

Relationship between iron deficiency and anemia in children younger than 4 years

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Abstract

Objective: To evaluate the prevalence of anemia, iron deficiency and iron deficiency anemia in a cohort of children.

Methods: A cohort study nested in a randomized field trial. Children were recruited at birth at the maternity unit of the only public hospital in the city of São Leopoldo, southern Brazil. This study assessed iron status (hemoglobin and serum ferritin) when children were 12-16 months old and later at the age of 3-4 years. Anemia was defined as hemoglobin concentration < 11.0 g/dL; iron deficiency as serum ferritin < 15.0 µg/L; and iron deficiency anemia as hemoglobin concentration < 11.0 g/dL with iron deficiency.

Results: At age 12-16 months, the overall prevalence of anemia, iron deficiency and iron deficiency anemia was 63.7, 90.3 and 58.8%, respectively. The values for age group 3-4 years were 38.1, 16.1 and 7.4%, respectively. At age 12-16 months, 95% of anemia cases were associated with iron deficiency against only 19.3% of cases at age 3-4 years.

Conclusions: Iron deficiency was the main cause of anemia in the second year of life, but not at age 3-4 years. Thus, we point out that anemia in preschool children may have other causes and deserves careful assessment.

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Introduction

The World Health Organization (WHO),¹ in a recent publication, presented a comprehensive analysis of the worldwide prevalence of anemia between 1993 and 2005. Globally, anemia affects 1.62 billion people (95%CI 1.50-1.74 billion). Preschool children are the most affected, with prevalence of 47.4% (293 million). Furthermore, the prevalence of iron deficiency (ID) is estimated to be 2.5 times that of anemia.²

Data from the 2006 Brazilian Demographic and Health Survey,³ the first nationwide anemia prevalence survey in children, revealed that 20.9% of children aged zero to 59 months had anemia, i.e., approximately 3 million Brazilian children. The occurrence of anemia among children has been explored by several studies in Brazil over the last 20 years. A systematic review conducted in Brazil summarizes the results of 53 studies carried out between 1996 and

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2007, at various scopes, and shows that the median data for anemia prevalence in this age group were 53%, and the highest prevalence was found in children younger than 24 months.⁴

In Brazil, there are no data on the national or regional prevalence of ID and few studies have assessed the prevalence of ID or ID anemia (IDA) in children. The results show an IDA prevalence of approximately 50%.⁵⁻⁸ However, the few studies that included other parameters to determine ID did not specify the different age groups. Therefore, the present study aimed to evaluate the prevalence of anemia, ID and IDA in a cohort of children followed from birth at ages 12-16 months and 3-4 years.

Methods

This is a cohort study nested in a randomized field trial of children recruited at birth at Hospital Centenário, the only hospital in the city of São Leopoldo, southern Brazil, only in the sectors providing care through the Brazilian public Unified Health System (SUS). Newborns eligible for the study had birth weight greater than 2,500 g and gestational age greater than 37 weeks. Mothers were invited to participate after being provided with detailed information on the study protocol, which included differences between intervention and control groups for implementation of a nutrition intervention program during the infant's first year of life. Sample size was calculated for a larger study, based on a frequency of exclusive breastfeeding up to 4 months old of 21.6% in the control group and an estimated 65% difference in the frequency of this practice between groups after intervention. Other parameters for this calculation were: 80% power and 95% confidence interval, which determined a sample size of 177 children in each group, totaling 354 children. Assuming 25% of losses, 500 mother-child pairs were recruited to reach the minimum sample size required. The initial study methodology has been described in detail elsewhere.⁹

The intervention program did not influence the prevalence of anemia among children, allowing them to be analyzed for this purpose regardless of to which group the child belonged. Children were assessed at age 12-16 months ($n = 397$) and later at age 3-4 years ($n = 354$). The sample for assessment of iron status was smaller due to losses caused by parents' refusal or because blood collection was not possible. Between the first and second assessments, no nutritional intervention was performed that could influence the nutritional status of children.

Assessment of iron status

All children evaluated at age 12-16 months and later at age 3-4 years were invited to undergo testing for assessment of iron status. Thus, whenever possible, fasting blood

samples were drawn by venipuncture from each child. In the first evaluation, 369 samples of hemoglobin (Hb) and 289 samples of serum ferritin (SF) were obtained. At 4 years of age, Hb was assessed in 354 children, SF in 321 children and C-reactive protein (CRP) in 318 children. Hb was measured with a Coulter counter, SF concentrations were analyzed by using a commercial ELISA kit, and CRP levels were measured by a high-sensitivity assay (detection limit 0.2 mg/L) using the Behring Turbitimer System.

