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Association of vitamin A with anemia and serum hepcidin levels in children aged 6 to 59 mo

Amanda de Paula Silva [a], Alessandra da Silva Pereira [b], Bruno Francisco Teixeira Simões [c], Juliana Omena [a], Cláudia dos Santos Cople-Rodrigues [a], Inês Rugani Ribeiro de Castro [a], Marta Citelli [a,*]

[a] Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Escola de Nutrição, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[c] Centro de Ciências Exatas e Tecnologia, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

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ABSTRACT

Objective: This study evaluates the association of serum retinol, hepcidin levels, and anemia in children.
Methods: This cross-sectional study included 312 children, ages 6 to 59 mo, from Rio de Janeiro, Brazil. The association between hepcidin and retinol levels, hematologic parameters, and body mass index (BMI) was analyzed using a generalized linear model with and without adjustment for C-reactive protein (CRP) level. Logistic regression analysis was used to test anemia as an outcome and serum retinol level as a predictive variable using the odds ratio (OR) function.
Results: Anemia was present in 14.6% of the children, 5.8% presented iron deficiency anemia, and 9.6% had vitamin A deficiency. The increase in serum retinol levels reduced the chances of anemia (OR = 0.13; confidence interval = 0.29–0.59). When CRP level was not adjusted for in the multiple regression analyses, retinol, ferritin levels, and BMI/age were predictors of serum hepcidin levels ($\beta = -3.36, 0.14, 1.02$, respectively; $P = 0.032$). Accordingly, serum retinol levels were inversely associated with CRP levels ($\beta = -0.025$ and $P < 0.001$).
Conclusions: The association between serum retinol and hepcidin levels in children ages 6 to 59 mo seems to be dependent on inflammation. Taken together, the results reinforce the need for the development of further studies to better understand the relationship between vitamin A and anemia of inflammation.

Introduction

Anemia and vitamin A deficiency (VAD) are major public health problems [1–3]. Worldwide, anemia affects approximately 273 million children (42.6%), and VAD is detected in approximately 190 million preschool-aged children (33.3%) [2,3]. In Brazil, a preliminary report of the National Study on Child Food and Nutrition (ENANI 2019) has shown that the national prevalence of anemia and VAD is 10.0% and 6.0%, respectively, among children under 5 y of age [1].

The main causes of anemia are believed to be iron deficiency and inflammation, and the high-risk groups are infants, preschoolers, women of reproductive age, and pregnant women [2,4,5]. Iron deficiency anemia can cause functional changes in the body, such as delayed development, impaired cellular immunity, and decreased intellectual capacity [6]. Iron deficiency may occur because of several causes, such as inadequate intake, increased loss, changes in absorption pattern, or changes in bioavailability of iron.

It is possible that a functional deficiency of iron develops even when the iron stores in the body are adequate because of changes in iron homeostasis, as it occurs in the presence of inflammation [5,7]. Anemia of inflammation has been characterized as mild to moderately severe anemia, with hemoglobin concentrations ranging from 7 to 12 g/dL [5]. It develops in the context of systemic inflammation, due to the decrease in red blood cell production, and it is accompanied by a modest reduction in half-life of red blood cells. Unlike iron deficiency anemia, in anemia of inflammation, iron stores are preserved. Thus, anemia of inflammation is primarily a disorder of iron distribution [5].

Iron homeostasis is regulated by two main mechanisms: intracellular and systemic. Systematically, iron balance requires communication between absorption, use, and storage, which is carried

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*Corresponding author: Departamento de Nutrição Básica e Experimental, Universidade do Estado do Rio de Janeiro, 20550-900, Rio de Janeiro, Brazil. Tel.: +55 21 23340679
E-mail address: martacitelli@gmail.com (M. Citelli).

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out through hepcidin; hepcidin is a hormone that acts as a negative regulator of serum iron concentration and plays a key role in iron homeostasis in the body. Inflammatory processes activate its synthesis, and its action consists of blocking the duodenal absorption of iron and efflux of iron from reticuloendothelial macrophages that recycle senescent red blood cells [8–10]. Therefore, the relationship between hepcidin and inflammation can contribute to the development of anemia of inflammation [5,7]. Relatively mild systemic inflammatory states, such as obesity, are clinically relevant and may induce the increase in serum hepcidin levels [5,11].

