Iron Supplementation in Previously Anemic Bolivian Children Normalized Hematologic Parameters, But Not Immunologic Parameters

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Summary

Iron deficiency anemia (IDA) is considered to be the most prevalent micronutrient deficiency in the world. Estimates indicate that 1.2 billion people suffer mild to severe forms of anemia and that up to 46% of schoolchildren in developing countries are affected. In 2003, ENDSA, the national demographic and health survey of Bolivia showed that 60% of children under five and 72% of children under 2 years old were anemic. Micronutrient deficiency has been suggested to impair cell-mediated immunity. In particular, iron, zinc and vitamin A deficiencies have an impact on the immune system. *In vitro* and *in vivo* laboratory studies indicate a link between iron deficiency and impaired T-lymphocyte proliferation. The exact effects or mechanisms of iron deficiency on maturation and proliferation of T-lymphocytes *in vivo* are, however, not yet known. This study investigated the effects of iron on the maturation of T-lymphocytes in anemic but otherwise healthy schoolchildren (no apparent protein-energy deficiency or other morbidity). Anemic children of a poor peri-urban school of Cochabamba city, Bolivia, were given iron treatment for three consecutive months. We chose to look at CD1a+ lymphocytes, which are immature thymocytes. The proportions of CD1a+ lymphocytes in the peripheral circulation measured at baseline and after treatment were compared with a reference group of age-matched non-anemic children controls from the same school. The immunologic parameters, although improved, did not reach the proportions of the control group. Overall, the proportion of circulating immature T-lymphocytes decreased from 18.3% to 9.2% in the treated following iron supplementation in anemic children, compared with 3.4% in non-anemic children.

Key words: iron deficiency, CD1a lymphocyte, school children.

Introduction

Iron deficiency anemia (IDA) is considered to be the most prevalent micronutrient deficiency in the world. Estimates indicate that 1.2 billion people suffer mild to severe forms of anemia and that up to 46% of schoolchildren in developing countries are affected [1]. In 1994, the national demographic and health survey of Bolivia showed that 34.7% of children between 6 to 12 years old were anemic.

Micronutrient deficiency has been suggested to impair cell-mediated immunity. In particular iron, zinc and vitamin A deficiencies all have an impact on the immune system [2]. But while the protective effect of zinc and vitamin A against infection is well established, there remains considerable controversy over the effects of iron [3]. Still, iron deficiency seems to have an effect on the immune status [4]. Humoral immunity seems to be largely spared in studies of iron deficient mice, but bactericidal activity of polymorphonuclear leukocytes and T-lymphocyte function appears to be impaired [5, 6].

Laboratory studies on mice and *in vitro* indicate a direct link between iron deficiency and impaired T-lymphocyte proliferation [7, 8]. The effects of iron deficiency on maturation and proliferation of T-lymphocytes in human are, however, less well documented. Inconclusive results could be attributed to the inability to control for interfering infections or other health problems [9]. Furthermore, most studies

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use markers of the mature stages of T-lymphocyte subsets, only indirectly indicating problems during T-cell maturation.

Iron deficiency induces thymus atrophy in laboratory animals and very likely in humans by unknown mechanism, possibly by reduced cell proliferation or T-cell differentiation which is associated with iron deficiency [10]. Studies *in vitro* also show that iron is necessary for lymphocyte proliferation [8, 11, 12]. Although there is a positive effect of iron on cellular immunity, the discrepancies in the literature, particularly among the results of human studies make it difficult to conclude: first, if the abnormalities of immune function can be attributed specifically to iron deficiency, and second the ultimate biological and clinical relevance of any abnormalities detected [5]. However, there are a number of studies that try to prove the effects of iron deficiency on immune status, especially cell-immunity [5, 9, 10, 13, 14].

The soluble transferrin receptor CD 71 (sTfR) is a clinical marker of erythropoietic activity, also used in the diagnosis of iron deficiency [15]. Also, TfR represents a valuable quantitative assay of marrow erythropoietic activity as well as a marker of tissue iron deficiency [16]. Recently the soluble serum transferrin receptor (sTfR) has been introduced as a promising new tool for diagnosing iron depletion [17].

The same authors have defined CD1a as a marker of the most immature thymocyte populations, and there is agreement among the studies that CD1a is therefore a useful marker of post-selection and post-thymic T-cell maturation [18, 19]. Past studies have found in malnourished children a higher proportion of circulating immature T-lymphocytes (CD1a+) and a lower proportion of mature T lymphocytes (CD4+ and CD8+), and that values have been reversed after adequate micronutrient and macronutrient repletion [20, 21].

