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Nutritional assessment of iron status and anemia in children under 5 years old at public daycare centers

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Abstract

Objective: To assess nutritional iron status and anemia prevalence in children less than 5 years old at public daycare centers in the city of Recife, PE, Brazil.

Methods: A cross-sectional study, with a systematic random sampling of 162 children aged 6 to 59 months. Nutritional iron status was assessed in terms of body iron reserves (serum ferritin), transferrinemia (serum iron, total iron binding capacity, and transferrin saturation %), erythropoiesis (free erythrocyte protoporphyrin) and hemoglobin production (hemoglobin).

Results: The prevalence of anemia (hemoglobin < 11.0 g/dL) was 55.6% (95%CI 47.3-63.5), evidence was found of depleted iron stocks (serum ferritin < 12.0 ng/mL) in 30.8% (95%CI 22.9-39.3), low transferrinemia levels (transferrin saturation % < 16) in 60.1% (95%CI 51.7-68.0) and deficient erythropoiesis (free erythrocyte protoporphyrin > 40 µmol/mol heme) in 69.6% (95%CI 61.0-77.1) of the children. Iron parameters were not correlated with sex ($p > 0.05$). However, children < 24 months exhibited lower hemoglobin concentrations ($p < 0.00$) and higher levels of free erythrocyte protoporphyrin ($p < 0.000$) and total iron binding capacity ($p < 0.001$) when compared with children > 24 months. The significant correlation observed between reserves, transferrinemia and erythropoiesis is a finding that is compatible with the expected lifecycle of iron in the body.

Conclusions: Iron deficiency and anemia appear to be an important public health problem among children less than 5 years old at public daycare centers in Recife. Therefore, effective actions aimed at the prevention and control of this deficiency are strongly recommended in this ecological context.

J Pediatr (Rio J). 2007;83(4):370-376 Anemia, iron deficiency, hemoglobin, serum ferritin, serum iron, total iron binding capacity, transferrin saturation, free erythrocyte protoporphyrin, preschool children.

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Introduction

Iron deficiency affects around 2.1 billion people worldwide, one third of whom exhibit clinical evidence of the problem. In Brazil[1-3] and in the state of Pernambuco, in particular,[4] studies with preschool children have found an elevated prevalence of anemia, with rates of around 40%. Additionally, studies of the tendency over time have demonstrated a significant increase in the prevalence of this nutritional disorder.[2,3]

Historically, several different parameters have been used to assess the iron status of individuals, including their dietary intake, hematological and biochemical parameters that are predictive of the body's iron reserves, transferrinemia and erythropoiesis.[5] The available laboratory parameters reflect different stages of deprivation, encompassing a wide spectrum that goes from subclinical deficiency to the onset of anemia. Nevertheless, the great majority of these indicators do not possess sufficient power of diagnostic discrimination, in terms of sensitivity, specificity and predictive values, since countless clinical conditions can alter their results without, in fact, reflecting the body's true nutritional iron status. Therefore, it has been recommended that a combination of these available indicators be used, taking into consideration the inherent characteristics of the individual or population group, prevalence and severity of deficiency, costs, methodological complexity and susceptibility to laboratory error.[6]

The objective of this article is to assess nutritional iron status and anemia prevalence among children under 5 years of age attending public daycare centers in the city of Recife, PE, Brazil, in terms of abnormally low body iron reserves, low transferrinemia, deficient erythropoiesis and anemia.

Methods

Cross-sectional study design, including children aged 6 to 59 months, of both sexes, enrolled at public daycare centers run by the Recife City Council (Prefeitura) in 1999. Children were excluded if they had consumed vitamin or mineral supplements during the 30 days prior to data collection. These daycare centers were attended by children with low socioeconomic status were spending around 7 hours a day 5 days a week at the institutions. The diet provided comprised five meals which, in principle, met dietary recommendations.

In order to determine the sample size, a census was taken of the 34 public daycare centers in Recife, finding a total of 2,500 eligible children. Iron deficiency prevalence was estimated at 40%,[4] with a precision of 9% and a level of confidence of 95. The sample size thus defined was 114 individual. In recognition of the possibility of losses and with the objective of developing an analysis of the indicators, with varying degrees of disaggregation, it was decided to correct the sample size by 40%, making a total of 162 children, selected by means of a systematic random sampling process, using a random number table.

