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Full Length Research Paper

Evaluation of serum copper level during *Giardia intestinalis* infection

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Alterations of serum copper concentrations are commonly found in patients suffering from gastrointestinal infections and with hepatic, renal, cardiovascular and malignant diseases. In this study, we aimed to investigate the changes of total content of the essential element of copper level in patients infected with *Giardia intestinalis*. Copper concentration was measured in 64 patients who were positive for intestinal parasite of *G. intestinalis*. Scores obtained for the positive samples, their age and sex, matched 60 *G. intestinalis* negative healthy controls. The mean concentration of copper in serum was significantly higher in *G. intestinalis* positive subjects than in their controls both in females ($p < 0.05$) and males ($p < 0.05$). No correlation could be demonstrated between age and mean value of copper in *G. intestinalis* positive in both females and males control ($p > 0.05$). Copper level was found to be clearly increased in the patients infected with *G. intestinalis* compared to controls. Although, our findings suggest altered element metabolism due to the pathophysiologic changes of *G. intestinalis*, a detailed biochemical and molecular studies on large sample size are needed to put forward a relationship between *G. intestinalis* (may be other parasitic diseases) and serum element level changes.

Key words*: Giardia Intestinalis*, copper, atomic absorption.

INTRODUCTION

Giardia intestinalis is a worldwide cause of intestinal infection. It is a flagellated enteric protozoon that infects humans. It exists in trophozoite and cyst forms and the infective form is the cyst. Trophozoites of *G. intestinalis* are found in the upper part of the small intestine, where they live closely attached to the mucosa. They are found at times in the gallbladder and in biliary drainage. *G. intestinalis* infection causes severe intestinal disorder, most commonly, diarrhea and related symptoms due to malabsorption (Bogitsh et al., 2005). In many patients who are not treated, however, infection can last for several months to years with continuing symptoms. Identification of characteristic cysts (sometimes trophozoites) in the stool is used in diagnosis of this parasite. Either saline or iodine smears can be employed for initial diagnosis, but a concentration method is commonly used to enhance detection. In developed countries, giardiosis is considered as a travel related disease (Mehlotra, 1996; Vesy and Peterson, 1999; Flohe et al., 1999; Harris et al., 2001).

Trace elements are used by all organisms and provide proteins with unique coordination and catalytic and electron transfer properties. Copper (Cu) is one relatively small metallic elements which is essential to human health. Cu deficiency is generally evidenced as anemia, edema and arthritis (Frieden, 1986; Linder and Hazegh-Azam, 1996; Harris, 2003; Biagini et al., 2001).

It is known that several element such as Cu, modulate immune function and influence the susceptibility of the host to several kinds of infections. Vital exchanges constantly occur involving many enzymatic systems activated by minerals or elements. All activities of human tissues and organs depend on mineral and element concentrations that act for enzyme biocatalyst such as: selenium copper zinc-superoxide dismutase (SeCuZn-SOD) and copper zinc-superoxide dismutase (CuZn- SOD), in other words, superoxide dismutases SODs (EC 1.15.1.1) play

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Table 1. Cu level of patients infected with *G. intestinalis* and control group.

	Age	Cu (µg/dl)
Patients		
Female (24)	31 ± 14	186 ± 30
Male (40)	21 ± 6	195 ± 22
Controls		
Female (50)	27 ± 15	122 ± 28
Male (10)	38 ± 14	89 ± 34

important role in the protection of the parasites against cellular oxygen mediated killing of the hosts (Li et al., 2005).

Our study was aimed to evaluate and characterize the relationship between intestinal parasite of *G. intestinalis* infection, which can cause serious pathology and serum Cu levels which is one of the main element that plays important roles in human health.

MATERIALS AND METHODS

Patients and sera

We assayed serum Cu level of 124 subjects aged between 12 and 69 years (50 males and 74 females). None of them were smokers, which had any known pathologies and taking steroids or medications such as iron for anemia at the time of sampling. Serum samples for control group were obtained from healthy people who have come to the different clinics for regular check-up. All subjects fasted at least 12 h before blood collection. 64 patients and 60 controls were examined in this study. To determine the presence of *G. intestinalis*, a smear of each fecal sample was analyzed with wet mount preparations in 0.9% sodium chloride (NaCl), diluted Lugol's iodine and examined microscopically using 400x magnifications. Besides, fecal preparations (formalin-ether concentrations) were prepared and examined for the presence of *G. intestinalis* cysts using 400x magnifications (Yazar et al., 2001).

Copper level assay

All venous blood samples taken between 8 and 9a.m after 12 h of fasting were collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at 500x g for 15 min. Sera were then removed and stored at -20°C for analysis.

