Diagnosis of iron deficiency anemia in children of Northeast Brazil

Diagnóstico de anemia por deficiência de ferro em crianças do Nordeste do Brasil

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ABSTRACT

OBJECTIVE: To diagnose iron deficiency anemia in children.

METHODS: The study was conducted with a sample of 301 children aged six to 30 months attending public daycare centers in the city of Recife, Northeast Brazil, in 2004. The diagnoses of anemia were based on a combination of different hematological and biochemical parameters: hemoglobin, mean corpuscular volume, ferritin, C-reactive protein, transferrin saturation and transferrin receptor. The chi-square test and ANOVA were used in the statistical analysis.

RESULTS: Of all children studied, 92.4% had anemia (Hb <110 g/L) and 28.9% had moderate/severe anemia (Hb <90 g/L). Lower levels of hemoglobin were found in children aged 6–17 months. Iron deficiency was found in 51.5% of children using ferritin (<12 µg/L) as parameter. Taking into consideration the combination of hemoglobin level, ferritin and transferrin receptor, 58.1% had anemia with iron deficiency, 34.2% had anemia without iron deficiency and 2.3% had iron deficiency without anemia. Mean ferritin concentration was significantly higher in children with high C-reactive protein when compared with those with normal levels (22.1 vs. 14.8 µg/L).

CONCLUSIONS: The use of several biochemical and hematological parameters allowed to diagnosing iron deficiency anemia in two thirds of children, suggesting a need to identify other determinants of anemia without iron deficiency.

DESCRIPTORS: Anemia, Iron Deficiency, diagnosis. Ferritins, blood. Laboratory Techniques and Procedures. Hematologic Tests. Iron Deficiency, prevention & control. Infant Nutrition. Child Nutrition.

RESUMO

OBJETIVO: Diagnosticar anemia por deficiência de ferro em crianças.

MÉTODOS: O estudo foi desenvolvido com uma amostra de 301 crianças com idade entre seis e 30 meses, usuárias de creches públicas de Recife, PE, em 2004. Para o diagnóstico da anemia utilizou-se a combinação de diferentes parâmetros hematológicos e bioquímicos: hemoglobina, volume corpuscular médio, ferritina, proteína C-reativa, saturação da transferrina e receptor da transferrina. Para a análise estatística empregou-se o teste do qui-quadrado e ANOVA.

RESULTADOS: Do total de crianças, 92,4% tinha anemia (Hb < 110g/L) e 28,9% apresentou anemia moderada/grave (Hb<90g/L). Níveis mais baixos de hemoglobina foram observados em crianças de seis a 17 meses. Encontrou-se deficiência de ferro em 51,5% das crianças, utilizando-se a ferritina (< 12µg/L) como parâmetro. Considerando a combinação da concentração de hemoglobina, ferritina e do receptor de transferrina, 58,1% tinha anemia com deficiência de ferro, 34,2% anemia sem déficit de ferro e 2,3% deficiência de ferro sem anemia. A concentração média de ferritina foi significativamente maior em crianças com proteína C-reativa aumentada quando comparada com aqueles com níveis normais (22,1 versus 14,8 µg/L).

CONCLUSÕES: A utilização de diversos parâmetros bioquímicos e hematológicos possibilitou diagnosticar anemia por deficiência de ferro em dois terços das crianças, revelando a necessidade de identificar outros determinantes de anemia sem deficiência de ferro.

DESCRITORES: Anemia Ferropriva, diagnóstico. Ferritinas, sangue. Técnicas e Procedimentos de Laboratório. Testes Hematológicos. Deficiência de Ferro, prevenção & controle. Nutrição do Lactente. Nutrição da Criança.

