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Research Article

Effectiveness of the Treatment of Helminth Infections (*S. mansoni, Ancylostomidae, T. trichiura, A. lumbricoides*) on Hemoglobin Concentration in School-Children and Adolescents

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- Helminthiasis
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- Copper anemia

Abstract

Background: Anemia remains a leading cause of morbidity in young children in developing countries.

Objective: In order to investigate the increase in hemoglobin concentration in individuals treated for helminth infections (*S. mansoni*, *A. lumbricoides*, *T. trichiura*, Ancylostomidae) of mild and moderate intensity.

Design: A prospective study of six months' duration was carried out in 644 individuals of 7-17 years of age in Jequié, Bahia. Oxamniquine (Mansil®) and albendazole were administered in the form of syrup in single doses of 20 mg/kg of weight and 400 mg, respectively. Data were collected on hemoglobin concentration, dietary intake (24-hour recall) and fecal parasitology prior to and six months following treatment. Data on environmental and socioeconomic conditions were obtained at baseline.

Results: The subgroup analyses resulted in significant interaction of the intervention with the dietary intake of copper and family income. The increase in hemoglobin concentration in those not infected six months after treatment was statistically significant when dietary intake of copper was adequate (from 12.2 g/dl to 12.9 g/dl; increase in hemoglobin was 0.7 g/dl [p=0.00]), but not in those whose dietary intake of copper was inadequate (from 12.4 g/dl to 12.4 g/dl, there was no increase in hemoglobin [p=0.53]). There was a statistically significant increase in hemoglobin concentration in those not infected (from 12.2 g/dl to 12.6 g/dl; increase in hemoglobin was 0,4 g/dl; [p=0.02]) and in individuals infected by only one intestinal helminth (from 12.2g/dl to 12.5 g/dl; increase in hemoglobin was 0,3 g/dl [p=0.03]) with per capita income ≤ ¼ of a minimum salary

Conclusions: In the short term, the adoption of mass treatment as an intervention strategy may cause a positive impact on hemoglobin concentration in groups more vulnerable.

INTRODUCTION

Anemia constitutes a significant health problem in the population of school-age children in developing countries, affecting half the children of some countries of Africa and Asia [1] and approximately one-quarter of the children of Latin America [2]. In Brazil alone it is estimated that 41.7 million people are infected with *A. lumbricoides*, 18.9 million with *T. trichiura*, and 32.3 million with hookworms (2). Studies undertaken in the Brazil demonstrated a reduction in the prevalence of these geohelminths and were associated with the improvement of the community's sanitation conditions. The prevalence of geohelminths (*A. lumbricoides, T. trichiura,* and *Ancylostomatidae*) reduced significantly from 81.3% in 1992–1996 to 23.8% in 2010-2011, in children in urban communities, in Fortaleza, Brazil [3], similarly to the findings in Caxias do Sul (Rio Grande do Sul, Brazil) [4] and in Salvador (Bahia, Brazil) [5]. Although, there have been a few reports of infection rates of intestinal parasites in some local areas of Bahia state, study data conduced in a public school, Jequié city, in 2010, showed that enteroparasitoses prevalence was of 74,08%, being the most frequent enteroparasites: *Schistosoma mansoni* (44,44%), *Trichuris trichiura* (21,48%), *Entamoeba coli* (20,74%), *Ascaris lumbricoides* (16,30%), *Giardia lamblia* (7,41%), *Ancilostomídeos* (6,67%). Polyparasitism was found in 45.19% of the samples [6].

The greater vulnerability of school-children to anemia is explained by the increased demands for nutrients in this age-group, such as those that occur during periods of intense growth and those resulting from physiological loss (menstruation). Anemia has been the target of much research, principally because of the systemic changes it provokes in the organism, negatively affecting resistance to infection [7], weight-height growth [8], cognitive development and learning [9]. Among the causes of the high prevalence of anemia in developing countries, the roles of the inadequate dietary intake of bioavailable iron [10,11], chronic diseases [12] and parasitic infections [13] are the most significant. Nevertheless, the interaction between these factors may lead to various epidemiological scenarios with respect to the occurrence of anemia that demand different strategies for the management of the disease depending on the age-group and the exposure of the individual to different environmental, social and cultural factors.

Dietary supplements based on iron-rich foods and pharmacological iron supplementation, have been the principal interventions used both by international and Brazilian entities, particularly in preschool children and pregnant women. However, whenever control measures for anemia in school-children are under discussion, intestinal helminth infections must be taken into consideration, particularly because these infections are frequent in this age-group, occur generally in the form of multiple infections and frequently involve a high parasitic load [14,15]. A recent study carried out in a sample of school-children in Zanzibar suggests that 25% of all cases of anemia, 35% of cases of iron-deficiency anemia and 73% of cases of severe anemia may be attributed to hookworm infection [14]. In the Tanga region of the United Republic of Tanzania, the percentage of school-children estimated to be infected by hookworm and schistosomiases was 6% and 15%, respectively. In this region, the prevalence of both infections has been estimated at more than 50% [15].

