

10P45 # 031

## Original Paper

# Asymptomatic Giardiasis Does Not Affect Iron Absorption in Children With Iron Deficiency Anemia

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**Key words:** giardiasis, iron deficiency, hypochromic anemia, malabsorption syndromes

**Objective:** Malabsorption of iron has been reported in children with symptomatic giardiasis. The aim of this study was to evaluate intestinal absorption of iron in children with asymptomatic giardiasis and iron deficiency anemia.

**Subjects:** Based upon results of blood hemoglobin and stool examination, two groups were established: asymptomatic giardiasis and anemia, and anemia without intestinal parasitosis (control group). Patients were aged 1–6 years. There was no difference in age, weight, height, or iron nutritional status between the asymptomatic giardiasis and control groups on admission to the study.

**Measures:** Intestinal absorption of iron was evaluated using the iron tolerance test and the hemoglobin response to iron therapy. The serum iron tolerance test was based on the increment of iron level 2 hours after administering an iron load of 1 mg/kg of elemental iron in the form of ferrous sulfate, in comparison to the fasting iron level. Hemoglobin response to oral iron therapy was determined by the increment of hemoglobin on day 30 of therapy with ferrous sulfate (5 mg/kg/day of elemental iron).

**Results:** There was no statistical difference between the asymptomatic giardiasis and control groups with reference to the iron tolerance test ( $159.1 \pm 73.1 \mu\text{g/dl}$  and  $154.5 \pm 76.5 \mu\text{g/dl}$ , respectively) and to the hemoglobin response to iron therapy ( $1.5 \pm 0.7 \text{ g/dl}$  and  $1.8 \pm 1.1 \text{ g/dl}$ , respectively). The presence or absence of trophozoites of *Giardia lamblia* on duodenal aspirate did not affect intestinal absorption of iron.

**Conclusion:** Asymptomatic giardiasis did not affect the intestinal absorption of iron and the hemoglobin response to oral iron therapy in iron-deficient anemic children.

## INTRODUCTION

*Giardia lamblia*, the most common human enteropathogenic protozoan [1], can cause acute and chronic diarrhea [1,2]. However, most human beings with giardia infection do not present symptoms despite eliminating *Giardia lamblia* cysts in the stool for several months [3,4]. Abnormalities of intestinal absorption of fat, D-xylose, vitamin A, vitamin B12, and folic acid [5–7] as well as deconjugation of bile salts [6,8] and bacterial overgrowth in the upper portion of the small intestine [8,9], have been reported associated with symptomatic and asymptomatic *Giardia lamblia* infection. Moreover, morphologic abnormalities in the small intestine have been described in both symptomatic and asymptomatic giardiasis [2,5,6].

Iron deficiency is still a very important problem in public health, especially among children living in developing coun-

tries [10], where the incidence of giardiasis is higher than in developed countries [2]. Despite evidence of iron malabsorption in symptomatic giardiasis demonstrated with the serum iron tolerance test [11,12], there are no data pertaining to iron absorption in asymptomatic giardiasis.

In this study, we evaluate the intestinal absorption of iron in children with iron deficiency anemia and asymptomatic giardiasis using the serum iron tolerance test and the hemoglobin response to oral iron therapy.

## MATERIAL AND METHODS

### Patients

The study was approved by the Ethics Committee of the Escola Paulista de Medicina and was carried out with the

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consent of the parents of children in the outpatient clinic of the Pediatric Gastroenterology Division, Escola Paulista de Medicina, São Paulo, Brazil. Of 240 children aged 1–6 years referred for clinical manifestation of iron-deficiency anemia, 50 patients who met the following criteria were enrolled in the study: 1) hemoglobin level  $<11.0$  g/dl; 2) three stool examinations on non-consecutive days with negative results for intestinal parasites or positive only for *Giardia lamblia*; 3) negative occult blood test in the stool; 4) no diarrhea, abdominal pain or abdominal distension in the previous 2 months; 5) no iron therapy or use of anti-*Giardia* drugs in the previous 4 months; and 6) weight for age, height for age and weight for height between the 5th and 95th centiles of the NCHS tables [13].

Children who exclusively excreted cysts of *Giardia lamblia* were assigned to the asymptomatic giardiasis group, while those with three negative stool examinations were allocated to the control group (iron-deficiency anemia without intestinal parasitosis). Because trophozoites may be present in the intestinal secretion even when stool examination is negative [14,15], duodenal aspirate was examined for trophozoites of *Giardia lamblia* on admission to the study in patients of both giardiasis and control groups.

Children with other intestinal parasitosis or hemoglobin less than 6.0 g/dl were not enrolled in the study.

