Serum Iron, Zinc and Copper Levels and Lipid Peroxidation in Children with Chronic Giardiasis

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ABSTRACT

This study investigated the levels of iron, zinc, and copper and their demolishing effects against lipid peroxidation in chronic giardiasis. Serum iron, zinc and copper levels, erythrocyte cytosolic superoxide dismutase activity, and malondialdehyde levels were measured in 34 children with chronic giardiasis and were compared with controls. The serum iron and zinc levels and erythrocyte superoxide dismutase activity were significantly lower, and malondialdehyde levels were significantly higher among the children with chronic giardiasis compared to the control group (p<0.001). There was no significant difference in copper levels between the two groups (p>0.05). Consequently, the oxidant-antioxidant balance may tilt towards the oxidative side due to weakness of the antioxidant system in giardiasis. If early and proper treatment is not performed, free radical-mediated damage might occur in children with chronic giardiasis.

Key words: Giardiasis; Giardia intestinalis; Iron; Copper; Zinc; Superoxide dismutase; Malondialdehyde; Child; Case-control studies; Turkey

INTRODUCTION

Giardia intestinalis, a parasitic flagellated protozoan, is the most common causative agent of parasitic diarrhoeal illness worldwide (1). It has been reported that blood levels of iron, zinc, and copper might decrease in children with chronic giardiasis (2,3).

Copper and zinc are cofactors of cytosolic superoxide dismutase, and their decreasing levels affect the activity of cytosolic superoxide dismutase (4). Superoxide dismutase is a metalloenzyme capable of scavenging superoxide radicals by catalyzing their dismutation to reactive oxygen species (5). Reactive oxygen species can cause peroxidation of lipids leading to damage of membrane permeability, loss of enzyme activity, DNA damage leading to mutagenesis, carcinogenesis, and apoptosis of cell (6).

MATERIALS AND METHODS

Study population

In this case-control study, 34 (19 female and 15 male) children with chronic giardiasis and 34 (18 female and 16 male) healthy children as a control group were included. The mean ages of children with chronic giardiasis and the control group were 8.05±1.39 and 7.94±1.43 years (range: 4-12 median: 8 years; 4-10 median: 7.5 years) respectively. The children with chronic giardiasis consisted of cases who were referred to the paediatric clinic of the Süleyman Demirel University Hospital for diarrhoeal symptoms which continued for at least one month during one year (June 2001-May 2002). The control group consisted of children who were referred to the paediatric outpatient clinic for a routine checkup and had normal physical examination and laboratory result. These children were of middle-
class status on the national standard. Height and weight percentiles of the children with chronic giardiasis and of the control group were 124.06±3.63 cm, 23.82±2.81 kg and 124.88±4.13 cm, 24.05±2.93 kg respectively. Children who had any other diseases and/or parasite eggs or cysts in the stool or were taking either vitamins or any other drugs were excluded from the study.

Parasitologic examination

Stool samples were examined for cysts and/or trophozoites of *G. intestinalis* by a wet mount of fresh specimen by direct saline and lugol preparation. Stools were concentrated to examination using the formalin-ethyl acetate sedimentation technique and were also stained by acid-fast staining for cryptosporidiosis and cyclosporiasis. Slides were prepared from fresh and concentrated specimens, pieces of 22×40-mm cover-glass were used, and the entire areas of cover-glass using a 10× and 40× objective were examined for identification of parasites (7). Stained specimens were also examined using a 100x objective. Stool cultures of these specimens were also done, and no bacteriologic pathogens were detected.

Biochemical parameters

Blood samples for iron, zinc, and copper were taken from all cases in the morning after overnight fasting by venipuncture into polystyrene tubes. Sera were stored at -20 °C until assay.

A polarized atomic absorption spectrophotometer (Z-8000 polarized Zeeman Absorption Spectrophotometer, Hitachi Ltd., Tokyo, Japan) was used for determining the iron, copper and zinc levels.

Blood samples for malondialdehyde levels and superoxide dismutase activity were centrifuged, and plasma was discarded. Erythrocyte packets were prepared immediately by washing erythrocytes three times with cold isotonic saline. Concentration of haemoglobin was determined by the cyanmethemoglobin method from washed erythrocytes (8). The erythrocytes were then stored at -20 °C, and all measurements were taken within a week.

Malondialdehyde, as a marker for lipid peroxidation, was determined by the double-heating method of Draper and Hadley (9). The principle of the method is the spectrophotometric measurement of colour produced during reaction to thiobarbituric acid with malondialdehyde. The concentration of malondialdehyde was calculated by the absorbance coefficient of malondialdehyde-TBA complex 1.56×10^5 cm^-1 M^-1 and expressed in nmol/gHb.

The measurement of superoxide dismutase was based on the principle that xanthine reacts with xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction (10).

Statistical analysis

Student’s *t*-test was performed to test the difference between the groups. The level of significance selected was *p*<0.05. Pearson’s correlation was used for testing the association between the zinc levels and both superoxide dismutase activity and malondialdehyde levels.