The outcome variables used were anemia, ID and IDA. Anemia was defined as Hb < 11 g/dL, ID as ferritin < 15.0 µg/L, and IDA as the concomitant presence of Hb < 11 g/dL and ferritin < 15.0 µg/L. At 4 years of age, CRP concentration was used as a marker of inflammation, since inflammation-induced infection can increase ferritin concentrations regardless of iron status. Signs of infection or inflammation were defined as CRP concentration ≥ 6 mg/L, and children below this cutoff value were excluded from the analysis.^{2,10}

Statistical analyses

All data were double entered on Epi-Info version 6.4 for subsequent validation. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 13. Since this study is nested in a randomized field trial,⁸ the prevalence of anemia, ID and IDA was compared between intervention (I) and control (C) groups to demonstrate that the intervention program had no effect on iron status. Confirmation of this hypothesis allowed children from both groups to be analyzed together, thus increasing statistical power for this study. Prevalence did not differ between groups at age 12-16 months – anemia (I: 66%; C: 60.1%), ID (I: 89.5%; C: 91%), and IDA (I: 56.8%; C: 52%) – and also did not differ at age 3-4 years – anemia (I: 41%; C: 40.7%), ID (I: 15.1%; C: 17.1%), and IDA (I: 7.9%; C: 7.4%).

Univariate analysis expresses the frequency of variables as proportion. Pearson's chi-square test was used for categorical variables, which evaluated the association between sex and the outcomes assessed. P values < 0.05 were considered to be statistically significant.

Ethical considerations

The research project was approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. At the time of home interview, the parents or legal guardians received a consent form with detailed information on the procedures to be employed in the study, as well as the assurance of confidentiality of the information provided and the possibility of refusing to participate in the investigation. The collection was carried out only after the parents or legal guardians agreed to participate and signed the consent form. In both evaluations, all children were

assessed (anthropometric evaluation and measurement of serum iron) and developmental examinations were performed. Children who were overweight, with short stature, malnutrition, dental problems or developmental problems were referred to primary care professionals for evaluation and treatment.

Results

The baseline socioeconomic characteristics of children's families revealed that 18.9% (75/397) of mothers were adolescents, 58.5% had less than 8 years of schooling, and 65.7% (257/391) had no paid occupation. Regarding family income, 71.9% of families had a monthly income \leq three minimum salaries. Among children, 56.4% were male. In the first evaluation of study outcomes, the mean age of the children was 12.95 ± 1.15 months (range: 11.84 to 16.25 months); in the second evaluation, the mean age of the children was 47.74 ± 2.68 months (range: 37.68 to 58.18 months).

At age 12-16 months, the prevalence of anemia, ID and IDA was 63.7, 90.3 and 58.8%, respectively (Table 1). Among children with anemia, in this age group, 95% (171/180)

had ID. At age 3-4 years, the prevalence of anemia, ID and IDA was 38.1, 16.1 and 7.4%, respectively. Among children with anemia, 19.3% (22/114) had ID. Eighteen children (5.7%) with elevated CRP concentration were excluded from the analysis. The results according to sex showed that the prevalence of ID and IDA was statistically higher among boys compared to girls only for age group 12-16 months (Table 2).

Discussion

The results of the present study showed that ID was associated with anemia in 95% of cases of children assessed at age 12-16 months, although at age 3-4 years this proportion was below 20%. The WHO^{1,2} estimates that half of anemia cases are caused by ID and that the prevalence of ID is 2.5 times that of anemia. However, our study showed that this concept does not apply to children older than 2 years. Studies conducted with children in countries other than Brazil exhibited results similar to those presented in this article, with low serum retinol and folic acid levels being observed among children with anemia.^{11,12} Research conducted in Northeast Brazil with pregnant women

Table 1 - Prevalence* of anemia, iron deficiency, iron deficiency anemia and microcytosis in low-income children, according to age group

Age	Anemia (n)	ID (n)	IDA (n)	Microcytosis (n)
12 to 16 months	63.7 (235/369)	90.3 (261/289)	58.8 (170/289)	51.1 (187/366)
3 to 4 years	38.1 (114/299)	16.1 (48/298)	7.4 (22/298)	32.8 (98/299)

ID = iron deficiency; IDA = iron deficiency anemia.
* %.

Table 2 - Prevalence* of anemia, iron deficiency and iron deficiency anemia in children, according to age and sex

Age/sex	Anemia (n)	p	ID (n)	p	IDA (n)	p
12 to 16 months						
Male	68.8 (148)	0.017	91.0 (152)	0.635	59.1 (107)	0.039
Female	56.8 (88)	89.3 (109)	47.4 (63)			
3 to 4 years						
Male	41.2 (70)	0.213	19.5 (33)	0.066	8.3 (14)	0.496
Female	34.1 (44)	11.6 (15)	6.2 (8)			

ID = iron deficiency (ferritin < 15 μ g/L); IDA = iron deficiency anemia (hemoglobin < 11 g/dL and ferritin < 15 μ g/L);
p = Pearson's chi-square test.
* %.

revealed that ID was not the primary cause of anemia in this population. The prevalence of anemia was 56.6%, and that of IDA was 10.7%.¹³

It is well established that deficiencies of vitamin B12 and folic acid are determinants of anemia in the absence of ID, but deficiencies of vitamin A, riboflavin and vitamin C have also been described as possible causes of anemia, although the pathophysiological mechanisms involved are not yet fully understood.^{14,15} Two studies conducted with children showed deficiency of other micronutrients, such as vitamin B12, folic acid and vitamin A, after intervention with iron supplementation alone.^{16,17} However, in our study, anemia in preschool children does not seem to be associated with deficiency of vitamin B12 and folic acid due to the high prevalence of microcytosis observed. In Thai schoolchildren, hemoglobinopathies, suboptimal vitamin A status and age were the major predictors of Hb concentration.¹²

