; 7.27-54.3 mmol/L) and the control group (12.2; 11.5-33.5 mmol/L) were not statistically different.

Conclusion: Serum zinc concentrations decreased in patients with *Entamoeba histolytica* rectocolitis, but a significant difference was not observed for serum copper concentrations.

Key words: Copper, zinc, dysentery, *entamoeba histolytica*

Entamoeba histolytica (*E. histolytica*) is the third leading parasitic cause of death in developing countries and one of the important health risk to which travelers are exposed. It is estimated that more than 10% of the world's population are infected by *E. histolytica* (1). The prevalence of infection is as high as 50% in underdeveloped areas and, serological studies indicate that up to 5% of the population may be reinfected every two years (2-5). Intestinal diseases associated with *E. histolytica* include diverse clinical syndromes, asymptomatic infection, symptomatic noninvasive infection, acute rectocolitis (dysentery) and fulminant colitis. Asymptomatic intestinal infection occurs in 90 to 99% of the infected individuals, most eliminate the parasite from the gut within 12 months, the mechanism of such are unknown (6-8). Diarrhea is a frequent presentation of acute amebic rectocolitis that occur in 90-100% of the patients (9,10). Epidemiological studies indicate an association between relatively low zinc concentrations and increased diarrheal morbidity (11). Moreover, zinc supplementation provides therapeutic benefits; reduces the duration,

treatment failure or death rate and stool mass in acute and persistent diarrhea (12,13).

In various experimental studies, it is suggested that zinc alter the functionality of *E. histolytica* in vitro as reflected by decrease in replication and adhesion, and in vivo as manifested by inhibition of amebic pathogenicity (14). Although a relation of copper deficiency and diarrheal morbidity has not been shown, *E. histolytica* acid phosphatase activity is significantly inhibited by Cu^{2+} suggesting a possible role in amebic intestinal diseases (15).

In this study, we determined serum concentrations of zinc and copper in patients with acute amebic rectocolitis together with a healthy control group matched in age and gender.

Material and Method

This study was conducted at Gaziantep University, Faculty of Medicine, Department of Infectious Diseases and Department of Biochemistry and Clinical Biochemistry in the year 2000. Informed consent was obtained from all subjects according to the Helsinki Declaration as revised in 1996. Thirty-one patients (20 men/ 11 women, median; min-max : 28; 16-45 years) with acute *E. histolytica* rectocolitis that were otherwise healthy were enrolled for this study. Amebiasis cases were collected in three consecutive weeks and the control group was collected in one weeks' period. Diagnosis was made by identification of trophozoites in fresh stool, that were performed by microscopic examination of trichrome-stained and unstained preparations. Presence of ingested erythrocytes was taken as criterium for discrimination of trophozoites from *E. dispar*. Twenty-six healthy subjects (16 men/ 10 women, mean 29; 16-49 years) who applied to the out-patient clinic for routine check-up purposes formed the control group.

All study participants were monitored and were excluded if symptoms of any infection (other than *E. Histolytica* rectocolitis) or systemic somatic illness were present.

Fasting venous blood samples were collected using standart venipuncture technique between 9:30-11:00 am after 12 hours of fasting. Sera were separated immediately by centrifugation at 3000 g for 10 min and stored at -20°C

Table I. Serum zinc and copper concentrations of the patients with acute *E. histolytica* rectocolitis and the healthy controls.

	<i>E. histolytica</i> Rectocolitis n:31	Healthy Control n:26	<i>p</i> *
Zinc (μmol/L)	9.9; 4.7-19.6	12.4; 7.9-20.0	0.0017
Copper (μmol/L)	19.7; 7.27-54.3	12.2; 11.5-33.5	0.959
Age (years)	28; 16-45	29; 16-49	0.802

**p* values obtained with non-parametric statistics.

until analysis. Hemolyzed specimens were excluded. Commercial reagents were used for determination of the trace metals. Serum copper concentration was determined with Bathocuproin with deproteinization method (Copper MPR 1, Catalog no: 124834, Boehringer Mannheim GmBh, Mannheim, Germany) and serum zinc concentration was determined with 5-Bromo-adenosine 3'-phosphate-5'-phosphosulfate (5-Br-PAPS) method (Catalog no: zinc-0100, Elitech Diagnostics, Sees, France) according to the manufacturer's instructions.

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.

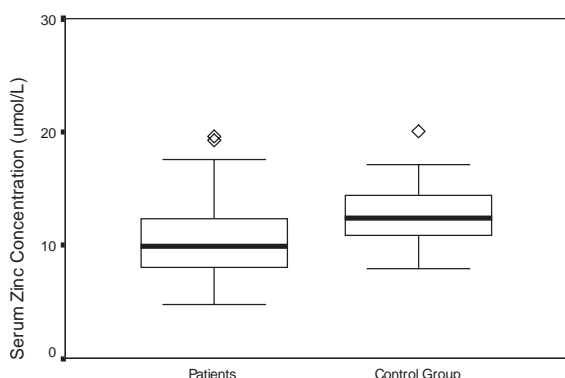


Figure 1. Serum zinc concentrations of the patients with acute *E. histolytica* rectocolitis and the healthy controls. The central box represents the values from lower to the upper quartile (25-75 percentiles). The middle line represents the median. A line extends from the minimum to the maximum value excluding the extreme values, which are displayed as separate points. An extreme value is defined as a value that is less (or greater than) the lower (or upper) quartile minus (or plus) 1.5 times the interquartile range.