Previous intervention studies have shown a positive effect of the treatment of heavy intestinal helminth infections on hemoglobin concentration in school-children [15-21]. However, there is still no consensus with respect to the pertinence of the treatment of mild and moderate intestinal helminth infections in the control of anemia. Mild and moderate infections are frequent and may represent a risk for the occurrence of anemia during periods of increased demand such as those that occur in infancy and adolescence.

The purpose of this study was, therefore, to investigate the increase in hemoglobin concentration in school-children and adolescents treated for mild and moderate helminth (*S. mansoni, A. lumbricoides, T. trichiura, Ancylostomidae*) infections. For the purpose of this evaluation, the presence of infection was tested at baseline and six months after treatment. Therefore, the primary aim of the current investigation is provides information might one day translate into the development of intervention strategies that guarantee better living and health conditions and nutrition of children and adolescents.

METHODS

A quasi-experimental study of six months duration was carried out in 644 individuals of 7-17 years of age living in the urban municipal district of Jequié, located in the southwest region of the Brazilian state of Bahia, 380 km from the state capital, Salvador. The climate is classified as tropical and temperatures range from 21°C at 36°C, with mean temperature of 23°C. Jequié has been one of the most important cities in Bahia in both economic and demographic aspects. In 2015, it was ranked eighth in population among the 417 Bahia cities (161.150 inhabitants, from which 37.5% belong to the age group 5 to 19 years), seventeenth in Gross Domestic Product, the local economy is based on agriculture and cattle breeding.

For the present study, a subsample of children and adolescents (n=644) was randomly selected from a census planned to evaluate the nutritional status and living conditions in this population segment [11]. The sample of 1,709 (12.4%) individuals was identified of stool examinations performed on 13,771 boys and girls between 7 and 17 years of age who attended public school and were include according to the following scheme: 1) all students infected with low to moderate-intensity *S. mansoni* (< 400 eggs/g of feces) associated with any intestinal helminth; 2) for those with isolated or associated low to moderate-intensity infection caused by hookworm (< 2,000 eggs/g of feces), *A. lumbricoides* (< 50,000 eggs/g of feces), or *T. trichiura* (< 20,000 eggs/g of feces), 14.5% were selected at random. For the present study, a minimum required sample size of 528 children and adolescents was estimated to provide 95 % power to determine with 95% confidence a hemoglobin mean difference of 0.3 g/dl or more (standard deviation = 0.8g/dl) according to the individual status of helminth infection six months after treatment [22]. Considering 20% dropout or migration, the total sample size needed was 634 individuals. A total of 692 individuals were enrolled. The reasons for drop-out were migration, incomplete data (n=48). However, there was no indication of differential attrition.

At baseline, data on environmental sanitation (water supply, sewage system, presence of toilet) and living conditions were obtained by observations of trained interviewers, supervised by field researchers. Level of income and other socio-economic variables such as education level of the head of household were obtained through a standardized questionnaire administered to the person responsible for meals in the household. An indicator of the environmental and household conditions was constructed from these variables. Variables describing household conditions and sanitation were dichotomized as favorable (0) or unfavorable (1) and tested for statistically significant associations with each of the infecting species. Those variables found to be significant were included in a logistic regression model for multivariable analysis. Those that remained with p value 0.15 were included in the index; the procedure has been fully described in a previously published paper [23]. Sex, age, and education of the head of household were included in the models as covariates. Thirteen variables were found to be significant, and the sum of these for each of the variables was used as the index of environmental and living conditions. The distribution of the scores in the community was used to define better (those with a score of 0-6) or worse (score 7-13) living conditions based on the 50th percentile of the index.

The treatment was managed in single dose, by a researcher of the project, on the basis of the result of the parasitological examination. Individuals infected with intestinal helminths were treated with albendazole (400 mg). Individuals infected with *S. mansoni* were treated with oxamniquine (40 mg/kg in a single dose). After receiving the drug, each one of them was closely monitored for 20 minutes. When someone presented some sensation of malaise, he or she was immediately directed to the hospital of the city. At baseline was performed other parasitological to verify the effectiveness of treatment.

After a period of six months, the participants were reviewed for new evaluation, including dosage of the hemoglobin concentration, parasitological exams of 24-hour excrements and alimentary recall. Two stool samples were collected, at different moments, and each specimen was mounted on two slides, making a total of four stool smears per individual, in each phase of the study. The identification of the *Schistosoma mansoni* and other parasites as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm was carried through the quantitative technique of Kato- Katz [24].

