Effect of Iron Supplementation on Development of Iron Deficiency Anemia in Breastfed Infants

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Summary

This trial aimed to investigate the effect of iron supplementation on the development of iron deficiency anemia. The study encompassed 6-month-old infants who had been exclusively breastfed in the first 4 months of life. Infants in the supplemented group were given 1 mg $kg^{-1}day^{-1}$ ferrous sulfate for 6 months starting at 6 months of age. Blood samples were taken at age 12 months. A 3-day-diet was evaluated at 1 year of age. Data of 51 infants in the supplemented and 54 infants in the control group were analyzed. Mean hemoglobin values were similar in the two groups at the age of 12 months. Mean ferritin level of the supplemented group was significantly higher than that of the control. There was a significant positive correlation between dietary iron intake and hemoglobin levels. Nutrition might be more important than iron supplementation in preventing iron deficiency anemia during infancy.

Key words: breastfeeding, iron deficiency anemia, iron supplementation, infants, anemia.

Introduction

For countries where prevalence of anemia among 12-month-old children is above 40%, iron supplementation starting from 6 months of age is recommended [1]. There are quite a number of studies on the prevention of iron deficiency anemia by iron supplementation [2–5]. Their results are inconsistent with each other. Few had sample sizes or study designs that were adequate for drawing conclusions. There are also some concerns with the early universal iron supplementation of breastfeeding infants [6, 7]. Therefore, it is acknowledged that new studies are necessary on assessment of strategies for preventing iron deficiency anemia in infancy [5].

The goal of the present study was to investigate the effect of iron supplementation on prevention of iron deficiency anemia in breastfed infants at 1 year of age.

Materials and Methods

This prospective randomized controlled trial was conducted among infants attending the Well Child Unit of the Istanbul School of Medicine, Istanbul University. The approval of the Ethics Committee of the Istanbul School of Medicine was obtained. The study was carried out between February 2007 and April 2009. Families attending the Unit are well above the poverty line as assessed by their ability to bring their baby to a center and pay for the service albeit modest. Families of infants who were eligible for the inclusion to the study were informed about the study. Written informed consent was taken from all families.

Inclusion criteria for the study are given in Table 1. Blood samples were drawn from infants who met the inclusion criteria and whose families accepted to participate in the study. Infants were recruited into two groups by simple randomization. The supplemented group had 1 mg $kg^{-1}day^{-1}$ of ferrous sulfate between

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TABLE 1
Inclusion criteria for the study

- Gestational age ≥37 weeks and birthweight >2500 g
- Singleton birth
- No history of gestational diabetes
- An uneventful perinatal history
- No chronic disease
- No known genetic disease or major congenital malformation
- No history of hospitalization >7 days during the neonatal period
- No iron supplementation before 6 months of age.
- Exclusive breastfeeding for the first 4 months of life.

6 and 12 months of age. Iron supplementation was provided free of charge. Infants in both groups continued to receive vitamin D supplementation. Mothers of the infants in the control group were told not to give any supplementation besides vitamin D. Mothers of the infants in the supplementation group were cautioned to give the iron supplement 1 h before or after feedings other than breast milk. All mothers were recommended to continue breastfeeding and give complementary foods according to the WHO Guidelines. At the beginning of the study, all mothers were asked to give a sample of their blood for complete blood count determination.

Information on sociodemographic characteristics of the families and the anthropometric measurements of their infants was retrieved from personal files at the Well Child Unit. Exclusive breastfeeding indicated that the infant did not take any supplementary food, not even water. Detailed physical examination of all infants was carried out at the Well Child Unit at ages 6, 9 and 12 months. Mothers were asked to bring documents showing their hemoglobin (Hb) values during pregnancy, if available. Venous blood samples were taken from all infants for complete blood analysis and ferritin levels at 12 months of age when the infant had no acute health problem. For a complete blood count, 2 ml of blood was drawn into the tubes with EDTA. For ferritin levels 2 ml of blood was drawn into tubes without EDTA. Blood count analysis was conducted by an ABX pentra 120-HORIBA device. Serum ferritin was determined by a modular analytic E 170 device, using the electro-chemiluminescence immunoassay method. The coefficient of variation (CV) of the measurements was always below 5%. The control samples were within the manufacturer's reference ranges.