INTRODUCTION

Iron deficiency is a main determinant of anemia, the most widespread nutritional disorder worldwide, representing a major public health problem.[a] It is estimated that more than two billion people are iron–deficient. This deficiency affects populations of almost all countries at different degrees, corresponding to approximately one third of the world's population. In Brazil, it has been increasing especially in vulnerable population groups such as infants, pre-school children, adolescents and women of childbearing age, including pregnant women.[19,20]

In Brazil, the short duration of exclusive breastfeeding, low intake of foods with bioavailable iron, diarrhea, poor protein energy deficiency, low maternal education level and lack of basic sanitation are reported as having a significant impact on reducing hemoglobin concentration.[17] Iron deficiency anemia impairs psychomotor development and growth, and reduces physical activity and resistance to infection.[5,15]

Hemoglobin level is a parameter universally used to assess anemia in population-based epidemiological studies. However, this parameter does not have good sensitivity and specificity to assess iron deficiency anemia because of the different stages of deficiency and specific characteristics of each population group. Moreover, hemoglobin concentration varies between individuals by sex, age, ethnic group and altitude.[6,25,26]

In addition, although internationally adopted, the cut-off point to classify anemic infants is not a consensus.[8] Changes in hemoglobin concentration occur gradually but the parameters recommended for diagnosing anemia are fixed for each age group, and the higher rate of growth during infancy is not taken into account, making it difficult to determine exclusively based on this indicator whether iron deficiency is due to physiological variations during infancy.[23]

[a] World Health Organization. Iron deficiency anemia: assessment, prevention, and control: a guide for program managers. Geneva; 2001 [cited 2005 Jan 5]. (WHO/NHD/01.3). Available from: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.3.pdf

The objective of the present study was to diagnose iron deficiency anemia in children based on a combination of different biochemical and hematological parameters.

METHODS

The study was conducted in a sample of 335 children aged six to 30 months attending 13 public daycare centers of five health districts in the city of Recife, Northeastern Brazil, from July to August 2004. The 13 daycare centers were selected through simple random sampling out of a total of 20 centers that provided care to children who met the inclusion criteria (age and no iron supplementation). These day care centers are attended by children with similar socioeconomic conditions, with a uniformity of services provided by the Health Secretariat of the municipality regarding to diet and health care. The children were recruited for an intervention study aimed to assess the efficacy of iron supplementation for treatment of anemia. Out of 335 recruited children, 34 (10%) had no assessment of ferritin and/or transferrin receptor.

Two trained research assistants collected 5 ml of blood through peripheral venous puncture with disposable syringes and needles in children fasting at least eight hours. Blood was collected in tubes labeled with the child's code, and with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The following tests were performed: hemoglobin (Hb), mean corpuscular volume (MCV), ferritin, C-reactive protein (CRP), transferrin saturation (TS) and transferrin receptor (TfR).

Information on family income, children's biological characteristics and feeding profile were collected through pre-coded standardized questionnaires applied to mothers by trained interviewers.

Hemoglobin and mean corpuscular volume were electronically determined by an hematology analyzer (ABX Pentra model ABX 120 VEP 0027) at room temperature. Ferritin was measured using an automated chemiluminescence system (ACS, 180 Bayer) with direct chemiluminescence and Bayer controls. Transferrin saturation was measured through spectrophotometer (CELM model E-225 D). Transferrin receptor concentration was measured through enzyme immunoassay (ELISA). CRP was measured using an analyzer (Cobas Mira) with a turbidimetric-latex high sensitivity method at room temperature.

Anemia was defined based on hemoglobin concentration <110 g/L. Anemia with iron deficiency was defined by the combination of the following indicators: hemoglobin <110 g/L, serum ferritin <12 µg/L and transferrin receptor >8.3 mg/L based on criteria established by World Health Organization (WHO).[a] The normal level of C-reactive protein is defined as <1.45 mg/L for boys and <1.90 mg/L for girls (Biosystems kit, Costa Brava, Barcelona, Spain).

The anthropometric evaluation was conducted in compliance with WHO recommendations by two trained technicians. Weight was measured using a digital scale (Marte, model DM 160, Brazil) with 50g precision. Length was measured using a 100cm long anthropometer with 0.1cm subdivisions (Raven Equipment, England). Two length measurements were taken and when the difference between them exceeded 0.5 cm, a third measure was taken, and the two measures with the smallest difference were recorded. For the analysis of nutritional status, a mean of the two measures recorded was considered. Nutritional status was assessed through weight-for-age, length-for-age and body mass index (BMI) using the WHO reference curves (Anthro-2005).

