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# SERUM LEVELS OF ZINC, COPPER, IRON, $B_{12}$ & MAGNESIUM IN CHILDREN DIAGNOSED WITH GIARDIA INTESTINALIS AND ENTEROBIOSIS VERMICULARIS

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## ABSTRACT
Parasitic infections are highly prevalent in the general population. A relation between a parasitic infection and absorption of minerals is not an easy task. Serum levels of copper, $B_{12}$, zinc, Iron and magnesium were prospectively measured in 60 children with intestinal parasitic infection. Thirty-nine children with Enterobius vermicularis were treated with pyrantel pamoate and 25 children with Giardia lamblia with tinidazole and metronidazole. Three months after treatment, significant differences in serum copper, zinc and magnesium were seen in patients with E. vermicularis infection and in serum magnesium levels in patients with G. lamblia. Although the pathogenic mechanism is not clear, these findings could reflect a deficiency related to malabsorption due to mucous affection. Early detection and treatment of intestinal parasitosis could avoid these serum mineral deficiencies. Thus, knowledge of eating habits nutritional value in tropical infectious zone is essential to fight effectively against infections and avoid the loops in course of infections or food intake. In this study we investigated about micronutrients as iron, copper, manganese and zinc. Overall, sauces showed a predominantly iron compared to zinc, manganese and copper; the mean content of iron in ingredients and sauces green leafy vegetables and other foods like fruits ($P < 0.0001$). Considering the exogenous origin of these micronutrients, and the effect of their interaction, it would be suitable to evaluate their content in other foods and to study their effects in the host-parasite interaction to avoid the loss and fight effectively against infections. **Background:** There are scant evidences between giardiasis and enterobiasis with human mal-absorption of micronutrient. **Objectives:** The aim of the present study was to found out the changes in the serum zinc, copper, magnesium and vitamin $B_{12}$ levels in schoolchildren infected with Giardia intestinalis and Enterobius vermicularis. **Patients and Methods:** This case-control study was carried out among 60 children from aged 6 - 12 years old. Three stool examinations were done using standard techniques for identification parasitic infection. Fifty E. vermicularis and 37 G. lamblia infected individuals were enrolled as study groups. Serum levels of copper, zinc, magnesium were assessed by auto analyzer and vitamin $B_{12}$ levels were measured using gamma counter. ANOVA and paired t-test analyses were used to determine the association between infections and trace elements changes. **Results:** The prevalence of G. intestinalis and E. vermicularis infections were 10.3% and 13.9%, respectively. The serum zinc, copper and magnesium levels showed a significant decrease in individuals infected with G. intestinalis and E. vermicularis ($P < 0.05$). Mean values of Giardia positive and negative groups for copper 143.65 ± 16.51 and 176.26 ± 17.6µg/dL, zinc 62.26 ± 16.06 and 80.66 ± 23.58 µg/dL and magnesium 1.82 ± 0.23 and 2.01 ± 0.16 mg/dL, respectively. Mean values of Enterobius positive and negative groups for copper 145.55 ± 26.84 and 176.26 ± 17.6 µg/dL, zinc 72.7 ± 17.92 and 80.66 ± 23.58 µg/dL and magnesium 1.93 ± 0.11 and 2.01 ± 0.16 mg/dL, respectively.

**KEYWORDS:** Giardia intestinalis and Enterobius vermicularis.

## INTRODUCTION
Intestinal parasites are still important a worldwide public health problem. Giardia lamblia is one of the important intestinal parasites that cause both acute and chronic diarrheal diseases in human. Symptomatic giardiasis is also characterized by epigastric pain, nausea, vomiting and weight loss. Giardia is a flagellated protozoa, has two forms, cyst and trophozoite. Infection is initiated by ingestion of cysts from contaminated food and water and by person-to-person contact.[6] Trophozoite of Giardia colonizes the lumen of the small intestine but do not invade the epithelium or deeper layers of the mucosa. Infections with Giardia are usually self-limited in immune competent individuals. Mucosal defenses against Giardia must act in the small intestine. Intestinal parasitic infections have a worldwide distribution and

