ORIGINAL ARTICLE

Effects of Iron Supplementation on Hemoglobin Concentration in 12-18 Month Old Iron-Deficient Anemic Infants

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ABSTRACT A randomized double blind clinical trial was conducted to study the effects of iron supplementation on hemoglobin values of 97 iron-deficient anemic (IDA) and iron-sufficient (IS) infants, 12 to 18 months of age. IDA (n=50) infants were assigned randomly to either receive dietary ferrous sulfate or placebo for 4 months. Similar treatment randomization was done among IS (n=47) infants. Before intervention, the mean hemoglobin value of the IDA infants was significantly ($p<0.01$) lower than that of the IS group. After intervention, there was an increase in the hemoglobin values of the IDA infants who had received iron, and was significantly ($p<0.01$) higher than those of the placebo-treated IDA group, IS group, as well as placebo treated IS group. Hemoglobin values of the IDA infants who had received iron, increased up to the normal level as refer to the WHO's criteria used for normal hemoglobin values. **[Paediatr Indones 1994; 34: 179-186]**

Introduction

Iron deficiency anemia (IDA) is still a public health problem in the developing countries as well as in affluent societies.[1] It is by far the most common form of nutritional deficiency and represents the most common cause of a subnormal hemoglobin values.[2] It is particularly prevalent among infants and young children. In Indonesia, the prevalence of IDA among children between 6 months and 6 years of age belonging to a low socioeconomic but well nourished group ranged from 37.8 to 73.0%.[3] It has a peak prevalence during the first two years of life because the rapid growth in that period imposes large iron needs while most of infant diets used in general population contain marginal supply of iron.[2]

Deficiency in total body iron ranges in severity from a clinically occult state re-

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cognizable only by biochemical assays to a life threatening condition characterized by severe anemia, exercise intolerance, and exhaustion of tissue iron containing enzymes. In addition to its recognized role in oxygen transport (hemoglobin) and iron storage (myoglobin), iron is also a structural part or co-factor for enzymes critical in oxidative metabolism, DNA synthesis, neurotransmitter synthesis, and also in mitochondrial cytochromes in many organ systems of the body.[4]

The consequences of iron deficiency other than anemia are non-hematologic alteration attributed to the exhaustion of tissue iron. In infants, adverse effects on behavior are of special concern because the later part of the brain growth spurt and the unfolding of fundamental mental, and motor processes coincide with the period in which IDA is most prevalent.[5] Iron deficiency anemia in infancy might thus have more serious and less reversible effects than comparable iron deficiency occurring later in life.

Because of its high prevalence and its long-term consequences due to either anemia or non-hematologic effects, iron treatment could be performed immediately, i.e., by iron supplementation. Iron supplementation ideally be maintained for a longer period to allow accumulation of storage iron. This objective can be accomplished by means of continuing treatment for a total of about 4 to 5 months.[2]

This randomized clinical trial was designed to evaluate the effect of iron supplementation on hemoglobin values in twelve to 18-month old iron-deficient anemic infants.

Methods

Subjects

The study was approved by the Ethics Committee of the Medical School, Padjadjaran University, Bandung, Indonesia. Ninety-seven 'healthy' infants aged 12 to 18 months attending (over a 3-month period) the Under Five Clinic, Department of Child Health, Medical School, Padjadjaran University-Hasan Sadikin Hospital, Bandung, were considered as potential subjects. These infants met the following criteria:[5] (1) birth weight equal or greater than 2500 g; (2) had no major congenital anomalies; (3) no hospital admission or supplementation with micronutrient in the last six months prior to enrollment, (4) no clinically identified neuromotor delay, (5) no major acute or chronic illness; (6) no folic acid deficiency; (7) hemoglobin at least 80 g/L; (8) no signs of abnormal hemoglobin or thalassemia; (9) weight, length, and head circumference within 2 standard deviations of the reference standards from the US National Center for Health Statistics (1977).

