

Original Research

Vitamin B₁₂ and Folic Acid in Children with Intestinal Parasitic Infection

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Objective: To determine prospectively plasma levels of vitamin B₁₂ and folic acid in children with intestinal parasitic infection before and three months after antiparasitic treatment.

Methods: 3036 stool samples were collected from 1959 children and 939 cello-tape anal swabs were taken from 688 children for intestinal parasite investigation. Of these, 155 children were identified as having a parasitic infection; however, only 86 were followed up during this study: 26 children with *Giardia lamblia* infection were treated with tinidazole and metronidazole, pyrantel pamoate was used in the treatment of 40 children with *Enterobius vermicularis*, and 20 patients infected with *Cryptosporidium parvum* received only symptomatic treatment. Vitamin B₁₂ and folic acid levels were measured by radioimmunoassay, before and three months after the completion of treatment.

Results: Vitamin B₁₂ serum concentrations did not show any significant differences among the three groups. There was a significant increase in vitamin B₁₂ serum concentrations after three months of anti-parasitic treatment (630.57 ± 200.97 vs. 667.97 ± 181.55 pg/dL, $p = 0.002$, $n = 86$). Paired analysis in each group showed only significant increases for vitamin B₁₂ in the *Giardia lamblia* group and in the *Enterobius vermicularis* group. No statistically significant differences were found for folic acid serum concentrations before and three months after treatment.

Conclusions: Patients with symptomatic infection by *Giardia lamblia* and *Enterobius vermicularis* have lower vitamin B₁₂ levels than asymptomatic patients. This could reflect a more affected intestinal mucous. These results could present the opportunity to treat these parasitic infections and to use vitamin B₁₂ supplementation in symptomatic children with *Giardia lamblia* and *Enterobius vermicularis* infection.

INTRODUCTION

Intestinal parasitosis remains an important public health concern world-wide because of the high frequency reached in several countries as well as its nutritional consequences [1–3]. Although childhood vitamin B₁₂ and folic acid deficiency is rather unusual, recent studies suggest that pre-school and school-aged children, adults and pregnant and lactating women suffer from folic acid deficiency more frequently than previously reported, mainly in certain populations [4–6].

Vitamin B₁₂ and folic deficiencies are characterised by unspecific symptoms like irritability, failure to thrive, muscular weakness and growth retardation. Early diagnosis and treatment by pediatricians is very important to avoid neurological and developmental damage [7].

The aim of this study was to investigate vitamin B₁₂ and folic acid serum concentrations during the acute *Giardia lamblia*, *Enterobius vermicularis* and *Cryptosporidium parvum* parasitisation and their changes after parasite elimination.

MATERIALS AND METHODS

Patients

Three thousand thirty-six fecal stools were collected from 1959 Spanish children from Aragon (Northeast Spain) during a 12-month period. From the whole population studied, 155 children were infected, 69 of them were excluded from the study: 12 (7.70%) with polymicrobial infection, (1 *Escherichia*

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coli, 3 *Campylobacter yeyuni*, 3 *Entamoeba coli*, 2 *Endolimax nana*, 3 *Blastocystis hominis*) and 57 of them because of no adequate clinical or microbiological control.

Eighty-six children were diagnosed with one unique parasite infection and were finally included in the study. From these 86 cases, 26 were infected by *Giardia lamblia*, 20 by *Cryptosporidium parvum* and 40 by *Enterobius vermicularis*. Fifty-six patients came from an urban area (Zaragoza) and 30 from rural areas. Most of these children were of a medium socio-economic status. Seventy of them complained of abdominal pain, acute diarrhoea, anorexia, anal itching and fever. The 16 remaining patients were asymptomatic. Ages at diagnosis ranged from 10 months to 15 years, mean 6.85 ± 4.16 years.

Identification of Parasites

For *Giardia lamblia* and *Cryptosporidium parvum* identification, concentrations on fecal stools were performed by the method described by Ritchie [8], using ether instead of acetyl acetate, the method of Young *et al.* [9] and Erdman [10]. The method of Ziehl-Neelsen, modified by Henricksen and Pohlenz [11], was performed for *Cryptosporidium* oocyst identification. Stool samples were covered with fuscine, washed, decoloured with sulphuric acid and washed again. Finally malachite green was employed to stain the specimens. Identification of *Enterobius vermicularis* was carried out by the Graham technique [12].

