

Effects of Three Different Iron Supplementations in Term Healthy Infants after 5 Months of Life

by Bahri Ermis,^a Fatma Demirel,^a Nejat Demircan,^b and Ahmet Gurel^c

^aDepartment of Pediatrics, ^bDepartment of Family Medicine, ^cDepartment of Biochemistry, School of Medicine, Zonguldak Karaelmas University, Turkey

Summary

The aim of this study was to investigate the effects of different doses of iron on haematological status of breastfed infants. One hundred and thirteen infants were randomized into four groups at 5 months of age. Iron supplementation was given at doses of 1 mg/kg/day, 2 mg/kg/day, and 2 mg/kg/every other day in the first three study groups, respectively, and the last group received placebo. The hematological values, except hemoglobin, were higher in the group supplemented with iron at a dose of 2 mg/kg/day, and ferritin values were statistically higher in the group supplemented with iron at a dose of 2 mg/kg/every other day than in the group supplemented with iron at a dose of 1 mg/kg/day. We suggest that intermittent iron supplementation is more effective than a daily regimen in equal dosages.

Introduction

Iron deficiency anemia (IDA) remains the most common nutritional deficiency in children worldwide^{1,2} and millions of children in the world suffer from irreversible brain injury as a result of iron deficiency that affects mental development and motor functions irreversibly despite iron therapy.³⁻⁵ Although, healthy term infants are born with sufficient iron stores to meet the needs during the first 4-6 months of life,⁶ after this age, iron supplementation is required to maintain adequate iron status and normal psychomotor development.^{1,6-8} However, the effect of prophylactic iron supplementation on healthy, term, and breastfed infants starting at 4-6 months of age is controversial.^{1,2,6-8}

Some authors have suggested that intermittent iron therapy has an effect on iron status similar to that of daily therapy in children.⁹⁻¹⁸ These results have great importance due to the potential adverse effects of iron supplementation.⁸ Iron is essential for the growth of micro-organisms and malignant cells and may increase the prevalence of sepsis and malaria.^{5,8,19,20} However, one prospective study found no effect of enteral iron on infections in term infants.⁸

Because of this uncertainty, the efficacy and safety of iron supplementation and the lowest dose of iron that prevents IDA should be evaluated carefully. In this study, we investigated the effects of iron supplementation at doses of 1 mg/kg/day, 2 mg/kg/day and

2 mg/kg/every other day on hematological values of breastfed infants.

Materials and Methods

This study was a prospective, randomized, placebo-controlled trial, conducted at the Research Hospital of Karaelmas University, Faculty of Medicine in Zonguldak, Turkey. The Ethical Committee of the University approved the study. The parents were informed and their consents were obtained before enrolment.

One hundred and thirteen infants aged 5 months receiving routine pediatric care were randomized into four groups. Iron supplementation was given at the doses of 1 mg/kg/day, 2 mg/kg/day and 2 mg/kg/every other day to the subjects in group I ($n_1 = 30$); group II ($n_2 = 30$) and group III ($n_3 = 30$), respectively, and the last group received placebo ($n_4 = 23$). Ferrous sulfate drops (Ferro-sanol: 1 mg~1 drop, Adeka, Turkey) were used for iron supplementation and the dosages were adjusted monthly according to infants' weights. The supplement was given by the mother each morning, just before or after breastfeeding and at least 1 h before or after any other food intake. Infants with Hb < 9.5 g/dl, MCV < 74 fl and serum ferritin < 12 ng/ml were accepted as IDA at 5 months²¹ and were not included in the study. Infants with Hb in normal ranges, but MCV < 74 fl and serum ferritin < 12 ng/ml were accepted as having iron deficiency. The cut-off values for iron status at 9 months were 10.5 g/dl Hb, 70 fl MCV and 12 ng/ml ferritin.²¹ Since 12 infants had iron deficiency and four of them also had IDA, these 12 infants were excluded from the study. The criteria used to accept the subjects into the study were as

