Original Research Article

Weekly iron supplementation in 2-year-olds is effective in combating anaemia

ABSTRACT

Introduction: Iron deficiency anaemia is a serious public health problem in developing countries, especially among children, as it is associated with serious developmental problems. **Objective:** To assess the effects of weekly ferrous sulfate supplementation on haemoglobin (Hb) levels and the prevalence of anaemia in children aged 2 to 3 years. **Methods:** A cluster-randomized clinical trial was conducted;two schools were randomly chosen. In the first school, the children received 6mg/kg of elemental iron in the form of iron sulfate once a week (intervention). In the other school, the children received a placebo (control). The intervention group had 44 participants at the end of the study, and the control group had 48 children. Blood samples were taken at baseline and at the end of the study to assess serum Hb levels and anaemia prevalence. The intervention lasted 14 weeks. **Results:** There was a mean increase in Hb of 0.85g/dL (p=0.0003) in the intervention group and a decrease of 0.74g/dL (p=0.0001) in the control group. The prevalence of anaemia significantly decreased in the weekly supplementation group with p=0.0002. **Conclusion:** Weekly iron supplementation in preschool-age children promoted a significant increase in Hb levels and a decrease in the prevalence of anaemia.

Keywords: Iron-deficiency anaemia; Haemoglobins; Ferrous sulfate; Preschool child; Clinical trial.

1. INTRODUCTION

The World Health Organization (WHO) defines anaemia as a condition in which the serum concentration of haemoglobin is below the reference values, to the point of not meeting the physiological needs according to age, sex, pregnancy and altitude(1).Of the causes of anaemia, approximately 50% are attributed to a diet deficient in iron, which is considered the most prevalent nutritional deficiency in the world, affecting mainly children under fiveyears of age (preschoolers), women of childbearing age, pregnant and lactating women, in greater numbers in developing countries (2,3).

Iron is a widespread metalin the human body, playing a crucial role in all phases of protein synthesis, cellular respiration, andoxidative and immunological processes (4,5,6). Iron deficiency is associated with bone fragility and distortions, hepatosplenomegaly (possibly from extramedullary hematopoiesis), delayed growth and puberty, neurodevelopmental changes, cardiomegaly, and electrocardiographic abnormalities (7). A recent study associated iron deficiency in childhood and adolescence with the increased prevalence of Attention Deficit Hyperactivity Disorder (ADHD), Anxiety Disorder and Bipolar Mood Disorder, highlighting the long-term importance of iron deprivation (8).

In this sense, one of the strategies recommended by the WHO to control iron deficiency is the use of weekly (intermittent) supplementation for risk groups, infants and preschoolers, which worldwide have a prevalence of anaemia close to 40% (9,10). Our

study used weekly iron supplementation in public schools as a strategy to try to improve children'shematimetric levels without the need for family adherence since it is often difficult to understand how important iron is for the development and homeostasisof these children.

2. METHODOLOGY

2.1. Study design

The authors designed and implemented a cluster randomized clinical trial study to address the research purpose. The study sample was derived from the population of preschoolers aged between 24 and 36 months, from public Infant Education Centers, in the municipality of Sobral - Ceará, a middle-sized city, in northeast Brazil, between August and December 2019.

Prior to intervention, three public Infant Education Centers were chosen using a table of randomized numbers; the first formed Group A, the second Group B. Group A received 6 mg/kg of elemental iron once weekly (intervention); and Group B was designated as control.

All preschoolers aged 24 to 36 months from the two Infant Education Centers were invited to participate in our study. Exclusion criteria were parents'refusal to participate and infants already using iron supplementation.

2.2. Intervention

The preschoolers in Group A received 6 mg/kg elemental iron once weekly (Mondays); intervention was administered using an individual plastic medical syringe with a scale, previously prepared according to child'sweight, to gently squirt the solution into the side of the child's mouth by graduate medical trainees. The interventionlasted 14 weeks, beginning and ending on the same date for all groups.

2.3. Primary outcomes and other variables

The study included twoprimary outcome variables: 1) change in Hb concentration measured in g/dL; and 2) anaemia prevalence before and after theintervention. Hb concentration <11.0g/dL was used as acutoff point to define anaemia (10).

