

IMPACT OF AMOEBIASIS AND GIARDIASIS ON TRACE ELEMENTS AMONG EGYPTIAN CHILDREN

BY

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Abstract

Giardia lamblia and *Entamoeba histolytica* are protozoan parasites causing marked morbidity in Egyptian children. A case-control random study was conducted from September to January 2020 and included 49 children (1-18 years) attending Al Hussein University Hospital, Cairo, Egypt. They had amoebiasis (30 cases) and giardiasis (19 cases) and considered as the study group. Another 50 healthy persons were considered as a control group. All cases were subjected to the estimation of serum levels of Zinc, Copper, and Iron.

Serum Zinc level was significantly decreased in the infected group than the control group. The mean and standard deviation in the study group was 66.83 ± 8.60 (amoebiasis was 65.64 ± 8.26 & giardiasis was 68.71 ± 9.01) compared to the control group 95.67 ± 8.34 . Serum Copper was significantly increased in infected group than control group. The mean and standard deviation was 129.93 ± 19.45 , for the amoebiasis was 130.63 ± 20.17 and giardiasis was 128.81 ± 18.76 as compared to the control group 85.07 ± 6.29 .

Serum Iron level was significantly decreased in the infected group than the control group. The mean and standard deviation in the study group was 50.63 ± 5.23 (amoebiasis was 49.96 ± 5.61 & giardiasis was 51.69 ± 4.50) compared to the control group 82.87 ± 9.28 .

Keywords: Children, Amoebiasis, Giardiasis, Trace elements

Introduction

Giardia lamblia and *Entamoeba histolytica* are protozoan parasitic agents that significantly cause diarrheal disease morbidity in developing countries including Egypt (Charles *et al*, 2004). *Giardia lamblia* is a flagellate that inhabits the gut lumen in close association with enterocytes. It is a major cause of malabsorptive diarrheal disease throughout the world mainly in children younger than 10 years with peak prevalence rates of 15-20% (Pennardt *et al*, 2008).

Entamoeba histolytica is an amoeba that preferentially inhabits the mucosal region of the gut lumen, and under certain circumstances becomes invasive, and more than 100,000 individuals died annually from invasive amoebiasis, was considered as the third cause of death among the parasitic infections after malaria and schistosomiasis (Charles *et al*, 2004).

Alteration in the concentrations of serum

trace elements as Iron, Zinc, and Copper is commonly found in patients with intestinal parasitic infections mainly giardiasis. These trace elements are important micronutrient elements for optimal human metabolic functions including catalytic structural and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones, and biological membranes. They also play an important role in reproduction, growth, and development of the immune system (Salgueiro *et al*, 2001). Zinc is the essential element required to maintain the normal physiological function of all forms of life. It is essential for growth, reproductive functions, and glucose metabolism (Ciftci *et al*, 2003). Copper is an essential trace element that plays an important role in mitochondrial cytochrome oxidase, cytoplasmic superoxide dismutase (SOD), and ceruloplasmin, which are known to have antioxidant functions (Mahboob *et al*, 2005).

Iron is a physiologically essential trace element and considered a useful component of cytochromes and oxygen-binding molecules. However, iron is also a biochemically dangerous element. It can damage tissues by catalyzing the conversion of H₂O₂ to free-radical ions that attack cellular membrane lipids, proteins, and DNA (Prasad, 2003).

Also, some defense mechanisms that counteract the effect of oxidant radicals which prevent the production of free radicals, decrease their activities, or destroy them need trace elements. The antioxidant Glutathione peroxides contain Selenium, Cu, and Zn, while the superoxide dismutase system contains copper and zinc in their structure which diminish the harmful effects of free oxygen radicals. Catalase needs iron as a cofactor as well as ceruloplasmin which is the carrier of Cu. Decreasing these trace elements reduce the action of the antioxidant systems which leads to hyperactivity and inflammation procedures (Schmidt et al., 1996).

The present study aimed to determine the impact of amoebiasis and giardiasis on trace elements among Egyptian children.

Patients and Methods

A case-control random study was conducted on the patients suffering from gastrointestinal manifestations who attending the outpatient clinics of Pediatrics and Tropical Medicine at Al Hussein University Hospital, Cairo, Egypt aging (1-18 years), during the period from early September to the end of January 2020. A randomly selected 49 patients suffering from amoebiasis (30 cases) and giardiasis (19 cases) as diagnosed by stool examination as well as Copro-antigen detection was chosen and considered as the infected study group. Another 50 healthy persons, free from intestinal parasites of the same age and gender were chosen and considered as the control group.

