Iron status biomarkers and C-reactive protein in children aged 19 to 72 months in Chile

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Abstract

Background. *The Chilean Ministry of Health has combated iron deficiency through the delivery of fortified milk by the National Complementary Feeding Program (NCFP).*

Objective. *To assess iron status and associations between biomarkers of iron status and serum C-reactive protein (CRP) in 218 beneficiaries of the NCFP aged 19 to 72 months in Santiago and Valparaiso, Chile.*

Methods. *Blood was collected from a cross-sectional representative sample. Iron status (measured by hemoglobin, zinc protoporphyrin, and serum ferritin levels) and inflammation (according to CRP level) were determined.*

Results. *Serum CRP level was positively associated with serum ferritin and zinc protoporphyrin levels (*r = *0.16 and* r = *0.15;* p = *.0168 and* p = *.0290, respectively). Serum ferritin was higher among children with high CRP (> 10 mg/dL) than among those with low CRP (≤ 10 mg/dL) (*p = *.003). After adjustment for 10, 6, and 5 mg/L CRP, the prevalence of low serum ferritin changed from 56.4% without adjustment to 60.6%, 61.5%, and 42.7%, respectively, and the prevalence of high zinc protoporphyrin changed from 22.9% to 21.6%, 17.4%, and 17.9%, respectively. There were no differences between regions in biomarkers of iron status. There was no association between consumption of fortified milk and the prevalence of abnormal serum ferritin (< 15 μg/L) after adjustment for sex, age, and breastfeeding (OR, 1.00; 95% CI, 0.99 to 1.01;* p = *.288). After adjustment for 10 mg/L CRP, 5.5% were classified as having iron-deficiency anemia, 42.7% as having iron-deficiency erythropoiesis, 17.9% as having depleted iron stores, and 35.8% as having normal iron status.*

Conclusions. *CRP level was positively associated with serum ferritin and zinc protoporphyrin levels. Chilean children aged 19 to 72 months from Santiago and Valparaiso who were beneficiaries of the NCFP had a low prevalence of iron-deficiency anemia, a high prevalence of iron-deficiency erythropoiesis, and a moderate prevalence of depleted iron stores.*

Key words: Anemia, Chile, C-reactive protein, ferritin, inflammation, iron deficiency, iron-deficiency anemia

Introduction

Iron deficiency is the most prevalent nutritional deficiency around the world, primarily affecting infants, young children, and women of childbearing age [1]. Iron deficiency adversely affects the cognitive development of children, increases maternal and infant mortality, and lowers physical work capacity in adulthood [2–6]. The main cause of iron deficiency among young children is a lack of bioavailable iron in the diet. Complementary foods targeted at improving nutritional status of children can be fortified with micronutrients; fortification of milk with iron is one example of this strategy [7]. Since 2000, the Chilean Ministry of Health has combated iron deficiency through the delivery of iron-fortified foods by the National Complementary Feeding Program (NCFP). With this purpose in mind, the milk delivered was replaced by powdered whole cow's milk (26% fat) fortified with 10 mg of iron as ferrous sulfate, 5 mg of zinc as zinc acetate, 0.4 mg of copper as copper sulfate, and 70 mg of ascorbic acid per 100 g of powder. The bioavailability of iron in this milk is 10.5% [8]. The milk is delivered for consumption by spontaneously weaned children up to the 18th

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month of life. For children from 19 to 72 months of age, the program delivers another milk, a powdered, low-fat (18% fat) formula based on cow's milk and cereals, fortified with 6.2 mg of iron as ferrous sulfate, 6 mg of zinc as zinc acetate, 0.3 mg of copper as copper sulfate, and 50 mg of ascorbic acid per 100 g of powder. The bioavailability of iron in this formula is 12.4% [8]. Nearly 80% of young Chilean children are covered by this program [9]. Each child receives 2 kg of powdered milk every month. Assuming a daily intake of 50 g of the fortified milk powder, 0.6 and 0.4 mg of absorbed iron will be supplied to spontaneously weaned children up to the 18th month of life and from 19 to 72 months, respectively, an amount covering nearly 80% of the recommended daily requirements [10].