VAD has also been linked to inflammatory processes [12–15]. Inflammation can cause or be caused by VAD [16]. The existence of a relationship between VAD and anemia is well recognized [17–19]. It is also known that vitamin A supplementation reduces the prevalence of anemia [14,20,21]. This relationship can be explained by several vitamin A–related biological mechanisms, such as the increase in the growth and differentiation of erythrocyte progenitor cells [14,22], potentiation of immunity, reduction of inflammation [23,24], and modulation of bioavailability and mobilization of tissue iron stores [25–27], which may be influenced by inflammation [5,14,20,21]. However, the pathogenesis of anemia due to VAD has not been well characterized.

Experimental studies have shown that vitamin A induces the synthesis of proteins responsible for iron transport in duodenal cells and that VAD increases hepcidin gene expression [25,27] and compromises its signaling pathway [23]. Therefore, it is plausible that in humans retinol may affect iron homeostasis through hepcidin modulation and that VAD can lead to the accumulation of this mineral in tissues.

The present investigation aimed to study the association between retinol and hepcidin levels in children under 5 y of age. It is suggested that VA is predictive of anemia because of its influence on hepcidin levels. The results of this study can contribute to strategies for the prevention and treatment of anemia.

Methods

This cross-sectional study was carried out in a subsample of children ages 6 to 59 mo who were under the primary health care of public health system (Sistema Único de Saúde) in the city of Rio de Janeiro, from a larger study "Anemia and vitamin A Deficiency in Preschoolers: Magnitude in a Large Metropolis and Validation of Diagnostic Methods (VITANEMIA)" [30] that studied a probabilistic sample of this group. Children with infectious diseases such as pneumonia and otitis, sickle cell disease, and liver diseases were excluded from the study.

The size of the subsample used in the present study was calculated using the STATA v.13 program. Correlation values between retinol and hepcidin levels were considered continuous variables with the following parameters: R1 (null value) of 0.00, R2 (value observed in the literature) of 0.40 [24], absolute study precision of 0.10, and 95% confidence interval (CI) [28]. By applying these parameters, the calculated sample size was 62 children. For the development of this study, a database of 312 children that included the variables of interest was used.

The Rio de Janeiro Municipal Health Office Ethics Committee for Research with Humans (no. 203A/2013) approved this study. The study was conducted only on children whose parents or guardians agreed to their participation and signed a free and informed consent form.

Data collect

Data were collected from July to December 2014. The guardians who agreed to participate in the study attended the primary health care and answered a questionnaire about sociodemographic characteristics, their child's health status, and other data of interest related to the larger study.

Blood samples were collected by venipuncture by trained clinical pathology technicians with experience in collecting blood from children. After collection, the samples were placed in two tubes: one with a gel clot activator for retinol and hepcidin levels and other serum analyses, and the other with ethylenediamine tetraacetic acid for hemoglobin analysis. The gel clot activator tube was centrifuged for 10 min in a Centribio portable centrifuge (model 80-2B, speed of 4000 rpm), was kept at 8°C for up to 4 h after blood collection, and was protected from light. The serum was then aliquoted in amber tubes and stored at −80°C until analysis. Weight was measured using a portable Tanita scale. Length was measured using a Sanny anthropometer (São Bernardo do. Campo, SP, Brazil), and height was measured using Alturexata stadiometer (Belo Horizonte, MG, Brasil). Trained professionals measured the weight, length, and height. Nutritional status was assessed using the following indices: weight/age (W/A), height/age (H/A), and body mass index for age (BMI/A) were calculated using the growth reference data [29]. More details are available from previously published studies [30].