Study Design

Tinidazole (50 mg/kg/day, two doses, separated by two weeks) was used to treat *Giardia lamblia* infected children. When parasitisation persisted after this treatment, metronidazole (25 mg/kg/day, seven days) was employed. Pyrantel pamoate (10 mg/kg/day, two doses separated by two weeks) was the treatment for *Enterobius vermicularis*. Because patients suffering from *Cryptosporidium parvum* infection, without associated immunodeficiency, do not receive any specific treatment but diet, in our study children with this parasitic infection were not treated.

A new fecal stool sample was collected two to three weeks after the completion of treatment. A new clinical control was done at the same time. Vitamin B₁₂ and folic acid values were determined three months after treatment when patients were asymptomatic and stools were not infected.

Measurement of Vitamin B₁₂ and Folic Acid

Vitamin B₁₂ and folic acid levels were assessed by radioassay (Solid Phase No Boil, Dualcount) using vitamin B₁₂ 57Co and folic acid 125I labeled. Values were measured by a gamma counter system [13, 14]. Children were evaluated twice, first at diagnosis and in a second control three months after the treatment and without active infection.

Statistical Analysis

Kolmogorov-Smirnov (Lilliefors modification) was applied to assess normality of each variable. To analyse changes in vitamin B₁₂ and folic acid serum concentrations during follow-up, repeated measures analysis of variance (ANOVA) were used to analyse differences between the three groups before and after treatment, changes between the two times (before *vs.* after therapy) and the interaction term group-time. Differences between paired sets of time were identified by a *t* test for each group. Statistic programs SPSS for Windows (SPSS Inc.), SOLO and BMDP Dynamic Version.7.0 (BMDP Statistical Software Inc.) were employed. Statistical significance was defined as $p < 0.05$.

RESULTS

Prevalence of parasite infections was 2.65% for *Giardia lamblia*, 1.53% for *Cryptosporidium parvum* and 10.61% for *Enterobius vermicularis*. Polymicrobial infection was found in 7.70% cases. Distribution by gender was: 44 boys and 42 girls. Mean age at diagnosis was 6.85 ± 4.16 years (from 10 months to 15 years). *Cryptosporidium parvum* infection was more frequent among the youngest children.

Low vitamin B₁₂ levels (< 200 ng/mL) were found just in one *Giardia lamblia* infected patient. There were no significant differences among the three types of parasites. Vitamin B₁₂ serum concentrations were significantly different before and three months after antiparasite treatment in the overall group (630.57 ± 200.97 *vs.* 667.97 ± 181.55 pg/dL, $p = 0.002$, $n = 86$). Paired analysis in each group showed only significant increases for vitamin B₁₂ in the *Giardia lamblia* group ($p = 0.045$) and in the *Enterobius vermicularis* group ($p = 0.004$). No significant differences were found in the *Cryptosporidium parvum* group ($p = 0.338$) (Table 1) (Fig. 1).

Folic acid was decreased (< 3 ng/mL) in two cases, (*Giardia lamblia* and *Enterobius vermicularis* infected children), and those values were restored to normal after treatment. No statistical difference was found between folic acid levels after and before treatment (10.79 ± 4.53 *vs.* 11.26 ± 4.38 pg/mL, $p = 0.088$, $n = 86$) nor among the parasite groups (Table 2).

DISCUSSION

Intestinal parasitic infection is, still nowadays, an important public health problem, mainly in specific geographical areas and among groups with specific socio-economic status. In Spain in recent years, the prevalence of these infections has decreased [2, 15]. Because of their special biological characteristics, these parasites find in the human gastrointestinal tract a good environment for their development. Carbohydrates, lipids, amino acids, iron, and the like are used by parasites to

Table 1. Plasma Vitamin B₁₂ Data of 86 Patients with Parasite Intestinal Infection before and Three Months after Treatment*

Type of Parasite	Patients	Vitamin B ₁₂ before Treatment ^a (pg/mL)	Vitamin B ₁₂ after Treatment ^b (pg/mL)
<i>Giardia lamblia</i>	26	631.92 ± 195.29	669.00 ± 173.97
<i>Cryptosporidium parvum</i>	20	658.05 ± 229.59	672.55 ± 190.19
<i>Enterobius vermicularis</i>	40	615.95 ± 193.10	665.00 ± 186.54
Total	86	630.57 ± 200.97	667.97 ± 181.55

* Values are expressed as means ± SD and were analyzed using repeated measures ANOVA.