It is well known that inflammation induces changes in biomarkers of iron status [11]. Even apparently healthy subjects may have elevated inflammatory biomarkers [11]. Serum ferritin is a recognized acute phase protein [11]. The US Centers for Disease Control and Prevention (CDC) recommends measuring at least one inflammatory biomarker, such as C-reactive protein (CRP), when determining iron status [12]. The present study determined the iron status and associations between biomarkers of iron status and serum CRP in Chilean children from Santiago and Valparaiso 19 to 72 months of age who were beneficiaries of the NCFP.

Methods

Study population and sample selection

Chile is divided into 15 regions. Between April and December 2009, anemia was assessed in a cross-sectional sample. This sample was representative of beneficiaries of the NCFP from the Santiago metropolitan and Valparaiso regions. These regions included eight health services accounting for 144 outpatient health centers. The sample size calculation was performed with a confidence of 95% and with a maximum error of 0.029 based on an 8% prevalence of anemia, yielding a sample size of at least 219 subjects. We recruited a total of 224 children. The method used to generate the random sample was to randomly enlist all health centers from each health service to be sequentially visited for recruiting 28 subjects aged 19 to 72 months in each health service, resulting in 224 subjects.

Mothers with young children attending health centers were contacted and invited to participate. Among those who accepted, anthropometric data were recorded from the healthy baby control registry, and current food intake was obtained from the mothers. Hemoglobin and mean cell volume were measured with a CELL-DYN 1700 (Abbott Diagnostic, Abbott Park, IL), zinc protoporphyrin with a hematofluorometer (Model 206D, AVIV Biomedical), serum ferritin with an enzyme immunoassay of double sandwich (INACG) [13], and CRP with a Turbox (Orion Diagnostica) in 5 mL of venous blood obtained from the children. Red blood cell distribution width was calculated by the following formula: SD of red blood cell volume (fL)/mean cell volume (fL) × 100.

Inclusion criteria

Apparently healthy children with birthweights over 2,500 g were included.

Ethical approval and informed consent

This study was approved by the Ethics Committee at the Institute of Nutrition and Food Technology, University of Chile, before its execution, in accordance with the Helsinki Declaration. Participation was voluntary and no remuneration was provided. The procedures were performed after the parents had read and signed the informed consent form.

Definitions of iron status and inflammation

The following values were used to define the lower limits of normal: hemoglobin, 11 g/dL for children under 5 years of age and 11.5 g/dL for those 5 years or more of age; mean cell volume, 77 fL for those 1 or 2 years old, 79 fL for those 3 to 5 years old, and 80 fL in those > 5 years; and 15 μg/L for serum ferritin. The upper limits of normal for zinc protoporphyrin and red blood cell distribution width were 80 μg/L red blood cells (RBC) and 14%, respectively [12]. Anemia was defined as hemoglobin below normal and iron-deficiency anemia as hemoglobin below normal plus two or more abnormal laboratory measurements. Iron-deficiency erythropoiesis was defined as normal hemoglobin plus two or more abnormal laboratory results; depleted iron stores was defined as serum ferritin below normal. Iron status was considered to be normal when all of these laboratory indexes were within the reference ranges. Mean cell volume was only used to determine microcytic anemia.

CRP adjustment and exclusion

Continuous variables were categorized into 5, 6, and 10 mg/L CRP intervals, which are commonly used in young children [14]. Biomarkers of iron status associated with CRP were adjusted using correction factors based on each CRP cutoff mentioned above. The adjustments were ratios of geometric means of iron biomarkers in the group with normal CRP to those in the group with abnormal CRP [14]. The continuous values and abnormal prevalence of biomarkers of iron status were recalculated and compared with those before adjustment and after exclusion of cases with abnormal CRP.