Exclusion criteria: included patients below one year as well as above 18 years, non-intestinal protozoal infections, and chronic diseases and did not take vitamins or miner-

al supplements for the last 3 months as well as immunocompromised patients.

All children were subjected to the following: 1- A questionnaire filled out by an interview with the patient and/or one of his/her parents or guardians. 2- Fresh stool samples were collected in clean, labeled, and wide-mouthed plastic containers that had tight-fitting overlapping lids. 3- Collection of aseptically 5 ml of venous blood samples using metal-free and stainless-steel needles into appropriately coated tubes (Becton Dickinson Laboratories, Franklin Lakes, NJ, USA) for measurement of serum levels of Zinc, Copper, and Iron, using Atomic Absorption Spectrophotometer according to the manufacturer.

Stool samples were examined macroscopically and microscopically by: 1- Direct wet smear method (Garcia, 2007). 2- Concentration methods by simple floatation and formal ether concentration techniques (Garcia, 2007). 3- Copro-antigen detection by ELISA was performed using a RIDASCREEN *Giardia and Entamoeba* kits (R-Biopharm AG, Darmstadt, Germany). The test was done according to the manufacturer's instructions. Statistical analysis: Data were collected, tabulated and statistically analyzed.

Ethical approval was obtained through the Ethical Committee of Al-Azhar University's Hospitals before the commencement of the study and only patients whose parents/ guardians signed the informed consent were included in the present work.

Results

The distribution of the chosen protozoal infection among the study group was presented in (Tab.1).

The age distribution among both study and control groups was presented in (Tab. 2 & Fig. 1). Sex and residence distribution were presented in (Tabs. 3 & 4) while serum trace elements level among both groups was presented in (Tab.5). Statistical comparison of serum trace elements level among both groups was presented in (Tabs. 6, 7, & 8).

Table 1: Distribution of the chosen protozoal infection among the study group

stage	Amoebiasis group (n =30)	Giardiasis group (n = 19)	Total Infected Group (n = 49)
Cysts	21 (70%)	16 (84.21%)	37 (75.51%)
Trophozoites	4 (13.33%)	1 (5.2%)	5 (10.20%)
Cysts and Trophozoites	5 (16.66%)	2 (10.52%)	7 (14.28%)

Table 2: Age distribution among both study and control groups

Age Group	Amoebiasis	Giardiasis	Total	Control
1 - <3	2 (6.7%)	1 (5.2%)	3 (6.1%)	3(6%)
3 - <6	9 (30%)	6 (31.6%)	15 (30.6%)	14(28%)
6 - <9	9 (30%)	5 (26.3%)	14 (28.6%)	14(28%)
9 - <12	5 (16.7%)	4 (21.1%)	9 (18.4%)	10(20%)
12 - <15	3 (10%)	2 (10.6%)	5 (10.2%)	6(12%)
15 - 18	2 (6.6%)	1 (5.2%)	3 (6.1%)	3(6%)
Total	30 (100%)	19 (100%)	49 (100%)	50(100%)
Mean±SD	7.47±3.58	7.32±3.74	7.41±3.61	7.58±3.84

Table 3: Sex distribution among both groups

Sex	Amoebiasis	Giardiasis	Total infected	Control	P-value
Male	16 (53.3%)	12 (63.2%)	28 (57.1%)	29(58%)	1.00
Female	14 (46.7%)	7 (36.8%)	21 (42.9%)	21(42%)	1.00
Total	30 (100%)	19 (100%)	49 (100%)	50(100%)	1.00

Table 4: Residence distribution among both groups

residence	Amoebiasis	Giardiasis	Total infected	Control	P-value
Rural	23(76.7%)	16(84.2%)	39(79.6%)	38(76%)	0.95
Urban	7(23.3%)	3(15.8%)	10(20.4%)	12(24%)	0.90
Total	30 (100%)	19 (100%)	49(100%)	50(100%)	0.94

Table 5: Serum trace elements level among both groups

Trace Elements	Amoebiasis (n= 30)		Giardiasis (n=19)		Total infected (n=49)		Control Group (n=50)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Zn (mcg/dl)	65.64	8.26	68.7	9.01	66.83	8.60	95.67	8.34
Cu (mcg/dl)	130.63	20.17	128.81	18.75	129.93	19.45	85.07	6.29
Fe (mcg/dl)	49.96	5.61	51.69	4.50	50.63	5.23	82.86	9.28

