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Prevention and Treatment of Anemia in Infants through Supplementation, Assessing the Effectiveness of Using Iron Once or Twice Weekly

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ABSTRACT

Background: The objective of this study was to compare the effect of once weekly iron supplementation (IS) *versus* twice weekly, on hemoglobin (Hb) levels and anemia prevalence.

Methods: In this cluster-randomized clinical trial study, we evaluated infants aged 6–18 months. Length of intervention: 16 weeks. Infants were cluster randomized to either 25 mgelemental iron once weekly (Group-A) or twice weekly (Group-B). Primary outcome variables were change in Hb concentration and anemia prevalence. Two biochemical evaluations were performed to determine Hb concentrations, before and after intervention.

Results: For Group-A, at baseline, mean Hb concentration was 10.8 ± 1.18 g/dl and after intervention 11.2 ± 1.07 g/dl, $p = 0.12$; anemia prevalence was 52.5% at baseline and 37.5% after intervention, $p = 0.18$; Group-B, mean baseline Hb was 10.7 ± 1.04 g/dl, and 11.3 ± 0.91 g/dl after intervention, $p = 0.002$; anemia prevalence reduced from 57.9 to 36.8%.

Conclusions: Both once and twice weekly IS increased mean Hb concentration; however, twice weekly supplementation provided more significant results.

KEYWORDS: anemia, hemoglobins, iron, infants.

INTRODUCTION

Iron deficiency (ID) is the most common and widespread nutritional disorder in the world and a public health problem in developing countries. ID is the result of negative balance of this mineral over time. Iron deficiency anemia (IDA) is the most serious form of ID, occurring after a long period of deficiency, when stores have already been depleted, and after a reduction in biochemical iron [1].

Groups at risk for the development of IDA are children, adolescents, pregnant women and women of reproductive age. This occurs owing to increased nutritional needs during this period in qualitative and/or qualitative manner [2, 3]. Adequate iron intake could be achieved through the consumption of naturally iron-rich foodstuffs [4]. However, this is not always the case, and new approaches to prevent ID in infants are necessary [5].

In a meta-analysis by Ramakrishnan *et al.* [6], iron supplementation (IS) has proven effective in increasing hemoglobin (Hb) levels and reducing anemia prevalence. The most common form of iron supplements are iron sulfate drops, chewable tablets and sprinkles with or without additional nutrients. Up to date, several studies have investigated different schemes to optimize IS in different populations, some of them with conflicting results [7–12]. As a result, further research is necessary to assess and validate findings.

This study investigated the effects of two different IS regimens for the prevention/treatment of anemia. In this investigation, we compared the effect of ferrous sulfate oral solution given once weekly *versus* twice weekly, on Hb concentrations, in infants aged 6–18 months.

MATERIALS AND METHODS

This cluster-randomized clinical trial study was conducted in the municipality of Sobral, northeast of Brazil, between September and December 2013. The study population was composed of infants aged 6–18 months, from four randomized public infant education centers; the first two formed Group-A, and the latter Group-B. Group-A was allocated to 25 mg elemental iron once weekly ($n = 55$), and Group-B to 25 mg elemental iron twice weekly ($n = 53$).

All infants from the centers were invited to participate in our study. Exclusion criteria were parents' refusal to participate and infants already using IS (Fig. 1).

The study included two primary outcome variables: change in Hb concentration; and anemia prevalence before and after intervention. Hb concentration <11.0 g/dl was used as cutoff point to define anemia [1]. According to information provided by parents, a standardized data sheet was filled in containing information on (other study variables) age, gender, exclusive breastfeeding (EBF) <6 months of age, mother's schooling and family income.

Two biochemical evaluations were performed, to determine Hb concentrations, before and after intervention, with portable HemoCue B-hemoglobin photometer (Hb301-HemoCue AB, Ängelholm, Sweden). Finger prick capillary blood was collected under aseptic conditions. Data collection team was blinded to different interventions.