Slide preparation was by the method of Katz and others (1972). By this method, hookworm species cannot be distinguished, because their eggs have similar microscopic appearance. Slides were examined 2 hours after preparation to allow for identification of hookworm eggs for each child examined. The estimated number of eggs per gram was obtained by multiplying the mean number of eggs for the two slides by 24.

At baseline and after a period of six months, hemoglobin concentrations were measured using the Hemocue photometer (Hemocue, Laguna Hills, CA). Finger stick blood was collected using disposable sterile lancets. Trained nutritionists obtained dietary information. Dietary intake was determined at baseline and after a period of six months. Bioavailable iron was quantified through an equation developed by Monsen and Balintfy [25]. For this measurement, the total iron, heme iron, non-heme iron, and the quantity of ascorbic acid and meat composition at each meal was considered. To adjust for bioavailability, the iron content of each food was first separated into its heme and non-heme components. The assumed proportion of total iron in each food that was non-heme was 60% for meats and 100% for non-meats. Heme iron availability was assumed to be 23%. Non-heme iron availability for each eating occasion was adjusted for meat and vitamin C based on equations developed by Monsen and Balintfy [25] and ranged from 3% to 8%.

Calculation of the diets was carried out using a program designed by the Department of Nutrition of the University of São Paulo (Virtual Nutri, version 1.0) [26]. The adequacy of dietary intake of protein, iron, copper, vitamin A, vitamin E, vitamin B6, vitamin B12 and zinc was calculated in conformance with the 2006 Dietary Reference Intakes (DRI) of the National Research Council [27]. The Estimated Average Requirement (EAR), the standard deviation of the requirement (10% of the EAR) and the intrapersonal standard deviation of intake were used to calculate the adequacy of each nutrient in accordance with the sex and age of the individual. The "Subcommittee for the Use and Interpretation of DRIs" recommends use of the estimated intrapersonal variability obtained from population studies. Since no population-based data are available on the variability of intrapersonal dietary intake in Brazil, data from the US were used [28]. The classification of the adequacy of nutrients was based on the probability of a correct classification obtained from the ratio between D/DPo where D is the difference between the observed intake and the average requirement (EAR) and DPo is the variance of requirement + (variance in intake/number of days of evaluation of intake). Intake of the nutrient was considered adequate when the ratio obtained was a positive value with a probability of correct classification ≥ 95% [29]. The study population was classified according to whether the intake of bioavailable iron, copper, vitamin A was adequate (reference group) or inadequate (risk group).

For statistical analysis, the geometric mean of egg counts per gram of feces was used. Analysis of covariance (ANCOVA) was chosen along with the Scheffé correction to compare the mean egg counts, stratified by the number of different infecting species. The chi-square test was used to evaluate the differences between the categorical variables and ANCOVA for the differences between mean hemoglobin concentrations.

For the simultaneous evaluation of confounders (income, sex, age, environmental, and household conditions) and effect modifier (consumption of bio-available iron), multivariable analyses were carried out using the logistical regression model and ANCOVA. A significance level of 0.05 was defined for this study. The statistical packages used were Epiinfo (version 6.0) and Stata (version 7.0) [30]. The study was approved by the ethics committees of the Gonçalo Moniz Research Center and Case Western Reserve University. Detailed information about the project and study protocols was provided to the community. All parents or persons responsible for each participating child provided written consent for inclusion in the study. Parasitologic and hemoglobin concentration results were provided to persons responsible for each participant. Additionally, an explanatory note about the prevention of anemia and parasitic infections was provided. All school-children infected with intestinal helminths, at the end of follow-up and those with anemia (at baseline and at the end of segment) were referred for further evaluation and appropriate treatment in the Health Department of the municipality. To facilitate access the children's and adolescents the health practitioners were informed and oriented on the proposed project.

RESULTS

The study sample consisted of 644 children and adolescents of 7 to 17 years of age of which 50.1% were boys, more than half

(54.4%) reported a per capita family income ≤ ¼ of a minimum salary, 42% lived in homes with inadequate environmental conditions and more than 50% had an inadequate dietary intake of copper and bioavailable iron (Table 1).

With respect to the subjects who were lost-to-follow-up between the baseline and the six-month evaluations, no statistically significant difference was found between those who were lost-to-follow-up and those who remained in the study in terms of mean hemoglobin concentration, age, sex and the other variables evaluated at baseline. Therefore, any possibility that the final results could have been affected by a bias caused by baseline differences in the cases that were lost-to-follow-up was eliminated.