Table 6: Statistical comparison of serum zinc level among both groups

Comparison	Mean difference	Value of q *	P-value **
Amoebiasis vs. Giardiasis	-3.06	1.75	ns P>0.05
Amoebiasis vs. Control group	-30.03	21.77	P<0.001
Giardiasis vs. Control group	-26.96	16.75	P<0.001
Total study group vs. control group	-28.84	16.94	P<0.001

*Tukey-Kramer: If value of q greater than 3.373 then P-value less than 0.05, ** P-value = < 0.001, considered extremely significant. Variation among column means = significantly greater than expected by chance

Table 7: Statistical comparison of serum copper level among both groups

Comparison	Mean difference	Value of q	P-value
Amoebiasis vs. Giardiasis	1.82	0.60	ns P>0.05
Amoebiasis vs. Control group	45.56	19.29	P<0.001
Giardiasis vs. Control group	43.74	15.87	P<0.001
Total study group vs. control group	44.85	15.49	P<0.001

Table 8: Statistical comparison of serum Iron level among both groups

Comparison	Mean difference	Value of q	P-value
Amoebiasis vs Giardiasis	-1.73	1.10	ns P>0.05
Amoebiasis vs Control group	-32.90	26.61	P<0.001
Giardiasis vs Control group	-31.16	21.60	P<0.001
Total study group vs control group	-32.23	21.22	P<0.001

Discussion

Amoebiasis and giardiasis are among the most common intestinal protozoa associated with diarrhea (Boughattas *et al*, 2017). The Global Burden of Disease Study (GBDS) estimated that amoebiasis was associated

with more than 55,000 deaths and 2.2 million disability-adjusted life years (Lozano *et al*, 2012). In developed countries, *Giardia intestinalis* is considered as one of the most common causes of water-borne outbreaks of gastroenteritis. While in developing coun-

tries, the infection was highly endemic and contributes to malnutrition, which may affect growth and later cognitive function in children (Mørch *et al*, 2009). The infection was regarded as a serious public health problem, as it causes iron deficiency anemia, micronutrient deficiencies as Zinc, Copper, Magnesium, and Selenium (Simsek *et al*, 2004).

The present study investigated the impact of amoebiasis and giardiasis on trace elements (Zn, Cu, and Fe) among Egyptian patients where a case-control random study was conducted on 49 patients (1-18 years old) suffering from amoebiasis (30 cases) and giardiasis (19 cases) and considered as the infected study group. Another 50 healthy persons (of the same age), free from intestinal parasites of the same age and gender were chosen and considered as the control group.

Among stool samples of the study group (n=49), 37 of them were positive for cysts (75.51%) and trophozoites were detected in 5 samples (10.20%), while both cysts and trophozoites were detected in 7 samples (14.28%). As regard amoebiasis group (n=30); cysts were detected in 21 samples (70%) with semi-formed stool, while among loose stool, trophozoites were detected in 4 samples (13.33%) and both are detected in 5 samples (16.66%).

While, in the giardiasis group (n=19) cysts were detected in 16 samples (84.21%) with semi-formed stool, while in loose stool, trophozoites were detected in one sample (5.2%) and both were detected in 2 samples (10.52%). These results coincided with Hill (2001) who reported that liquid stool may yield motile trophozoites while semi-formed stool will contain only cysts.

In the present study, the age of the total infected group (n= 49) ranged from 1-18 years, the highest level of infection is in the age group (3 - <9 years) (59.2%) and the mean \pm standard deviation of the age was (7.47 \pm 3.58) for amoebiasis group and (7.32 \pm 3.74) for giardiasis group which coin-

cided with that obtained by Volotao *et al*. (2007).

This fact was explained by Culha and Sangün, 2007 who reported that children under 6 years of age are more frequently infected with *Giardia lamblia* due to immature immune system and frequent exposure to infection. On the other hand, Azazy *et al*. (2002) stated that giardiasis is commonly seen in children aged 4-12 years in developing countries.

In the present study, the total infected group was 28 were males (57.1 %) and 21 were females (42.9 %), in amoebiasis group; 16 were males (53.3%) and 14 were females (46.7%) and in giardiasis group; 12 were males (63.2%) and 7 were females (36.8%). Besides, Lucas and Upcroft (2001) found that amoebiasis is more frequent in males than females.