In this study, we used ferrous sulfate oral solution (orange flavor) (Far-Manguinhos/Fiocruz). Intervention was administered using a plastic medical syringe to gently squirt solution into the side of the child's mouth. Length of intervention: 16 weeks, beginning/ending on the same date for both groups.

Anemia prevalence in study population was estimated at 40%. To achieve a reduction in global anemia prevalence from 50 to 25%, with 80% power, two-sided, type I error of 5%, accounting for 10% losses to follow-up, each group required a minimum of 43 participants [13].

At baseline, to identify statistical significance between groups, we used unpaired *t*-test for age and mean Hb concentration, and Fisher's exact test for gender, EBF, mother's schooling and family income.

To compare means, we used paired student's *t*-test to assess difference in Hb concentration within the groups, and Fisher's exact test to assess the difference between good and bad outcomes (absence or presence of anemia). Data had normal distribution. The statistical software package SPSS for Windows, version 17.0, was used for all analyses (SPSS Inc., Chicago, IL). 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 at the State University *Vale do Acaraú* 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 on request. After intervention, anemic children were referred for treatment.

RESULTS

At baseline, the other study variables were analyzed. However, there were no statistically significant differences between the groups (Table 1). Before second biochemical evaluation, there were 19 dropouts (Fig. 1).

In Group-A, mean Hb concentration before intervention was 10.8 ± 1.18 g/dl and 11.2 ± 1.07 g/dl after intervention, $p = 0.12$; anemia prevalence was 52.5% (21 of 40) at baseline and 37.5% (15 of 40)

