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Serum Iron, Zinc and Ferritin in 6 to 12-Year-Old Children with *Giardia lamblia* Positive and Healthy Children in Urmia City

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Abstract

Background: *Giardia lamblia* is the most common cause of acute non-viral diarrhea worldwide and resides in the small intestine, leading to Steatorrhea, growth and cognitive retardation, and trace element deficiencies. This study aimed to compare serum iron, zinc, and ferritin levels between 6- to 12-year-old children infected with *Giardia lamblia* and healthy controls in Urmia, West Azerbaijan Province, Iran.

Materials and Methods: A case-control study was conducted with 35 children infected with *Giardia* (cases) and 35 healthy children (controls), aged 6–12 years, selected from 325 children referred to health centers in Urmia. Blood samples (5 mL) were collected from all participants and stored for later analysis. Serum concentrations of iron and zinc were measured using atomic absorption spectrophotometry, and ferritin levels were assessed by ELISA. Data were analyzed using mean, standard deviation (SD), and variance, and statistical comparisons were performed. The relationship between serum ferritin and zinc concentrations was evaluated using Student's t-test.

Results: Serum iron levels were significantly lower in *Giardia*-infected children compared to healthy controls ($P = 0.03$). Serum zinc levels were also significantly lower in the infected group ($P = 0.001$). The mean ferritin level was 59 ± 34 ng/dL in children with *Giardia* compared to 110 ± 43 ng/dL in the control group, and this difference was statistically significant ($P = 0.001$).

Conclusion: These findings indicate that Giardia lamblia infection in children is significantly associated with lower serum levels of iron, zinc, and ferritin. Early diagnosis and treatment of giardiasis could help prevent nutrient deficiencies and related complications such as growth retardation and impaired immune function. Timely intervention may reduce long-term developmental and health risks in affected children.

Keywords: Ferritin, *Giardia lamblia*, Iron, Zinc

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Introduction

As a flagellated protozoan, *Giardia lamblia* is one of the most common intestinal parasites of humans, causing symptoms of infection in about 200 million people across Asia, Africa, and Latin America (1. 2). The prevalence of giardiasis varies between 2-5% in developed countries and 20-30% in developing countries (3).

Reports on the prevalence of giardiasis in Iran, from different economic and geographical regions, range from 1.4% to 59.6% (4). Giardiasis is of particular concern due to its public health impact, high prevalence, and its potential complications, especially its effects on growth and cognitive development in children. The susceptibility to giardiasis is

influenced by host factors such as nutritional status, immune function, and growth status, as well as parasite factors. *Giardia lamblia* has been detected in all age groups, with children being at higher risk for infection (5, 6). Its transmission occurs both directly and indirectly. Direct transmission happens through contact with infected individuals and animals, while indirect transmission occurs through the consumption of contaminated water (7). Clinical symptoms of giardiasis include greasy stools, abdominal bloating, diarrhea, and abdominal cramps, but many cases are asymptomatic (8, 9). The most important symptoms in children include impaired physical growth, malabsorption, and weight loss. In severe cases, there is also steatorrhea and deficiencies in folic acid, gamma globulin, and fat-soluble vitamins, along with deficiencies in trace elements such as zinc and iron (10, 11). Giardiasis leads to reductions in essential trace elements, such as ferritin, zinc, and iron, resulting in cellular dysfunction and a decline in physiological and enzymatic activities in children. While zinc levels may initially rise during acute infections due to rapid depletion, chronic giardiasis results in sustained low serum zinc levels (12). One of the notable symptoms of giardiasis is disturbances in iron metabolism, as the malabsorption of iron leads to decreased serum levels, despite *Giardia lamblia* having no direct effect on intestinal iron absorption (13, 14). Giardiasis is a leading cause of non-viral diarrhea in children under 5 years of age in developing countries, causing physical and mental retardation, early onset of Alzheimer's disease (15), and allergies in children under 15 years old (16). Investigating the extent, causes, and contributing factors of giardiasis prevalence is essential.