In order to perform analysis of biochemical parameters, 5mL of blood was taken by puncture of the cubital vein, in the morning with the child fasting for 12 hours. Samples were taken and analyzed by researchers from the Center for Micronutrients Investigation at the Universidade Federal da Paraíba. Hemoglobin concentrations (Hb) were determined using the cyanomethemoglobin method (Drabkin, Sigma Chemical Co. St. Louis, MI, USA), with readings taken by spectrophotometer (Spectrumlab, Spectrum Lab Products, Gardena, LA, USA), with children considered anemic when Hb < 11 g/dL.[6]

Serum ferritin (SF) was determined by enzyme immunoassay, modified in a complete automation system (System AxSym, Abbot Diagnostics, Abbot Park IL, USA). Values of SF < 12 ng/mL were considered deficient.[6] Serum iron (SI) and total iron binding capacity, (TIBC) were analyzed by the AAII-25 modified automatic colorimetric method (Alpkem TFA analyzer, Clackamas, OR, USA). Levels of SI < 50 μg/dL and TIBC > 400 μg/dL were adopted as indicative of deficiency.[6] The transferrin saturation percentage (%TSat) was obtained from the ratio between the SI and TIBC concentrations, with values < 16% considered deficient.[6] Analyses were carried out at the Clinical Analysis Laboratory at the Hospital Universitário Lauro Wanderley – UFPB. Free erythrocyte protoporphyrin (FEP) by the hematofluorometric method (ZPP Hematofluoremeter, Aviv Biomedical Inc., Lokewood, NJ, USA), by the Instituto de Patologia Clínica Hermes Pardini, with deficiency defined as values > 40 μmol/mol heme.[6]

Continuous variables were tested according to their normality of distribution. Neither SF nor a FEP had Gaussian distribution, and were transformed logarithmically ($\log_{10}$), and presented as geometric mean + confidence interval. Pearson's chi-square test was used to test the homogeneity of the variables sex, age and biochemical parameters. In order to describe proportions, the binomial distribution was approximated to normal distribution by the 95% confidence interval. Proportions were compared with Pearson's chi-square test with Yates' correction, where applicable, and the chi-square test for linear tendencies, with the respective odds ratios (OR). Associations between variables were evaluated with Pearson's correlation. Mean values were compared using Student's *t* test for unpaired samples, (2 means) and ANOVA (> 2 means), using Scheffé as the *a posteriori* test. A 5% significance level was adopted, and the database will was constructed and statistical analyses performed using the statistical software SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA).

The research project was approved by the Ethics Committee at the Hospital Universitário Lauro Wanderley –

UFPB and the Prince Lepold Institute of Tropical Medicine – Antwerp, Belgium, in accordance with established standards for research with human beings.

Results

One hundred and sixty-two children were studied. The 5.5% of children lost to the study was due to refusal of venous puncture and problems related to collection, processing and analysis of the biological material.

With reference to body reserves of iron and transferrinemia and erythropoiesis (Table 1), the data demonstrate that 30.5% (95%CI 22.9-39.3) of the children exhibited ferritin concentration < 12 ng/mL. With reference to transferrinemia, around 60% of the sample exhibited SI values < 50 µg/dL and TSat < 16%. Nevertheless, it should be pointed out that just 8.0% (95%CI 4.4-13.9) of the children exhibited TIBC > 400 µg/dL. Even more significant was the compromised erythropoiesis, represented by a FEP concentration > 40 µmol/mol/heme in 69.6% of the children assessed, characterizing a state of ferropenia without anemia necessarily having taken hold.

The prevalence of anemia (Hb < 11.0 g/dL) was 55.6% (95%CI 47.3-63.5), with a predominance of the mild form, with 40.6% (95%CI 48.4-32.8) of the children exhibiting Hb concentrations < 11.0 or > 9.0 g/dL. It should be noted that no cases of severe anemia (Hb < 7.0 g/dL)[6] were identified. The mean values of the iron assessment parameters were homogeneously distributed by sex (Table 2).

In contrast, it was observed that all parameters demonstrated a correlation with age, with the exception of SI concentrations (r = 0.16; p = 0.05), although the statistical validation criterion is on the threshold of rejection of the null hypothesis. Both Hb (r = 0.38; p = 0.00) and SF (r = 0.26; p = 0.00) demonstrated a direct correlation, whereas FEP (r = -0.49; p = 0.00) and TIBC (r = -0.28; p = 0.00) exhibited an inverse correlation with age.