Laboratory analysis

Blood samples were processed for hemoglobin analysis at the Lipids Laboratory of our University. The ethylenediamine tetraacetic acid tube was subjected to automated counting in an Automated Hematological Counter XS1000 i Sysmex using a Stromatolyser-4 DS-Sysmex on the same day that the blood was collected. Serum retinol was measured by high-performance liquid chromatography at the Physiopathology and Nutrition Biochemistry Laboratory of our University using an adapted extraction method [31]. Serum concentrations of ultrasensitive C-reactive protein (CRP) were analyzed using nephelometry (Biosystems). An immunoenzymatic assay was performed to analyze serum ferritin (Symbiosys kit, ALKA Tecnologia, São Paulo, Brazil) and bioactive serum hepcidin-25 levels (DRG Instruments GmbH, Marburg, Germany). The manufacturer's instructions were followed. The coefficient of variation of the measurements of retinol, hepcidin, ferritin, CRP, and hemoglobin levels were 7.7%, 5.4%, 2.5%, 8.8%, and 0.45%, respectively.

Variables of interest

Sociodemographic and health variables included were sex, age group, family income in American dollars, mother's education, presence of anemia (hemoglobin level <11 g/dL) [2], iron deficiency anemia (hemoglobin level <11 g/dL and ferritin level < 2 μg/L when CRP was ≤ 5 mg/L; or ferritin <30 μg/L when CRP was >5 mg/L) [7], and VAD (retinol level < .70 μmol/L) [32], or VAD adjusted for inflammation [33], W/A, H/A, and BMI/A. The primary outcome was hepcidin level (ng/mL), which was analyzed as a continuous variable. The following variables were tested as hepcidin level prediction variables: serum retinol level (μmol/L), hematologic and biochemical parameters (levels of ferritin [μg/L], hemoglobin [g/dL], hematocrit [%], mean corpuscular volume [fL], mean corpuscular hemoglobin [pcg], mean corpuscular hemoglobin concentration [%], and leukocytes [thousand/mm^3]), BMI (z-score), and CRP level (mg/L). Sex and age groups were included as adjustment variables.

Data analysis and statistical treatment

Data were analyzed using STATA software (version 13.0; StataCorp LLC, College Station, TX, USA). Descriptive analysis of the data was performed. The frequency distributions, measures of central tendency, and dispersion were calculated. A Shapiro-Wilk test was used to analyze the normality of the continuous variables.

As the outcome variable (hepcidin) presented positive asymmetrical distribution, we used a generalized linear model with gamma family identity function to assess the association between hepcidin levels, serum retinol levels, other hematologic parameters (levels of hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and ferritin), CRP level, and BMI. The analyses were performed with and without adjusting for CRP levels. In both analyses, sex, age, family income in American dollars, and mother's education were adjusted for, following the backward protocol.

Multiple logistic regression analysis was performed, in which anemia was tested as an outcome and VAD and retinol levels as predictor variables after adjusting for sex, age, BMI/A (z-score), CRP, family income and mother's education. In both logistic and generalized linear model analyses, variables that presented $P <$ 0.2 in the simple analyses were included in the multiple regression models. Subsequently, a significance value of 5% was adopted for the multiple regression analyses to obtain the final model.

Simple linear regression analyses were performed in which CRP (mg/L) was considered as the dependent variable and retinol (μmol/L) or VAD (presence) as the independent variables. The adjustment variables were age, sex, family income, maternal education and Z-BMI/A. A significance value of 5% was adopted.

Results

In this study, 312 children aged 6 to 59 mo were included. Of these, approximately 67% were older than 24 mo, and 52% were male (Table 1). The prevalence of anemia and VAD was 14.6% and 9.6%, respectively, and 5.8% of the children presented iron deficiency anemia. Because of the study design, in which all children were users of the Sistema Único de Saúde, maternal education was primarily at secondary school level and the family income was mostly less than $619.

Table 1
Distribution of selected characteristics of children ages 6 to 59 mo from Brazil.