RESULTS

In this study, the serum iron and zinc levels decreased significantly in children with chronic giardiasis compared to healthy controls. There was no significant difference in serum copper levels between the two groups. The results are presented in the table.

<table>
<thead>
<tr>
<th>Table. Serum iron, zinc, copper and erythrocyte superoxide dismutase, malondialdehyde levels in chronic giardiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
</tr>
<tr>
<td>Zinc (µg/dL)</td>
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<tr>
<td>Copper (µg/dL)</td>
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<tr>
<td>Superoxide dismutase (U/gHb)</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/gHb)</td>
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</tbody>
</table>

* Not significant  
** Significant
The erythrocyte superoxide dismutase activity also decreased significantly in children with chronic giardiasis compared to healthy children. The erythrocyte malondialdehyde levels increased significantly in children with chronic giardiasis compared to healthy children.

A significant correlation was found between the zinc levels and both SOD activity (positive correlation) and malondialdehyde levels (negative correlation) in children with chronic giardiasis \((r:0.713, r:-0.595, p<0.001)\), whereas no correlation was found between the iron levels and both SOD activity and malondialdehyde levels \((r:0.107, r:0.048, p>0.05 \text{ for both})\).

**DISCUSSION**

The study showed that the serum zinc levels and erythrocyte superoxide dismutase activities decreased significantly \((p<0.05)\) in children with chronic giardiasis. This result is in agreement with the finding of Shaheen and El-Fattah (4). Chen and Young demonstrated that zinc deficiency caused an increase in the generation of free radicals and lipid peroxidation in blood and liver of mice and rats, and decreased superoxide dismutase activity in the liver of mice (11). Yousef et al. reported that dietary zinc deficiency induced an increased lipid peroxidation in the liver, brain, and testes in growing rats (5).

Since a significant correlation was found between the zinc levels and both superoxide dismutase activity and malondialdehyde levels in children with chronic giardiasis, it is possible to speculate that the increased production of reactive oxygen species may depend mainly on the decreased superoxide dismutase activity, which is likely to be affected by the zinc levels. The production of reactive oxygen species can be grossly amplified in response to various pathophysiological conditions, such as inflammation, immunologic disorders, hypoxia, hyperoxia, metabolism of drug or alcohol, exposure to ultraviolet or therapeutic radiation, and deficiency in antioxidant vitamins. The uncontrolled production of reactive oxygen species often leads to damage of cellular macromolecules (DNA, protein, and lipids) and of other small antioxidant molecules (6).

Despite significant recent advances in the knowledge of the biochemistry and molecular biology of *G. intestinalis*, little is known about the pathogenesis of giardiasis. A combination of parasitic factors and host responses seems to be involved in the pathogenesis of giardiasis (1,12). It has been suggested that *G. intestinalis* releases cytopathic substances that damage the intestinal epithelium (13). Alterations in concentrations of serum iron, zinc, and copper are commonly found in patients with gastrointestinal infections and with hepatic, renal, cardiovascular and malignant diseases (2). Iron deficiency is common among patients affected by malabsorpive states, such as coeliac disease, cow’s milk intolerance, Crohn’s disease, and giardiasis (3). Cheek et al. showed that concentrations of zinc and iron are lower in children with a high prevalence of giardiasis compared to normal children (14). This suggests that the reasons for poorer growth in this region may reside in poor nutrition, or repeated bowel infections in postnatal life leading to malabsorption, or both. In our country, Karakas et al. reported a significant decrease in serum zinc levels in children with giardiasis and amoebiasis (2). De Vizia et al. reported that malabsorption of iron was a complication of giardiasis (3). Evaluation of absorption of iron by the oral iron load test demonstrated a subnormal response in patients with giardiasis and iron deficiency.

The present study found a low significant correlation between the iron levels and both superoxide dismutase activity and malondialdehyde levels in children with chronic giardiasis; it is, thus, possible to speculate that iron deficiency may depend on both poor nutrition and malabsorption due to giardiasis. Iron is an important transient metal and plays a role in the formation of hydroxyl radicals. Since its free form is so harmful, proteins, such as transferrin and ferritin, bind this metal. When superoxide anions are produced in excessive amounts, they may separate iron from ferritin. H$_2$O$_2$ may also cause formation of free iron by attacking haem groups and the production of hydroxyl radicals. Iron can start lipid peroxidation that abolishes membrane structure and functions (15). In the present study, it was shown that the serum iron levels decreased significantly \((p<0.001)\) in children with chronic giardiasis compared to the control group. Iron deficiency in children with chronic giardiasis may be protective because iron plays a role in the formation of hydroxyl radicals. For this reason, giardiasis therapy should be initiated before treatment of iron deficiency in children with chronic giardiasis.

Consequently, the oxidant-antioxidant balance may tilt towards the oxidative side due to weakness of the antioxidant system in giardiasis. If early and proper treatment is not performed, free radical-mediated damage may occur in patients with chronic giardiasis.
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