According to information provided by parents, a standardized data sheet was filled in containing information on (other study variables): age, gender, exclusive breastfeeding (EBF) up to 6 months, mother'sschooling, and family income.

2.4. Sample size

According to previous studies conducted in this region, anaemia prevalence in the study population was estimated at 40-50% (11). To reduce global anaemia prevalence from 50 to 25%, with 80% power, 2-sided, type I error of 5 %, accounting for 10% losses to follow-up, each group required a minimum of 43 participants (12).

2.5. Data collection

Two biochemical evaluations were performed to determine Hb concentrations before and after theintervention. A technician promptly analyzed Hb concentrations with a portable HemoCue B-haemoglobin photometer (Hb 301 - HemoCue AB, Ängelholm, Sweden). Finger prick capillary blood was collected under aseptic conditions using Carelet® Safety Lancets (Facet Technologies, Atlanta, GA, USA). Members of the study team who collected outcome data were blinded to the different interventions.

2.6. Data analyses

To compare means,the investigators used the paired student'st-test to assess the difference in Hb concentration before and after theintervention within the groups and Fisher'sexact test to assess the difference between good and bad outcomes (absence or

presence of anaemia). Data had anormal distribution. The statistical software package SPSS for Windows, version 17.0, was used for all analyses (SPSS Inc., Chicago, IL). The limit for statistical significance was set at p=0.05. Analyses were by intention to treat.

This study was approved by the Ethics Committee for Research of the Universidade Federal do Cearáaccording tothe ethical principles established by the National Health Council Resolution #466/2012.Prior written consent was obtained from school directors and parents/guardians. Medical support was available upon request. After theintervention, anaemic children were referred for treatment.

3. RESULTS

At baseline, 14 preschoolers were excluded before blood analysis, nine from group A (3 refused and six already using iron supplementation), and five from group B (3 refused and twoalready using iron supplementation) (Figure 1).

Before the second biochemical evaluation (at the end of the intervention), there were ten dropouts from Group A (5 left Infant Education Center, two absentees, three non-compliant); in Group B, there were 11 dropouts (7 left Infant Education Center, two absentees, two non-compliant) (Figure 1).