Data were coded, double entered and validated using EpiInfo software version 6.04. For categorical variables, differences were assessed by chi-square test adopting Yates' correction for dichotomous variables. For continuous variables, one-way analysis of variance (ANOVA) was employed. Statistical significance was set at a p-value ≤0.05. All analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 12.0.

The study was approved by the Research Ethics Committee of Universidade Federal de Pernambuco Health Science Center. A written informed consent was obtained from the mothers or guardians and they were informed of the test results. Children diagnosed with anemia were properly treated.

RESULTS

The children studied had low socioeconomic condition with a monthly per capita family income of less than two monthly minimum wages (1 MMW = US 117). Seven percent were low birthweight (<2500 g); current weight-for age and length-for-age deficits (< –2 z-scores) were 5% and 11%, respectively, and 11% of them were overweight (BMI ≥ 2 z-scores). Most (64%) were 12 to 23 months old, 7% had never been breastfed and 22% were still receiving breast milk at the time of data collection.

Means and standard deviations of biochemical and hematological parameters according to age groups are presented in Table 1.

Of all subjects (n=301), 92.4% had anemia, 58.1% had anemia with iron deficiency (49.8% with low ferritin and 8.3% with high transferrin receptor) and 34.2% had anemia without iron deficiency. Only 2.4% had iron deficiency without anemia (Table 2).

Table 1. Means and standard deviations of biochemical and hematological parameters by age in children of daycare centers. City of Recife, Northeastern Brazil, 2004.

Variable	Total n=301	Age (months) 6 to 11	12 to 17	18 to 23	24 to 30
		n = 56	n = 98	n = 96	n = 51
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Hemoglobin (g/L)*	95.43 (10.0)	92.89 (8.2)	94.45 (11.0)	96.10 (11.2)	98.78 (8.6)
MCV (fL)*	66.19 (6.6)	65.14 (5.8)	65.01 (6.7)	67.02 (7.2)	68.02 (5.50)
Ferritin	18.56 (24.8)	24.77 (32.6)	15.23 (18.0)	19.25 (28.8)	17.00 (16.1)
TfR (mg/L)**	7.78 (2.2)	7.51 (1.6)	8.45 (2.6)	7.56 (1.8)	4.85 (2.2)
TS (%)	18.12 (9.3)	17.65 (11.8)	17.75 (8.6)	18.13 (7.5)	19.33 (9.1)
C-reactive protein	5.34 (10.9)	4.66 (7.7)	6.26 (13.3)	5.1 (10.2)	4.76 (10.2)

MCV: Mean corpuscular volume, TfR: Transferrin receptor, TS: Transferrin saturation
*p<0.05; **p<0.01

Table 3 shows that all children aged six to 11 months had anemia while the rate of anemia in children of other age groups was greater than 88%. Moderate and severe anemia (hemoglobin <90 g/L) was found in 28.9% of subjects and was more frequent in children aged 6–11 and 12–17 months than in older children. Anemia with iron deficiency was around 60% in those aged 23 months.

About 51% of subjects had an inflammation. Only ferritin was significantly associated with CRP. High ferritin levels were significantly associated with high CRP levels. Moreover, mean ferritin concentration was significantly higher in subjects with inflammation when compared with those with normal CRP (22.1 vs. 14.8 μg/L, p = 0.01) (Table 4).

DISCUSSION

In most studies in Northeast Brazil, the prevalence of iron deficiency anemia has been determined only based on the measurement of hemoglobin levels. There are few studies on the content of body iron reserves in vulnerable groups. Paiva et al[18] (2000) claimed that biochemical and hematological parameters can be used alone or associated for the diagnosis of iron status in individuals or populations. However, when used alone, none of them is sufficiently sensitive or specific, especially in developing countries where common infections (such as diarrhea, respiratory infections, intestinal parasites and malaria) are often present.[4]

The sample of this study comprised children attending public daycare centers five days a week for eight hours a day, where they received five meals a day supposedly following a menu appropriately planned by nutritionists. However, food intake was not assessed and ⅔ of children had per capita family income less than two MMWs. Although 94% of the mothers reported breastfeeding, exclusive breastfeeding during the first six months of life was considered insignificant, which corroborates similar findings from other studies conducted in the same region.[13] Stunting was found in 14% of children and overweight in about 11% while the prevalence of weight-for-age deficit was low (3%).