The behavior of the parameters in the form of continuous variables in relation to age categorized into 24-month intervals did not demonstrate statistically significant variations (p = 0.08) for SF levels, although the data indicate tendency for mean concentrations to increase, *pari passu* as age increases. The behavior of SI also indicated lower values (35.4±25.3 µg/dL) in children less than 24 months old, when compared with those observed for children in the other age groups, although without statistical significance (p = 0.2). Concentrations of FEP and TIBC was significantly higher in children less than 24 months old, and 80% (95%CI 58.7-92.4) of the children in that age group exhibited TSat < 16%. Nevertheless, the tendency for the percentage of children with deficiency to decrease as age increased could not be validated from a statistical point of view (p = 0.06). This greater vulnerability to iron deficiency in children of lower age was also observed when the behavior of Hb was investigated, with a lower mean value (9.5±1.3 g/dL) (p = 0.000) in the age group 0 to 24 months (Table 3), in relation to the group of older children. This tendency is accentuated when we evaluate the prevalence of severe

Table 1 - Prevalence of iron deficiency and anemia < 5 year-olds, in public daycare centers in Recife, PE, Brazil, 1999

Parameters (cutoff points)*	N	n	%	95%CI
Hb (< 11 g/dL)	153	85	55.6	47.3-63.5
SF (< 12 ng/mL)	130	40	30.8	22.9-39.3
FEP (> 40 µmol/mol/heme)	135	94	69.6	61.0-77.1
SI (< 50 µg/dL)	148	92	62.2	53.8-69.9
TIBC (> 400 µg/dL)	150	12	8.0	4.4-13.9
TSat (< 16%)	148	89	60.1	51.7-68.0

95%CI = 95% confidence interval; FEP = free erythrocyte protoporphyrin; Hb = hemoglobin; SF = serum ferritin; SI = serum iron; TIBC = total iron binding capacity; TSat = transferrin saturation.
* World Health Organization (2001).

Table 2 - Iron parameters, by sex, in children < 5 years old in public daycare centers in Recife, PE, Brazil, 1999

	Sex				
	Male		Female		
Parameters	**n**	**X ± SD**	**n**	**X ± SD**	**p***
Hb (g/dL)	87	10.5±1.5	66	10.8±1.4	0.26
SF (ng/mL)	72	3.3 (3.0-3.7)†	58	3.3 (3.0-3.7)†	0.32
FEP (µmol/mol heme)	76	5.5 (5.3-5.7)†	59	5.5 (5.2-5.8)†	0.24
SI (µg/dL)	83	42.0±25.5	65	48.4±32.1	0.18
TIBC (400µg/dL)	85	317.1±64.6	65	297.9±52.7	0.05
%TSat (<16%)	50/83‡	60.2±10.2§	39/65‡	60.0±12.3§	0.98‖

FEP = free erythrocyte protoporphyrin; Hb = hemoglobin; SD = standard deviation; SF = serum ferritin; SI = serum iron; TIBC = total iron binding capacity; %TSat = transferrin saturation percentage.
* Student's *t* test for unpaired samples.
† Geometric mean + confidence interval.
‡ Total male-female.
§ 95% confidence interval.
‖ Chi-square test.

anemia (Hb < 9.5 g/dL), where approximately half of the cases diagnosed (47%; 95%CI 29.5-65.0) were in this same age group.

Odds ratios demonstrate that children less than 24 months old exhibited a significantly greater chance of being ferropenic, when compared with those older than 48 months, with OR = 14.9 (95%CI 3.3-77.0) for Hb, OR = 6.3 (95%CI 1.6-26.2) for SF and OR = 10.3 (95%CI 1.8-76.2) for FEP.

Iron parameters reflecting reserve levels demonstrated a significant correlation with erythropoiesis. In contrast, Hb only correlated with FEP (Table 4).

Discussion

The elevated prevalence of anemia detected in the study (55.6%), confirms that this is an important public health problem in the institutionalized child population of the city of Recife. This prevalence, to a certain extent, is in agreement with data from earlier studies carried out in Pernambuco and other states in the Northeast region of Brazil, for example Paraíba[3] and Sergipe.[7]

These data are extremely worrying due to the fact that the study population, being institutionalized, in principle should have guaranteed access to a balanced diet, in addition to general healthcare. One would, therefore, expect that the prevalence of anemia would be lower than reported in other strata of the population. According to Silva et al.,[8] among the factors that could be contributing to this picture of deficiency figure a lack of knowledge of the nutritional status of these children, before enrolling at the daycare centers, making it possible that many children were already anemic and had not had sufficient time to recover from their state of deprivation; a probable absence or low proportion of foods rich in iron in the menus offered by the daycare centers; the presence of inhibitory agents and/or a reduced proportion of facilitating agents in the diet, leading to low absorption of the bioavailable iron. Account should also be taken of the unfavorable social economic conditions of these children, which in homes living conditions that make them more vulnerable to diarrhea, respiratory infections and intestinal parasitosis, which can compromise, to a great extent, the intake, absorption and biological utilization of iron.[9]