Sociodemographic and health variables	n (%)
Sex	
Female	151 (48.40)
Male	161 (51.60)
Age range (months)	
6–23	104 (33.33)
24–59	208 (66.67)
Weight/age (z score)*	
< −3.0	2 (0.64)
−3.0 ┤ −2.0	2 (0.64)
−2.0 ┤ 2.0	(92.31)
> 2.0	20 (6.41)
Height/age (z score)*	
< −3.0	2 (0.64)
-3.0 ┤ −2.0	13 (4.17)
≥ −2.0	297 (95.19)
BMI/age (z score)*	
< −3.0	2 (0.64)
−3.0 ┤ −2.0	0 (0.00)
−2.0 ┤ 1.0	204 (65.38)
1.0 ┤ 2.0	74 (23.72)
2.0 ┤ 3.0	23 (7.37)
>3.0	9 (2.88)
Family income in American dollars	
<155	8 (2.58)
155–309	29 (9.35)
310–619	136 (43.80)
620–929	59 (19.03)
≥930	53 (17.10)
Did not know how to inform	25 (8.06)
Mother's education	
Lower primary school	64 (20.71)
Primary school	99 (32.04)
Secondary school	133 (43.04)
Higher education	11 (3.56)
Prevalence of anemia†	45 (14.56)
Iron deficiency anemia‡	18 (5.80)
Prevalence of VAD§	30 (9.62)
Prevalence of VAD, adjusted for inflammation‖	29 (9.32)
CRP >5 mg/L	1 (0.32)

*Classification according to World Health Organization [29].
†Presence of anemia: hemoglobin level <11 g/dL [2].
‡Presence of iron deficiency anemia: hemoglobin level <11 g/dL and ferritin <12 μg/L when CRP is ≤5 mg/L or ferritin is <30 μg/L when CRP is >5 mg/L [7].
§Presence of VAD: retinol level <0.70 μmol/L [32]. n = 312.
‖Prevalence of VAD, adjusted for inflammation [33].BMI, body mass index; CRP, C-reactive protein; VAD, vitamin A deficiency

Table 2
Retinol status, iron and inflammatory markers of children ages 6 to 59 mo

Variables	Median (IQR)	P25	P75
Hepcidin (ng/mL)	6.57 (6.24)	4.48	10.72
Hemoglobin (g/dL)	11.90 (1.30)	11.30	12.60
Ferritin (μg/L)	31.00 (29.00)	16.00	45.00
Hematocrit (%)	35.80 (3.70)	34.00	37.70
Retinol (μmol/L)	1.00 (0.32)	0.84	1.16
CRP (mg/L)	0.04 (0.22)	0.01	0.23
Leukocytes (thousand/mm^3)	8.70 (3.90)	7.00	10.90

Table 3
Logistic regression analyses with anemia as the dependent variable.

Independent variables	β	OR	95% CI	*P*
Retinol (μmol/L)	−2.08	0.13	0.029–0.56	<0.001
Presence of VAD	0.473	1.61	0.578–4.46	0.364

Adjustment were made for sex, age, BMI/A (z-score), CRP, family income and mother's education.
CI, confidence interval; BMI, body mass index; OR, odds ratio; VAD, vitamin A deficiency

As shown in Table 2, the studied children did not present high levels of inflammation, which was expected based on the exclusion criteria. Retinol P25 levels were found to be above the value considered adequate, corroborating the low prevalence of VAD. In addition, the median ferritin and P25 values were above the lower limit, corroborating the finding of a low prevalence of iron deficiency among the children.

Logistic regression showed that an increase in serum retinol level of 1 μmol/L reduced the chances of anemia occurring by 87% (OR = 0.13) in children aged 6 to 59 mo (Table 3). On the other hand, VAD was not significantly associated with anemia (Table 3). Similarly, serum retinol levels were inversely associated with serum CRP levels (β = −0.2317, P = 0.006; CI = −0.398 to −0.065; data not shown) but VAD was not significantly associated with CRP concentrations (β = −0.4429, P = 0.070; CI = −0.9221 to 0.0362; data not shown).

The simple analyses showed a direct and significant association between hepcidin level and CRP level (β = 11.33, P < 0.001), ferritin level (β = 0.15, P < 0.001), leukocyte counts (β = 0.81, P < 0.001), W/A (β = 0.78, P = 0.08), BMI/A (β = 1.17, P < 0.01), and an inverse and significant association with retinol (β = −3.67, P = 0.06) (data not shown).