The aim of this study was to investigate the effects of iron on the maturation of T-lymphocytes in anemic but otherwise healthy schoolchildren (no apparent protein-energy deficiency or other morbidity). Anemic children of a poor peri-urban school of Cochabamba city, Bolivia, were given iron treatment during 3 consecutive months. The proportions of CD1a+ lymphocytes in the peripheral circulation were measured at baseline and after treatment and compared with a reference group of age-matched non-anemic children of the same school.

Material and Methods

Population and intervention

Hemoglobin concentrations of all 368 children of a school in a poor peri-urban area of Cochabamba City—Bolivia, were measured. Anemic children received a daily iron supplement (3–4 mg FeSO4/kg body weight) for 5 consecutive days (Monday to Friday) during 12 weeks, one and a half hours after breakfast, and under direct supervision. The conventional cut-off value for anemia was defined as 12.7 mg/dl Hb, to compensate for the altitude (2500 m above sea-level), using the formula proposed by Ray Rip: $Hb = -0.032\ (alt \times 3.3) + 0.22\ (alt \times 3.3)(alt \times 3.3)$. Controls were age-matched children with a hemoglobin concentration ≥ 14.5 g/dl.

Exclusion criteria were: (i) Children with a history of having taken any iron, multivitamin or other mineral supplements under any form (breakfast biscuits at school or prescription by their physician, etc.) within the last four months, as reported by the teachers, the parents or the children. (ii) Children with any type of acute or chronic disease, as reported by the teachers, the parents or the children. (iii) Weight-for-height of ≤ -2 SD of the mean NCHS (National Centers for Health Statistics). (iv) Children with a Hb below 9.0 g/dl. All these children excluded, were given a treatment appropriate to their condition.

The study was approved by the Technical Committee of the Biomedical Research Institute of the Faculty of Medicine, of University of San Simon of Cochabamba. The Ministry of Education, all school directors and teachers and the children's parents (the father) gave their written consent.

Laboratory methods

Blood samples were taken at the start of the study and after 12 weeks of iron supplementation. 6 ml venous blood was drawn via venipuncture and evacuated in three tubes:

- Total of 0.5 ml of blood was drawn into tubes containing 20 µl of EDTA (ethylene-diamino-tetra-acetate) as anticoagulant for determination of hemoglobin and hematocrit levels. Hemoglobin concentration was determined by the cyanomethemoglobin method (Eagle Diagnostics—hemoglobin procedure from USA) and Hematocrit by centrifuging at a speed of 10 000 rpm for 5 min.
- Total of 1.5 ml of blood was drawn into a dry tube for determination of serum ferritin, assessed by micro-ELISA, serum transferrin assessed by simple radial immuno-diffusion and serum iron assessed by colorimetry on total blood.
- Four milliliters of blood in a test tube containing heparin as anticoagulant for determination of cellular transferrin receptors, (CD71) and CD1a lymphocytes. Lymphocytes CD71 and CD1a were isolated from blood by Ficoll-Hypaque gradient density centrifugation. Monoclonal anti-T cell antibodies of the OKT series (DAKO Denmark and Ficoll Hypaque of Pharmacia-France) were used to evaluate the proportion of T-lymphocyte subsets by indirect immunofluorescence [21].

TABLE 1
Comparison of study groups at baseline

	Cases ($n = 38$) Mean (SD)	Control ($n = 33$) Mean (SD)	p[a]
Boys (%)	65.8	51.5	0.22
Age, months	107.0 (21.5)	96.36 (16.0)	0.22
Weight, kg	24.6 (6.7)	23.2 (4.2)	0.03
Height, cm	124.3 (9.6)	121.1 (7.9)	0.14
Weight-for-age, *Z*-score	−0.5 (1.2)	0.03 (1.1)	0.09
Height-for-age, *Z*-score	−1.2 (0.8)	−1.0 (1.0)	0.20
Hemoglobin, g/dl	11. 89 (1.1)	15.08 (0.9)	<0.0001
Transferrin, mg%	5.9 (1.1)	3.68 (0.6)	<0.0001
Serum iron, μg/dl	82.9 (53.6)	118.4 (31.9)	0.0004
Serum ferritin, ng/dl	35.6 (31.6)	160.2 (79.4)	<0.0001
Total number of lymphocytes, count/ml	1482.1 (577.0)	1522.1 (570.9)	0.77
Proportion of CD71 lymphocytes, %	16.2 (3.5)	2.8 (1.5)	<0.0001
Proportion of CD1a lymphocytes, %	18.3 (3.9)	3.4 (1.7)	<0.0001

[a]Brownie, *et al.*'s modified *t*-test.

Counting was performed on at least 200 cells under a UV fluorescence microscope.

The children were weighed and their height was measured at the beginning and at the end of the study. Body height was measured twice with a precision of 0.1 cm and the mean value calculated. Body weight was measured to the nearest 0.1 kg with a mechanic scale (Health Balance Bean Scale—USA). Age was calculated from the date of birth as indicated in the school register.