When the infants reached age 12 months, their parents were asked to fill a standard 3-day diet form for the infant. Standard measurements such as teaspoons and cups were used to indicate the amounts. Iron supplements were not included in the estimates. The amount of breast milk ingested by the infant was roughly measured according to ALSPAC references [8, 9]. According to these references breast milk amount taken for each minute of breastfeeding was calculated as 10 ml. A feed lasting 10 min or longer was assumed to be 100 ml in volume. Diet lists were evaluated with the Nutrition Information System (BeBis) Program (Epispro for Windows, Stuttgart, Germany: Turkish version BeBis, Version 8, Data bases. Bundeslebelmittelschlüssel, 11.3, USDA_ sr19 and other sources). The Turkish database updated in 2006 was used. The database included infant foods fortified with iron. Daily iron intake of infants by their diet was calculated as its ratio to the recommended daily allowance (11 mg day^{-1}) for that age.

Iron deficiency was defined as a ferritin level lower than 10 μg l^{-1}, and anemia was defined as a Hb level lower than 10.3 g dl^{-1} [10]. Using existing data, we assumed prevalence of anemia at 1 year of age as 30% in the control group and 5% in the supplemented group with a confidence interval of 95%. Given an α-level of 5% and power of 80%, we needed to obtain data on 43 infants in each group. To take into account an expected dropout rate of 25%, we calculated that 60 infants would need to be enrolled in each group.

Data were analyzed by using the SPSS 10.0 program. Kolmogorov–Smirnov, Anderson–Darling, Student's *t*-test, Fisher's exact test, Mann–Whitney test and Pearson correlation tests were used for the analysis. Statistical significance was defined as $p < 0.05$ (two-sided). Variables that did not show a normal distribution were analyzed by the Mann–Whitney test.

Results

During the enrollment period, parents of 1120 infants aged 6 months were invited to the study. Of these, 620 infants had an exclusion criteria. Initially, the parents of 120 infants gave consent for the study. After initial complete blood count analysis, 12 infants (6 from each group) who were anemic (Hg <10.3 g dl^{-1}) were excluded from the study.

Families of three infants did not want to use iron supplementation and left the project. The study was completed with a total of 105 infants (51 in the supplemented group and 54 in the controls). At 12 months of age, blood samples for complete blood cell count were drawn from 105 infants and for ferritin levels from 80 infants.

The majority (72.4%) of mothers was between 18 and 34 years of age. None of the infants had

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TABLE 2
Initial characteristics of infants and their families in the two groups

	Supplemented group ($n=51$)	Control group ($n=54$)	p-values
Gender (male)	56.9%	38.9%	0.07
Mode of delivery (caesarean section)	64.7%	51.9%	0.10
Mean Birthweight (g)	3407.7	3500.9	0.27
Mean Gestational age (weeks)	39.4%	39.1%	0.20
Mother's age (>35)	18.8%	31.5%	0.14
Father's education level (university)	56.3%	33.3%	0.06
Mother's education level (university)	52.1%	31.5%	0.11
Exclusive breastfeeding for 6 months	45.1%	55.6%	0.64
Being breastfed at 12th month	80.4%	81.5%	0.89
History of acute disease	5.9%	1.9%	0.35

TABLE 3
Feeding type at 6 months of age in the iron-supplemented and control groups

	Groups		Total, n (%)
	Supplemented ($n=51$), n (%)	Control ($n=54$), n (%)	
Only breast milk	23 (45.1)	30 (55.6)	53 (50.5)
Breast milk+water	1 (2)	0	1 (1)
Breast milk+ fruit juice	1 (2)	2 (3.7)	3 (2.9)
Breast milk+ formula	2 (3.9)	2 (3.7)	4 (3.8)
Breast milk+ mixed[a]	24 (47.1)	20 (37.0)	44 (41.9)

[a]Mixed: Home-made complementary foods.

a detectable chronic disease during the study period. Sociodemographic characteristics of supplemented and control groups were similar (Table 2).

There was no difference between groups in terms of breastfeeding status (Tables 2 and 3). Only 23 mothers presented their Hb levels during pregnancy. Complete blood count could be performed in 39 mothers in the supplemented group and in 46 mothers in the control at the beginning of the study. Mean Hb levels of mothers during pregnancy and at the beginning of the study were similar in both groups (Table 4).

Of all the infants, 7.6% had Hb levels below $10.3\,g\,dl^{-1}$ at 12 months of age. This figure was 7.8% in the supplemented group and 7.4% in the controls. The difference was not statistically significant (Fisher's exact test, $p=1$). Mean Hb values at 12 months of age were not significantly different in two groups (Table 4).