^{a,b} There were no significant differences among the three groups, before and after treatment, $p = 0.889$.

^{a-b} There were significant differences when we compared serum concentrations, before and after treatment, with the three groups taken together, $p = 0.002$.

There was no significant effect of the interaction term group time, $p = 0.403$.

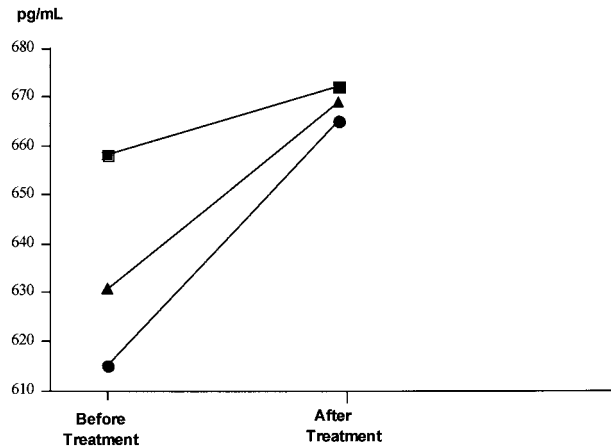


Fig. 1. Plasma vitamin B₁₂ in 86 children with intestinal parasitosis, before and three months after treatment. Analysis showed a significant increase for *Giardia lamblia*, $p = 0.045$ (triangle) and *Enterobius vermicularis*, $p = 0.004$ (circle). No significant differences were found for *Cryptosporidium parvum*, $p = 0.388$ (square).

grow and develop and they also use macromolecules that they can break down using their own enzymes.

The all vitamin B₁₂ comes from the diet, with vitamin B₁₂ present in all animal foods. After being ingested, vitamin B₁₂ is bound to the intrinsic factor, a protein secreted by the gastric parietal cells. Other cobalamin-binding proteins (called R factors) compete with the intrinsic factor for vitamin B₁₂. Vitamin B₁₂ bound to R factors cannot be absorbed. The vitamin B₁₂-intrinsic factor complex travels through the intestine and is absorbed in the terminal ileum by cells with specific receptors for the complex. It is then transported through plasma and stored in the liver. Three plasma transport proteins have been identified. Transcobalamins I and III (differing only in their carbohydrate structure) are secreted by the white cells. Although approximately 90% of plasma vitamin B₁₂ circulates bound to these proteins, only transcobalamin II is able to transport vitamin B₁₂ into cells. Vitamin B₁₂ is a cofactor for two key metabolic reactions, methylation of homocysteine to methionine and conversion of methylmalonyl coenzyme A to succinyl CoA. It is necessary for tetrahydrofolate production, an important factor for hematopoiesis and DNA synthesis [7,16].

Vitamin B₁₂ deficit is extremely rare. Information about the prevalence of vitamin B₁₂ deficiency is limited. Normal serum B₁₂ levels range from 200 to 900 pg/mL, and levels below 80 are indicative of deficiency, except for inborn errors of metabolism. One of each 200 children has serum levels less than 200 pg/mL [7].

Vitamin B₁₂ deficit may be due to dietary deficiency, breast-fed infants of mothers with vegetarian diets, poorly controlled phenylketonuria, inborn errors of B₁₂ absorption, transport and metabolism [17,18]. The pathogenetic mechanism could be the result of an absent or abnormal intrinsic factor, failure of R-protein degradation, lose of the ileum absorptive surface or an abnormal receptor [7,19].

Competition for B₁₂ in the intestinal lumen may cause malabsorption. Low vitamin B₁₂ levels have been seen in cases of infection with *Giardia lamblia*, *Plasmodium falciparum*, *Diphyllobothrium latum* and *Strongyloides stercoralis* [20,21]. Hjelt *et al.* [22], in 1992, noted an abnormal fractional absorption of vitamin B₁₂ and folic acid in children with chronic diarrhea due to giardiasis. In the initial investigation, these values were below normal in one-third and one-sixth of patients, respectively. From two to twelve months after treatment of giardiasis and cessation of diarrhea, the concentrations and the fractional absorption increased significantly, but serum vitamin B₁₂ and folic acid levels remained low.

Springer *et al.* [23], in 1997, studied vitamin B₁₂ plasma levels and subclinical infection with *Giardia lamblia* in an adolescent with agammaglobulinemia of Bruton and neurological symptoms. Metronidazole normalized vitamin B₁₂ levels and neurological manifestations disappeared. Brieva *et al.* [24], in 1998, described a polyneuropathy caused by vitamin B₁₂ deficiency due to a chronic atrophic gastritis and giardiasis, that improved after anti-parasite treatment.