Dietary survey

A previously validated dietary survey was applied for those mothers who agreed to participate in this study. This information was obtained once at the time of sample selection. Consumption of iron-fortified milk delivered by NCFP and breastfeeding were determined in accordance with the Feeding Guide for Young Children of the Chilean Ministry of Health [15]. Regular consumption was defined as daily consumption of iron-fortified formula delivered by this program since 19 months of age.

Statistical methods

Only data from children with complete measurements for biomarkers of iron status and serum CRP were analyzed. We assessed normality by the Shapiro Wilk test. The values of serum CRP, zinc protoporphyrin, and ferritin were not normally distributed. Continuous variables were reported as means (standard deviations) and medians with interquartile ranges (25th and 75th quartiles) for normal and non-normal distributions, respectively. Categorized variables were described as frequencies (percentage). Correlations between CRP and concentrations of iron status biomarkers were assessed by Spearman's correlation coefficient. Significant correlations were further explored. Global differences between stratified levels of biomarkers of iron status across categories of CRP levels were assessed by the Kruskall–Wallis test. The Wilcoxon and chi-square tests were used to compare continuous and categorical paired variables. Simple linear regression analysis was performed to determine the relation between serum ferritin and age, breastfeeding, and consumption of iron-fortified milk.

Multiple logistic regression models were used to examine the association between consumption of fortified milk and the prevalence of abnormal serum ferritin after adjustment for potential confounding factors (sex, age, and breastfeeding). The level of statistical significance was set at $p < .05$. All statistical analyses were performed with StataIC, version 1.0.

TABLE 1. Demographic, nutritional, and biochemical characteristics of young children in Chile, 2009 ($n = 218$)

Characteristic	Value[a]
Age (yr)	2.6 (2.1–4.0)
Birthweight (g)	3,366 (481)
Birth height (cm)	49.6 (2.6)
Sex	
Male	129 (59.2%)
Female	89 (40.8%)
Hemoglobin (g/dL)	12.3 (1.0)
Hemoglobin < 11 g/dL (< 5 yr) or < 11.5 g/dL (≥ 5 yr)	8 (3.7%)
Zinc protoporphyrin (μg/dL RBC)	68.6 (59.9–79.9)
Zinc protoporphyrin > 80 μg/dL RBC	50 (22.9%)
Mean cell volume (fL)	77.2 (4.3)
Mean cell volume < 77 fL (1–2 yr), < 79 fL (3–5 yr), or < 80 fL (> 5 yr)	113 (51.8%)
Red blood cell distribution width (%)	14.6 (1.5)
Red blood cell distribution width > 14%	132 (62.4%)
Serum ferritin (μg/L)	13.2 (8.6–21.8)
Serum ferritin < 15 μg/L	123 (56.4%)
Serum CRP (mg/L)	6.7 (5.5–10.1)
CRP > 10 mg/L	55 (25.3%)
CRP > 6 mg/L	145 (66.5%)
CRP > 5 mg/L	188 (86.3%)

CRP, C-reactive protein; RBC, red blood cells

a. Values are mean (SD), median (25th–75th percentile), or number (%).

Results

Eighty-one outpatient health centers were visited until the sample size of 224 subjects was reached. Data for biochemical biomarkers were complete for 218 children, who were included in the analysis. The median (25th and 75th quartiles) age was 2.6 (2.1 and 4.0) years. Fifty-nine percent were boys. The mean (SD) birthweight was 3,366 (481) g and birth height was 49.6 (2.6) cm. The prevalence of anemia was 3.7%, and a high percentage had microcytosis (51.8%) and anisocytosis (62.4%) (according to mean cell volume and red blood cell distribution width, respectively). Serum ferritin was less than 15 μg/L in 56% and zinc protoporphyrin concentration was greater than 80 μg/dL RBC in 23%. The percentages of children with CRP concentrations greater than 10, 6, and 5 mg/L were 25%, 67%, and 86%, respectively (**table 1**).