The mean ferritin level in the iron-supplemented group was significantly higher than that of the control (Table 4). The proportion of infants whose ferritin levels were lower than $10\,\mu g\,l^{-1}$ was 16.3%. These figures were 8.1 and 23.3% in the supplemented and control groups, respectively. The difference was not statistically significant (Fisher's exact test $p=0.078$). Of all the infants, 10.0% had ferritin level lower than $7\,\mu g\,l^{-1}$. These rates were 2.7 and 16.3% in the supplemented and control groups, respectively, and the difference was not statistically significant (Fisher's exact test, $p=0.063$). There was no difference between two groups with respect to number of infants whose Hb values were below $10.3\,g\,dl^{-1}$ and ferritin levels below $7\,\mu g\,l^{-1}$. There was no infant in the supplemented group with these values, but there were two such infants in the control group. No difference was observed between the two groups in terms of growth and development at 9 and 12 months of age. No increase in frequency of diarrhea and pneumonia was observed among infants receiving iron supplementation.

Diet records of 35 infants from the supplemented group and of 37 infants from the control were suitable for evaluation. Iron intake by diet at 12 months of age was high in the control group but the difference was not statistically significant (Table 3). The main source of iron was red meat products. Consumption of iron-fortified cereals was not

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TABLE 4
Some characteristics of infants and their mothers in the two groups

Variable	Group	*N*	Mean (SD)	*p*
Hb in pregnancy (g dl^{-1})	Supplemented	12	12.0 (1.6)	0.45
	Control	11	12.2 (0.9)	
Mother's Hb (g dl^{-1}) at 6 months	Supplemented	39	13.3 (0.8)	0.30
	Control	46	13.5 (0.7)	
Hb at 6 months (g dl^{-1})	Supplemented	51	11.3 (0.6)	0.89
	Control	54	11.4 (0.5)	
Hb at 12 months (g dl^{-1})	Supplemented	51	11.4 (0.9)	0.33
	Control	54	11.2 (0.8)	
Ferritin (μg l^{-1})	Supplemented	37	31.2 (24.9)	0.003
	Control	43	20.6 (16.8)	
Iron intake in diet (% of RDA[a])	Supplemented	35	43.3 (19.3)	0.11
	Control	37	53.1 (24.0)	

[a]RDA: 11 mg day^{-1}.

a common practice. A significant positive correlation between iron intake by food and Hb value at 12 months of age was identified (Pearson correlation value = 0.306, $p = 0.009$).

Discussion

In this prospective randomized-controlled study, mean Hb levels at age 1 year were similar among infants who received iron supplementation and those who did not. The mean ferritin level was observed to be significantly higher in the iron-supplemented group than that of the control. Maternal iron level, birthweight, gestational age and umbilical cord clamping time are factors that largely determine body iron content in the first 6 months of life [11]. There was no reported difference between iron-supplemented and control groups in this respect except for umbilical cord clamping time. No information was gathered for this aspect.

Domellöf *et al*. [4] reported that iron supplementation significantly decreased iron deficiency anemia in Honduran infants but a similar effect was not observed among Swedish infants. They also reported that iron supplementation at age 4 months was not superior to supplementation started at 6 months of age. In a study from USA, a group of exclusively breastfed infants received iron supplementation starting from the first month of life until they were 5.5 months old; the other group had placebo. The iron store was higher in the supplemented group, but no differences were observed in hematological parameters [12]. Iron supplementation during the first year of life did not have any impact on healthy well-nourished infants in another study [13]. These results have similarities with our findings although the starting age of iron supplementation was different.

In a study, 4-month-old infants were randomly assigned to three groups, the first group receiving daily iron supplementation, the second a weekly iron supplementation and the third group no supplementation. In all groups, the mean levels of ferritin increased significantly from 6 to 7 months of age. The authors concluded that proper nutritional supplements in the diet had a positive effect on iron stores of infants, independent of iron supplementation [14]. We also found a significant positive correlation between iron intake by food and Hb value at 12 months of age in our study.

Our study had some limitations. No placebo was used and we noted that the parents of the control group have paid special attention to the diet of their infants in terms of iron-containing foods. The adherence to the iron supplementation was not assessed objectively. We evaluated iron intake by diet in the two groups but we were not in a position to compare the data on the use of iron-fortified infant formula *per se* in the two groups, since consumption of iron-fortified infant formula was not a common practice among our families. All infants in our study received exclusive breastfeeding until 4 months of age with home-based complementary food after 4–6 months of age. We also could not calculate the real amount of iron that the infants ingested. The real supplementation might be $<1\,mg\,kg^{-1}\,day^{-1}$ and this also could affect the negative results.

We could not find any effect of iron supplementation on the development of anemia in our study. However, mean ferritin levels were significantly high in the iron-supplemented group, and there was a significant positive correlation between iron intake by food and Hb value at 12 months of age. When difficulties experienced in iron-supplementation practice are also taken into account, it may be speculated that diet-based approaches could be a more effective way to prevent iron deficiency.

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