Table (1) describes the sociodemographic and environmental characteristics and dietary intake of the school-children and adolescents according to whether or not they were found to have a helminth infection six months after treatment, and according to the prevalence of anemia. No statistically significant differences were found between the groups with respect to age, environmental conditions or dietary intake of bioavailable iron and copper. Nevertheless, the school-children and adolescents who were found to have an infection six months after the intervention pertained principally to the group of individuals with a per capita family income ≤ ¼ of a minimum salary. This proportion was significantly greater compared to the group of children and adolescents who had no intestinal helminth infection six months after treatment. In the group infected by two or more helminths six months after treatment, there was found to be a significantly greater number of male children and adolescents compared to the non-infected group. The prevalence of anemia was found to be significantly higher in the group of school children and adolescents who belonged to the lower economic stratum group (per capita family income < ¼ of a minimum salary) and those whose dietary intake of vitamin A was inadequate compared, respectively, to school-children and adolescents who belonged to the higher income group and those whose dietary intake of vitamin A was adequate.

The school-children and adolescents infected with helminths six months after treatment were found to have significantly lower geometric mean egg counts (*Schistosoma mansoni, Ascaris lumbricoides, Trichuris trichiura* and *Ancylostomidae*) compared to those recorded at baseline. A statistically significant decrease was found in the prevalence of *Schistosoma mansoni* (58.3%), *Ascaris lumbricoides* (84.3%), *Ancylostomidae* (65.6%) and *Trichuris trichiura* (12.5%) in those individuals infected with only one of these helminths six months after the intervention. In the group found to be infected by two or more helminths at six months, the results pointed to a non-significant reduction in the prevalence of *Schistosoma mansoni* (7.8%), a statistically significant increase in the prevalence of *Ancylostomidae* (91.5%) and an increase that was not statistically significant in the prevalence of *Trichuris trichiura* (3,6%) and *Ascaris lumbricoides* (0.9%), (Table 2).

Table (3) describes the distribution of hemoglobin concentration and the prevalence of anemia at baseline and at the end of the study according to the individual status of helminth infection six months after treatment. Although an increase was registered in the hemoglobin concentration of the three groups studied, a statistically significant increase was found only in the group that was not infected six months after treatment. The increase in hemoglobin in this group (0.30 mg/dl) was two-fold that found in the group infected with one helminth at six months (0.13 mg/dl) and in the group infected by two or more helminths (0.14 mg/dl), even after adjustment for potentially confounding variables. Moreover, no significant reduction was found in the prevalence of anemia in the period studied, a reduction of 5.4% being recorded in the group that was not infected by helminths, while the prevalence remained unchanged in the group infected by two or more helminths six months after the intervention. In addition, an increase in the prevalence of anemia was found in 17.3% of those who were infected by only one intestinal helminth six months after treatment.

A strong interaction was found between the status of helminth infection six months after treatment and the following variables: per capita family income ($p<0.01$) and the adequacy of dietary intake of copper ($p=0.01$). Table (4) shows the variation in hemoglobin concentration according to the status of helminth infection at different per capita income levels and according to dietary intake of copper. Findings revealed that in the group of individuals with a per capita income ≤ ¼ of a minimum salary, there was a statistically significant increase in hemoglobin concentration in those not infected (from 12.2 g/dl to 12.6 g/dl [p=0.02]) and in individuals infected by only one intestinal helminth (from 12.2 g/dl to 12.5 g/dl [p=0.03]). In the group infected by only one intestinal helminth at baseline, a statistically significant difference was found in hemoglobin concentration according to income level, hemoglobin concentration being significantly higher in the higher income group (per capita income > ¼ of one minimum salary) ($p<0.01$). Six months after treatment, the difference in hemoglobin concentration between the two income groups failed to reach statistical significance ($p=0.43$). In the group of individuals infected by two or more helminths six months following the intervention, a statistically significant increase in hemoglobin concentration was found only for the higher income group (from 12.4 g/dl to 12.8 g/dl [p=0.04]). With respect to the group of individuals infected by two or more intestinal parasites at six months, there was no statistically significant difference in hemoglobin concentration between the two income levels at baseline. At six months of treatment, the differences between the income levels increased, and hemoglobin concentration were significantly higher in the school-children and adolescents that had a per capita family income > ¼ of a minimum salary compared to those of the group of individuals with a per capita income ≤ ¼ of a minimum salary.

Among the group of school-children and adolescents whose dietary intake of copper was adequate, a statistically significant increase in hemoglobin concentration occurred in those who were not infected six months after treatment (from 12.2 g/dl to 12.9 g/dl [p=0.00]) and in those infected by just one species of helminth (from 12.4 g/dl to 12.7 g/dl [p=0.03]). In the case of the school-children and adolescents whose dietary intake of copper was inadequate, no statistically significant increase in hemoglobin concentration was found irrespective of the status of helminth infection six months after treatment (Table 4).