Correspondence: Bahri Ermis MD, School of Medicine, Department of Pediatrics, Zonguldak Karaelmas University, 67600 Kozlu/Zonguldak, Turkey. Tel. +90 (372) 261 0169; Fax +90 (372) 261 0155. E-mail <bahriermis@yahoo.com>.

follows: no gestational problem (hypertension, pre-eclampsia, ablatio placenta, infection), no congenital anomalies, no neonatal complications, no emergency cesarean section, no jaundice requiring phototherapy, no hospitalization, no chronic illness, no iron therapy, no formula feeding and having exclusive breastfeeding, birthweight > 3.0 kg, gestational age > 37 weeks. Complementary food was started at 4 months of age and mothers continued breastfeeding until 9 months. The infants who had an infection during iron supplementation were excluded from the study.

Data collection and analysis

We took two venous blood samples from infants at 5 months of age on each morning before feeding, and repeated at 9 months. One of two blood samples was placed in an EDTA test tube and used to measure hemogram by cell counter analysis method (Coulter Micro Diff 18, Miami, USA). The other blood sample was placed in a clear test tube and was used to calculate serum ferritin (SF) level by the electrochemiluminescence immunoassay (ECLIA) method (Hitachi Elecsys 2010, Tokyo, Japan).

Statistical methods

All statistical analyses were performed with SPSS software version 9.0 (SPSS Inc, Chicago, Ill). Student's *t*-test was used for comparing means and χ^2 for comparing proportions. Analysis of variance was used for multivariate analyses and the Tukey HSD technique for multiple comparisons. An alpha-level of 0.05 was used in two-tailed tests of statistical significance.

Results

At 5 months of age, 113 infants (male = 57, female = 56) were randomized into four study groups. The four groups were similar with respect to baseline hematological values (Hb, MCV, ferritin), infant weight, infant sex, maternal age, and educational status of their mothers (Table 1). The infant weights were at the 50th percentile according to Turkish standards. There were no significant differences in the mothers' educational status between the groups and 58.6–74.1 per cent of the mothers included in the study graduated from high school or university. Compliance with the iron supplementation was checked by controlling the amount of medicine remaining in the bottles each month. If the received iron drops were less than 75 per cent of the expected amount of the monthly time intervals, these cases were excluded from the study. For these reasons, two cases in group I, three cases in group II and one case in group III were excluded from the study. The causes of non-compliance were infection during iron usage, refusing iron drops due to unpleasant taste, or mothers forgetting to use iron drops. The effects of

the three different regimens of the iron and placebo on infants' hematological status with baseline values are shown in Table 2. Iron deficiency was not observed in groups supplemented with three different doses of iron at 9 months of age. However, 17 per cent of the placebo group had iron deficiency and 50 per cent of them had IDA. There were significant increases in Hb, MCV, and ferritin values at 9 months compared with baseline values in the first three study groups but not in the placebo group, by paired-samples *t*-test. The mean values of Hb, MCV and ferritin were compared at 9 months by ANOVA analysis and significant differences were found ($p < 0.001$). Multiple comparisons were done by Tukey HSD technique (Table 3). There were no significant differences in Hb values between groups I and II ($p = 0.89$), groups I and III ($p = 0.92$), groups II and III ($p = 0.54$), but significant differences were found by comparing the first three groups with the placebo ($p < 0.001$). There were no significant differences in MCV values between groups I and III ($p = 1.0$), but there were significant differences between groups II and III ($p = 0.021$), groups I and II ($p = 0.023$) and between the first three groups and the placebo ($p < 0.001$). On the other hand, there were significant differences in ferritin values between groups I and II ($p < 0.001$), groups I and III ($p = 0.038$), groups II and III ($p = 0.014$) and between the first three groups and the placebo ($p < 0.001$).

According to these results, the Hb values were statistically similar at 9 months of age in the three groups supplemented by different doses of iron, but MCV values were superior in group II than in group I ($p = 0.023$) and in group II than in group III ($p = 0.021$); no significant differences were found between groups I and III ($p = 1.0$).