### Evaluation of Iron Intestinal Absorption

The serum iron tolerance test is a simple method to evaluate intestinal iron absorption based on the increment of iron in the serum after administration of a small dose of iron. This method in adults provides good correlation with radioisotope techniques [16,17], and can identify the increment of iron absorption in children with iron deficiency [11,12,18,19].

Hemoglobin response to oral iron therapy is another method to evaluate iron absorption, but it is applicable only to overtly anemic children with iron deficiency [20]. The hemoglobin response to oral therapy is also considered the gold standard for the diagnosis of iron deficiency [21–23].

### Study Design

On day 0, after an overnight fast, a blood sample was collected to assess the iron status and to function as the baseline for the serum iron tolerance and D-xylose absorption tests. One mg/kg of elemental iron in the form of ferrous sulfate (Fer-in-sol<sup>R</sup>, Mead Johnson) was then administered and 1 hour later, D-xylose (15 g/m<sup>2</sup> of body surface) was administered in a 10% water solution [18]. Blood was collected to determine 1-hour D-xylosemia and the increment of iron in the serum 2 hours after the administration of the test dose of ferrous sulfate [18]. On the same day, oral iron therapy was started as ferrous sulfate (Fer-in-sol<sup>®</sup>, Mead Johnson) 5 mg/kg/day of elemental iron in two doses. The medication was given to the patients' parents,

and treatment compliance was confirmed on return visits by the volume of medication consumed. No anti-*Giardia* drugs or dietary guidance were given. On day 30, a blood sample was collected to determine the hemoglobin response to oral iron therapy, and a sample of stool was examined for intestinal parasites.

### Exclusion Criteria

The development of infectious disease during oral iron therapy was a criterion for exclusion because it may produce iron malabsorption [24]. Other exclusion criteria were consumption of less than 80% of the prescribed doses of ferrous sulfate, or acquisition of intestinal parasitosis during the oral iron therapy.

### Methods

Stool examination was performed using Hoffman (sedimentation method) and Ritchie (formol-ether method) concentration techniques [25]. Occult blood test was investigated by the Feca-Cult method. Trophozoites of *Giardia lamblia* in duodenal aspirate were investigated by the Kamath and Murugasu method [26]. Erythrocyte number and mean corpuscular volume were determined by a Coulter/Coulter T890. Hemoglobin was determined by the cyanometahemoglobin method and sedimentation rate by the Westergren method [27]. Serum iron was measured by the spectrophotometric method of batofenantroline using reagents from Merck (Merckotest-iron 3307R). Serum transferrin was determined by radial immunodiffusion employing Nor-Partigen-Behring plates. Serum ferritin was measured by enzyme immunoassay (Ferrizyme-Abbott). Blood xylose concentration was determined by Roe and Rice method [28]. Statistical analysis was performed using the Minitab 6.1 software [29].

### RESULTS

All patients in both groups presented at least two of the following criteria thus confirming that iron deficiency was the etiology of the anemic status: transferrin saturation  $<15\%$ , serum ferritin  $<12$  ng/ml, and/or an increase in the hemoglobin level ( $>1.0$  g/dl) after 1 month of oral iron therapy started on admission to the study [22].

Of the 50 patients who started the study, 38 completed the 30-day study protocol, including 19 in the giardiasis group and 19 in the control group. Both groups had the same number of boys ( $n = 10$ ) and girls ( $n = 9$ ). Twelve patients were excluded, including two who developed a respiratory-tract infection during iron therapy, four who acquired intestinal parasitosis after admission to the study, and six due to lack of compliance with the iron therapy.

## Absorption of Iron in Asymptomatic Giardiasis

Table 1 shows patients' data upon admission to the study. Both groups had similar data concerning age, sex, weight, height and iron status.

Table 2 depicts the results of the serum iron tolerance test, the hemoglobin level prior to and on day 30 of oral iron therapy as well as the increment of hemoglobin, and 1-hour D-xylosemia. There was no statistical difference between the giardiasis and control groups.

None of the patients in the control group presented trophozoites of *Giardia lamblia* in duodenal aspirate, but they were identified in eight (42%) of 19 patients in the asymptomatic giardiasis group. Table 3 presents hemoglobin levels, hemoglobin response to oral iron therapy, serum iron tolerance test and 1-hour D-xylosemia in the giardiasis group based on the presence or absence of trophozoites in intestinal secretion.