Table 1: Sociodemographic, environmental and dietary intake data by status of helminth infection six month after treatment and prevalence of anemia in children and adolescents in Jequie, Bahia, Brazil, 1997-1998.

Variables		**Status of helminth infection six month after treatment**			**Anemia %**
		Not infected(a)	**Infected**		
			1 helminth (b)	**2 or more helminths (c)**	
Age (years)	N (%)				
7- 9	213 (33.1)	35.5	30.9	33.7	33.8
10 – 14	347 (53.9)	51.3	56.1	53.4	26.8
15 – 18	84 (13.0)	13.2	13.0	12.9	22.6
P value[1]			a-b= 0.56	a-c= 0.91	0.09
***Per capita* family income (MW)[2]**					
> ¼	297 (46.1)	53.4	44.3	36.8	23.2
≤ ¼	347 (53.9)	46.6	55.7	63.2	33.1
P value			a-b= 0.02	a-c= 0.00	0.01
Sex					
Female	329 (51.1)	46.6	48.5	59.1	32.8
Male (784)	315 (48.9)	53.4	51.5	40.9	24.1
P value			a-b= 0.69	a-c= 0.01	0.02
Environmental and household conditions[3]					
Adequate	379 (58.9)	62.4	62.6	50.3	26.1
Inadequate	265 (41.1)	37.6	37.4	49.7	32.1
P value			a-b= 0.97	a-c= 0.02	0.10
Dietary intake					
Copper (baseline)					
Adequate	249 (38.7)	39.7	42.4	32.6	24.5
Inadequate	395 (61.3)	60.3	57.6	67.4	31.1
P value			a-b= 0.57	a-c= 0.15	0.07
Copper (six months)[3]					
Adequate	264 (41.0)	41.3	45.4	34.7	25.4
Inadequate	380 (59.0)	58.7	54.6	65.3	30.8
P value			a-b= 0.38	a-c= 0.18	0.14
Bioavailable iron (baseline)					
Adequate	288 (44.7)	45.0	48.9	38.9	26.7
Inadequate	356 (55.3)	55.0	51.2	61.1	30.1
P value			a-b= 0.41	a-c= 0.23	0.35
Bioavailable iron (six months)[4]					
Adequate	219 (34.0)	37.0	35.1	29.5	29.2
Inadequate	425 (66.0)	63.0	64.9	70.5	28.2
P value			a-b= 0.68	a-c= 0.12	0.79
Vitamin A (baseline)					
Adequate	394 (61.6)	61.3	65.9	56.0	27.4
Inadequate	246 (38.4)	38.7	34.1	44.0	30.5
P value			a-b= 0.31	a-c= 0.29	0.40
Vitamin A (six months)[4]					
Adequate	357 (56.2)	63.3	58.3	46.3	24.9
Inadequate	278 (43.8)	36.7	41.7	53.7	33.1
P value			a-b= 0.29	a-c= 0.00	0.02

[1]χ^2 test was used to test the differences
[2]MW = minimum wage calculated as monthly minimum equivalent to at the time of the study.
[3]Environmental and household conditions= adequate environmental score of 0 – 6 and inadequate of 7 - 13
(a)= reference group
[4]Prevalence of anemia six months after treatment

Table 2: Geometric mean egg count and prevalence of helminth infections (*S. mansoni, A. lumbricoides*, *T. trichiura,* Ancylostomidae) at baseline and at the end of the study according to the individual status of helminth infection six months after treatment in Jequie, Bahia, Brazil, 1997-1998.

Intestinal helminths	Status of helminth infection six month after treatment		Geometric mean egg (SD)		P value[1]	% reduction or increase	Prevalence		McNemar's test P value[2]	% reduction or increase of prevalence
			Baseline	Six months after treatment			Baseline	Six months after treat-ment		
Schistosoma mansoni	Not infected (189)		63.1 (2.6)	0		- 100.0	36.5	0		-100.0
	Infected	1 helminth (262)	64.6 (2.4)	38.9 (3.3)	0.00	- 39.8	43.9	18.3	0.00	-58.3
		2 or more helminths (193)	72.4 (2.6)	47.9 (3.5)	0.00	- 33.8	51.3	50.3	0.63	-7.8
Ascaris lumbricoides	Not infected (189)		1258.9 (5.0)	0		- 100.0	69.3	0		-100.0
	Infected	1 helminth (262)	1380.4 (6.5)	660.7 (6.6)	0.00	- 52.1	53.8	8.4	0.00	-84.3
		2 or more helminths (193)	3090.3 (5.8)	676.1 (9.8)	0.00	- 78.1	63.7	64.3	0.54	+0.9
Trichuris trichiura	Not infected (189)		125.9 (3.2)	0		- 100.0	51.3	0		-100.0
	Infected	1 helminth (262)	182.0 (3.9)	134.9 (3.2)	0.00	- 25.9	79.0	69.1	0.00	-12.5
		2 or more helminths (193)	199.5 (4.1)	199.5 (3.8)	0.94	0	87.1	90.2	0.32	+3.6
Ancylostomidae	Not infected (189)		120.2 (3.8)	0		- 100.0	7.9	0		- 100.0
	Infected	1 helminth (262)	177.8 (4.3)	87.1 (6.0)	0.00	- 51.0	12.2	4.2	0.00	- 65.6
		2 or more helminths (193)	134.9 (4.9)	58.9 (3.9)	0.00	- 56.3	17.6	33.7	0.00	+ 91.5