Those 97 infants also met the criteria for one of two iron-status classes, i.e., iron deficient anemia class (IDA) with hemoglobin level < 105 g/L, transferrin saturation < 10%, and serum ferritin < 12 μg/L, or iron sufficient class (IS) with hemoglobin level > 120 g/L, transferrin saturation > 10%, and serum ferritin > 12 μg/L). Infants with a hemoglobin level between 106 and 119 g/L were excluded. Infants with hemoglobin level of < 80 g/L were also excluded from the study but treated appropriately. Likewise, once

the study was completed, all those subjects who persisted to be classified as iron deficiency anemia were treated with ferrous sulfate following the schedule and dosage used during the trial.

Because parents of 6 infants declined to complete the course of the study, only 91 subjects were investigated. Random assignment of those 91 infants to either receive the iron treatment or placebo was done separately for each iron-status class by a table of random numbers.[6]

Intervention

Iron treatment consisted of ferrous sulfate in syrup form at a dose of 3 mg elemental iron per kg for 4 consecutive months. The placebo was a syrup similar in appearance to the ferrous sulfate, and both had a sweet, cherry flavor. Either the ferrous sulfate or the placebo were produced by P. T. Kimia Farma, an Indonesian pharmaceutical company. Parents gave the syrup to infants at home. Compliance was checked weekly by a nurse who visited infant's home and determined the amount of syrup consumed. A hemoglobin response of at least 10 g/L to iron treatment among IDA infants was used as a reference criterion to determine the sensitivity and specificity of the diagnostic criteria with the reference yielded a sensitivity of 100% and specificity of 77%.

Hematological Methods

Venipuncture blood specimens were collected by the technician after obtaining a given child's history and completing the physical examination. Both the technician and pediatrician remained unaware of each child's hematological status until their participation in the study was completed. All assays were conducted by other laboratory personnel, who had no direct contact with the families.

Hemoglobin determinations were done by cyanmet-hemoglobin method using Merck hemoglobin standard. Children whose blood was collected by venipuncture also had the following additional assays on 8 ml of blood: peripheral smear stained with Giemsa stain, serum iron (Ferritin Kit, Bio Merieux), total iron binding capacity (TIBC Kit and Ferrimat kit, Bio Merieux), serum ferritin (Enzyme-immuno assay method with serum ferritin kit, Bio Merieux), vitamin B_{12} or folic acid,[7] hemoglobin eletrophoresis (Helena Laboratories), and HbA_2 by microcolumn chromatography (Helena Laboratories). Transferrin saturation was calculated by the ratio of serum iron to total iron binding capacity, x 100. Iron measures were repeated 4 months later.

Variables: Background Factors

To aid in the interpretation of the effects of iron supplementation on iron status, information on background factors were collected, i.e., sex, age, birth weight, and anthropometric measurements.

Statistical Analysis

All analyses were performed using standard statistical packages.[8] Differences between groups on continuous background variables were evaluated by analysis of variance; differences between the groups on categorical variables were

assessed by X^2 tests. Two sets of analysis tested for the effects of the iron intervention on the hematological outcomes. First, to maximize the advantages of randomization, anova with repeated measures was calculated (SAS statistical software, general linear model type III, SAS, Cary, North Carolina, USA). Thus, each iron-status class was analyzed for: (1) differences between treatment groups (i.e., ferrous sulfate and placebo) in changes over the period of intervention, and (2) post-treatment differences between treatment groups after controlling for possible pre-treatment differences. The second set of analyses was primarily concerned with differences between iron-status classes and was a backup for the first set of analyses. Anova with repeated measures was calculated on hematological variables, including iron status class and intervention as between group variables and time as the within group variable. Interactions of the predictor variables were also included in the model.

Results

A reference criterion for the diagnosis of IDA is a response to iron treatment as indicated by a change of hemoglobin value (i.e., > 10 g/L).[9] This criterion was used to assess the sensitivity and specificity of the criterion used to classify the subjects. A comparison of the criterion with the reference yielded a sensitivity of 100% and specificity of 77%.

Study Group Characteristics

Infants in each group were compared on measures of sex, age, birth weight, and growth (Table 1). The groups were comparable in all respects, i.e., sex, age, birth weight, weight, length, and weight for-length percentile.