When performing multiple regression analysis, retinol was not significantly associated with hepcidin (P = 0.36). However, in the crude model, without adjustment for CRP level, serum retinol, ferritin, and BMI/A (β = −3.36, 0.14, 1.02, respectively) remained in the final model (Table 4). The socioeconomic indicators included in the model did not influence hepcidin levels in the study population. Alternatively, VAD was tested as an independent variable, replacing retinol concentrations. However, in both the crude and the CRP-adjusted model, VAD was not significantly associated with hepcidin (data not shown).

Discussion

In this study, serum retinol levels were associated with serum hepcidin levels, and this association was dependent on inflammation. To the best of our knowledge, this is the first study to assess this association in children ages 6 to 59 mo. Inflammation is known to greatly increase hepcidin synthesis, which is important in the pathogenesis of anemia of inflammation [5]. The results presented in this study suggest that vitamin A may play a role in the pathophysiology of anemia of inflammation, in which hepcidin is an important mediator, corroborating findings from previous experimental studies [23,25,26,27].

We observed that an increase in serum retinol level reduced the chances of anemia. Although the biological mechanisms by which VA can modulate the development of anemia are not completely clear, it is known that VA is necessary for hematopoiesis [22]. Furthermore, previous data indicated that VAD can induce and aggravate inflammation [16,36,37]. It is known that the production of hepcidin is increased in the presence of inflammation as a defense mechanism of the human body to decrease the extracellular availability of iron. This may lead to functional iron deficiency, and consequently, anemia of inflammation [5,34,35]. These data supported a line of reasoning in which VAD could modulate hepcidin and cause anemia. However, in the present study, VAD was not significantly associated with CRP, hepcidin or anemia, but serum retinol concentrations were inversely and significantly associated with CRP, hepcidin and anemia. In the multiple regression model without adjustment for CRP level, an inverse and statistically significant association between retinol and hepcidin levels was observed. This association did not occur when the model was adjusted for CRP level. This result is in agreement with the possibility that

Table 4
Generalized linear model analyses with hepcidin as the dependent variable

	Not adjusted for CRP			Adjusted for CRP		
	β	95% CI	*P*	β	95% CI	*P*
Retinol (μmol/L)	−3.36	−6.38 to 0.33	0.03	−1.43	−4.19 to 1.33	0.309
Ferritin (μg/L)	0.14	0.09–0.19	<0.001	0.10	0.06–0.15	<0.001
BMI/A (z-score)	1.02	0.43–1.61	<0.001	0.70	0.20–1.21	0.010
CRP (mg/L)	–	–	–	8.06	3.72–12.40	<0.001

GLM to assess the association between hepcidin levels, serum retinol levels, hematologic parameters, CRP level, and BMI/A. The analyses were performed with and without adjustment for CRP, both adjusted for sex and age, family income, and mother's education. AIC = 6.5183. AIC, Akaike information criterion; BMI, body mass index; CRP, C-reactive protein

inflammation might modulate the association between retinol and hepcidin. It is worth noting that relatively mild systemic inflammatory states may induce the increase in serum hepcidin levels [5,11].

As far as we know, only one study previously investigated the association between hepcidin and retinol. A cross-sectional study carried out in Mexico with 783 individuals over 60 y of age showed that hepcidin levels, after adjusting for sex, age, and inflammation, were significantly higher in the group with VAD than in the group without VAD, showing that VAD status was inversely associated with hepcidin levels [24].

Further studies are needed to elucidate how the iron regulatory system works in children and to identify factors that can lead to anemia of inflammation in preschoolers. It is worth mentioning that the children studied here had at most low-grade chronic inflammation, which, nevertheless, appeared to influence serum hepcidin levels. Furthermore, hepcidin serum levels can vary during human growth and development [3,38,39,40].

In the present study, BMI/A was directly associated with serum hepcidin levels, independent of inflammation. Previously, a study carried out in the state of Bahia, Brazil, with 376 children showed that overweight children had a higher prevalence of tissue iron deficiency, as measured by serum ferritin level (30.6 versus 12.5%, respectively; $P = 0.002$) and chronic inflammation (α-acid glycoprotein-1 > 25 μmol/L) (18.9 versus 10.0%, respectively; $P = 0.025$), compared with their normal-weight counterparts. Tissue iron deficiency in those preschoolers was associated, at least in part, with adipose-related inflammation. The role of adiposity-related inflammation in tissue iron deficiency should always be considered, even in groups of children with a relatively low prevalence of overweight [11].