[1]Scheffe´ test was used to test the differences between geometric mean of eggs of parasite species
[2] was used to compare prevalences of parasite species

Table 3: Hemoglobin concentration and prevalence of anemia in children at baseline and at the end of the study according to the individual status of helminth infection six months after treatment in Jequie, Bahia, Brazil, 1997-1998.

Status of helminth infection six months after treatment		Mean hemoglobin (SE)[1,2]		P value[3]	Prevalence of anemia (95% IC)[3]		McNemar's test P value	% reduction or increase of prevalence
		Baseline	Six months after treatment		Baseline	Six months after treatment		
Not infected (189)a		12.3 (0.09)	12.6 (0.09)	0.01	28.0 (21.6 – 34.5)	26.5 (20.1 – 32.8)	0.72	- 5.4
Infected								
	1 helminth (262)b	12.4 (0.07)	12.5 (0.08)	0.34	26.0 (20.6 – 31.3)	30.5 (24.9 – 36.1)	0.24	+ 17.3
	2 or more helminths (193) c	12.4 (0.09)	12.5 (0.09)	0.43	32.6 (26.0 – 39.3)	32.6 (26.0 – 39.3))	1.0	0
P value		a-b = 0.79[3] a-c= 0.89	a-b = 0.79 a-c= 0.89		a-b = 0.62[4] a-c= 0.32	a-b = 0.34 a-c= 0.18		

a=reference group
[1]Mean hemoglobin concentrations at baseline adjusted for income, sex, age, helminths infections at baseline, dietary intake (zinc, bioavailable iron, vitamin A, protein) and environmental conditions.
[2]Mean hemoglobin concentrations at the end of the study adjusted for income, sex, age, helminths infections at baseline, dietary intake (zinc, bioavailable iron, vitamin A, protein), hemoglobin concentration at baseline, environmental conditions.
[3]ANCOVA used to test the differences of the average of hemoglobin between groups.
[4]χ^2 test was used to test the differences

Table 4: Hemoglobin concentration in children at baseline and at the end of the study according to the individual status of helminth infection six months after treatment in Jequie, Bahia, Brazil, 1997-1998.

Variables	Status of helminth infection six month after treatment								
	Not infected (N=189)			Infected (N=455)					
	Mean hemoglobin (SE)[2]			Mean hemoglobin (SE)[2]			Mean hemoglobin (SE)[2]		
				One helminth (N=341)			2 ou more helminths (N=114)		
	Baseline[2]	Six months after treatment[3]	P value	Baseline[2]	Six months after treatment[3]	P value	Baseline[2]	Six months after treatment[3]	P value
***Per capita* family[1] income (MW)**									
≤ ¼ (350)	12.2 (0.11)	12.6 (0.14)	0.02	12.2 (0.10)	12.5 (0.10)	0.03	12.3 (0.10)	12.3 (0.11)	0.30
> ¼ (294)	12.5 (0.13)	12.7 (0.13)	0.27	12.6 (0.10)	12.6 (0.11)	0.98	12.4 (0.14)	12.8 (0.14)	0.04
P value[4]	0.12	0.70		<0.01	0.43		0.59	0.01	
Copper dietary intake[5]									
Inadequate (373)	12.4 (0.11)	12.4 (0.12)	0.53	12.4 (0.11)	12.5(0.11)	0.52	12.3 (0.11)	12.4 (0.15)	0.59
Adequate (271)	12.2 (0.14)	12.9 (0.15)	0.00	12.4 (0.12)	12.7(0.12)	0.03	12.4 (0.15)	12.6 (0.11)	0.28
P value[4]	0.37	0.02		0.97	0.94		0.43	0.30	

[1]MW = minimum wage calculated as monthly minimum equivalent to at the time of the study.
[2]Mean hemoglobin concentrations at baseline adjusted for income, sex, age, helminth infections at baseline, dietary intake (zinc, bioavailable iron, vitamin A, E, B6, B12 and protein) and environmental conditions.
[3]Mean hemoglobin concentrations at the end of the study adjusted for income, sex, age, helminth infections at baseline, dietary intake (zinc, bioavailable iron, vitamin A, E, B6, B12 and protein), hemoglobin concentration at baseline, environmental conditions.
[4]ANCOVA used to test the differences of the average of hemoglobin between groups.
[5]Dietary intake = mean of two 24-hour recall (one at baseline and other at the end of the study).