Table 1. Sex mean (SEM) age, birth weight, weight, and weight for length percentile by iron status classification (randomization)

	IDA (n=50)	IS (n=47)
	x (SEM)	x (SEM)
Sex (% male)	54	43
Age (mo)	14.4 (0.3)	14.3 (0.3)
BW (g)	3105 (63.4)	3166 (65.4)
Weight (kg)	9.27 (0.12)	9.31 (0.12)
Length (cm)	75.9 (0.4)	76.0 (0.4)
Weight for length percentile	24 (3.0)	27.0 (3.0)

SEM : Standard error of mean; IDA = iron deficiency anemia; IS = iron sufficient

Before treatment, hemoglobin, transferrin saturation, and serum ferritin of IDA infants were significantly ($p<0.01$) lower than that of the IS group (Table 2).

The mean and standard errors for hemoglobin for each class and treatment group before and after treatment are presented in Table 3. When analyzed by iron-status class, mean pre-treatment values of hemoglobin did not differ significantly in infants received iron compared to the placebo group (Table 3).

The Anova with repeated measures of the hemoglobin values showed that the main effects of iron status, treatment, and time were statistically significant ($p<0.01$). However, the three bivariate interactive terms as well as three-way interaction were also significant ($p<0.01$).

Table 2. Mean (SEM) hemoglobin, transferrin saturation, and serum ferritin by iron status classification (before randomization)

	IDA x (SEM)	IS x (SEM)	F	p
Hb (g/dl)	96.5 (0.6)	127.4 (0.7)	692.3	0.0001
Transferrin saturation (%)	7.6 (0.9)	24.3 (0.9)	106.9	0.0001 **
Ferritin serum (µg/dl)	6.9 (1.4)	28.8 (1.4)	68.6	0.0001**

* : Analysis of variance ('one way Anova')
** : Using log transformation

Table 3. Mean (SEM) hemoglobin before and after administration of ferrous sulfate (Rx) or placebo (Pl) by iron status classification

	IDA		IS	
Hb	Rx (n=24)	Pl (n=23)	Rx (n=22)	Pl (n=22)
Before (g/dl)	95.7 (1.1)	98.0 (1.0)	126.1 (0.7)	128.8 (1.1)
After (g/dl)	129.4 (1.9)	107.1 (0.9)	131.4 (1.5)	128.0 (1.7)

The changes (T_2-T_1) produced by ferrous sulfate on hemoglobin values (i.e., from 95.7 to 129.4 g/L) in the IDA subjects was significantly ($p<0.01$) larger than those in any of the other 3 subgroups, i.e., from 98.0 to 107,1 g/L for placebo treated IDA class, from 126,1 to 131,4 g/L for iron treated IS class, and from 128,8 to 128,0 g/L for placebo treated IS class infants. After 4 months of iron supplementation, the mean hemoglobin of iron-treated IDA subjects (129.4 g/L) was no longer significantly different when compared to that of the IS infants.

Table 4. Summary of between and within analysis from Anova with repeated measures: Hemoglobin

	Hemoglobin	
	df	F
Between groups		
Iron status (Fe)	1	269.12*
Treatment (Rx)	1	37.38*
Fe x Rx	1	11.75*
Error	87	
Within groups		
Time (T)	1	135.28*
(T2-T1)		
T x Fe	1	77.68*
T x Rx	1	82.38*
T x Fe x Rx	1	15.77*
Error	87	

*p < 0.01

The post-treatment distribution of hemoglobin of the iron-treated IDA infants is presented at Figure 1. Post-treatment, all of the iron-treated IDA infants (100%) and only 5 (21.7%) of the placebo-treated IDA infants had hemoglobin value above 110 g/L, the usual cutoff point for definition of anemia in this age group.[1] This difference was statistically significant ($p<0.01$). Twenty-one (84%) of the iron-treated IDA infants had hemoglobin value above 120 g/L, while none of the placebo treated infants had achieved such level of hemoglobin above 120 g/L, the criterion for inclusion in the IS class.