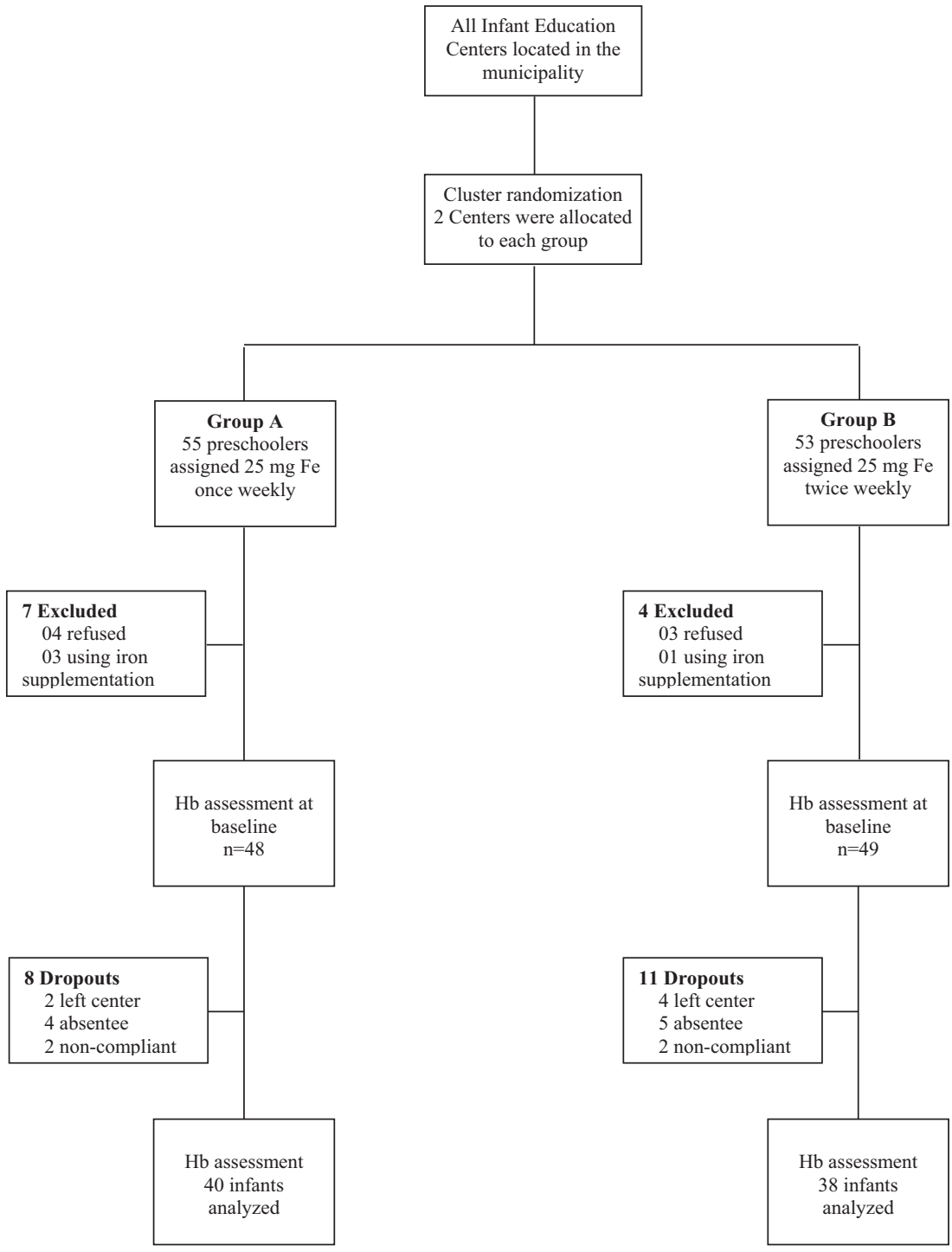

Fig. 1. Study profile.

Table 1. Baseline characteristics of study participants, by intervention group

Variables	Group A ($n = 48$)	Group B ($n = 49$)	p
Age (months) Mean ± SD	11.3 ± 2.47	12.1 ± 2.74	0.16[a]
Gender M:F	21:27	25:24	0.54[b]
EBF	20	18	0.68[b]
Mother with ≤ 9 year schooling	28	33	0.40[b]
Family income ≤ 300 USD	38	35	0.48[b]
Hb (g/dl)	10.8 ± 1.25	10.6 ± 0.99	0.60[a]

Note. 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 *t*-test.
[b]Based on Fisher's exact test (two-tailed).

Table 2. Effect of iron supplementation, 25 mg elemental iron once weekly and twice weekly, and anemia prevalence before and after intervention

Variables	Group-A—Once Weekly ($n = 40$)			Group-B—Twice Weekly ($n = 38$)		
	Before	After	p	Before	After	p
Hb (g/dl) Mean ± SD	10.8 ± 1.18	11.2 ± 1.07	0.12[a]	10.7 ± 1.04	11.3 ± 0.91	0.002[a]
CI	10.46, 11.21	10.84, 11.52		10.32, 11.01	10.98, 11.58	
Anemia[b]	21 (52.5)	15 (37.5)	0.26[c]	22 (57.9)	14 (36.8)	0.11[c]

Note. All numbers are absolute except numbers in brackets, which represent percentages.
SD = standard deviation.
[a]Based on paired Student's *t*-tests.
[b]Anemia defined as Hb concentration < 11.0 g/dl.
[c]Based on Fisher's exact test (two-tailed).

after intervention, without statistical significance, $p = 0.18$. In the twice weekly group (Group-B), mean baseline Hb concentration was 10.7 ± 1.04 g/dl, and after intervention mean Hb concentration improved significantly to 11.3 ± 0.91 g/dl, $p = 0.002$; anemia prevalence reduced from 57.9 to 36.8%, without statistical difference (Table 2).