Serum CRP concentration was positively associated with serum ferritin and zinc protoporphyrin concentrations ($r = 0.16$ and $r = 0.15$; $p = .0168$ and $p = .0290$, respectively). Serum CRP concentration was not associated with red blood cell distribution width ($p = .6583$) or with hemoglobin concentration ($p = .2716$) (**table 2**). Serum ferritin and zinc protoporphyrin concentrations were different across CRP intervals ($p < .001$). There were significant differences in both serum ferritin and

TABLE 2. Spearman's correlations between serum C-reactive protein (CRP) and biomarkers of iron status ($n = 218$)

Pair of variables	Spearman's coefficient	*p*
CRP–ferritin	0.16	.0168
CRP–zinc protoporphyrin	0.15	.0290
CRP–red blood cell distribution width	0.03	.6583
CRP–hemoglobin	–0.08	.2716

zinc protoporphyrin concentrations across CRP intervals ($p < .001$ and $p < .05$, respectively). Serum ferritin was significantly ($p < .001$ and $p = .003$, respectively) lower in > 5–6 mg/dL and > 6–10 CRP intervals than in the 0–5 mg/L CRP interval. Zinc protoporphyrin concentrations were significantly ($p = .038$) lower in the > 5–6 mg/dL CRP interval than in the 0–5 mg/L CRP interval (**table 3**).

Those with > 5 mg/L CRP had lower ($p = .029$) serum ferritin concentrations than those with ≤ 5 mg/L CRP, while those with > 10 mg/L CRP had higher ($p = .003$) serum ferritin concentrations than those with ≤ 10 mg/L CRP. There were no differences on zinc protoporphyrin concentrations for any CRP cutoff as categorical variable (**table 4**). **Table 5** shows medians, geometric median concentrations, and prevalence rates of abnormal serum ferritin (< 15 μg/L) and zinc protoporphyrin (> 80 μg/dL RBC) with and without adjustment by CRP cutoffs. The median serum ferritin and zinc protoporphyrin concentrations without adjustment were 13.2 μg/L and 68.6 μg/dL RBC, with 56.4% and 22.9% prevalence of abnormal concentrations, respectively. Exclusion of cases with CRP > 6 mg/L resulted in medians for serum ferritin and zinc protoporphyrin of 11.5 μg/L and 65.9 μg/dL RBC, with a 63.0% and 16.4% prevalence of abnormal concentrations, respectively. After adjustment using > 5 mg/L CRP there were no changes in median concentrations of serum ferritin or zinc protoporphyrin compared with values before adjustment. Also, with > 6 and >

TABLE 3. Serum ferritin and zinc protoporphyrin concentrations within CRP intervals ($n = 218$)[a]

CRP interval (mg/L)	No.	Ferritin (μg/L)	Zinc protoporphyrin (μg/dL RBC)
0–5	30	16.3 (9.4–22.3)[b]	67.1 (57.9–76.5)[b]
> 5–6	43	11.3 (7.2–15.4)***	62.9 (54.4–74.3)**
> 6–10	90	12.5 (9.1–20.2)**	71.4 (59.9–82.2)
> 10	55	17.4 (10.2–27.6)	68.6 (64.3–80.0)
p[c]		< .001	< .05

CRP, C-reactive protein; RBC, red blood cells
*$p < .05$; ** $p < .01$; *** $p < .001$ (p values by the two-sample Wilcoxon test).
a. Values are median (25th–75th percentile).
b. Reference group for pairwise comparison of serum ferritin and zinc protoporphyrin concentrations.
c. *P* values by the Kruskal–Wallis test for global comparisons.

TABLE 4. Serum ferritin and zinc protoporphyrin concentrations within groups defined by binary CRP levels ($n = 218$)

	Low level		High level		
CRP level	*n*	Median (25th–75th percentile)	*n*	Median (25th–75th percentile)	p[a]
Serum ferritin (μg/L)					
≤ 5 vs > 5	30	16.3 (9.4–22.3)	188	12.8 (8.6–20.7)	.029
≤ 6 vs > 6	73	12.5 (7.9–18.8)	145	13.9 (9.6–22.8)	.078
≤ 10 vs > 10	163	12.5 (8.5–19.8)	55	17.4 (10.2–27.6)	.003
Zinc protoporphyrin (μg/dL RBC)					
≤ 5 vs > 5	30	67.1 (57.9–76.5)	188	68.6 (59.9–79.9)	.355
≤ 6 vs > 6	73	65.6 (57.1–74.4)	145	68.7 (60.1–80.1)	.278
≤ 10 vs > 10	163	68.6 (57.2–79.9)	55	68.6 (64.3–80.0)	.638

CRP, C-reactive protein; RBC, red blood cells
a. P values were calculated by the Wilcoxon test for pairwise comparisons.