DISCUSSION

The findings of the present study show that the efficacy of the treatment of helminth infections (absence of helminth infection six months after the intervention) was important in increasing hemoglobin concentration in individuals of the low-income group whose dietary intake of copper was adequate. It is noteworthy that similar results were seen in the individuals infected by only one species of helminth six months following treatment. These findings are consistent with data from randomized clinical trials that have reported a positive effect of the treatment of mild and moderate intestinal parasite infections on the hemoglobin concentration of preschool and school-children [21,31]. Nonetheless, based on the data from randomized clinical trials, no consensus has yet been reached regarding the role of the treatment of mild and moderate infections in the occurrence of anemia. Studies carried out among school-children showed a positive effect of the treatment on hemoglobin concentration only when infection was heavy [15, 18,19,32,33].

Nevertheless, it is difficult to compare the results obtained in the present study with the data originating from randomized clinical trials, principally because of methodological differences. In the present study, the efficacy of the treatment of helminth infections six months after therapy was found to represent an important predictor variable for an increase in hemoglobin concentration. Bearing in mind that re infection with intestinal helminths is common following treatment, principally in the case of hookworm infections [34], it is important to evaluate whether there are differences in the benefits of treatment after six months in individuals with no intestinal helminth infection and in those who become re infected. However, in randomized clinical trials, the effect of the treatment of intestinal helminth infections, including data from subjects who become re infected, is compared with subjects who received placebo. It is therefore possible that the effect of treatment on hemoglobin concentration is underestimated as a result of the rates of re infection that may occur in the treatment group.

The number of species of intestinal parasites in the individual six months after treatment may represent an important differential in the risk of anemia. The significant increase in hemoglobin concentration found in individuals infected with two or more helminths six months after treatment occurred only in the group with a higher income level, suggesting that this finding may be due to other protective factors for anemia in this group. It is important to remember that simultaneous infection by various species of helminth may represent a greater risk for the health of the individual than isolated single infections. As well as the additional damage inflicted on digestion, absorption and the loss of nutrients that may occur in cases of multiple infections, the infections may also involve higher parasite loads [11]. Studies show that children treated for multiple infections of *A. lumbricoides* and *T. trichiura* have a statistically significant increase in appetite compared to a control group [35,36]. Furthermore, in the group with two or more infections at six months, although the reduction in the geometric mean helminth egg counts was significant, there was a statistically significant increase in the prevalence of hookworm in this group. Hookworms are the most important of all intestinal parasites and infection may be caused by two different species: *Ancylostoma duodenale* and *Necator americanus*, which lodge in the small intestine, causing chronic blood loss. Although part of the iron lost may be reabsorbed [37], studies show that the significant loss of blood caused by heavy parasite infections may result in iron deficiency and consequently may lead to anemia in children [38,39].

The control of helminth infections may result in an increase in hemoglobin concentration by correcting the different mechanisms that facilitate the onset of anemia, such as: anorexia, competition for nutrients [40,41], chronic micro-hemorrhages resulting from lesions to mucosa [13], reduction in dietary intake [42,43], reduction in absorption [44,45] and the release of endogenous cytokines, which, in turn, induce alterations in the metabolism of iron and a consequent reduction in hemoglobin synthesis [12].