In this study, we evaluated and compared serum levels of iron, zinc, and ferritin in 6 to 12 year-old children with *Giardia lamblia* infection and healthy controls in Urmia, West Azerbaijan Province, Iran.

Materials and Methods

Study population

This case-control study was conducted on 35 children, aged 6-12 years, who were infected with *Giardia* (case group) and 35 healthy children, aged 6-12 years, who were not infected (control group). The participants were selected from 325 children referred to health centers in Urmia in 2016, based on a questionnaire. The children were compared according to their age, gender, height, and place of residence. Case-control matching was performed based on age, sex, and socioeconomic conditions. Normal serum levels of zinc, iron, and ferritin have been reported as 104-205 µg/dL, 80-120 µg/dL, and 70-142 ng/dL, respectively. The study was registered in the Iranian Registry of Ethical Codes under the code IR.SSU.MEDICINE.REC.1395.146.

Sample selection

According to the article by Dr. Mahdi Sharif and colleagues (17), and considering the standard deviation of serum iron levels in the case and control groups as 25 and 23 respectively, with a mean difference of 14, a 95% confidence level, and an 80% power, we consider 35 samples in each group.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

Case-control matching and sampling

The feces of all the participants were freshly examined using parasitological methods (direct smear and formalin-ether concentration) in three shifts. To investigate *Giardia* infection, 5 mL of blood was collected from each child. After centrifugation at 3000 rpm for 6 minutes, the serum was separated, transferred into a special polymer tube, and stored in a freezer at -18°C.

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Measurement of ferritin, zinc, and iron serum levels

The concentrations of ferritin, iron, and zinc in serum were measured using the relevant kits (Biomide Iran, Biokit Iran, and Liaison Italy) at Dr. Kamali's Laboratory (Urmia, West Azerbaijan Province, Iran.

Data analysis

The data obtained from the serum measurements were recorded. The mean, standard deviation (SD), variance (Z), and concentrations of ferritin, zinc, and iron were calculated for both the case and control groups and tabulated. The comparison of serum ferritin, zinc, and iron concentrations between the case and control groups was analyzed using the student's t-test.

Results

Of the 35 (100%) healthy children selected after matching, 16 (45.71%) were female, and 19 (54.29%) were male. Additionally, of the 35 (100%) *Giardia lamblia* positive children, 12 (34.29%) were female, and 28 (65.71%) were male. The highest percentage of children infected with *Giardia* was in the 9–10-year age group, with 17 cases (48.28%), while the lowest was in the 11–12-year age group, with 9 cases (25.72%). In terms of gender, 21 cases (60%) of the infected children were male, and 14 cases (40%) were female. The highest percentage of healthy children was also in the 9–10-year age group, with 14 cases (48.28%), while the lowest was in the 11–12-year age group, with 10 cases (28.57%) (Table I).

In *Giardia* lamblia positive children, the mean serum iron level was 65.82 µg/dL, with the highest serum iron level being 68.11 µg/dL and the lowest at 63.43 µg/dL. In *Giardia* negative children, the mean serum iron level was 107.87 µg/dL, with the highest serum iron level being 111.23 µg/dL and the lowest at 68.49 µg/dL. Based on the Student's t-test, serum iron levels in children aged 6 to 12 years with *Giardia lamblia* infection were significantly lower than in healthy children ($p = 0.03$).

In *Giardia lamblia* positive children, the mean serum zinc level was 68.63 µg/dL, with the highest serum zinc level at 69.77 µg/dL and the lowest at 67.14 µg/dL. In *Giardia* negative children, the mean serum zinc level was 143.99 µg/dL, with the highest serum zinc level at 144.96 µg/dL and the lowest at 143.16 µg/dL. According to the Student's t-test, the serum zinc level in 6–12-year-old children with *Giardia lamblia* infection was significantly lower than in healthy children ($p = 0.001$).