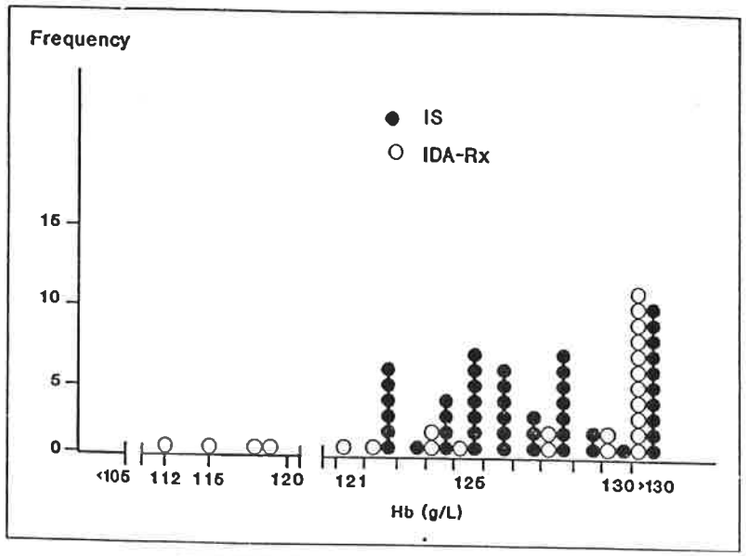

Figure 1. Distribution of hemoglobin of the IDA-Rx infants post-treatment and IS infants before treatment

Discussion

Data on iron-status classes revealed that during intervention, changes in hemoglobin values among iron-treated IDA infants was significantly larger ($p < 0.01$) than that of the changes among placebo-treated IDA infants (3-way interaction, $p<0.001$), and the mean post-treatment hemoglobin values of the iron-treated IDA subgroup was not significantly different from the values in either subgroup of IS infants.

The increment in the mean hemoglobin of the iron-treated IDA subgroup was 33.7 g/L. The same increment was 9.1 g/L in the hemoglobin of the placebo-treated IDA subgroup. The randomized blinded design used in this study makes it is unlikely that the significant difference between iron and placebo-treated IDA infants in changes during intervention in hemoglobin could depend on errors associated with subjects selection or laboratory examination. Difference is explained by the different treatment given to the two subgroups of IDA infants. In fact, based on these findings and maintaining all other variables constant, it is to be expected that after iron supplementation the hemoglobin of IDA infants will be similar to that of infants without signs of iron-deficiency anemia. Thus, treatment with iron in IDA infants results in the increment of their hemoglobin concentration reaching values which were equal to that of the IS infants. The improvement in hemoglobin concentration of iron-treated IDA infants with iron treatment has important clinical implications. Moreover, the improvement in hemoglobin concentration and in replenishing iron stores caused the acceleration of weight gain and reversal of developmental delays among IDA infants.[10]

The distribution of the normal hemoglobin values in the iron-treated IDA infants was significantly higher than that of the placebo-treated IDA subgroup. Furthermore, ferrous sulfate intervention resulted in normalization of iron status (transferrin saturation >10% and ferritin serum > 12 µg/L in 91.7% of the iron-treated IDA subjects, and replenishment of iron stores in all cases of iron-treated IDA subjects.[11] This result indicated that ferrous sulfate intervention resulted in normalizing hemoglobin levels (Hb > 110 g/L) and replenishing iron stores in all cases of the iron-treated IDA group. Only a small number of placebo-treated IDA subjects had a normal hemoglobin level after treatment. These results show that dietary iron could not generally cover the physiological iron requirement of the infants. In developing countries, it might be due to the negligible amount of heme iron consumed. Thus, non-heme is the main type of dietary iron in which its absorption is low.[12]

A closer look at the individual hemoglobin value of iron-treated IDA and IS subjects post-treatment showed that 1 (4.5%) infants belonged to the IS group had hemoglobin value of 148 g/L. This hemoglobin value is still lower than that of the upper limit of the normal range for the age group of 6-24 months as described by Viteri.[13]

Those findings, however, might not be generalized to more severe forms of anemia. The lowest level of anemia accepted for case inclusion in this study was 80 g/L. Likewise, the external validity of the finding does not extend to other age groups in infancy of childhood periods.

In conclusion, in infants 12 to 18 months of age with iron deficiency anemia, iron supplementation with ferrous sulfate at a dose of 3 mg elemental iron per kg for 4 consecutive months results in an increase of hemoglobin value up to the normal level as referred to the WHO's criteria used for normal hemoglobin values.

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