Considering only anemic participants, both interventions significantly increased mean Hb levels. In Group-A, mean Hb concentration was 9.93 ± 0.79 g/dl at baseline and 10.92 ± 0.84 after intervention, $p = 0.001$; from the 21 participants who were anemic at baseline, 12 were no longer anemic after intervention, $p < 0.0001$. In Group-B, mean Hb concentration was 9.93 ± 0.67 at baseline and 11.06 ± 0.92 after intervention, $p < 0.0001$; at baseline, 22 participants were anemic, and after intervention this number reduced to 11, $p = 0.0002$ (Table 3).

In this study, we compared once weekly with twice weekly IS, for a favorable (absence of anemia) or adverse (anemic) outcome. After intervention, adverse outcome was present in 37.5% of participants in Group-A and 36.8% of participants in Group-B. The difference, the reduction of absolute risk, was 0.66%. The 95% confidence interval for this difference ranges from −20.80 to 22.11%. The number needed to treat was 153.

DISCUSSION

The objective of this study was to compare two different IS regimens, once weekly and twice weekly, in infants aged 6–18 months. In both groups, participants presented an increase in mean Hb concentration and a reduction in anemia rates; however, the increase in Hb levels was only statistically significant in the twice weekly supplementation group.

Table 3. Effect of iron supplementation in anemic participants, 25 mg elemental iron once weekly and twice weekly on hemoglobin levels, and anemia prevalence before and after intervention

Variables	Group-A—once weekly ($n = 21$)			Group-B—twice weekly ($n = 22$)		
	Before	After	p	Before	After	p
Hb (g/dl) Mean ± SD	9.93 ± 0.79	10.92 ± 0.84	0.001[a]	9.93 ± 0.67	11.06 ± 0.92	<0.0001[a]
CI	9.47, 10.29	10.54, 11.31		9.63, 10.22	10.65, 11.47	
Anemia[b]	21	9	<0.0001[c]	22	11	0.0002[c]

Note. All numbers are absolute.
Hb = hemoglobin; SD = standard deviation; CI 95% = confidence interval.
Comparison between the groups for anemia based on Fisher's exact test (two-tailed) $p = 0.76$.
[a]Based on paired Student's *t*-tests.
[b]Anemia defined as Hb concentration < 11.0 g/dl.
[c]Based on Fisher's exact test (two-tailed).

Furthermore, there was a greater reduction in anemia prevalence in the twice weekly group; however, this reduction was not significant.

If we consider only anemic participants, both interventions significantly increased mean Hb concentrations. Additionally, the number of anemic individuals also reduced, from 21 to 9 (Group-A), and 22 to 11 (Group-B). These results suggest that the two interventions were efficacious in the treatment of anemia.

Several studies have compared the effectiveness of IS in infants. Three clinical trials in preschoolers have demonstrated that supervised weekly IS had the same effectiveness as daily supplementation [14–16].

Meta-analyses by Beaton and McCabe [17] and De-Regil *et al.* [18] compared the effectiveness of daily and weekly IS regimens; it was observed that both supplementation regimens were effective in favorable conditions, with daily supplementation providing better results. Greater effectiveness from daily supplementation was witnessed from higher Hb and serum ferritin values, and a reduction in relative risk for anemia. In these studies, weekly supplementation was effective when compared with placebo; nevertheless, effectiveness was inferior to that of daily supplementation. De-Regil *et al.* [18] concluded that weekly supplementation should only be considered for preschoolers and schoolchildren when there is a strong guarantee of supervision and high adherence, or when daily supplementation cannot be implemented.

Another recent meta-analysis analyzed the effectiveness of daily and weekly regimens of ferrous sulfate supplementation for the prophylaxis of IDA in children <5 years. This study also showed that the daily dose of ferrous sulfate was more efficient in increasing Hb levels than the weekly administration of this supplement; however, there was no difference between the reductions in the prevalence of anemia [19].

Yet, despite these meta-analyses, other studies in infants aged 6–18 months, using a similar dose to our 25 mg of elemental iron weekly, during a 4 month period, did not obtain significant increases in mean Hb concentrations [20–22].