In *Giardia lamblia* positive children, the mean serum ferritin level was 68.63 ng/mL, with the highest serum ferritin level at 69.77 ng/mL and the lowest at 67.14 ng/mL. In *Giardia* negative children, the mean serum ferritin level was 143.99 ng/mL, with the highest serum ferritin level at 144.96 ng/mL and the lowest at 143.16 ng/mL. According to the Student's t-test, the serum ferritin level in 6–12-year-old children with *Giardia lamblia* infection was significantly lower than in healthy children ($p = 0.001$) (Table II and Figure 1).

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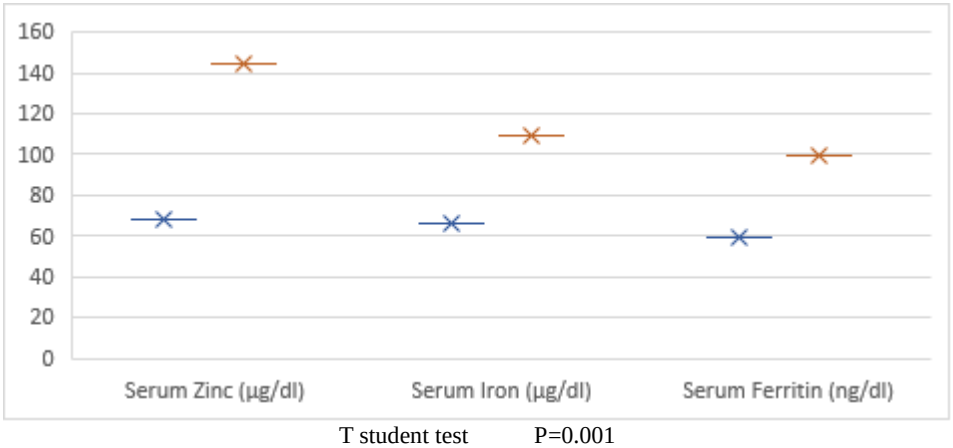
Table I: Frequency distribution and age group percentage in 6- to 12 year- old Giardia positive and healthy children in Urmia, West Azerbaijan Province, Iran

Age (Year)		6-8	9-10	11-12	Total
Case	Number	14	17	9	35
	Percent	40	48.28	25.72	100
Control	Number	11	14	10	35
	Percent	31.43	40	28.57	100

Table II: Mean serum levels of zinc and iron between 6-12-year-old Giardia positive and healthy children in Urmia, West Azerbaijan Province, Iran

Types of serum / Cases	Serum Zinc (µg/dl)		Serum Iron (µg/dl)		Serum Ferritin (ng/dl)	
	Giardia Positive	Giardia Negative	Giardia Positive	Giardia Negative	Giardia Positive	Giardia Negative
Maximum	69.77	144.96	68.11	111.23	70.05	133.87
Minimum	67.14	143.16	63.46	86.49	52.76	77.45
Mean ± SD	68.63±11.5	143.99±11.95	65.82±10.90	107.87±13.35	59.32±10.61	99.67±18.14
P value T student test	P=0.001		P=0.03		P=0.05	

Student's t-test



T student test P=0.001

Figure 1. Mean serum levels of zinc and Iron between 6-12-year-old Giardia positive and healthy children in Urmia, West Azerbaijan Province, Iran

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Discussion

Gardia duodenalis, as one of the most common causes of waterborne diseases worldwide, is a globally distributed parasitic protozoan with a prevalence of 0.4–7.5% in developed countries and 8–30% in developing countries (18, 19). Giardiasis is a neglected protozoan-caused disease reported in both developing and developed nations. The parasite is estimated to cause more than 28.2 million cases of diarrhea each year due to food contamination (20, 21, 22).