In Brazil, a recent nationwide study showed low frequency of sickle cell anemia in the population studied.³ It is also noteworthy that microcytosis is not present in sickle cell anemia, except in cases of co-inheritance of thalassemia. A study conducted in Rio Grande do Sul, the southernmost state of Brazil, analyzed blood samples from newborns screened by the National Newborn Screening Program and revealed that, of the 437,787 samples analyzed, 6,391 showed an abnormal Hb pattern. These included 48 cases (0.01%) of sickle cell anemia, one neonate who was homozygous for β thalassemia, 6,272 (1.4%) newborns who were heterozygous for Hb S, C, or D, and 71 (0.02%) neonates who were carriers for rare Hb variants.¹⁸ Thus, the low prevalence of sickle cell anemia and thalassemia observed nation and statewide could not justify the high prevalence of anemia without ID observed among preschoolers in this study.

Other non-nutritional factors implicated in the etiology of anemia include parasitic infections, such as malaria and hookworm disease. However, in Brazil, 99.5% of malaria cases occur in the North and Midwest of the country,¹⁹ and the presence of hookworm disease among preschoolers is associated with ID, a condition not found in our study. Thus, these factors could not serve as a basis for explaining the results described herein. Another aspect worth mentioning is the presence of anemia and microcytosis with adequate iron stores among low-income preschool children. Iron mobilization is reduced in the presence of vitamin A deficiency.²⁰ This may therefore be a potential hypothesis to explain the results observed in this study, since the 2006 Brazilian Demographic and Health Survey showed that 17.4% of Brazilian children younger than 5 years had deficient vitamin A concentrations.³ Another possible hypothesis may be related to the role of hepcidin, which is produced in the liver for iron metabolism. When iron stores are replete, this peptide inhibits the release of iron from the reticuloendothelial system to the circulation along with

transferrin. If for some reason there is high hepcidin activity, this fact might explain why the stored iron would not be released to the circulation along with transferrin, resulting in high SF levels and low hematopoietic activity.^{21,22}

The absence of CRP measurement in the first evaluation of children could be a limitation of the study. CRP measurements aim to identify children with infections and to exclude these children from the analysis, since they may have increased ferritin levels as a result of infection and, thus, lead to a higher incidence of false-negative results for ID. Since ID prevalence in this age group (12-16 months) was 95%, not excluding children with infections did not affect the conclusions of this study. For ethical reasons, all children who were diagnosed with anemia at age 12-16 months received a prescription for ferrous sulfate for a 3-month period. Since there was no follow-up of children after this period, the level of treatment adherence was not assessed. Therefore, since the interval between the first and second anemia assessment was approximately 2 years, we do not believe that low ID prevalence as a cause of anemia in children aged 3-4 years can be attributed to this factor.

Another aspect that may explain the low prevalence of ID among preschoolers is their ability to consume larger amounts of food – and, consequently, higher amounts of iron, especially of food made from wheat flour and corn flour, which are currently fortified – than when they were 12-16 months old.

We also highlight the higher prevalence of anemia and IDA among boys aged 12-16 months, which may be explained by the accelerated growth velocity during the first year of life, since growth velocity is higher in boys when compared to girls during this period. This process increases iron requirement, which is not often supplied by the diet due to the low bioavailability of dietary iron. At age 3-4 years, growth velocity is constant and much lower, thus no longer being an additional risk factor for IDA.²³

The consequences of IDA are widely known, but anemia resulting from other nutritional deficiencies may also result in impaired health in children. Distinguishing between types of anemia resulting from causes other than ID represents a challenge to be overcome, as well as the consequences of unnecessary iron treatment. Data from this study highlight the importance of targeting interventions to children in the first year of life, before they become anemic or iron deficient, through the promotion of breastfeeding and adequate introduction of complementary foods.²⁴ After 6 months of life, the Brazilian Society of Pediatrics and the Brazilian Ministry of Health recommend prophylactic iron supplementation up to 2 years of age.^{25,26} However, prevention and control of nutritional anemia probably require the use of multiple micronutrients, since children with insufficient dietary intake of iron are likely to show inadequate intake of other micronutrients necessary for their proper growth. A recent review²⁷ and meta-analysis²⁸

assessed the effects of individual and multiple micronutrient supplements on the nutritional status of populations in different settings and showed better results with the use of multiple micronutrient supplements.

Conclusion

The results showed that ID, considered the most prevalent nutritional deficiency in the world, is the leading cause of anemia in children aged 12-16 months, but not at age 3-4 years. Therefore, it does not seem appropriate to use the prevalence of anemia as a proxy for IDA, regardless of age. Thus, further studies are warranted to explore the causes of low Hb levels in children with adequate iron stores and to elucidate other causes of anemia in children older than 2 years.

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