

Impact of *Giardia lamblia* on Growth, Serum Levels of Zinc, Copper, and Iron in Egyptian Children

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Received: 13 January 2010 / Accepted: 3 March 2010 /
Published online: 26 March 2010
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Abstract The aim of this study is to evaluate the serological levels of zinc, copper and iron in *Giardia lamblia*-infected children and to study the effect of giardiasis on their weight compared to controls. We studied 30 children, 1–10 years old, who attended the outpatient clinics of Cairo university pediatric hospital, with gastrointestinal complaints and diagnosed as having giardiasis by stools examination, they were enrolled as a study group. The control group consisted of 30 age- and sex-matched healthy children, free of gastrointestinal complaints and free of giardiasis. Serological levels of zinc, copper, and iron were measured by atomic absorption spectrophotometer. The infected group had significantly lower weight, serum iron, and zinc than controls ($P=0.035$, <0.001 , and <0.001 respectively) and 63.3% of patients infected with giardiasis were 1–5 years old. In the infected cases, 60% suffered from of abdominal pain, 50% from weight loss, and 40% had intermittent diarrhea. Infected cases with weight percentiles below the fifth had significantly lower serum iron than those with normal percentiles (>5 th). In conclusion, most giardiasis-infected children were between 1 and 5 years, with significant affection of weight, abdominal pain, and/or intermittent diarrhea. Serum zinc and iron levels were significantly decreased in the infected group compared to control ($P<0.001$).

Keywords *Giardia* · Iron · Copper · Zinc · Children · Weight loss

Introduction

Giardiasis is a common protozoan infection with clinical manifestations in children ranging from asymptomatic carriage to persistent diarrhea with malabsorption. It can lead to growth

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and developmental retardation [1]. Over several million cases of malabsorptive diarrhea by giardiasis are estimated to occur annually worldwide and waterborne outbreaks of giardiasis are frequent in areas where no disinfected water is routinely contaminated [2].

Giardia lamblia can produce a wide spectrum of clinical manifestations ranging from asymptomatic to acute or chronic diarrhea with malabsorption syndrome and weight loss [3]. The majority of infected individuals (60–80%) have few symptoms [4]. Symptomatic infections are more common in children than adults because children have lower immunity and are more liable to have repeated infections. Symptoms develop in nearly 40–80% of infected children. Symptoms depend on whether the patient is suffering from acute or chronic *Giardia* infection [5].

Alterations in concentrations of serum iron, zinc, copper, malabsorption syndrome, and growth retardation are commonly found in patients with gastrointestinal infections, mainly giardiasis [6]. Zinc, copper, and iron are all vital micronutrient elements for reproduction, growth, and development. Low serum levels of these elements may result in serious affection of cellular, physiological, and enzymatic function [7].

The aim of this study is to evaluate the serological levels of iron, copper and zinc in *G. lamblia*-infected children and to study the effect of giardiasis on the weight of these children compared to controls.

Patients and Methods

Patients

The study was carried out at the outpatient clinics of Cairo University Pediatric Hospital. The study was conducted on 50 children 1 to 10 years old complaining of gastrointestinal symptoms such as abdominal pain, diarrhea, and flatulence who were enrolled, of whom, 30 were positive for giardiasis and were set as the study group. Thirty healthy children free of gastrointestinal symptoms, giardiasis, or any other parasitic infection were considered as a control group. Both groups did not take vitamin–mineral supplements for the prior 3 months so as not to affect the serum levels of the tested trace elements.

Methods

1. Stools samples were collected from all cases and controls included in the study. Samples were collected in sterile clean stool cups labeled with the patient's serial number and date of collection. Then parasitological examination was immediately processed.
Examination of fecal samples for detection of *Giardia* cyst and/ or trophozoite was done by direct wet smear and by formol-ether sedimentation concentration technique.
 - (a) Direct wet smear: wet films were examined using saline and iodine.
 - (b) Formalin-ethyl acetate sedimentation concentration method: done according to Garcia [8].
2. Biochemical estimation: Serum samples were collected from cases and control groups for estimation of zinc (Zn), copper (Cu), and iron (Fe) levels by atomic absorption spectrophotometer. They were determined by electrothermic atomization ET-AAS in a graphite cuvette. We used a Solaar M-6 atomic absorption spectrometer with GF 90 Zeeman graphite cuvette and FS 95 furnace autosampler [9].

- (a) Sample preparation: The samples for Fe, Cu, and Zn were prepared by centrifugation of blood samples to obtain serum then diluting the samples (0.5 ml of serum with 2 ml deionized water).
- (b) Calibration solution: Prepared by serial dilution of parent stock, which contained 1,000 µg/L using deionized water.
- (c) Optimization technique: For determination of metal concentration in both standard solution and the samples, proper wave length, and lamp current band were chosen for optimization of the wave used for each metal.
- (d) Calculation of results: By plotting standard curve, the readings of absorbance of sample and standard were plotted in semilog curve; the concentration of each metal in samples was interpreted from this curve.

Statistical Methods

Statistical Package for Social Science (SPSS 10.0) was used. Categorical variables were presented by number and percent. Continuous variables were presented by mean and standard deviation. In all tests, *P* value was considered significant if it was less than 0.05.

Results

Children enrolled in the study were classified as follows: Infected group with *Giardia* cyst or trophozoite included 30 cases and control group included 30 children age and sex matched and were negative for *Giardia* cyst and trophozoite or any other parasites. The demographic, clinical data, and serum iron, copper, and zinc levels of infected and control groups are presented in Table 1. In the infected group, 56.7% of cases were below the fifth weight percentile, compared to 26.7% in the control group. The infected group had significantly lower weight percentiles, serum iron, and zinc than controls ($P=0.035$, <0.001 , <0.001), respectively, while no difference was detected in copper.