According to previous studies, the serum levels of zinc, iron, and ferritin in serum and cerebrospinal fluid decrease during infectious diseases, and this finding has been well-documented. The reduction in the incidence of chronic infectious-parasitic diseases is particularly significant in children (23, 24).

Similarly, Andriastuti M et al. (2023) conducted a study on children aged 2–14 years and showed that giardiasis increased serological copper levels, similar to other infectious agents. However, zinc and iron levels decreased during giardiasis due to malabsorption (25).One of the most important symptoms of giardiasis in children is growth retardation, poor absorption, and weight loss. In severe infections, in addition to steatorrhea, deficiencies of gamma globulin, folic acid, fat-soluble vitamins (K, E, D, A), and essential trace elements such as zinc and iron occur (26–28). Recent studies and prospective cohort studies have consistently identified a direct relationship between intestinal function and child growth. Even in animal models, *Giardia lamblia* infection disrupts various pathways of intestinal function, resulting in impaired growth (28). Since zinc is not stored in large amounts in the body, its serum level decreases during infection in children with low zinc intake. The reduction in zinc levels can disrupt cellular function, leading to impairments in physiological and enzymatic activity (29). During the acute stage of giardiasis infection, serum zinc levels increase due to the rapid depletion of zinc stores; however, a continuous decrease in serum zinc levels is observed during the chronic stage (30–32). Disruption in iron metabolism is another consequence of *Giardia lamblia* infection (33, 34). Although Giardiasis does not affect intestinal iron absorption, malabsorption of iron, leading to deficiency in serum levels, can be expected in individuals infected with *Giardia* (35–37).

Children infected with Giardiasis may exhibit symptoms such as paleness, weakness, lethargy, lack of concentration, and anorexia, which can be attributed to malnutrition and deficiencies of essential minerals (38). Carrilho GF et al. (211) and Nxasana N et al. (2013) identified a variant-specific surface protein (VSP) of *Giardia lamblia*. There is a theory that VSP has a tendency to bind with divalent metals in the culture medium. Different VSP types have varying affinities for metals, with zinc being the most commonly targeted (38, 39).

In the study conducted by Ngui R et al. (2012), it was shown that 85% of active zinc compounds inhibit the growth of *Giardia*. Moreover, the trophozoite of *Giardia lamblia* has the ability to bind with metals, which means the parasite removes zinc from the body, leading to malnutrition in the infected person (40). Numerous investigations have been conducted on this topic. Fatahi Bafghi et al. (2012) (41), Currie SL et al. (2017) (42), Fattahi Bafghi et al. (2018) (43), and Fattahi Bafghi A et al. (2015) (44) all found a decrease in ferritin, zinc, and serum levels during *Giardia lamblia* infection. This finding is consistent with the present study, which suggests that *Giardia lamblia* infection leads to a decrease in the serum levels of ferritin, zinc, and iron. This should be considered

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one of the important and effective factors in causing malabsorption and the decreased trace elements of ferritin, zinc, and iron in *Giardia lamblia* sufferers (45).

Conclusion

Based on our findings, early diagnosis of giardiasis in children is crucial to prevent intestinal damage and related clinical symptoms, including deficiencies in essential vitamins and trace elements. Timely treatment of these deficiencies may significantly improve the overall health and development of affected children.

Ethical Considerations

The study received approval from the Ethics Committee of the School of Medicine, Shahid Sadoughi University of Medical Sciences. The study is observable at https://ethics.research.ac.ir/ IR.SSU.MEDICINE.REC.1395.146

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Authors' Contributions

A FB: Conceptualization (supporting). M V & A E data collection and analysis. A FB: writing the original draft. A FB & A E: Methodology, review and editing. A FB & M V: Methodology, review and editing: A FB: Conceptualization (leading), methodology and supervision, KB writing the English Manuscript.

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Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper. Ethical approval of this study was bestowed by the Ethics Committee of the School of Medicine, Shahid Sadoughi University of Medical Sciences.

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