Results and Discussion

Of the 368 children in the school, 43 proved to be anemic. Five of these children had Hb level below 9 g/dl and thus were excluded from the study. A total of 98 children had Hb levels ≥14.5 g/dl. Of these 33 were age-matched as a reference group.

The prevalence of anemia defined as Hb < 12.7 g/dl was 11.7%. At baseline there were no differences between the treatment group and the control group for their age, sex distribution for their nutritional status. Both groups were well nourished. All hematologic and immunologic parameters, except for total lymphocyte count, were significantly different in both groups (Table 1).

After 12 weeks of iron supplementation, hematological and immunological parameters improved significantly (Table 2). The average improvement in hemoglobin was +2.4 g/dl, serum iron +34.8 μg/dl and serum ferritin 94.8 ng/dl. There were also significant improvements in the CD1a+ and CD71+ lymphocyte counts. Both subsets of lymphocytes decreased almost half. (Table 2).

Compared with the control group, all hematologic parameters (hemoglobin, transferrin, serum iron, serum ferritin and transferrin receptor CD71 lymphocytes) were normalized. CD1a as immunologic indicator, although it improved, did not reach the same level as the control group. On average, the proportion CD71 lymphocytes was still at 8.4% compared with 2.8% in the control group and the CD1a lymphocyte proportion was at 9.2% compared with 3.4% (Table 3).

TABLE 2
Changes in immune and other parameters in the treatment group after supplementation with iron (paired t-test)

	Cases ($n = 38$) Mean change (SD)	p
Weight-for-age	+0.45 (1.3)	0.05
Hemoglobin, g/dl	+2.4 (1.7)	<0.0001
Transferrin, mg%	−1.3 (1.0)	<0.0001
Serum iron, μg/dl	+34.8 (60.2)	0.002
Serum ferritin, ng/dl	+94.8 (59.1)	<0.0001
Total number of lymphocytes, count/ml	+366.0 (586.6)	0.005
Proportion of CD71 lymphocytes, %	−7.8 (4.7)	<0.0001
Proportion of CD1a lymphocytes, %	−9.1 (4.9)	<0.0001

This study shows that iron deficiency in school children has a significant effect on the levels of the circulating immature lymphocyte subpopulations. This was demonstrated by determining the numbers of CD1a+ cells in peripheral blood, which were significantly decreased in iron deficient subjects.

The results of the present study clarify the relationship between iron deficiency and the inability of lymphocytes to mature in anemic schoolchildren without any other illness.

The fact that in our study the proportions of CD1a and CD71 markers were increased in the anemic group and decreased following iron supplementation

TABLE 3
Comparison of treatment group after three months of iron supplementation with control group at baseline

	Treated cases ($n = 38$) Mean (SD)	Controls ($n = 33$) Mean (SD)	p[a]
Weight-for-age	−0.03 (1.3)	0.03 (1.1)	0.29
Hemoglobin, g/dl	13.9 (2.6)	15.08 (0.9)	0.11
Transferrin, mg%	4.63 (0.7)	3.68 (0.6)	<0.0001
Serum iron, μg/ml	113.3 (53.6)	118.4 (31.9)	0.55
Serum Ferritin, ng/ml	130.4 (71.4)	160.2 (79.4)	0.16
Total number of lymphocytes, count/ml	1948.1 (541.0)	1522.1 (570.9)	0.006
Proportion of CD71 lymphocytes, %	8.4 (2.8)	2.8 (1.5)	<0.0001
Proportion of CD1a lymphocytes, %	9.2 (2.3)	3.4 (1.7)	<0.0001

[a]Brownie, *et al.*'s modified *t*-test.

in the same group suggests that iron has a positive effect on proliferation and maturation of T-lymphocytes. But the fact that the proportions did not normalize up to the levels of the reference group may indicate that other factors were not optimal in the study population. Height and weight measurements did not suggest any protein-energy deficiencies. Further study on deficiency in zinc and/or vitamin A is needed in this population.

We found high levels of CD1a+ immature lymphocytes in peripheral blood of anemic children. It has been described that as CD1a+ increases, the mature CD4 lymphocytes decrease and the function is altered in relation to immune status [21], in severely malnourished children [9]. We provide one more argument towards understanding the relationship between anemia by iron deficiency, immunity and infection and developing comprehensive programs to reduce iron deficiency and infections in developing countries, as Bhaskaram [3], wrote.

One of the most interesting ideas this study emphasizes is the debate over whether or not iron deficiency (without anemia) should be corrected to prevent disease or other functional impairments, such as cognitive development and performance.

The fact that the proportions of circulating immature lymphocytes were not normalized raises the idea of an added effect of supplementation with more than one micronutrient. We are continuing to address this question, and are looking at the efficacy of weekly vs. daily iron supplementation on hematologic and immunologic parameters.

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