The low body iron reserves (SF < 12 ng/mL) of the children studied (30.5%) demonstrate that this deficiency is an important specific nutritional deprivation and among preschool children at public daycare centers in Recife. The comparability of the magnitude of in adequate iron reserves with other reports in the specialized literature has been made difficult by the scarcity of data, the use of different cutoff points and the diversity of the study population. One example of this diversity, in terms of the adoption of critical points of normality and deficiency, can be observed in studies undertaken in the 1980s and 1990s, with reference values for SF oscillating between 10 and 15 ng/mL.[10,11]

The high prevalence of deficient erythropoiesis, where 70.0% of the children exhibited elevated FEP, 62.0% lower levels of SI and around 60.0% low %TSat, demonstrates that transferrinemia is seriously compromised in the study population. Nevertheless, one fact which merits better investigation is that TIBC concentrations recorded an extremely low prevalence of in adequate levels of transferrinemia (8.0%). A study carried out by Rettmer et al.[12] with children and adolescents from the USA

Table 3 - Means and standard deviations for iron parameters, by age, in children < 5 years old at public daycare centers in Recife, PE, Brazil, 1999

	Age (months)			
	0-24	24-48	≥ 48	
Parameters	**X ± SD**	**X ± SD**	**X ± SD**	**p***
Hb (g/dL)	9.5±1.3[a]	10.8±1.4[b]	11.3±1.2[b,c]	0.00
SF (ng/mL)	3.0 (2.5-3.7)†	3.3 (3.0-3.7)†	3.7 (3.3-4.1)†	0.08
FEP (µmol/mol heme)	6.7 (6.1-7.4)†a	5.5 (5.3-5.7)†b	5.0 (4.8-5.2)†b,c	0.00
SI (µg/dL)	35.4±25.3	47.1±28.5	45.2±30.5	0.2
TIBC (µg/dL)	340.3±73.9[a]	305.4±56.9[b]	293.8±49.7[b,c]	0.01

FEP = free erythrocyte protoporphyrin; Hb = hemoglobin; SD = standard deviation; SF = serum ferritin; SI = serum iron; TIBC = total iron binding capacity.
* p = ANOVA + Scheffé (different letters indicate different means between age groups at a level of 5%).
† Geometric mean + confidence interval.

Table 4 - Matrix of correlations between iron assessment parameters , in children < 5 years old at public daycare centers in Recife, PE, Brazil, 1999

Parameters	Hb	FerS	FEP	FeS	TIBC	%TS
Hb	-	-0.07	-0.49*	0.17	-0.05	0.16
SF	-0.07	-	-0.35*	0.37*	-0.53*	0.44*
FEP	-0.49	-0.35*	-	-0.40*	0.51*	-0.54*
SI	0.17	0.37*	-0.40*	-	-0.49*	0.90*
TIBC	-0.05	-0.53*	0.51*	-0.49*	-	-0.65*
%TSat	0.16	0.44*	-0.54*	0.90*	-0.65*	-

* p < 0.05, Pearson's correlation test (r).
FEP = free erythrocyte protoporphyrin; Hb = hemoglobin; SF = serum ferritin; SI = serum iron; TIBC = total iron binding capacity; %TSat = transferrin saturation percentage.

demonstrated a variable prevalence of deficient erythropoiesis, according to the laboratory test employed. The authors observed that the proportion of inadequate %TSat reached the figure of 27.9%, whereas the proportion of in adequate FEP values was extremely low (3.1%). Nevertheless, it is important to point out that the cutoff point adopted for FEP was 80 µmol/mol heme, irrespective of the age group assessed. Hamedani et al.,[10] assessed children less than 6 years old in Pakistan, observing low concentrations of FeS in 57.8% of the children, although they employed 36.7µg/dL as the critical level for depletion. In contrast, 43.9% of the Pakistani children exhibited high TIBC values (> 398 µg/dL).