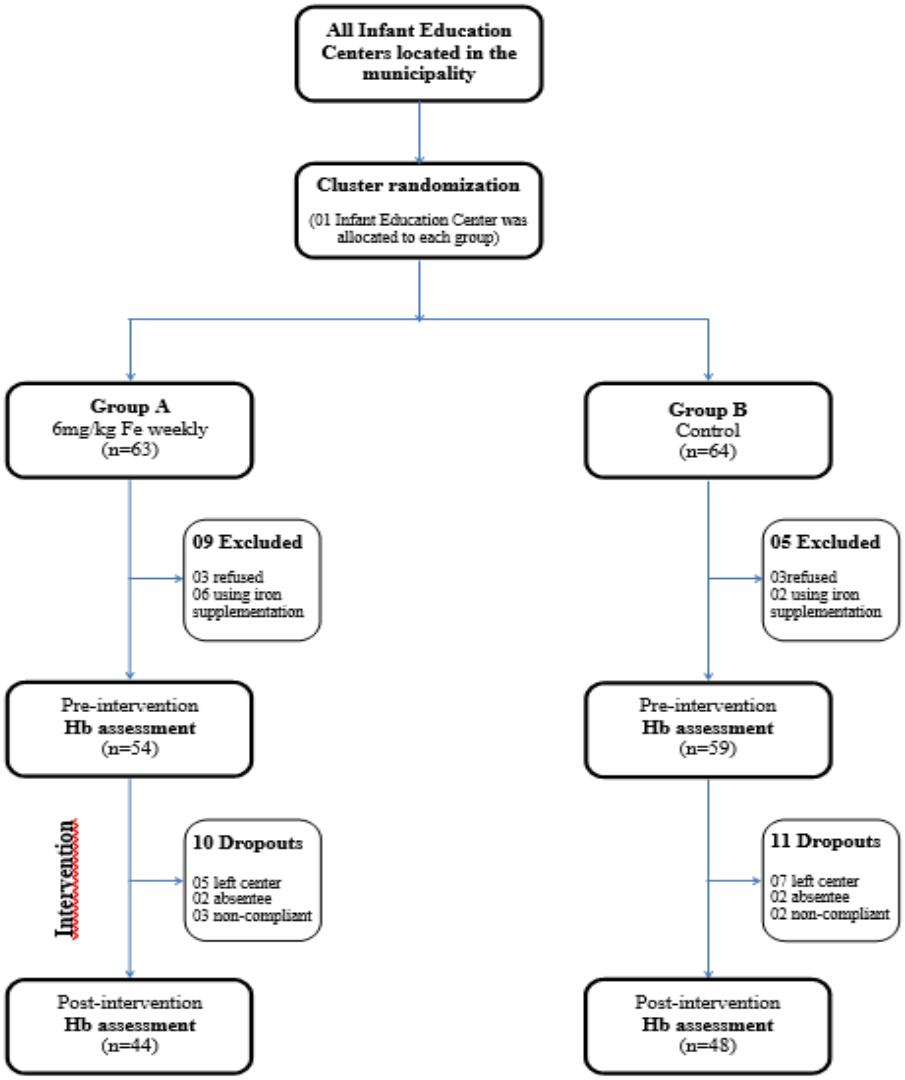

Figure 1. Flow chart of study design

At baseline, Hb concentration and the other study variables were analyzed. There were no statistically significant differences for age, gender, EBF, mother'sschooling, and family income. Mean age (in months) for group A was 29.9±3.51for group A and 30.4±3.36 for group B, p= .30; in group A, 27 participants were male, and 27 were female. In group B, 28 were male and 31were female, p= .96. The p-values between groups for EBF, mother'sschooling and family income were .96 and .90, respectively. However, there was a significant difference between the groups for mean Hb values; themean Hb for group A was 11.34±1.31 for group A and 11.88±0.78 g/dL for group B, p= .003 (Table 1).

Table 1. Baseline characteristics of study participants by intervention group and control

Variables	**Group A** (n=54) *Weekly iron*	**Group B (**n=59) *Control*	p-value[a]

Age (months) *Mean±SD*	29.9±3.51	30.4±3.36	.30[a]
Haemoglobin (g/dL)	11.34±1.31	11.88±0.78	**.003**[a]
Gender M:F	27:27	28:31	.96[b]
EBF	25	24	.46[b]
Mother with ≥9y schooling	22	23	.96[b]
Family income ≥300USD	23	25	.90[b]

All numbers are absolute; SD standard deviation; M: F male: female; EBF exclusively breastfed up to 6 months of age; [a] Based on unpaired Student's *t*-tests; [b] Based on Fisher's exact test (2-tailed).

In Group A, themean baseline Hb concentration was 11.19±1.42 g/dL, and after intervention mean Hb concentration increased to 12.04±0.96 g/dL, p= .0003; and anaemia prevalence was 20 out of 44, 45.5% at baseline, and 4 out of 44 (9.1%) at the end of the study, p= .0002. In the control group (Group B), themean baseline Hb concentration was 11.85±0.86 g/dL, and after intervention mean Hb concentration decreased to 11.11±0.87 g/dL, p< .0001; and anaemia prevalence was 8 out of 48, 16.7% at baseline, increasing to 12 out of 48, 25.0% at the end of the study, without astatistical difference, p= .452. (Table 2).

Table 2. Effects of weekly iron supplementation and control on haemoglobin levels and anaemia prevalence before and after the intervention.

	Group A (n=44) *Weekly iron*			**Group B** (n=48) *Control*		
Variables	Before	After	*p*	Before	After	*p*
Hb (g/dL) *Mean±SD*	11.19±1.42	12.04±0.96	**.0003**[a]	11.85±0.86	11.11±0.87	**<.0001**[a]
CI	10.76, 11.62	11.75, 12.33		11.60, 12.10	10.85, 11.36	
Mean increase in Hb *Mean±SD*		0.85±1.42			-0.74±0.96	**<.0001**[a]

CI		0.413, 1.278			-1.020, -0.463	
Anaemia[b]	20 (45.5)	4 (9.1)	**.0002**[c]	8 (16.7)	12 (25.0)	.452[c]

All numbers are absolute except numbers in brackets, which represent percentages; Hb Haemoglobin; SD standard deviation; CI 95% Confidence interval; [a] Based on paired Student's*t*-tests; [b] Anaemia defined as Hb concentration <11.0 g/dL; [c] Based on Fisher's exact test (2-tailed).