TABLE 5. Concentrations of serum ferritin and zinc protoporphyrin and the prevalence of their abnormal levels before and after adjustment ($n = 218$)

	Serum ferritin (μg/L)				Zinc protoporphyrin (μg/dL RBC)			
Adjustment[a]	CF	Median	GMC	< 15 μg/L (%)	CF	Median	GMC	> 80 μg/dL RBC (%)
5 mg/L	1.18	13.2	12.9	42.7	0.96	72.9	69.5	17.9
6 mg/L	0.84	12.1	11.4	61.5	0.92	64.4	65.8	17.4
10 mg/L	0.73	12.6	11.9	60.6	0.97	68.5	69.0	21.6
Excluding CRP > 5 mg/L		16.3	14.8	46.7		67.1	67.2	20.0
Excluding CRP > 6 mg/L		12.5	11.5	63.0		65.6	65.9	16.4
Excluding CRP > 10 mg/L		12.5	11.9	61.3		68.6	69.2	22.1
No adjustment		13.2	12.9	56.4		68.6	69.5	22.9

CF, correction factor; CRP, C-reactive protein; GMC, geometric mean concentration; RBC, red blood cells

10 mg/L CRP there were relatively similar abnormal serum ferritin concentrations being 61.5% and 60.6%, respectively. In the case of zinc protoporphyrin, after adjustment using CRP > 6 and > 10 mg/L, 17.4% and 21.6% presented abnormal concentrations, respectively.

There were no statistically significant differences in biomarkers of iron status or CRP between regions (data not shown). Sixty-eight percent of children regularly consumed iron-fortified milk delivered by NCFP, with a median daily consumption of 50 (40–75) g of powder, diluted at the recommended dilution (10%) supplying 3.1 (2.5–4.7) mg of iron. Based on a bioavailability of iron of 12.4% as determined by our laboratory, the estimated iron absorption was 0.4 (0.3–0.6) mg/day [8]. Among children who consumed this milk regularly, 60.4% had abnormal serum ferritin (< 15 μg/L), compared with 85.7% of children who did not consume this milk regularly ($p < .001$) (**table 6**). However, consumption of iron-fortified milk did not show a positive significant relationship with serum ferritin ($r = .02$, $p = .893$). In the same way, multiple logistic regression models did not show an association between consumption of fortified milk and the prevalence of abnormal serum ferritin (< 15 μg/L) after adjustment for sex, age, and breastfeeding (OR, 1.00; 95% CI, 0.99 to 1.01; $p = .288$). Serum ferritin was positively associated with age ($r = 0.11$, $p = .018$).The median duration of breastfeeding was 12 (6–22) months. At the time of the survey, 21% of children were still being breastfed. There was no association between hemoglobin and breastfeeding ($r = –0.13$, $p = .263$).

After adjustment for zinc protoporphyrin and serum ferritin using a CRP cutoff of 10 mg/L in the overall sample, 5.5% of children were classified as having iron-deficiency anemia, 42.7% as having iron-deficiency erythropoiesis, 17.9% as having depleted iron stores, and 35.8% as having normal iron status (**figure 1**).