Discussion

The aim of this study was to investigate the effects of different supplementation of iron to breastfed infants on their hematological status and whether intermittent iron prophylaxis was more effective than daily iron prophylaxis. It is suggested that breastmilk, in which iron is low but highly bioavailable, is sufficient to prevent iron deficiency during the first 4–6 months of life.^{1,2,6–8} McMillian, *et al.*,²² Duncan, *et al.*,²³ and Saarinen²⁴ reported that breastfeeding until 6 months of life is sufficient to prevent anemia, while Garry, *et al.*²⁵ and Kim, *et al.*²⁶ stressed the need for supplementary iron at 6 months. Pizarra, *et al.*²⁷ suggested that iron supplementation should start at 6 months for infants who were exclusively breastfed, and continue up to 9 months of age. Domellof, *et al.*⁶ suggested that iron drops have a significant influence on iron status between 6 and 9 months and there is no significant advantage of giving the iron supplement from 4 to 9 months compared with 6 to 9 months. The American

TABLE 1
Demographic characteristics of the study groups

	Group I	Group II	Group III	Placebo	<i>p</i> value
Maternal education (> 11 years, %)	67.9	74.1	58.6	69.6	0.65
Maternal age (years)	26.3 (2.6) ^a	27.4 (2.5)	26.4 (3.1)	27.8 (3.1)	0.17
Sex (% male)	53	48	48.2	52	0.96
Birthweight (kg)	3.41 (0.28)	3.39 (0.25)	3.37 (0.29)	3.38 (0.26)	0.90
Weight at 5 months (kg)	7.1 (0.44)	7.1 (0.44)	7.1 (0.33)	7.0 (0.5)	0.75
Weight at 9 months (kg)	9.0 (1.0)	9.2 (0.93)	9.0 (0.84)	9.1 (1.0)	0.75

p value: there were no significant differences between groups with respect to maternal education, maternal age, infants' sex and weights of infants at birth, 5 and 9 months of age.

^a Numbers in parentheses are standard deviations.

TABLE 2
Hematological findings of groups at 5 and 9 months of age

	Hb (g/l)		MCV (fl)		Ferritin (ng/ml)	
	5 months	9 months	5 months	9 months	5 months	9 months
Group I	11 (0.36) ^a	11.6 (0.31)	78 (2.1)	80 (1.9)	29.3 (6.6)	38.5 (7.3)
Group II	10.9 (0.38)	11.7 (0.29)	78.1 (2.0)	81.6 (1.4)	29.2 (7.4)	52.4 (8.3)
Group III	11 (0.33)	11.6 (0.29)	77.9 (1.6)	80 (1.5)	29.2 (7.2)	45 (12.7)
Placebo	10.9 (0.2)	11.3 (0.4)	77.6 (1.8)	74.3 (2.9)	28.8 (5.1)	17.2 (4.6)

There were significant differences between groups at 9 months of age with respect to Hb, MCV and ferritin values by ANOVA analysis (*p* < 0.001).

^a Numbers in parentheses are standard deviations.

TABLE 3
Multiple comparisons by Tukey HSD technique at 9 months of age

		Group II	Group III	Placebo
Hb	Group I	0.89	0.92	0.001
	Group II		0.54	<0.001
	Group III			0.004
MCV	Group I	0.023	1.0	<0.001
	Group II		0.021	<0.001
	Group III			<0.001
Ferritin	Group I	<0.001	0.038	<0.001
	Group II		0.014	<0.001
	Group III			<0.001

The numbers shown in the table are *p* values obtained by multiple comparisons using Tukey HSD technique.

Academy of Pediatrics Nutritional Committee suggested that iron supplementation should be given at a dose of 1 mg/kg/day after 4 months of life.²⁹ On the other hand, Makrides, *et al.*²⁸ found 1.4 per cent of infants who were exclusively breastfed had IDA at 6 months of age. In our clinic, 1.7 per cent of infants who were exclusively breastfed had IDA at 5 months and were excluded from the study. In our study, iron

was started at 5 months and continued up to 9 months of age. There was no iron deficiency and/or IDA in the study groups except placebo (17 per cent of the placebo had iron deficiency and 50 per cent of them had IDA). These findings and previous studies suggest that iron supplementation should be started after 4–6 months of age to prevent iron deficiency and/or IDA.