When considering alterations in mean Hb concentrations, there was an increase in mean Hb values for Group A (0.85±1.42); however, Group B registered a reduction in mean Hb concentration (-0.74±0.96), p< .0001 (Table 3).

When considering only the anaemic participants, in Group A (n=20), themean Hb concentration was 9.82±0.60 at baseline and 11.58±0.45 after theintervention, p< .0001; at baseline, 20 participants were anaemic; however, after theintervention, this number reduced to 4, p< .0001. In the control group (Group B), themean Hb concentration was 10.78±0.14 g/dL at baseline, decreasing to 10.60±1.27 after theintervention, without statistical significance, p= .077. The interventiongroup presented an increase in mean Hb concentration, 1.76±0.85 g/dL, while the control group (Group B) slightly decreased mean Hb concentration, p= .677. (Table 3).

Table 3. Effects of weekly iron supplementation and control on haemoglobin levels and anaemia prevalence for anaemic preschoolers, before and after the intervention.

	Group A (n=20)			**Group B** (n=8)		
	Weekly iron			*Control*		
Variables	Before	After	*p*	Before	After	*p*
Hb (g/dL) *Mean±SD*	9.82±0.60	11.58±0.45	**<.0001**[a]	10.78±0.14	10.60±1.27	.677[a]
CI	9.58, 10.06	11.34, 11.82		10.09, 11.46	9.91, 11.29	
Mean increase in Hb *Mean±SD*		1.76±0.85			-0.18±1.14	**<.0001**[a]
CI		1.363, 2.157			-1.126, 0.776	
Anaemia[b]	20	4	**<.0001**[c]	8	4	.077[c]

All numbers are absolute; Hb Haemoglobin; SD standard deviation; CI 95% Confidence interval; [a] Based on paired Student's*t*-tests; [b] Anaemia defined as Hb concentration <11.0 g/dL; [c] Based on Fisher's exact test (2-tailed).

The following indicators were compared in this study: intervention group A versus control, for a favourable or adverse outcome (absence of anaemia versus anaemia). The adverse outcome was present at the endpoint in 100% of control subjects and 20% (group A) of experimental subjects. The Reduction of Absolute Risk (RAR) difference was 80% for

group A. This difference 95% confidence interval ranged from 62.5 to 97.5% (group A). Relative Risk (RR) was 0.36 for theweekly supplementation group. The numberNeeded to Treat (NNT) was 2 for group A. This means that one in every twopreschoolers in the intervention group benefited from the intervention. The 95% confidence interval for the NNT ranged from 1.0 to 1.6 (group A).

4. DISCUSSION

In Brazil, in 2005, the Ministry of Health implemented the National Iron Supplementation Program, which aimed to reduce the prevalence of iron deficiency anaemia, through preventive iron supplementation in children aged sixmonths to 2 years, andpregnant women, and women in the postpartum period (13). This type of program has been conducted for more than 60 years in developed countries, but only in the last decade has it been implemented on a larger scale (14,15).

Even with these interventions, we still have iron deficiency anaemiaas a public health problem in our country. In our study, the prevalence of iron deficiency anaemia was 30.4%, classified as moderate, unlike in a recent systematic review on the prevalence of iron deficiency anaemia in pre-school children in Brazil, 40.2%, a level considered to be considered be a severe public health problem (10).Despite being conducted in a poor region, our study presented a lower prevalence of iron deficiency anaemia than the more developed regions of the country (38.7%) (11). This may be explained by effective local public policies, iron-rich school lunch menus, and extra-governmental interventions (16-18).

In the present study, the weekly use of iron led to a significant decrease in the prevalence of iron deficiency anaemia in children, from 45.5 to 9.1%. Whereas there was a non-significant increase in the anaemic population from 16.7 to 25.0% in the control group. When analyzing only anaemic participants, a large reduction of iron deficiency anaemia was observed, 80%, in the group that had weekly iron supplementation. We achieved an NNT of 2; that is, for every twochildren exposed to the intervention, onechild was recovered from the condition of anaemic. Such data show that weekly iron supplementation in the anaemic participants was very effective.