## DISCUSSION

Our study was designed to investigate whether the iron tolerance test indicates iron malabsorption in asymptomatic giardiasis as it has previously been described in symptomatic giardiasis [11,12], and if asymptomatic giardiasis can decrease the hemoglobin response to oral iron therapy. Our results did not show any evidence of intestinal malabsorption of iron in children with asymptomatic giardiasis and iron-deficiency anemia compared to the control group of iron-deficient anemic children without intestinal parasitosis. We studied only children with iron-deficiency anemia because the presence of anemia is essential in order to analyze the hemoglobin response to oral iron therapy and facilitates the interpretation of the oral iron tolerance test. Additionally, iron-deficient patients should present increased intestinal absorption of iron compared to iron-sufficient individuals.

We previously analyzed the efficacy of the serum iron

**Table 1.** Giardiasis Group and Control Group on Admission to Study

	Giardiasis (n = 19)	Control (n = 19)
Age (months)	37.7 ± 18.8	36.6 ± 19.6
Weight (kg)	14.3 ± 4.0	13.9 ± 4.6
Height (cm)	94.1 ± 11.4	92.0 ± 12.7
Erythrocytes (ml)	4.5 ± 0.5	4.4 ± 0.6
VCM (fl)	73.3 ± 9.8	75.3 ± 10.6
Hematocrit (%)	33 (31-34)	33 (31-34)
Hemoglobin (g/dl)	10.2 ± 1.0	9.7 ± 1.3
Sedimentation rate (mm)	15 (6-21)	9 (5-20)
Serum iron (μg/dl)	44.7 (31.0-64.9)	34.5 (19.3-67.3)
Transferrin (mg/dl)	352 (290-419)	387 (323-499)
Transferrin saturation	14.9 (8.4-19.8)	9.1 (4.9-20.4)
Serum ferritin (ng/ml)	7.9 (2.3-18.0)	3.7 (0.9-17.8)

Mean ± SD, t test.

Median and (P<sub>25</sub>-P<sub>75</sub>), Mann-Whitney test.

Giardiasis × control: p > 0.05 for all.

**Table 2.** Serum Iron Tolerance Test, Hemoglobin on Day-0 and Day-30 of Oral Iron Therapy, Hemoglobin Response to Oral Iron Therapy and 1-Hour D-Xylosemia on Asymptomatic Giardiasis and Control Groups

	Giardiasis (n = 19)	Control (n = 19)
Serum iron tolerance test (μg/dl)	159.1 ± 73.1	154.5 ± 76.5
Hemoglobin day 0 (g/dl)	10.1 ± 1.0	9.7 ± 1.3
Hemoglobin day-30 (g/dl)	11.5 ± 1.2	11.4 ± 0.9
Hemoglobin response to iron therapy (g/dl)	1.5 ± 0.7	1.8 ± 1.1
1-Hour D-xylosemia (mg/dl)	26.9 ± 10.4	30.6 ± 6.1

Mean ± SD, t test.

Giardiasis × control: p > 0.05 for all.

**Table 3.** Serum Iron Tolerance Test, Hemoglobin on Day-0 and Day-30 of Oral Iron Therapy and 1-Hour D-Xylosemia on Asymptomatic Giardiasis With and Without Trophozoites of *Giardia lamblia* on Duodenal Aspirate Examination

	Present (n = 8)	Absent (n = 11)
Serum iron tolerance test (μg/dl)	157.4 ± 81.0	160.5 ± 70.6
Hemoglobin day-0 (g/dl)	10.3 ± 0.9	10.1 ± 1.0
Hemoglobin day-30 (g/dl)	11.7 ± 0.8	11.2 ± 0.8
Hemoglobin response to iron therapy (g/dl)	1.3 ± 0.8	1.6 ± 0.6
1-Hour D-xylosemia (mg/dl)	25.5 ± 12.1	28.0 ± 9.4

Mean ± SD, t test.

Present × absent: p > 0.05 for all.

tolerance test in detecting the increment of intestinal iron absorption in iron deficiency children [19]. The increment of iron in the serum after 1 hour of administering 1 mg of elemental iron as ferrous sulfate was 164 ± 80 μg/dl in 38 tests performed on patients with iron deficiency anemia (low hemoglobin and serum ferritin), and 151 ± 58 μg/dl in 11 tests in children with iron-deficiency but without anemia (low ferritin and normal hemoglobin) which were statistically higher than the value of 89 ± 57 μg/dl obtained in 40 tests performed on iron-sufficient children (normal ferritin and normal hemoglobin). In the present study, the mean and standard deviation of the iron tolerance test were very similar in both asymptomatic giardiasis and control groups. These results were also very similar to the oral tolerance test which we previously performed in iron-deficient anemic children and described above [19].