Data are presented as median; minimum-maximum. Analysis of groups was performed with Mann Whitney-U test. Chi-square test was performed to consider case-control differences in nominal data (i.e. gender). SPSS 9.0 (SPSS Inc. Chicago, Illinois, USA) program was used for statistical analyses and illustration.

Results

Because of matching criteria, characteristics of the study groups were not different. Serum zinc and copper levels of the patients and the control group are given in Table I. Serum zinc concentration was significantly lower in patients with *E. histolytica* rectocolitis (median; minimum-maximum, 9.9; 4.7- 19.6 mmol/L) than the healthy control group (12.4; 7.9-20.0 mmol/L), (*p*: 0.017). Serum zinc concentration of the patients with *E. histolytica* rectocolitis and the control group are presented in Figure 1. Serum copper concentrations of the patients (19.7; 7.27-54.3 mmol/L) and the control group (12.2; 11.5-33.5 mmol/L) were not statistically different.

A significant correlation between serum zinc and copper concentrations was not observed in patients with amebic dysentery (*r*: 0.292, *p*: 0.139).

Discussion

The data from this study demonstrate that serum concentrations of zinc is decreased in patients with acute *E. histolytica* rectocolitis whereas a significant difference was not observed for serum copper concentrations. Similar findings were observed in patients with diarrhea of various etiologies (16, 17). It is suggested that acute phase action causes increased metallothionein mediated hepatic uptake of serum zinc, leading to hepatic zinc accumulation and decreased serum zinc levels via interleukin 1 mediated mechanisms (18-20). On the other hand acute phase response upregulates ceruloplasmine gene and synthesis in liver and subsequently the level of ceruloplasmin-Cu complexes in the blood. As ceruloplasmin contains approximately 95% of total serum copper, a concomitant increase in the serum copper concentrations is an expected finding in infectious/ inflammatory conditions (21).

However the increase occurs relatively slowly, peaking at 4 to 20 days after a single, acute insult (22). The group with acute *E. histolytica* rectocolitis was at a relatively early phase of the disease and this may at least partly explain lack of a significant increase in serum Cu levels.

On the other hand dietary zinc deficiency or the relative zinc deficiency state associated with acute phase response may increase severity of the disease and/or symptoms in acute *E. histolytica* rectocolitis i.e. altered immune status (23-25), impaired antioxidant system (26,27) elevated intestinal uroguanylin levels (28) and enhanced *E. histolytica* pathogenicity (14,29).

Zinc deficiency rapidly diminishes antibody and cell-mediated responses and is associated with increased susceptibility to infectious diseases. Conversely, zinc supplementations substantially improve immune defense in individuals with moderate zinc deficiency (23,24). Regarding lymphocyte subsets, zinc supplemented children had a significantly higher rise in CD3, CD4 and CD4/CD8 ratio with no difference in CD8 and CD20 (25).

Increased lipid peroxidation was observed in duodenum and jejunum associated with zinc deficiency. Glutathione peroxidase and catalase activities are decreased and CuZnSOD activity is increased which may collectively lead accumulation of hydrogen peroxide, may activate inflammatory molecules and worsen tissue damage (26). On the other hand exogenous zinc supplementation may promote intestinal epithelial wound healing by enhancement of epithelial cell restitution (27).

Northern blot analysis of RNA from rat intestine demonstrated that preprouroguanylin mRNA was 2.5 fold more abundant during zinc deficiency. Uroguanylin, a natriuretic peptide hormone, is an endogenous ligand for the same guanylate cyclase c that the *Escherichia coli* heat-stable enterotoxin binds when it causes secretory diarrhea. This suggests a mechanism whereby zinc deficiency could induce uroguanylin levels in the intestine and cause or potentiate diarrhea (28).

It is suggested that zinc at 100 mmol/L concentration does not affect amebic viability, however it does decrease amebic replication and adhesion and in vivo inhibits amebic pathogenicity (14). Cysteine-proteinases are thought to play an important role in *E. histolytica* pathogenicity. Franco et al. (29) have shown that ZnCl₂ specifically inhibited cysteine proteinase activities, blocked proteolysis and interfered with trophozoite adhesion, thus making amebiasis deficient in substrate degradation and cell damage. However, these effects are seen at supraphysiological zinc concentrations and may be important for management of the disease.

In conclusion, our study indicates that patients with *E. histolytica* rectocolitis have low serum zinc concentrations but no alteration in serum copper levels which is consistent with early phase of acute phase response.

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