Given that anemia and VAD are more prevalent in the lower socioeconomic strata [3,13], socioeconomic indicators were included in the multiple regression model. However, possibly owing to the socio-economic homogeneity of the studied sample, we were able to verify that the relationship between vitamin A and hepcidin levels seems to exist regardless of the influence of socioeconomic conditions.

In addition, the inverse association observed between serum retinol and CRP levels corroborates the protective effect that retinol seems to exert against anemia, wherein an increase of 1 μmol/L retinol reduced the chances of anemia by 87%. The association between VAD and anemia is well documented [19,41–43]. Serum retinol level has been shown to be directly associated with hemoglobin, hematocrit, transferrin saturation, and iron levels [41,44]. However, in the present study, VAD was not associated with hepcidin while retinol concentrations were. Inflammation is believed to reduce plasma retinol levels owing to a decrease in the concentration of retinol transport proteins 24 h after the onset of infection [33]. However, a reduction in the prevalence of anemia, an improvement in anemia after vitamin A supplementation, or both, has also been observed [14,20,21,45].

Here, the observed prevalence of VAD was 9.6%. Since 2005, the Ministry of Health of Brazil has been developing the National Vitamin A Supplementation Program in areas considered to be at risk. In 2012, the program was expanded to the entire country, including the Southeast region (area where the study is located). Important measures were implemented by the program for children: promotion of exclusive breast-feeding up to the sixth month and complementary breast-feeding up to 2 y of age or more; promotion of adequate and healthy diet, ensuring information to encourage the consumption of foods sources of vitamin A by the population; periodic and regular prophylactic supplementation of children ages 6 to 59 mo, with megadoses of vitamin A. Currently, the program has been less effective, but the data analyzed in this study were collected in 2014, when these public policies were more effective.

The results of the present study corroborate previous findings [46,47] regarding the association between hepcidin and ferritin levels or another indicator of iron stores in the body. Limitations of the present study include the cross-sectional design, which limits the ability to infer causality. Further studies are needed to explore this issue because the association between hepcidin and vitamin A is far from obvious. Vitamin A can reduce inflammation. Conversely, inflammatory processes can decrease vitamin A concentrations [16]. Therefore, it is worth noting that many chronic inflammatory diseases can reduce serum retinol concentrations. So, ultimately, the study design did not allow us to understand whether the decrease in retinol concentrations caused inflammation and, consequently, hepcidin modulation, or whether inflammatory conditions modulated both retinol and hepcidin. Regardless, the results suggested that vitamin A is associated with the key molecules of the anemia of inflammation.

The association between hepcidin level and inflammation evidenced in this study has been previously reported [48,49]. Given the importance of hepcidin in iron homeostasis, its level has already been suggested as a useful indicator for differentiating iron deficiency anemia and inflammation related anemia [5].

The observed association between retinol and hepcidin levels may explain why strategies to control anemia based solely on iron supplementation have a limited effect on the overall prevalence of anemia. Here, we observed that iron deficiency anemia was present in approximately only one-third of the children with anemia. Therefore, it is necessary to improve our understanding about the relationship between vitamin A, hepcidin levels, and anemia in children.

Conclusion

The association between serum retinol and hepcidin levels seems to be dependent on inflammation in children ages 6 to 59 mo. Taken together, the results reinforce the need to develop further studies to better understand the relationship between vitamin A and anemia of inflammation.

Authorship

APS wrote the first draft of the manuscript, participated in the analysis and interpretation of results. MC and ASP developed the concept and design of this study, participated in the analysis and interpretation of results, and supervised the writing of the manuscript. BFTS supervised the statistical analysis and critically revised the manuscript. JO performed laboratorial analysis and critically revised the manuscript. IRRC and CC participated in developing of the concept and design of this study and critically revised the manuscript. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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