Using the iron tolerance test, De Vizia et al [11,12] demonstrated decreased iron absorption of iron in patients with

symptomatic giardiasis presenting diarrhea and/or intestinal malabsorption syndrome, taking into consideration the results of the iron tolerance test before and after treatment of asymptomatic giardiasis [11] and the comparison of the iron tolerance test to normative values obtained in children without intestinal malabsorption syndrome [12]. Our results cannot be interpreted using the normative values described by De Vizia et al [12] because vitamin C was used in association with ferrous sulfate, probably providing a higher increment of iron in the serum after the test dose of ferrous sulfate.

The hemoglobin response to oral iron therapy was similar in both groups, demonstrating that asymptomatic giardiasis cannot be considered a common cause of lack of hemoglobin response during treatment of iron deficiency anemia. The hemoglobin increment mean of the control group was 0.3 g/dl greater than the mean of the giardiasis group. However, the mean of hemoglobin on day 0 of iron therapy in the control group was 0.4 g/dl lower than the mean of the asymptomatic giardiasis group, confirming that the lower the level of hemoglobin before iron therapy, the greater the hemoglobin response.

The presence of trophozoites of *Giardia lamblia* in intestinal juice in eight (42%) of 19 patients in the asymptomatic giardiasis group is in accordance with previous studies that demonstrated a relatively low positivity of duodenal aspirate examination for the diagnosis of giardiasis [30,31]. The data presented in Table 3 demonstrate that the iron tolerance test and the response of hemoglobin to iron therapy were very similar in asymptomatic giardiasis, with and without trophozoites in the intestinal aspirate.

The D-xylose absorption mean was similar in both groups, but it was lower than the mean of normal Canadian children ( $49.4 \pm 12.1$  mg/dl) subjected to this absorption test using the same D-xylose dose [32]. There are two possible explanations for this finding. First, despite the fact that the patients did not present any gastrointestinal manifestations, they could be suffering from asymptomatic environmental enteropathy which produces a decrease in the D-xylose intestinal absorption [33]. Second, it could be a consequence of iron-deficient anemia based on previous studies that demonstrated abnormalities of intestinal absorption [34,35] and intestinal permeability [36] in patients with iron-deficiency anemia.

In conclusion, despite decreases in the D-xylose intestinal absorption in both groups, asymptomatic giardiasis did not affect the intestinal absorption of iron and the hemoglobin response to oral iron therapy in iron-deficient anemic children.

## ACKNOWLEDGMENTS

We are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support for this study.

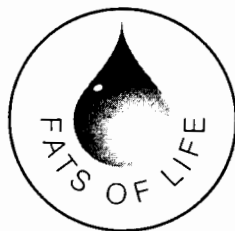
## REFERENCES

1. Wolfe MS: Giardiasis. *Pediatr Clin North Am* 26:295-303, 1979.
2. Farthing MJG: Pathogenesis of giardiasis. *Trans R Soc Trop Med Hyg* 87:17-21, 1993.
3. Ish-Horowicz M, Korman SH, Shapiro M, et al. Asymptomatic giardiasis in children. *Pediatr Infect Dis J* 8:773-779, 1989.
4. Pickering LK, Morrow AL: Commentary. *Pediatr Infect Dis J* 10:846-848, 1991.
5. Barbieri D, De Brito T, Hoshino S, et al. Giardiasis in childhood: Absorption tests and biochemistry, light, and electron microscopy of jejunal mucosa. *Arch Dis Child* 45:466-472, 1970.
6. Jove S, Fagundes-Neto U, Wehba J, Machado NL, Patricio, FRS: Giardiasis in childhood and its effect on the small intestine. *J Pediatr Gastroenterol Nutr* 2:472-477, 1983.
7. Gillon J: Giardiasis. *Q J Med* 209:29-39, 1984.
8. Tandon BN, Tandon RK, Satpathy BK, Shrinivas M: Mechanism of malabsorption in giardiasis: a study of bacterial flora and bile salt deconjugation in upper jejunum. *Gut* 18:176-181, 1977.
9. Tomkins AM, Drasar BS, Brandley AK, Williamson WA: Bacterial colonization of jejunal mucosa in giardiasis. *Trans R Soc Trop Med Hyg* 72:33-36, 1978.
10. Yip R: Iron deficiency: contemporary scientific issues and international programmatic approaches. *J Nutr* 124:1479S-1490S, 1994.
11. De Vizia B, Poggi V, Vajro P, Cucchiara S, Acampora A: Iron malabsorption in giardiasis. *J Pediatr* 107:75-78, 1985.
12. De Vizia B, Poggi V, Conenna R, Fiorillo A, Scippa L: Iron absorption and iron deficiency in infants and children with gastrointestinal diseases. *J Pediatr Gastroenterol Nutr* 14:21-26, 1992.
13. World Health Organization: Measuring changes in nutritional status: guidelines for assessing the nutritional impact of supplementary programmers for vulnerable groups. Geneva: WHO, 1983.
14. Ament M, Rubin CE: Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes. *Gastroenterology* 62:216-226, 1972.
15. Burke JA: Giardiasis in childhood. *Am J Dis Child* 129:1304-1310, 1975.
16. Heinrich HC, Fischer R: Correlation of postabsorptive serum iron increase and erythrocyte  $^{59}\text{Fe}$  incorporation with the whole body retention of absorbed  $^{59}\text{Fe}$ . *Klin Wochenschr* 60:1493-1496, 1982.
17. Kaltwasser JP, Werner E: Significance of postabsorptive serum iron increase for the valuation of oral iron preparations. *Blut* 51:227, 1985.
18. Greco L, Troncone R, De Vizia B, Poggi V, Mayer M, Grimaldi M: Discriminant analysis for the diagnosis of childhood celiac disease. *J Pediatr Gastroenterol Nutr* 6:538-542, 1987.
19. Morais MB, Suzuki HU, Machado NL, Fagundes-Neto U: Avaliação de um teste simples de absorção de um teste simples de absorção de ferro na deficiência de ferro. *J Pediatr (Rio J)* 68:48-53, 1992.
20. Werner E, Kaltwasser JP: Judgement of measured values of intestinal iron absorption. *Arzneim-Forsch/Drug Res* 37:116-121, 1987.
21. Garby L, Irneel L, Werner I: Iron deficiency in woman of fertile age in a Swedish community. III—Estimation of prevalence based on response to iron supplementation. *Acta Med Scand* 185:113-117, 1969.