constitute considerable public health problems especially in developing countries. Giardia lamblia and Enterobius vermicularis are one of the most common intestinal parasitic infections worldwide, that affect people particularly children in developing countries. Approximately 3.5 billion people are infected by intestinal parasites and more than 450 million children are ill because of this parasitic infection.[5] Current worldwide prevalence of giardiasis and enterobiasis among children under 10 years of age was to range from 15 - 20% and 11.4 - 61%, respectively.[10] Apart from causing morbidity and mortality, infection with pathogenic parasites of intestine has known to cause iron deficiency anemia, growth retardation in children and other physical and mental health problems. Also, chronic intestinal parasitic infections have been about the spreading and severity of other infectious diseases of viral origin, tuberculosis and malaria.[4] Low levels of health education, poor individual and public health, poor drinking water, overcrowded conditions and poor environmental sanitation increase the prevalence of giardiasis and enterobiasis.[7] The main impact of human intestinal parasitic infections is its relation to trace elements and vitamin deficiency, which may have effects at the community level as regards work and productivity in adults and learning and school performance in children. Children who have suffered these deficiencies may give less attention to education and social skills irrespective of IQ (intelligence quotient). Trace elements regulate metabolic pathways and the immune response, as well as suppress the incidence of various diseases.[8] The most important vital elements in the human body are zinc, copper, magnesium and vitamin $B_{12}$.[11] Zinc is especially vital for the immune system functions and its depletion is associated with decline in lymphocyte and thymus functions. Due to its role in immune system functions, zinc deficiencies make infants suffer from acute diarrhea. Zinc deficiency is another increasing public health problem. Its global prevalence was estimated at 31%, ranging from 4 - 73% across developing countries.[3] Zinc deficiency has been found to be caused by poor intake and malabsorption and has been associated with growth retardation, neurosensory changes, impaired cognitive function, abnormal immune functions and death. Zinc and magnesium also helps in the production of antibodies and T-cell and other blood cell activities.[9] Copper is essential for producing red blood cells, hemoglobin formation and absorption of iron and for the activity of various enzymes. Copper and zinc are cofactors for cytosolic superoxide dismutase and their decreasing levels affect on the activity of cytosolic superoxide dismutase. Superoxide dismutase is a metalloenzyme capable of scavenging superoxide radicals by catalyzing their dismutation to reactive oxygen species. 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. Vitamin $B_{12}$ and folate deficiency are related to cell proliferation and anemia. Also, vitamin $B_{12}$ is involved in DNA synthesis.[2] The association between trace elements deficiency and G. intestinalis and E. vermicularis infection has scarcely been investigated and links are controversial.

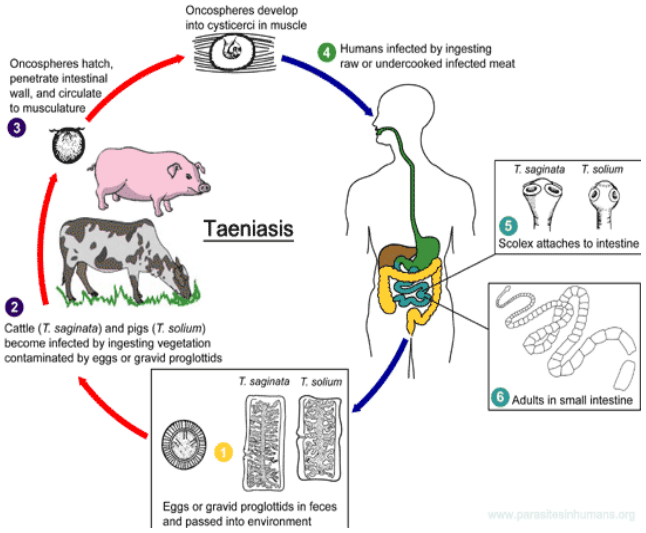
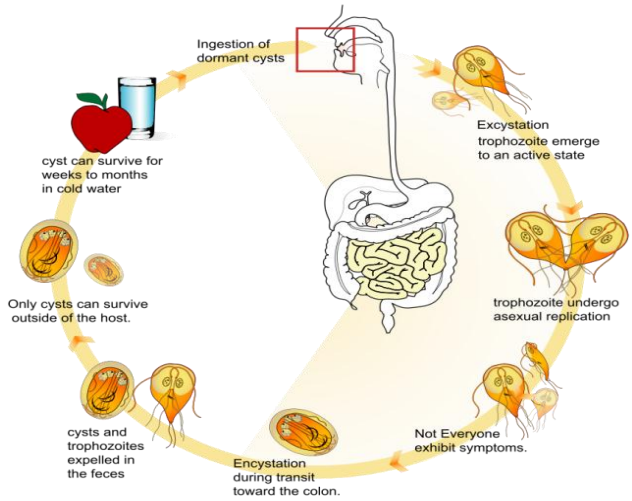