Still analyzing these same groups, a significant increase in serum Hb levels was verified in the group that received weekly ferrous sulfate supplementation (0.85g/dL) compared to the control group (-0.74g/dL) presented a decrease in Hb levels. This result agrees with the systematic review by De-Regil (2011), who found a mean increase in Hb of 0.5g/dL compared to placebo. In this review, greater adherence to treatment was found with weekly supplementation when compared to the daily use of iron (19). It is understood that intermittent supplementation may be an alternative to increase patients' patients' adherence to treatment and reduce the costs that daily supplementation demands.

Numerous studies have analyzed the specific benefits of preventive iron supplementation in children. The convergence in the reduction of the prevalence of anaemia, in the reduction of the morbidity of infectious diseases and infant mortality, ,and the contribution to the integral development of the tissues has been observed (7,9,10). However, few studies analyze practically the possible damage from this conduct, such as possible losses in the absorption of some micronutrients, such as zinc, and the possibility of excessive accumulation of iron in the body, which could be maximized with the daily use of iron and perhaps minimized with intermittent supplementation (20).

Most of the studies that relate iron supplementation with the reduction of anaemia do not specifically analyze the 2-year-old age group addressed in the present study. Generally, they assess older children (11,14,16-19,21). It is observed in these studies, including a systematic review, randomized clinical trials and community trials, that it is frequent to increase Hb levels and reduce iron deficiency anaemia with weekly

supplementation, as verified in the present study, in addition to increasing iron deposits (11,14,16-19,22-24).

As potential weaknesses to this study, the investigators make some considerations, first as the study was cluster randomized the prevalence of anemia would not necessarily be similar between the groups, it is believed that the group with lower levels of Hb and higher prevalence of anaemia may have presented a better response due to possible lower ferric status. This cannot confirmed as this study only analyzed Hb concentrations, iron deficiency through ferritin or transferritin receptors was not assessed, which would make iron assessment more reliable. Although in the anemic groups under intervention, there was a considerable decrease (from twenty to four) in the number of anemic children at the end of the study, the investigators believe that the short study duration (only 14 weeks) may have led to a smaller result, and perhaps a greater effect could be attained with a longer intervention period.

5. CONCLUSION

In our study, we have some approaches that make it innovative: low cost due to the weekly use of ferrous sulfate, use of supplementation in a community manner with the school lunch space in schools, reducing the chances of failures that could occur in the family environment, and intervention conducted with theintent to treat, with significant results in the short period of just 14 weeks. Thus, it becomes a plausible strategy to be implemented on a large scale in developing countries with a high prevalence of iron deficiency anaemia.

CONSENT

Informed consent forms (ICF) were distributed to parents or guardians, per Resolution Nº 466, of December 12, 2012. All students who signed the assent term and whose ICF was completed and signed by their parents or guardians were included in the study.

ETHICAL APPROVAL

This study was approved by the ethics committee for research at the Universidade Federal do Ceará following the ethical principles established by the National Health Council resolution #466/2012, with necessary prior written consent from school directors and parents/guardians. Medical support was available upon request. After theintervention, anaemic children were referred for treatment.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. de Castro TG, Silva-Nunes M, Conde WL, Muniz PT, Cardoso MA. Anemia e deficiência de ferro em pré-escolares da Amazônia Ocidental brasileira: prevalência e fatores associados [Anemia and iron deficiency among schoolchildren in the