Also, Al-Mekhlafi *et al*. (2005) reported in their study that giardiasis was more common in males than in females while Lengerich *et al*. (1994) reported that giardiasis was more common in females than in males. Also, Lebwohl *et al*. (2003) found the incidence of giardiasis was equal among men and women.

No doubt, the trace elements (Zn, Cu, Fe) are vital and essential micronutrients for reproduction, growth, and development with regulatory, immunologic, and antioxidant functions resulting from their action as essential components or cofactors of enzymes throughout metabolism. Low serum levels of these elements could cause impairment in cellular, physiological, and enzymatic functions (Shenkin, 2006).

Zinc is required as a cofactor for the function of intracellular enzymes that may be involved in protein, lipid, and glucose metabolism. It may participate as an integral component of several antioxidant enzymes as a superoxide dismutase enzyme. It is an important element for the immune system, energy production, and absorption of vitamins. So, zinc deficiency can lead to chronic diarrhea in the infants and children Çeliksöz

et al. (2005).

As regard copper, Wilson (2008) reported in his study that copper is vitally important for the immune system and the nervous system, production of energy in cells as well as the absorption of iron and hemoglobin formation. It has antioxidant function through superoxide dismutase and ceruloplasmin enzymes.

Generally, elevated copper levels are reported in response to infections. This is attributed to changes in the concentration of specific tissue proteins controlled by cytokines (Shenkin, 2006). Turnlund (2006) reported that the most common clinical signs of copper deficiency are anemia, impaired growth, abnormally low numbers of white blood cells a condition that may be accompanied by increased susceptibility to infection and neurological signs.

El Hioui *et al.* (2008) in their study found that iron was important for hemoglobin synthesis that carries oxygen to all parts of the body, important for the immune system, DNA synthesis and it has antioxidant function through catalase and peroxidases enzymes. They also reported that iron deficiency can exist with or without anemia that can lead to serious health problems, such as poor cognitive, motor development, and behavioral problems in children. Also, recurrent infections and delayed growth.

The exact mechanism of micronutrient deficiency in giardiasis is not clear. Some interacting factors as cytopathic substances are thought to be responsible. *Giardia* trophozoites attach to the microvillus-covered surface of the duodenum and jejunum. The attachment process damages the microvilli and causes villous atrophy and reduces the gut absorptive capacity (Hawrelak, 2003). The attachment of *Giardia lamblia* trophozoites by sucking disk to the intestinal epithelium can distort and disrupt microvilli, rapid multiplication of trophozoites can also lead to microvillus, crypt atrophy and affect the activity of digestive enzymes such as lipases, proteases, and disaccharidases which inter-

fere with digestion and absorption (Roxström-Lindquist *et al.*, 2006).

In the present study, serum zinc level is significantly decreased in the infected group when compared to the control group. The mean and standard deviation in the total study group was 66.83 ± 8.60 (amoebiasis group was 65.64 ± 8.26 and giardiasis was 68.71 ± 9.01) but in the control group was 95.67 ± 8.34 . Statistical comparison of serum Zinc by One-way Analysis of Variance (ANOVA) test shows that P-value is < 0.001 , considered extremely significant.

This may be due to dietary factors (intake of food low in antioxidant) so the body will use endogenous superoxide dismutase (SOD) to scavenge the overproduction of reactive oxygen species as a result of parasitic infection which leads to decrease serum zinc level. Also, it could be due to the antioxidant effect of zinc which plays a structural role in the maintenance of Cu-Zn-SOD structural integrity Çeliksöz *et al.* (2005).

Also, Demicri *et al.* (2003) showed that serum zinc level was significantly decreased in giardiasis when compared to controls which may be due to poor absorption.

Nevertheless, Kiliç *et al.* (2003) and Çulha and Sangün (2007) did not find any significant difference in zinc levels in patients with giardiasis compared to controls.

As zinc is not stored in the body in large amounts, serum zinc levels could easily decline during infections in children with low zinc intake (Yones *et al.*, 2015).

As regards copper, in the present study, the serum level was significantly increased in the infected study group when compared to the control group.

The mean and standard deviation in the total study group was 129.93 ± 19.45 (amoebiasis group was 130.63 ± 20.17 and giardiasis was 128.81 ± 18.76), but in the control group was 85.07 ± 6.29 . Statistical comparison of serum zinc by One-way Analysis of Variance (ANOVA) test shows that P-value is < 0.001 , considered extremely significant. The significant increase in the serum level of

copper demonstrates that inflammation initiates free radical generation.