**Figure 1**

## AIMS AND OBJECTIVES

The aim of the present study was to investigate the changes of the serum zinc, copper, magnesium G. intestinalis and E. vermicularis in comparison to normal subjects inhabiting Punjab. Patients were patients attending the GGS Medical College and Hospital, Faridkot. Controls were the friends and relatives accompanying the patients.[1] This case control study was carried out on among 60 patients & 60 controls. The study participants were selected using a multistage sampling method. Stool samples were collected in sterile clean stool cups from all individuals. Examination of fecal samples for detection of G. intestinalis cyst and/or trophozoite was done by direct wet smear and standard formol-ether concentration method immediately. E. vermicularis was diagnosed using the Graham technique. The control groups consisted of 60 (giardiasis) and 50 (enterobiasis) age and sex matched healthy volunteers according to the criteria described in the questionnaire.[10] Some questionnaires were completed by parents, including questions about records for malabsorption, analyzed parameter deficiencies, and infection with parasites. Both groups had no record of serum shortage of mentioned factors and malnutrition.[11] Blood samples were collected from the case and control groups after taking parent's permission. The serum was separated from the clotted blood in both groups and kept in freezer for further biochemical laboratory examination. All blood samples were measured for the amount of copper, zinc, magnesium and vitamin $B_{12}$. Copper, $B_{12}$, zinc and magnesium levels were assessed by calorimetric endpoint method by auto analyzer (AU 480 & Chemiluminescence Access of Beckman USA). Vitamin $B_{12}$ levels were measured by chemiluminescence. ANOVA and paired t-test analyses were used to determine the association between

giardiasis and enterobiasis and trace elements changes (SPSS-17 for windows, SPSS Inc. Chicago, USA). P value less than 0.05 was regarded as statistically significant.




**Life cycle of Giardia lamblia**
**Figure 2**

**Table 2 Mean Serum Levels of Biochemical Parameters in 100 Enterobius Positive and Negative Individuals**

| Parameters | Enterobiasis | Control | P Value |
|---|---|---|---|
| Serum Zinc, µg/dL | 72.7 ± 17.92 | 80.66 ± 23.58 | 0.05 |
| Serum Cu, µg/dL | 145.55 ± 26.84 | 176.26 ± 17.60 | 0.0001 |
| Serum Mg, mg/dL | 1.93 ± 0.11 | 2.01 ± 0.16 | 0.05 |
| Serum Vitamin $B_{12}$, pg/mL | 514.75 ± 142.96 | 538.00 ± 122.09 | 0.54 |

**OBSERVATION**

Totally, 24 (34.5%) individuals were found positive for at least one intestinal parasite. The prevalence of G. intestinalis and E. vermicularis infection were 10.3% (n = 37) and 13.9% (n = 50), respectively.[12] Sex was not associated with parasitic infections. No statistically significant difference of infection was noted among the age groups. The results of this study are summarized and. Mean serum levels of zinc, copper and magnesium in G. intestinalis positive is remarkably lower than *G.* intestinalis negative group (P = 0.05, P = 0.0001 and P = 0.05, respectively).[13] In addition, there was no significant difference in serum vitamin $B_{12}$ between both groups. Besides ranges of copper, zinc, magnesium and vitamin $B_{12}$ distributions of E. vermicularis positive and E. vermicularis negative individuals was shown in Table 2. Results clearly showed that the serum zinc, copper and magnesium levels decreased significantly in children with enterobiasis, but no significant difference in serum vitamin $B_{12}$ between both the groups.

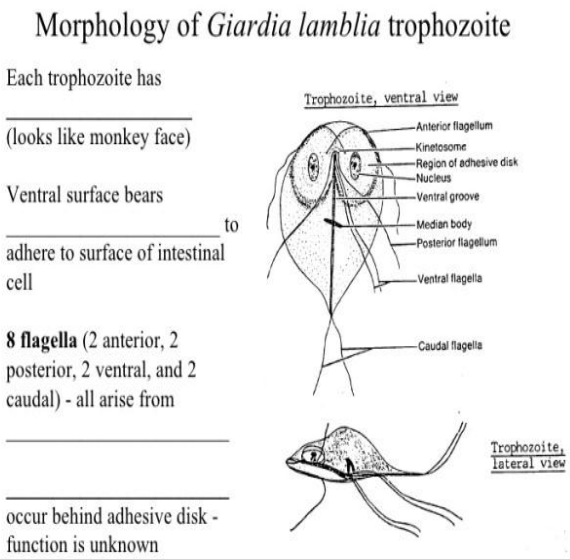



**Figure 3**

**Table1 Mean Serum Levels of Biochemical Parameters in 60 Giardia Positive and Negative Individuals**

| Parameters | Giardiasis | Control | P Value |
|---|---|---|---|
| Serum Zinc, µg/dL | 62.26 ± 16.06 | 80.66 ± 23.58 | 0.04 |
| Serum Cu, µg/dL | 143.65 ± 16.51 | 176.26 ± 17.60 | < 0.0001 |
| Serum Mg, mg/dL | 1.82 ± 0.23 | 2.01 ± 0.16 | 0.02 |
| Serum Vitamin $B_{12}$, pg/mL | 525.75 ± 138.40 | 538.00 ± 122.09 | 0.74 |