The Cu concentration of serum samples were determined by Zeeman atomic absorption spectrometry (Hitachi Z-8000 mode). Standard solutions were freshly made from standard stock solutions containing 1 g Cu/L. Serum samples were prepared by dilution with deionized distilled water (serum in a dilution 1:5). We matched the viscosity of the standard solutions to viscosity of diluted serum by adding an appropriate amount of glycerol. The total level of Cu in the samples was determined by the regression analysis of the sample absorption data on the standard curve.

Statistical analysis

Statistical analysis was performed with SPSS software package (Version 11.0 for Windows). Data were expressed as mean ± standard deviation (SD). For comparison of two groups of continuous variables, independent samples t test was used. A probability value of ($p < 0.05$) indicated a statistically significant difference.

RESULTS

The mean age of the patient group consist of 40 men (aged 26 ± 14 years) and 24 women (aged 21 ± 6 years). The mean age of the control groups included 10 men (aged 38 ± 14 years) and 50 women (aged 27 ± 15 years). Cu score found in this study was given in Table 1.

The mean concentration of Cu in blood was significantly higher in *G. intestinalis* positive patients than in their controls, both in females ($p < 0.05$) and males ($p < 0.05$). The average Cu concentration in *G. intestinalis* positive female patients was 186 ± 30 and 122 ± 28 µg/dl in controls, and the average Cu concentration in *G. intestinalis* positive male patients was 195 ± 22 and 89 ± 34 µg/dl in controls. No correlation could be demonstrated between age and mean value of copper in *G. intestinalis* positive females and males control ($p > 0.05$).

DISCUSSION

G. intestinalis infection is prevalent throughout the world and widely distributed in developing countries. Most infections are symptomatic but in some cases, acquisition of infection is followed by acute diarrhea after an incubation period of two weeks (Muller and Gottstein, 1998). A relationship between a parasitic infection and absorption of minerals is not proven enough. The aim of the present study was to clarify the relationship between intestinal parasite *G. intestinalis* infection and serum copper concentration.

It has been known that deficiency or serum level raise of Cu affects many different enzyme systems such as SODs (Jones et al, 1996; Kvietkauskaite et al., 2004). Cu is a transition metal that can generate oxygen radicals by the Fenton reaction. Cu is an essential trace element for all organisms, due to its action as a cofactor in many enzymes, but it is also potentially toxic because, it can donate or receive electrons (Rasoloson et al., 2004).

In the present study, we found that Cu level was significantly increased in both female and male patients infected with *G. intestinalis.* Although, the mechanism is unknown, this increase in Cu level in patients with *G. intestinalis* could possibly be explained by increase of copper containing enzyme systems. It has been known that Cu is an essential trace element with immune function that plays a role in immune response against parasites (Linder and Hazegh-Azam, 1996). It is also known that serum Cu concentration is maintained within a narrow range by the small intestine and kidney which both affect their fractional absorption under conditions of

Cu deprivation (Linder and Hazegh-Azam, 1996; Harris, 2003).

Nowadays, scientists from around the world are taking a closer look at the health benefits of Cu and other elements. Because an improper balance of Cu or other elements may lead to heart and circulatory problems, bone abnormalities and especially complications in the immune system (Kvietkauskaite et al., 2004). Both element deficiencies raise and infectious disease often coexist and show complex interactions leading to mutually reinforced detrimental clinical effects. Such a combination is predominantly observed in underprivileged people of developing countries, particularly in rural regions. Several elements, such as Cu modulate immune function and influence the susceptibility of the host to infection. Nevertheless, the effect of individual element on components of innate immunity is difficult to design and interpret. Element level in general has a widespread effect on nearly all components of the innate and maintains the immune response.

The present study with the similar and consistent increased values in both male and females show that Cu have seem to be diagnostic value of disease progression. However, we should emphasize that no correlation could be demonstrated between age and mean value of Cu in *G. intestinalis* positive in both females and males and controls.

The Cu value could be used as a valuable laboratory tool for the clinicians to assess response to therapy or effectiveness of the ongoing therapy, or it could be said that, it seems to be important to consider Cu value in such cases as an indication of increased immune activity. To the best of my knowledge, this may be the first clear report indicating that separately, male and female level of Cu increase in patients with *G. intestinalis*. This may possibly affect some specific enzyme systems, which can consequently exhibit pathology and may require therapy. High level of Cu may cause tendency to fail when confronted with parasitic challenge. Signs of high serum Cu can also be similar to serum Cu decrease and include nausea, diarrhea, appetite loss, muscle weakness, difficulty breathing and irregular heartbeat. Increased Cu concentration in progressive cases may be caused by inefficient immune activation but over accumulation of Cu by the growing parasite may also play a role.

In conclusion, although, our findings suggest altered element metabolism due to the pathophysiologic changes of *G. intestinalis*, a detailed biochemical and molecular studies on large sample size are needed to put forward a relationship between *G. intestinalis* (and may be other parasitic diseases) and serum element level changes.

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