In the infected group, 63.3% of cases were between 1 and 5 years old, and 60% (18 cases) suffered from abdominal pain, 50% (15 cases) suffered from weight loss, and 40% (12 cases)

Table 1 The Demographic, Clinical Data and Serum Iron, Copper and Zinc Level of Infected and Control Groups

	Infected group $n=30$ (%)	Control group $n=30$ (%)	<i>P</i> value
Mean age in years \pm SD	5.21 \pm 2.64	5.10 \pm 2.55	0.947
Sex distribution F:M	17:13	16:14	1.000
<3rd weight percentile	9 (30.0)	1(3.3)	0.035
3th–5th weight percentile	8 (26.7)	7 (23.3)	
5th–10th weight percentile	3 (10.0)	4 (13.3)	
10th–25th weight percentile	8 (26.7)	9 (30.0)	
25th–50th weight percentile	2 (6.7)	7 (23.3)	
50th–75th weight percentile	0 (0)	2 (6.7)	
Fe mean \pm SD	44.27 \pm 5.11	81.46 \pm 7.04	0.000
Cu mean \pm SD	80.29 \pm 6.83	82.62 \pm 5.66	0.155
Zn mean \pm SD	62.65 \pm 6.94	92.90 \pm 7.61	0.000

had intermittent diarrhea. The variation between those with or without abdominal pain, weight loss, and intermittent diarrhea regarding Fe, Cu, and Zn were not significant $P>0.05$ for all.

Table 2 showed that, in the infected group, the variation between weight percentiles (<5th and >5th) in Fe level was significant ($p<0.001$), while Cu level and Zn level were not significant. In the control group, the comparison between percentiles in Fe, Cu, and Zn level was not significant.

Discussion

In the present study, 63.3% of individuals in the infected group with giardiasis were below 5 years old. Reported rates of *Giardia* infection in some communities may be as high as 50% in children under the age of 5 years [10], which may be due to the immature immune system and frequent exposure to infection in young children [11].

Abdominal pain was the most common presenting symptom among the infected cases in the present study, followed by weight loss and intermittent diarrhea. Similarly, the occurrence of abdominal pain was reported in 50–80% of patients with *Giardia* [12] and severe watery diarrhea possibly as a consequence of malabsorption [13]. Others reported that giardiasis is a self-limited illness and that most often the infections are asymptomatic [14].

In the present study, the number of children below the fifth percentile for weight was 56.7% in the infected group, compared to 26.7% in the control. This means that giardiasis significantly affects the weight of children infected in the study since values below the fifth percentile of weight are considered abnormal. Similarly, some studies reported a significant association between *Giardia* infection and deficit in growth during the first 3 years of life [15], lower weight per age, and height for age than children who are not infected [16], as well as delay in psychomotor development [17]. On the other hand, many others did not find any association between infection by *Giardia* and nutritional status [18–21].

In the current study, serum zinc level was significantly decreased in the infected group when compared to the control group, which was also observed in Turkish children due to poor absorption of zinc in giardiasis [6] and aggravated by decreased antioxidant status due to Zn deficiency [7, 22]. As regards serum iron, in the present study, there was a significant decrease in serum Fe levels in the cases compared to the controls. These results are in concordance with many studies [7, 11, 22], were explained by the malabsorption, and were proved by finding no change in serum levels of Fe after oral intake of Fe supplements in giardiasis group. The lower serum Zn and Fe can be explained by the fact that *G. lamblia*

Table 2 Serum Iron Copper and Zinc Levels According to the Percentile Weight of Infected and Control Groups

		Weight <5th percentile	Weight >5th percentile	P value
Infected group	Fe mean \pm SD	40.91 \pm 3.5	48.65 \pm 3.0	0.00
	Cu mean \pm SD	78.90 \pm 6.7	82.10 \pm 6.7	0.210
	Zn mean \pm SD	62.78 \pm 6.4	62.46 \pm 7.8	0.903
Control group	Fe mean \pm SD	85.02 \pm 7.0	80.16 \pm 6.7	0.095
	Cu mean \pm SD	85.83 \pm 5.3	81.45 \pm 5.4	0.060
	Zn mean \pm SD	95.01 \pm 7.8	92.13 \pm 7.5	0.369

Percentiles approximated to the nearest tenth

produce variable degrees of mucosal injury, which could impair digestion and absorption. Rapid multiplication of trophozoites can also lead to microvillus and crypt atrophy and affect the activity of digestive enzymes such as lipases, proteases, and disaccharidases, which interfere with digestion and absorption [7, 23] and release cytopathic substances that damage the intestinal epithelium [24].

Copper is absorbed through the whole small intestine and, to a lesser extent, through the large intestine, and its lower recommended dietary intake is very close to the lower limit found in diet, so in the present study, as well as in Turkish studies [6, 22], there was no significant difference in serum copper between the giardiasis and control groups. One author reported an increased serum level of copper due to undulating levels of ceruloplasmin during infections [7].

Conclusion

Giardiasis is more prevalent in children 1–5 years old, with significant affection of weight. The most common clinical presentation in the infected group was abdominal pain, followed by weight loss and intermittent diarrhea. Serum levels of zinc and iron were significantly decreased in the infected group when compared to control, while serum copper was not significantly different between the two groups.

Acknowledgment Thanks to Prof. Dr. Amal El Saffy, professor of industrial medicine, Cairo University, Egypt, for carrying out most of the laboratory work of this study.

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