Nevertheless, Coutinho *et al.* [23] and Nogueira Arcanjo *et al.* [24] in randomized controlled trials were able to significantly increase Hb concentration; the first study used 25 mg of elemental iron once weekly, during 12 weeks, and the second used either 25 mg of elemental iron once weekly or 12.5 mg daily, both groups increased Hb levels when compared with control; it is important to acknowledge that in this study mean baseline Hb concentrations were low.

In contrast, studies conducted by Ferreira *et al.* [25] and Monteiro *et al.* [26] obtained favorable results with the use of 50 mg weekly supplementation regimens in infants. The first before-and-after study supplemented 293 children aged 24 months during a 24 week period, and the intervention was able to increase mean Hb concentration from 10.1 to 11.1 g/dl. However, in the study by Monteiro *et al.*

[26] with 1158 children using a 5 mg/kg/week dose of iron (approximately 50 mg/week), mean Hb levels increased significantly (>1 g/dl) from 11.1 to 12.1 g/dl.

Differing from the previous studies, there are researchers such as Zlotkin *et al.* [27] in a clinical trial with 40 mg of iron during 24 weeks, and Brunken *et al.* [28] in a before-and-after study with 6 mg/kg/week of iron during 16 weeks did not witness statistical differences after interventions; this in part may be explained by the high Hb values at the beginning of the studies, 11.0 and 11.2 g/dl, respectively. In an Iranian study by Khandemloo *et al.* [29], a dose of approximately 3–4 mg/kg/week was able to significantly increase Hb levels after 12 weeks ($p = 0.005$, mean Hb > 0.4 g/dl).

In Brazil, there is already a National Iron Supplementation Program, which distributes free iron supplements at health units within the Unified Health System to infants aged 6–18 months, who meet criteria for inclusion in the program. The program is based on a 25 mg dose of elemental iron weekly for infants <18 months [30]. However, the program has had problems with coverage: 1 year after implementation in 2006, the program covered only 19.4% of national territory, rising to 27.2% in 2010 [31]. In 2013, the program was reformulated with the decentralization of acquisition of supplements to municipal, district and state spheres [32]; another problem in the program concerns low adhesion—many mothers do not know of the program or the benefits that IS provides [33].

Some limitations need to be acknowledged and addressed regarding the present study. Many confounding factors affect the outcome of Hb concentrations and anemia prevalence, such as illness, inconsistent eating habits, periods of rapid growth. Furthermore, the number of children in each group was small; a larger number of participants in each group could have led to more expressive results, especially in the once weekly group. Another important fact is that the period of intervention was short (limited by the school semester); a longer period may have presented more expressive results in both groups. Another possible limitation is that this study depended exclusively on Hb concentrations to measure outcomes, without serum ferritin levels, which measure iron stores in the organism. However, the inclusion of these measurements would have implied operational difficulties and possibly a lower number of participants in the study. However, despite these limitations, our study was able to identify significant results.

Although mean Hb concentration increased significantly in the twice weekly group, the decrease in anemia prevalence was not statistically significant. This may have been caused by the fact that several children in the study were on the borderline between anemia and non-anemia (10.8/10.9 g/dl) after intervention; in other words, with a minimal increase in Hb concentration, more children would no longer have been classified as anemic; this may have been achieved if the intervention had lasted a little longer. However, both interventions were efficacious in increasing mean Hb concentrations in anemic infants.

IDA is a public health problem, which if left untreated can cause irreversible sequels especially in this age range. Therefore, there is an important and urgent need to find the best regimen to treat and/or prevent anemia. In our study, we witnessed that both once and twice weekly, IS with 25 mg of elemental iron increased mean Hb concentration; however, twice weekly supplementation provided more significant results. Furthermore, both interventions were similar in the treatment of anemic individuals. Simple and inexpensive IS programs constitute a useful strategy to treat highly anemic populations in public schools.

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