To evaluate anemia as a nutritional problem resulting from social causes, discussions on the structural problems that interfere with living conditions, health and nutrition of the population must be included in the analysis. In the group in which no infection was found six months after treatment, treating the helminth infections had a positive and more pronounced effect on the hemoglobin concentration of the school-children and adolescents with a per capita family income < ¼ of a minimum salary compared to those whose per capita family income was > ¼ of a minimum salary. A similar effect was observed in the group with only one intestinal helminth infection six months after treatment. The occurrence of anemia is known to vary, in general, according to the socioeconomic conditions of the population [46,47]. These conditions are not directly responsible for the occurrence of anemia; however, they indirectly predispose individuals to exposure to this pathology [48,49]. Therefore, part of the negative effect caused by precarious socioeconomic conditions on hemoglobin concentration may have been reduced in individuals who had no infection or infection by only one helminth six months after treatment. Six months after the intervention, the school-children and adolescents infected by two or more helminths, who had a per capita family income ≤ ¼ of a minimum salary had significantly lower hemoglobin concentration compared to those whose per capita family income was > ¼ of a minimum salary. It is noteworthy that even mild and moderate infections represent a factor to be added to the other multiple factors involved in the occurrence of anemia, which coexist in low-income populations. The low-income children and adolescents are probably more exposed to unhealthy environmental factors and to an inadequate dietary intake. It is important to remember that the school-children and adolescents belonging to the lower-income group initially had lower mean hemoglobin concentration compared to those found in the higher-income group. Data from other studies have shown greater benefits of the treatment of intestinal helminth infections in school-children with lower baseline hemoglobin concentration [19,35]. Data situated at a given moment at one of the extremes of a distribution curve are known to have a tendency to be less distant from the mean at a later moment even when no intervention has been carried out. Nevertheless, it may be assumed that the results of the present study were not influenced by regression to the mean, since the hemoglobin concentrations six months after the intervention were adjusted according to baseline hemoglobin levels.

In the school-children and adolescents with a higher income level, the efficacy of the treatment of mild and moderate helminth infections did not appear to play a significant role in hemoglobin concentration, bearing in mind that a statistically significant increase in hemoglobin concentration only occurred in the group infected by two or more intestinal helminths.

The status of infection six months following the intervention also had a different impact on the increase in hemoglobin concentration according to dietary intake of copper. The results showed that of the school-children and adolescents who had adequate dietary intakes of copper, the group with no helminth infection and the group infected by only one helminth had a statistically significant increase in hemoglobin concentration six months after treatment. It should be emphasized that there was no statistically significant increase in hemoglobin concentration in the group of individuals whose dietary intake of copper was inadequate, even in those who were not infected by helminths six months following the intervention. It could therefore be speculated that copper may play a role in protecting against re infection by intestinal helminths. This role of copper has been reported in studies carried out in animals. Results from these studies have shown that copper supplementation may constitute a significant strategy for reducing the use of antihelminthic medication for the control of parasitic gastroenteritis [50]. It has been suggested that the administration of copper oxide, as well as leading to an increase in the mortality of helminths in infected sheep, may also result in reduced fecundity of the females surviving in the host [51,52]. Copper oxide consists of a central nucleus of pure copper covered with a mixture of cuprous and cupric oxide. Following oral administration, the capsules of copper oxide are dissolved in the lumen and the particles are then lodged in the folds of the intestinal mucosa. In this acid environment, the copper is released over a long period of time, the dissolvability of the inorganic ions being dependent on pH [53]. According to Bang et al. (1990), copper supplementation in sheep resulted in benefits in the combat of intestinal parasites. Gonçalves & Echevarria (2004) carried out a study to determine the time of protection of copper supplementation against reinfection by intestinal helminths in sheep [54]. Findings indicated that the administration of copper contributed significantly to a lower rate of re infection by intestinal helminths, particularly over a period of 4 weeks following administration of capsules of copper oxide. In addition, copper is an essential mineral in dozens of enzymatic systems, and participates in processes such as energy and hemoglobin production. It is involved in hemoglobin synthesis by different mechanisms: as an essential cofactor for the action of enzymes that oxidize the ferrous iron in ferric iron and as an essential component of ceruloplasmin, also known as ferroxidase I, the enzyme that is involved in the formation of the transferrin that binds iron and transports it to the bone marrow where the erythrocytes are formed, and hemoglobin is synthesized [55]. Copper also plays an important role in the mechanisms of defense of the organism. Individuals with copper deficiency have impaired immunological response, principally due to a reduction in interleukin-2 and neutrophil levels [56-58]. Therefore, individuals with copper deficiency may be more susceptible to infectious diseases and, consequently, defects may be present in the mobilization of iron for the production of erythrocytes

[59]. However, the observation of an increase in hemoglobin concentration even in the group of individuals infected by one helminth six months after treatment may be explained in part by the reduction in parasite load that occurred over the same period. Moreover, the time of exposure to the intestinal helminth infections may have been insufficient to be detrimental to the evolution of hemoglobin concentration.

Studies carried out in Brazilian children and adolescents have shown that anemia constitutes a public health problem in this age-group, as also confirmed by the findings at the beginning of the present study. In this age-group, parasitic infections have been indicated as important determinants of anemia, particularly due to their high prevalence and often heavy parasitic load. Therefore, in addition to controlling these infections, it is also necessary to implement nutritional interventions and improvements in sanitary conditions. Hence, any intervention should not be restricted to compensating the deficits in hemoglobin concentration over a limited period of time, but should attempt to maintain the advances achieved during that period.

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