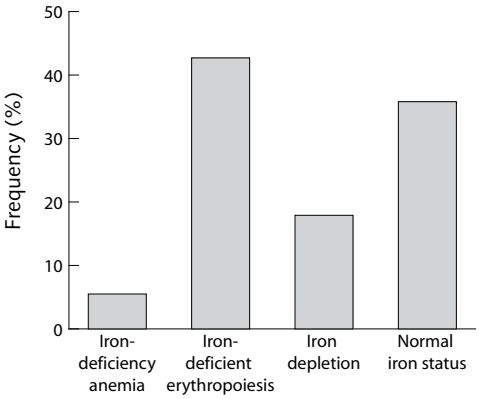

FIG. 1. Iron status prevalence among children aged 19 to 72 months in 2009 stratified by region ($n = 218$). There were no significant differences in iron status classification between regions. Zinc protoporphyrin and serum ferritin were adjusted using CPR cutoff >10 mg/L.

Discussion

This research was carried out to assess iron status and associations between biomarkers of iron status and serum CRP in young children who were beneficiaries of the NCFP in Santiago and Valparaiso, Chile. CRP was positively associated with serum ferritin and zinc protoporphyrin. Children aged 19 to 72 months had a low prevalence of iron-deficiency anemia but a high prevalence of iron-deficiency erythropoiesis and depleted iron stores after adjustment for inflammation.

Our sample had a low prevalence of iron-deficiency anemia. However, it is important to note the high frequency of children with iron-deficiency erythropoiesis (42.7%) and depleted iron stores (17.9%). It has been reported that in the United States, the prevalence of iron deficiency in this age group based on the body iron model fluctuates between 3% and 14% [16]. However, to compare iron deficiency based on the body iron model with our data has limitations, because we did

TABLE 6. Biomarkers of iron status and C-reactive protein stratified by children who regularly consumed and did not consume iron-fortified milk delivered by NCFP ($n = 218$)

	Consumed milk ($n = 149$)		Did not consume milk ($n = 69$)		
Biomarker	Mean (SD) or median (25th–75th percentile)	Abnormal (%)[a]	Mean (SD) or median (25th–75th percentile)	Abnormal (%)[a]	p^b
Hemoglobin (g/dL)	12.4 (0.9)	2.7	12.3 (1.1)	5.8	.634
Zinc protoporphyrin (μg/dL RBC)[c]	68.4 (59.6–77.7)	22.1	68.6 (59.9–77.5)	28.6	.355
Serum ferritin (μg/L)[c]	12.6 (8.5–19.3)	60.4	12.4 (7.1–20.4)	85.7	< .001
Red blood cell distribution width (%)	14.3 (13.7–15.0)	63.1	14.3 (13.9–15.1)	60.9	.870
CRP (mg/L)	6.5 (5.4–9.8)	24.8	6.8 (5.7–10.3)	36.7	.116

CRP, C-reactive protein; NCFP, National Complementary Feeding Program; RBC, red blood cells

a. Cutoffs for abnormal vaues were as follows: hemoglobin, 11 g/dL for children < 5 years and 11.5 g/dL for children ≥ 5 years; zinc protoporphyrin, 80 μg/dL RBC; serum ferritin, 15 μg/L; red blood cell distribution width, 14%; CRP, 10 mg/L.

b. *P* values were calculated by the chi-square test for differences in prevalence rates of abnormal biomarker values.

c. Adjusted by using CRP cutoff equal to 10 mg/L.

not conduct a study with a similar design and we did not include determinations of other biomarkers such as soluble transferrin receptor to perform the body iron model. Independently of these limitations, these large differences suggest that iron intake in United States is higher than in Santiago and Valparaiso even with the support of the NCFP.

It has been reported that inflammation influences biomarkers of iron status [17]. In this study, we observed a high prevalence of elevated conventional CRP. A study conducted in Guatemalan schoolchildren aged 9.0 (1.2) years found only 2.7% and 7.4% of children with elevated concentrations of high-sensitivity CRP of > 10 and > 5 mg/L, respectively [14]. Our study found much higher rates of 25.3% and 86.3%, respectively. This result may suggest that it is necessary to use a CRP cutoff > 10 mg/L, as the kit provider recommends, but it also indicates a high prevalence of inflammation. Chile has sanitation and hygiene standards that are far superior to those of other developing countries. However, we believe that these high rates may have been a consequence of a high prevalence of viral respiratory infections, independently of sanitation. Most of the children who participated in this research attended nursery schools, where the frequency of infection is greater. Moreover, approximately half of the samples were obtained during winter, increasing the risk of infection.