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22. Margolis HS, Hardison HH, Bender TR, Dallman PR: Iron deficiency in children: the relationship between pretreatment laboratory tests and subsequent hemoglobin response to iron therapy. *Am J Clin Nutr* 34:158-168, 1981.
23. Oski FA: Iron deficiency in infancy and childhood. *N Engl J Med* 329:190-193, 1993.
24. Weinberg ED: Infection and iron metabolism. *Am J Clin Nutr* 30:1485-1490, 1977.
25. Pessoa SB, Martins AV: *Parasitologia Médica*. Rio de Janeiro: Guanabara Koogan, 1978.
26. Kamath KR, Murugasu R: A comparative study of four methods for detecting *Giardia lamblia* in children with diarrheal disease and malabsorption. *Gastroenterology* 66:16-21, 1974.
27. International Committee for Standardization in Hematology: Recommendations for reference method for hemoglobinometry in human blood and specifications for international haemiglobincyanide reference preparation. *J Clin Pathol* 31:139-143, 1978.
28. Roe JH, Rice EW: A photometric method for the determination of free pentoses in animal tissues. *J Biol Chem* 173:507-512, 1948.
29. Minitab Inc: "Minitab Statistical Software 6.1." Boston: PWS, 1988.
30. Naik SR, Rau NR, Vinayak VK: A comparative evaluation of examinations of three stool samples, jejunal aspirate and jejunal mucosal impression smears in the diagnosis of giardiasis. *Ann Trop Med Parasitol* 72:491-492, 1978.
31. Heymans HSA, Aronson DC, Hoof MAJ: Giardiasis in childhood: an unnecessarily expensive diagnosis. *Eur J Pediatr* 146:401-403, 1987.
32. Buts JP, Morin CL, Roy CC, Weber A, Bonin A: One hour blood xylose test: a reliable index of small bowel function. *J Pediatr* 90:729-733, 1978.
33. Fagundes-Neto U, Viaro T, Wehba J, Patricio Fr, Machado NL: Tropical enteropathy (environmental enteropathy) in early childhood: a syndrome caused by contaminated environment. *J Trop Pediatr* 30:204-209, 1984.
34. Naiman JL, Oski FA, Diamond LK, Vawter GF, Shwachman H: The gastrointestinal effects of iron-deficiency anemia. *Pediatrics* 33:83-99, 1964.
35. Guha DK, Walia BNS, Tandon BN, Deo MG, Ghai OP: Small bowel changes in iron-deficiency anaemia of childhood. *Arch Dis Child* 43:239-244, 1968.
36. Berant M, Khourie M, Menzies IS: Effect of iron deficiency on small intestinal permeability in infants and young children. *J Pediatr Gastroenterol Nutr* 14:17-20, 1992.

Received October 1995; revision accepted April 1996.



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