This may be explained by the antagonistic effect of zinc and zinc deficiency in a patient with parasitic infection and increase copper absorption by the gastrointestinal tract. Zinc antagonize copper absorption by inducing the synthesis of thionine, which have a higher affinity to copper than zinc (Emerit *et al.*, 2001). Similarly, Ertan *et al.* (2002) have studied a total of 45 children aged between 2 and 14 years. They found that giardiasis increased the serum level of copper due to undulating levels of ceruloplasmin during infections. The study done by Eckersall and Conner (1988) stated that ceruloplasmin may be involved in the transfer of copper into cells, or as an antioxidant, as it is the major extracellular scavenger of oxygen-derived free radicals secreted by phagocytes. Ceruloplasmin level increased during infections, in 50% only of cases, and no increases at all found in some conditions where other reactants have increased.

However, Karakas *et al.* (2001) studied the serum copper in a total of 20 Turkish children with giardiasis, and 20 healthy children were selected as control. They showed that serum Cu levels were not significantly differed when compared to control. They stated that absorption of Cu through the whole small intestine and to a lesser extent through large intestine may be responsible, in addition to lower recommended dietary intake about 0.9 mg/day which is very close to the lower limit of 1 mg/day found in the diet. Also, Demicri *et al.* (2003) and Çulha and Sangün (2007) have investigated the level of Cu in chronic giardiasis and they found that there was no significant difference in copper between giardiasis and control groups. They stated that a small amount of Cu absorbed by skin and inhalation also the intestinal absorption of Cu varies with dietary intake, so it increased with low intakes of Cu and lowered with high intakes of it.

One of the main influences of infections in the hemopoietic system is concerning iron

metabolism. Malabsorption related iron deficiency is expected in patients with giardiasis, which could be protective considering the role of iron in the formation of the hydroxyl radical. However, asymptomatic giardiasis did not affect the intestinal absorption of iron and the hemoglobin response to oral iron therapy in a study conducted with iron-deficient anemic children aged between 1- and 6-years-old.¹⁵ Iron deficiency in patients with giardiasis could be treated successfully by anti-giardia compounds, not with iron supplementation (Taneli *et al.*, 1994).

As regards Iron, in the present study, serum iron (Fe) level was significantly decreased in infected study groups when compared to the control group. The mean and standard deviation in the total study group was 50.63 ± 5.23 (amoebiasis group was 49.96 ± 5.61 & giardiasis was 51.69 ± 4.50) but in the control group was 82.87 ± 9.28 . The statistical comparing of serum Zinc by (ANOVA) showed ($P = < 0.001$), considered extremely significant. There is a significant difference between all studied groups and the control group, but no significant difference statistically between the amoebiasis and giardiasis groups.

Also, Demicri *et al.* (2003) have tested serum Iron level among 34 children with chronic giardiasis and were compared with controls. They found that there was a significant difference in Iron between giardiasis and control groups. They stated that malabsorption was responsible for this and it was evidenced by finding no change in serum levels of Fe after oral intake of Fe supplements in giardiasis group. Also, Ertan *et al.* (2002) found that giardiasis significantly lowered the serum levels of Fe in the infected group than in the control group due to malabsorption. Also, Çulha and Sangün (2007) found there was a significant difference in Fe levels between the infected group with giardiasis and the control group.

However, Saba Adnan *et al.* (2009) have studied one hundred patients with parasitic infections (35 patients with toxoplasmosis,

32 patients with leishmaniasis, 24 patients with amoebiasis and 9 patients with giardiasis) while 130 were a control group. children, they found a significant increase in the serum level of the Iron inpatient group as compared with control subjects. This difference may be due to erythrocytes degradation after phagocytosis, also the release of ferritin into circulation (Salgueiro et al 2001).

Conclusion

The intestinal protozoal infection is an important cause of malabsorption in children. It can elevate serum copper levels and decrease serum zinc and iron levels. Low serum zinc is associated with a decline in lymphocyte and thymus functions, while iron deficiency can cause significant impairments in the hemopoietic system.

Consequently, it is essential to do proper-diagnose and treat pediatric patients to prevent potential damages to child growth and health.

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Explanation of figure

Fig. 1: Age distribution among infected group