## DISCUSSION

### Vermicularis lumbricoides

The present study showed 10.3% and 13.9% individuals were infected to *G. intestinalis* and *E. vermicularis*, respectively. The serum zinc, copper and magnesium levels showed a significant decrease in individuals infected with G. intestinalis and E. vermicularis. There was no significant difference between vitamin $B_{12}$ levels between the two groups. Intestinal parasitic infections such as Giardia and Enterobius are still an important public health problem and responsible for considerable morbidity and occasional mortality among the infected population throughout the world, mainly in specific geographical areas and among people with specific socioeconomic status.[14] However some intestinal parasites, like Enterobius are still ignored, even though it can defect the growth and learning of the young population. Intestinal parasites use carbohydrates, lipids, minerals, vitamin and other food sources of the host in order to essential energy of the life cycle.[15]

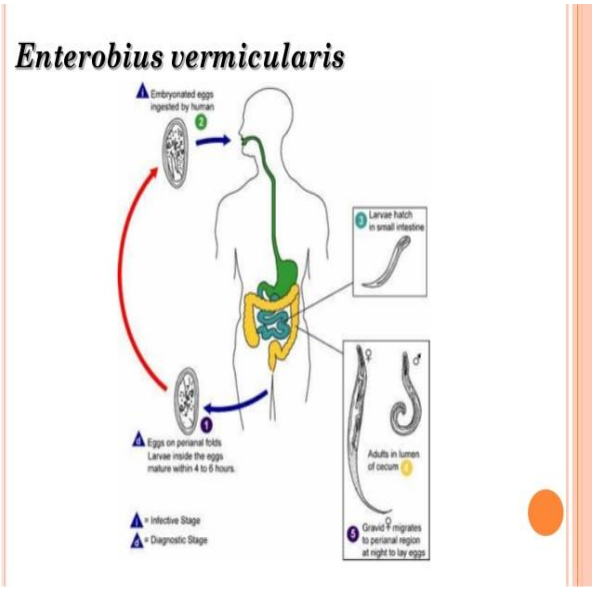



**Figure 4**

Minerals have an important role in the metabolic and physiology of the human body, especially in the growing children. Several studies have been reported that elements such as copper, zinc and magnesium are vital for growth and development.[16] They have a significant role in the structure of some cellular enzymes, different immunological process and the resistance to the free radical damage by stabilizing the cellular membrane. Low serum levels of copper and zinc could causes harm in cellular and enzymatic roles. Serum zinc levels were lower during protozoan infections. In addition, elevations of serum copper levels are noted in most of the acute and chronic parasitic infections. The association of giardiasis and enterobiasis with the malabsorption of zinc, copper, magnesium and vitamin $B_{12}$ remains controversial. In several studies conducted about trace elements in giardiasis has shown a significant decrease in zinc levels while there was an obvious increase in copper levels. However, in few reports there was no significant difference in serum levels of zinc, copper and magnesium elements between the two groups. In contrast, a study showed mean serum levels of copper in Giardia positive, was significant upper than the Giardia negative groups. Also in some investigation showed the mean serum levels of copper, zinc and magnesium in individuals with enterobiasis were significantly lower than in the control groups. The present study, showed a significant decrease in zinc, copper and magnesium levels in G. intestinalis positive and E. vermicularis positive individuals compare to the control groups. Results obtained in our study are compatible and agreeing with result of other researches. As it was mentioned above zinc cannot be stored in the body, therefore it could be easily declined in the serum. However, 90% of serum copper is stored in the bound form to the ceruloplasmin. Elevations of serum copper levels are observed in most of the infections in relation to fluctuations of ceruloplasmin as an acute phase reactant. Vitamin $B_{12}$ is involved in the synthesis of important biochemical transmitters in the brain and nervous system. Vitamin $B_{12}$ is involved in the synthesis of DNA. It is especially important during growth and cell proliferation. Vitamin $B_{12}$ is important in the transport and storage of folate in the cells. Folate is also important in cell division and DNA synthesis. Folate must be digested by pancreatic juice in the duodenum, where G. intestinalis usually colonizes. Damage of intestinal epithelium occurs by adherent trophozoite of G. intestinalis. It has been proposed as one important mechanism in the pathogenesis of infection. Giardiasis can cause vitamin $B_{12}$ deficiency, bowel inflammation and interfere with folate absorption. Few studies showed $B_{12}$ deficiency due to giardiasis. However, some reports found normal absorption of folate during G. intestinalis infection. In this study, no significant difference was viewed between the G. intestinalis positive and G. intestinalis negative individuals and E. vermicularis positive groups compared to control groups. Our results regarding trace elements, giardiasis and enterobiasis decreased serum, zinc and magnesium

levels, while they no significant effect on serum levels of vitamin $B_{12}$.

## CONCLUSIONS

The results showed that giardiasis and enterobiasis decreased serum levels of copper, zinc and magnesium. Further studies are needed to clarify the actual mechanism governing the zinc, copper, magnesium and vitamin $B_{12}$ giardiasis/enterobiasis interaction.

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