We observed a positive correlation between CRP and both serum ferritin and zinc protoporphyrin. In this context, a recent study conducted in Kenyan preschool children aged 6 to 35 months also showed the same associations [18]. Serum ferritin was higher in children with elevated CRP (> 10 mg/L). Serum ferritin was higher in children with CRP ≤ 10 mg/L than in those with CRP > 10 mg/L, but it was also higher in children with CRP ≤ 5 mg/L than in those with CRP > 5 mg/L. No significant changes were observed in zinc protoporphyrin levels within groups defined by binary CRP levels, We showed the prevalence of iron status adjusting by > 10 mg/L CRP, because there were inconsistencies with the use of > 5 mg/L and because there were no significant differences in the concentrations of serum ferritin neither zinc protoporphyrin concentrations within groups defined by > 6 mg/L binary CRP level.

Multiple variables affect iron status. We observed that serum ferritin was positively associated with age. This situation is consistent with the normal increases of serum ferritin at older ages [19]. Our study showed no significant differences in biomarkers of iron status between children from the Santiago Metropolitan and Valparaiso areas. In Chile, the economic development of the provinces is not very different from that of the capital; Chile has a small rural population. We observed a high prevalence of abnormal serum ferritin in those children who regularly consumed iron-fortified milk delivered by NCFP. However, there was no association between serum ferritin and consumption of this milk, and no association between the frequency of abnormal serum ferritin and consumption of iron-fortified milk after adjustment for confounding factors.

In Chile, 100% of children aged 0 to 5 years attending private and public schools have free access to NCFP. However, the 2008 census of the Chilean Ministry of Health found a national coverage of 82.4% (the percentage of mothers who received fortified foods from NCFP) [9]. The present research was conducted in the Santiago and Valparaiso regions, which contain 60% of the national population. At the time the study was designed, the Santiago and Valparaiso regions together had 144 health centers. In the present research, 81 health centers were visited until the total sample size was reached with an average of approximately three children participating for each health center. It is important to note that 100% of the children who participated in this research were recipients of NCFP. However, there were mothers who reported that their children did not consume the iron-fortified milk delivered by this program. Although we validated our dietary survey, we cannot rule out under- or overestimations of dietary intake due to the facts that this survey was conducted only once and that the response depended on the subjectivity of the mothers. There was no association between breastfeeding and serum ferritin. However, there are studies supporting an association between prolonged breastfeeding and lower iron status [20, 21]. Our study had the limitation that in most cases data were retrospectively obtained from the mother, increasing the possibilities of under- or overestimation. We could not determine the level of influence of supplementation with iron due to a lack of accuracy in the responses of the mothers.

This study was done under standardization and quality control in the biomarker assays, but we did not measure total iron-binding capacity, transferrin saturation, or soluble transferrin receptor; this constrains us from performing more advanced models and comparing our results with data from developed countries. However, the American Pediatric Association has reported that it is correct to establish a diagnosis of iron status as in our evaluation [19]. This research is limited by the use of conventional CRP instead of high-sensitivity CRP and also by the use of a single biomarker of inflammation. The use of conventional CRP did not allow us to use a higher range of CRP cutoffs to adjust for inflammation. Moreover, adjustment using a single biomarker of inflammation has lower precision than adjustment using combined biomarkers, i.e., α_1-acid glycoprotein plus high-sensitivity CRP.

Conclusions

CRP was positively associated with serum ferritin and zinc protoporphyrin in apparently healthy young children. Chilean children aged 19 to 72 months from Santiago and Valparaiso who were beneficiaries of NCFP had a low prevalence of iron-deficiency anemia, a high prevalence of iron-deficiency erythropoiesis, and a moderate prevalence of depleted iron stores.

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