Table 2. Rates of anemia and iron deficiency in children of daycare centers. City of Recife, Northeastern Brazil, 2004.

Anemia n = 278 (92.4%)				No anemia n = 23 (7.6%)			
Low ferritin[a] n = 150 (49.8%)		Normal ferritin n = 128 (42.5%)		Low ferritin n = 5 (1.7%)		Normal ferritin n = 18 (6.0%)	
TfR[b]		TfR[c]		TfR[d]		TfR[e]	
High	Normal	High	Normal	High	Normal	High	Normal
n = 65 21.6%	n = 68 22.6%	n = 25 8.3%	n = 103 34.2%	n = 2 0.7%	n = 3 1.0%	n = 2 0.7%	n = 16 5.3%

[a] 17 (5.6%) missing values for TfR (Transferrin Receptor)
[b] (Hb < 110g/L) + (FeS < 12 μg/L) or/and (TfR > 8.3 mg/L)
[c] (Hb < 110g/L) + (FeS ≥ 12 μg/L) and (TfR ≤ 8.3 mg/L)
[d] (Hb ≥ 110g/L) + (FeS < 12 μg/L) or/and (TfR > 8.3 mg/L)
[e] (Hb ≥ 110g/L)) + (FeS ≥ 12 μg/L) and (TfR ≤ 8.3 mg/L)

Table 3. Biochemical and hematological parameters by age in children of daycare centers. City of Recife, Northeastern Brazil, 2004.

Variable	Total n=301		Age (months) 6 to 11	12 to 17	18 to 23	24 to 30
	n	%	n = 56	n = 98	n = 96	n = 51
Hemoglobin (g/L)						
< 90	87	28.9	35.7%	36.8%	22.9%	17.6%
90 to 109	191	63.5	64.3%	56.1%	65.6%	72.6%
≥ 110	23	7.6	0.0%	7.1%	11.5%	9.8%
Mean (SD)	95.4 (10.3)		92.9 (8.2)	94.4 (10.8)	96.1 (11.2)	98.8 (8.6)
Anemia						
With ID[a]	175	58.1	60.7%	58.2%	60.4%	51.0%
Without ID[b]	103	34.2	39.3%	34.7%	28.1%	39.2%
Total	278	92.3	100.0%	92.9%	88.5%	90.2%
No anemia						
With ID[c]	7	2.3	0.0	3.0	4.2	2.0
Without ID[d]	16	5.3	0.0	4.1	7.3	7.8
Total	23	7.6	0.0	7.1	11.5	9.8

ID: Iron deficiency
[a] (Hb < 110g/L) + (FeS < 12 μg/L) or/and (TfR > 8.3 mg/L)
[b] (Hb < 110g/L) + (FeS ≥ 12 μg/L) and (TfR ≤ 8.3 mg/L)
[c] (Hb ≥ 110g/L) + (FeS < 12 μg/L) or/and (TfR > 8.3 mg/L)
[d] (Hb ≥ 110g/L)) + (FeS ≥ 12 μg/L) and (TfR ≤ 8.3 mg/L)

The prevalence of anemia was extremely high (92%), with mean hemoglobin concentration of 95.4 g/L (SD=10.3). Of all, 29% had moderate and severe anemia. The prevalence of anemia was higher than that reported in other studies conducted in Northeast Brazil,[12,17,24] other Brazilian regions,[7,10] and also other developing countries,[1] probably due to the fact that the children studied at these daycare centers were younger and came from the poorest families. Also, the cut-off point of 110 g/L is probably too high for infants, thus overestimating the prevalence of anemia in this age group.