Adults with human immunodeficiency virus infection may have low vitamin B₁₂ levels, possibly related to malabsorption. *Cryptosporidium* is found in the stools of 10% to 20% of patients with acquired immunodeficiency syndrome (AIDS) associated diarrhea. Goodgame *et al.* [25] affirm that *Cryptosporidium* infection in patients with AIDS causes malabsorption and intestinal injury proportionally to the number of microorganisms infecting the intestine. In this study, none of the

Table 2. Plasma Folic Acid Data of 86 Patients with Parasite Intestinal Infection before and Three Months after Treatment*

Type of Parasite	Patients	Folic Acid before Treatment ^a (ng/mL)	Folic Acid after Treatment ^b (ng/dL)
<i>Giardia lamblia</i>	26	11.13 ± 4.66	11.60 ± 4.72
<i>Cryptosporidium parvum</i>	20	11.34 ± 5.19	11.26 ± 4.59
<i>Enterobius vermicularis</i>	40	10.29 ± 4.15	11.03 ± 4.16
Total	86	10.79 ± 4.53	11.26 ± 4.38

* Values are expressed as means ± SD and were analyzed using repeated measures ANOVA.

^{a,b} There were no significant differences among the three groups, before and after treatment, $p = 0.850$.

^{a-b} There were no significant differences when we compared serum concentrations, before and after treatment, with the three groups taken together, $p = 0.088$. There was no significant effect of the interaction term group time, $p = 0.738$.

Cryptosporidium infected patients was diagnosed of immunodeficiency. No significant increase in vitamin B₁₂ and folic acid values was observed after three months of surveillance.

In our study, just one child showed a vitamin B₁₂ level lower than 200 pg/mL; however, vitamin B₁₂ concentrations were significantly increased after treatment. No significant differences between the type of infection, before and after treatment, were found.

Folic acid is present in most fruits and vegetables in form of polyglutamic acid. Hydrolysis to monoglutamic acid is necessary to its absorption, and it is carried out in the intestinal mucosa. After this, folic acid is absorbed in the proximal small intestine and then transported, bound to a binding-protein, to the cells. We considered serum folic acid levels normal when they were higher than 3 ng/mL [26].

By far the most common cause of folate deficiency is inadequate dietary intake. Reduced folate absorption is rarely seen, like in celiac disease or chronic enteritis. Drugs such as phenytoin, trimethoprim-sulfamethoxazole or sulfasalazine may interfere with folate absorption. Congenital dihydrofolate reductase may also give rise to a folate deficiency [5]. Ambrose *et al.* [27] demonstrated folate deficiency due to giardiasis. Hjelt *et al.* [17] found normal fractional absorption of folic acid during *Giardia lamblia* infection with a significantly increased folic acid level after anti-parasite treatment [17]. Heap *et al.* [28] refers to psychiatric symptoms in patients with chronic giardiasis and vitamin B₁₂ and folate deficiency.

No folate malabsorption in *Cryptosporidium parvum* or *Enterobius vermicularis* parasitization has been described. However, there is agreement about the use of effective drugs against these opportunistic protozoan pathogens. Folate metabolic enzymes and enzymes of the thymidylate cycle, particularly dihydrofolate reductase (DHFR), have been widely exploited as chemotherapeutic targets. Future chemical refinements of the potent and selective lead compounds have been identified as a potentially efficacious antifolate drugs for the treatment of cryptosporidiasis [29].

We have observed a non-significant increase in serum folic acid levels after anti-parasite treatment. Despite this, no significant differences were found among the types of infection.

As neurological symptoms are the most common in vitamin B₁₂ deficiency, psychiatric symptoms are more frequent when

a folate deficit is present. All these symptoms are more usual in chronic deficiencies or when a chronic disease or malnutrition is established, not in acute infections as those referred to in our study.

In conclusion, our data confirm the high intestinal parasitism prevalence in our area, in fact a public health problem. That plasma levels of both vitamin B₁₂ and folic acid increase after adequate antiparasite treatment, mainly for *Giardia lamblia* and *Enterobius vermicularis* infection, suggests that early detection and treatment of the parasites may prevent ulterior intestinal damage and appearance of clinical symptoms.

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