Western Brazilian Amazon: prevalence and associated factors]. Cad Saude Publica. 2011;27(1):131-142. doi:10.1590/s0102-311x2011000100014
2. World Health Organization. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization, 2012.
3. Capanema FD, Lamounier JA, Norton RDC, Jácome AADA, Rodrigues DA, Coutinho RL, et al. Anaemia ferropriva na infância: novas estratégias de prevenção, intervenção e tratamento. Rev Med Minas Gerais. 2003;13(Supl 2):30-34.
4. Grotto, H. Z. Metabolismo do ferro: uma revisão sobre os principais mecanismos envolvidos em sua homeostase. Rev Bras Hematol Hemoter. 2008;30(5),390-397.
5. Dunn LL, Suryo Rahmanto Y, Richardson DR. Iron uptake and metabolism in the new millennium. Trends Cell Biol. 2007;17(2):93-100. doi:10.1016/j.tcb.2006.12.003
6. Henriques GS, Cozzolino SMF. Ferro. In: Cozzolino SMF. Biodisponibilidade de nutrientes. 4 Ed. Barueri, SP: Manole; 2012. p. 462-482.
7. Allali S, Brousse V, Sacri AS, Chalumeau M, de Montalembert M. Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. Expert Rev Hematol. 2017;10(11):1023-1028. doi:10.1080/17474086.2017.1354696
8. Islam K, Seth S, Saha S, Roy A, Das R, Datta AK. A study on association of iron deficiency with attention deficit hyperactivity disorder in a tertiary care center. Indian J Psychiatry. 2018;60(1):131-134. doi:10.4103/psychiatry.IndianJPsychiatry_197_17
9. Dary O, Hurrell R. Guidelines on food fortification with micronutrients. Geneva: World Health Organization, Food and Agricultural Organization of the United Nations, 2006.
10. World health Organization. Nutrition – Micronutrient deficiencies: Iron deficiency anemia [Online]. Geneva: World health Organization, 2020. Available from: http://www.who.int/nutrition/topics/ida/en/
11. Islam K, Seth S, Saha S, Roy A, Das R, Datta AK. A study on association of iron deficiency with attention deficit hyperactivity disorder in a tertiary care center. Indian J Psychiatry. 2018;60(1):131-134. doi:10.4103/psychiatry.IndianJPsychiatry_197_17
12. Lwanga SK, Lemesshow S. Sample Size Determination in Health Studies: A Practical Manual. Geneva: World Health Organization; 1991.
13. Portaria nº 730/GM, de 13 de maio de 2005. Institui o Programa Nacional de Suplementação de Ferro, destinado a prevenir a anemia ferropriva, e dá outras providências. Diário Oficial da União, Brasília-DF, Seção 1, 14 maio 2005. Available from: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2005/prt0730_13_05_2005.html
14. Detzel P, Wieser S. Food fortification for addressing iron deficiency in Filipino children: benefits and cost-effectiveness. Ann Nutr Metab. 2015;66 Suppl 2:35-42. doi:10.1159/000375144
15. World health Organization. Nutritional Anaemias: Tools for Effective Prevention and Control. Geneva: WHO, 2017.
16. Arcanjo FPN, da Costa Rocha TC, Arcanjo CPC, Santos PR. Micronutrient Fortification at Child-Care Centers Reduces Anemia in Young Children. J Diet Suppl. 2019;16(6):689-698. doi:10.1080/19390211.2018.1474987
17. Nogueira Arcanjo FP, Santos PR, Arcanjo CP, Amancio OM, Braga JA. Use of iron-fortified rice reduces anemia in infants. J Trop Pediatr. 2012;58(6):475-480. doi:10.1093/tropej/fms021
18. Nogueira Arcanjo FP, Santos PR, Arcanjo CP, Amancio OM, Braga JA. Use of iron-fortified rice reduces anemia in infants. J Trop Pediatr. 2012;58(6):475-480. doi:10.1093/tropej/fms021
19. De-Regil LM, Jefferds ME, Sylvetsky AC, Dowswell T. Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. Cochrane Database Syst Rev. 2011;2011(12):CD009085. doi:10.1002/14651858.CD009085.pub2

20. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr. 2006;84(6):1261-1276. doi:10.1093/ajcn/84.6.1261
21. Arcanjo FP, Arcanjo CC, Amancio OM, Braga JA, Leite AJ. Weekly iron supplementation for the prevention of anemia in pre-school children: a randomized, double-blind, placebo-controlled trial. J Trop Pediatr. 2011;57(6):433-438. doi:10.1093/tropej/fmq119
22. Palupi L, Schultink W, Achadi E, Gross R. Effective community intervention to improve hemoglobin status in preschoolers receiving once-weekly iron supplementation. Am J Clin Nutr. 1997;65(4):1057-1061. doi:10.1093/ajcn/65.4.1057
23. Brunken GS, Muniz PT, Silva SMD. Weekly iron supplementation reduces anemia prevalence by 1/3 in preschool children. Rev Bras Epidemiol. 2004;7(2): 210-219.7
24. Berger J, Aguayo VM, Téllez W, Luján C, Traissac P, San Miguel JL. Weekly iron supplementation is as effective as 5 day per week iron supplementation in Bolivian school children living at high altitude. Eur J Clin Nutr. 1997;51(6):381-386. doi:10.1038/sj.ejcn.1600418