The extreme variability, in terms of magnitude, of the iron deficiency and assessment parameters, when analyzed in isolation, results in the issue of the need to use more than one parameter and so offer a wide spectrum that includes transferrinemia, body iron reserves and anemia. For Madanat et al.,[11] iron deficiency could be defined by a significant reduction in %TSat, associated with microcytosis and hypochromia. From this point of view, the inclusion of hematological parameters that assess erythrocyte morphology (MCV and RDW) and mean corpuscular hemoglobin concentration (MCHC) would be of fundamental importance for correct interpretation of the biochemical indicators.[5]

The homogenous distribution by sex of iron deficiency and anemia observed in our patients, has been confirmed in other studies.[1,8,13,14] However, certain findings have pointed to increased prevalence of anemia in male children.[2,3,15] This could possibly be caused by the greater growth velocity exhibited by boys at preschool ages, resulting in increased demand for iron by the body, which is not being supplied by the diet.[15]

The increased susceptibility of children under 24 months old to depleted body iron reserves, deficient erythropoiesis and anemia demonstrates that this is an age group at greater risk of iron deprivation. It is a well-known fact that iron deprivation affects, in a very significant manner, individuals who are in a phase of accelerated growth, with consequent increased iron requirements.[15] Associated with this are short duration of total and exclusive breastfeeding, the introduction of cow's milk to the diet, causing micro-hemorrhages in the gastrointestinal tract, allied to the introduction of a monotonous diet that is poor in iron and also a greater predisposition to infectious and parasitic diseases, are risk factors which could explain the increased vulnerability of this age group to iron deficiency and anemia.[4,16,17]

The inverse correlation between Hb levels with FEP concentrations, observed in the study population, is a fact also described by Serdar et al.[18] This association is, in principle, predictable, since the increase in unconjugated protoporphyrin in erythrocytes would be the result of an inadequate supply of iron at the cellular level and, therefore, be insufficient for the formation of Hb. Nevertheless, an absence of correlation was observed between Hb and the other indicators of the nutritional iron status assessment, when these parameters were compared as continuous variables. This finding merits consideration when analyzed according to the paradigm of the natural history of the deprivation. It should however be noted that, in the specific analysis of transferrinemia, %TSat demonstrated a discrete tendency towards a positive correlation with Hb, although this was not validated statistically. Considering that %TSat represents a synthesis of the combination of the parameters SI and TIBC, this indicator, in theory, should provide a more coherent reading when interpreting the transferrinemia results. It is in this manner that %TSat has often been employed, as has FEP, for evaluation of erythropoiesis.[11,19] A group of factors that have not yet been fully elucidated could provide a basis for a better understanding of these results. One element to take into consideration when analyzing the absence of correlation relates to the behavior of these biochemical parameters in a biological environment. In the specific case of SF, the low prevalence of children with insufficient iron reserves, with relation to the prevalence of anemia, would be an unexpected finding if viewed from the perspective of the natural history of deprivation, in which anemia would only have onset once the body's iron reserves had reached inadequate levels. Reports have shown the extreme variability of SF and Hb concentrations that result from pathological alterations, such as infectious processes.[19] It is known that SF is considered a positive protein of the acute phase, which increases significantly during infectious processes, even during subclinical stages, irrespective of the reserves of iron deposits in the body.[20] It is therefore of fundamental importance that analysis be undertaken of concentrations in the body of markers of infectious /inflammatory states no, for example, of C-reactive protein (CRP), in order that a correct interpretation can be made of the SF levels and, consequently, the body's iron reserves.

The significant correlation between the parameters that assess body iron reserves, transferrinemia and the availability of iron for erythropoiesis demonstrates that this association was compatible with the links in the metabolic chain that make up the iron cycle in the body. With respect to transferrinemia, the inverse relationship observed between SI and TIBC levels can theoretically be explained, since, when circulating mineral levels are reduced, there is an increase in the concentration of transferrin in serum, assessed by TIBC. On the other hand, the direct relationship observed between SI and %TSat is also obvious, as is its inverse relationship with TIBC. This finding is plausible since %TSat is a parameter arrived at from the relationship between SI and

TIBC. Therefore, when SF is reduced and/or TIBC increased, %TSat tends to exhibit reduced values, confirming deficiency. In turn, when the quantity of circulating iron increases and/or TIBC decreases, the %TSat will indicate that the proportions of transferrinemia levels are adequate. This association between body iron reserves, transferrinemia and erythropoiesis was also observed by Hershko et al.[21] in children between 1 and 6 years old from a rural area in Israel.

Summing up, iron deficiency anemia is an important health problem at the public daycare centers of Recife. Insufficient body iron reserves were detected in around 30% of the study population, and erythropoiesis status exhibited abnormal transferrinemia levels in the iron plasma cycle, with rates of deficiency that varied from 69.6% for FEP to 8.0% for TIBC. Children < 24 months old exhibit an increased susceptibility to iron deficiency, in terms of depleted reserves, deficient erythropoiesis and anemia. Therefore, timely and effective measures for the prevention and control of iron deprivation are to be recommended for this high-risk population.

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