On the other hand, it is suggested that intermittent iron reduces gastrointestinal side-effects, reduces transient overload of iron and has absorptive advantage over daily iron supplementation.¹² In experimental animals, the absorption of supplemental iron is greatest when it is administered at times of intestinal mucosal renewal, so that each dose is received by new cells.^{30,31} Thus, the absorption of iron is reduced in daily iron regimens because of the iron overload in intestinal cells.^{30,31} Infants who received excessive iron supplementation are not able to downregulate the absorption of iron; as Domellof, *et al.* suggested, extended iron supplementation might cause adverse effects such as impaired linear growth.⁶ On the other hand, daily vs. intermittent iron supplementation was investigated and identical results were found in children by some authors.^{9–18} These results have great importance due to the potential adverse effects of iron supplementation, such as infection, malignancies

and malaria.⁸ Because of the unpleasant taste of iron, the difficulty of daily iron usage, and lots of adverse effects observed with daily iron supplementation, the importance of intermittent iron usage is increased. In our study, the frequencies of side-effects were 4, 7, and 2.5 per cent in groups I, II and III, respectively. The cases of non-compliance excluded from study were found to be higher in the groups supplemented with daily iron regimens, (not shown here). Also, the expected remaining volume of syrup in the bottles was much more in groups supplemented with daily regimens (especially group II), proposing that intermittent regimens are tolerated better.

There were significant differences between groups in Hb, MCV, and ferritin values at 9 months of age, by ANOVA analysis. Tukey HSD technique was used for multiple comparisons. There were no significant differences of Hb values between groups except between the first three groups and the placebo (Table 3). These results suggest that there is no advantage over each other of giving iron supplementations on Hb values at a dose of 1 mg/kg/day, 2 mg/kg/day, or 2 mg/kg/every other day. There were no significant differences in MCV values between groups I and III ($p = 1.0$), but there were significant differences between groups II and III ($p = 0.021$), groups I and II ($p = 0.023$) and between the first three study groups and the placebo ($p < 0.001$). According to these results, MCV values were best in group II, but no significant differences were found between groups I and III. On the other hand, the effects of three different iron regimens on ferritin values were different ($p < 0.001$, group I vs. group II; $p = 0.038$, groups I vs. group III; $p = 0.014$, group II vs. group III). Studies comparing the effectiveness of intermittent and daily iron regimens showed that Hb values are similar in different regimens, whereas the ferritin values are higher in the groups of daily regimens than in the groups of intermittent regimen.⁹ Identical results were found in our prophylactic study. According to these results, we can conclude that different dosages of iron have similar effects on Hb values, but not on MCV and ferritin values. On the other hand, Domellof, *et al.*⁶ suggested that Hb and ferritin values are regulated independently at 4–9 months of age. Similarly, it has also been reported that Hb and MCV values are regulated separately being independent from each other as is the case for Hb and ferritin levels.⁶ The similar effects of different iron regimens on Hb values, which were observed in our study, might be related to this mechanism. However, our findings are not sufficient to explain this issue yet. In this study, we compared the daily vs. intermittent iron supplementation for prophylactic purposes. Although the amount of iron used in groups I and III were equal during the 4 months of period, ferritin values were higher in group III than group I. According to these results, although the non-compliance and adverse effects were higher in group II than the

other groups, the hematological values were the best in the group supplemented with 2 mg/kg/day iron. On the other hand, although the amount of iron given was equal during the study period, the ferritin values, which are an indicator of iron deposits, were higher in group III than in group I, suggesting that intermittent supplementation is more effective than daily supplementation.

Although The American Academy of Pediatrics Nutritional Committee recommend iron supplementation at a dose of 1 mg/kg/day after 4 months of life for prophylactic purposes,²⁹ we suggest that supplementation of iron at a dose of 2 mg/kg/every other day is more effective and has fewer adverse effects than 1 mg/kg/day dose of iron supplementation.

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