The analysis of anemia and iron deficiency by age showed that prevalence of severe and moderate anemia was higher in those age 6–17 months with a trend to decline as age increased but no difference was observed between males and females. The same was seen for iron deficiency, with the highest prevalence found in children aged 6–23 months as early introduction of complementary foods with low iron content predominates with marked reduction of breastfeeding.[12,13,17] Another important factor would be the use of in natura cow milk whose iron has low bioavailability and may interfere with iron absorption from other foods due to its direct effect on the intestinal mucosa, which can cause loss of occult blood in feces.[16]

The lower prevalence of anemia with iron deficiency in children aged 24–30 months could be explained by regular consumption of foods in the family diet, characterized by greater diversification and especially higher consumption of foods of animal origin.[9] In this age group, consumption of wheat and maize flours, which has been fortified with iron and folic acid in Brazil since 2004, may have also contributed to a greater iron intake. The same has been reported in developed and developing countries.[a]

Whereas 58% of anemic children were iron-deficient, 34% of anemic children had no biochemical evidence of iron deficiency, suggesting other causes for anemia. A study conducted by Muniz et al[14] (2007) found similar results in children from Northern Brazil.

The role of vitamin A in anemia is that it mobilizes iron from the liver, increasing the availability of iron for hemoglobin synthesis.[21] The prevalence of vitamin A deficiency is known to be high in this Brazilian region.[2] The prevalence of other micronutrient deficiencies that are know to play a role in anemia, such as vitamin B12 or folate deficiency, has not been documented in this population.

Besides nutritional causes, the role of infectious and parasitic diseases, common in the first years of life, particularly in children from low socioeconomic populations attending daycare centers[22] should also be taken into consideration. It is known that serum ferritin increases in infectious diseases not related to iron body store. Thus, it is necessary to assess markers of infectious and inflammatory processes, such as CRP, in

Table 4. Biochemical and hematological parameters according to inflammation status of children from daycare centers. City of Recife, Northeastern Brazil, 2004.

Variable	Total	High CRP n=154 (51.2%)		Normal CRP n=147 (48.8%)		p[a]
		n	%	n	%	
Hemoglobin	301					
<110 g/L		141	91.6	137	93.2	
≥ 110 g/L		13	8.4	10	6.8	0.75
MCV (fL)	301					
< 77 fl		146	94.8	136	92.5	
≥ 77 fl		8	5.2	11	7.5	0.56
Ferritin	301					
< 12 µg/L		63	40.9	92	62.6	
≥ 12 µg/L		91	59.1	55	37.4	<0.001
TfR (mg/L)	284					
≤ 8.3 mg/L		102	69.9	88	63.8	
> 8.3 mg/L		44	30.1	50	36.2	0.33
TS (%)	291					
< 16 %		64	43.0	55	38.7	
≥ 16 %		85	57.0	87	61.3	0.54

CRP: C-reactive protein, normal parameter: males <1.45mg/L; females <1.90 mg/L
MCV: Mean corpuscular volume, TfR: Transferrin receptor, TS: Transferrin saturation
[a] Yates chi-square test

iron deficiency anemia. In the present study, although a significant association between CRP with serum ferritin concentration was found, the latter may be underestimated, possibly due to its lower sensitivity as a specific marker of iron deficiency in infants.[10,24]

Chronic or recurrent infectious diseases such as malaria and diarrheal diseases can interfere with iron intake, absorption and metabolism. Although diarrhea has declined in Brazil, this reduction has been less pronounced in the Northeastern region. Diarrhea during childhood, even of moderate severity, has been associated to lower iron absorption and consequent reduction in hemoglobin concentration.[11] With regard to parasitic diseases studies with infants conducted by Batista Filho & Ferreira[3] (1996) found no association with iron deficiency anemia in the Northeastern region.

The high prevalence of iron deficiency anemia found in two thirds of children is concerning as these children are at greater risk of inadequate growth and development. There is a need for further studies to identify other determinants of non-iron deficiency anemia. Given the serious consequences of anemia with or without iron deficiency, more effective basic health actions should be promoted, particularly reinforcing feeding practices of infants and young children, such as the promotion of exclusive breastfeeding in the first six months of life and timely introduction of adequate complementary foods fortified with micronutrients or based on iron rich foods. Supplementation with iron salts and/or fortification of basic foods aiming at restoring iron reserves in vulnerable populations would be a complementary strategy to be implemented.

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