Iron Supplementation at Age 4 Months to Prevent Iron Deficiency Anemia in Infants: A Randomized Controlled Trial; Pilot study

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Objective: To study the effects of iron supplementation on hemoglobin and iron status in children aged 9 months old.

Method: This was a randomized control trial in a study group (n = 20) and a control group (n = 20) of infants aged 4 months. The study group was given iron supplement (15 mg;1.5-2 mg /kg/day). Meanwhile, the same dose of placebo was given to the control group. Hemoglobin and serum ferritin were measured at age 4 months and 9 months (after the 5-month medication). The primary outcome was the evaluation of hemoglobin and serum ferritin between the two groups. Whilst, the secondary outcome was the prevalence of iron deficiency and iron deficiency anemia at age 9 months.

Results: During 4-9 months, iron supplement significantly increased weight, serum ferritin, change of hemoglobin, and serum ferritin between both groups. The prevalence of iron deficiency anemia at age 9 months was decreased in the supplemented group.

Conclusion: After undergoing 5 months of iron supplementation, there was a statistically significant increase in hemoglobin levels and serum ferritin within the study group, compared to the control group, at the age of 9 months. The prevalence of iron deficiency anemia in the control group was 20%. However, the study group exhibited a lower occurrence of iron deficiency anemia, specifically at 5%.

Keyword: Iron deficiency anemia, Iron supplement, Hemoglobin, Serum ferritin

Introduction

Iron deficiency anemia (IDA) is a common condition that affects people of all ages, including young children. Iron deficiency anemia can have serious health consequences, such as impaired physical and cognitive development in children [1]. To prevent iron deficiency anemia, it is important to ensure that children have a diet rich in iron and receive iron supplements.

According to the World Health Organization (WHO), iron deficiency anemia is the most common nutrient deficiency affecting over two billion people worldwide, especially in low- and middle-income countries. Also, it becomes a significant health issue in non-industrialized preschool children with the prevalence of 39% [2].

In Thailand, according to the South East Asian Nutrition Survey of Infants and Children, the prevalence of iron deficiency (ID) with or without anemia in urban and rural Thailand ranged between 32.3-38.9%, while the prevalence of IDA ranges from 4.2-8.8%, These findings suggest that ID and IDA might impact the health and development

of children in Thailand, particularly inurban and rural areas [3].

Daily iron supplementation during infancy was recommended by the American Academy of Pediatrics (AAP) and World health organization (WHO), Ministry of Public Health, Thailand, and The Royal College of Pediatricians of Thailand. The recommendations were unequal drugsizes and small drug sizes (1 mg/kg/day), and some recommendations for intermittent iron supplementation, but there was issues with complaints about drug use in practice [4-6].

WHO recommends a hemoglobin level of 11 mg/dL or higher in children under 5 years of age as the threshold for normal hemoglobin levels. The diagnosis of iron deficiency is typically based on serum ferritin (SF) test to measure the amount of iron stored in the body. According to the Cochrane Database of Systematic Reviews (2021), the cut-offs between 15-30 mcg/L yielded 100% sensitivity and 98% specificity for the value of SF < 30 mcg/L [7].

This study aimed to determine whether iron supplementation could prevent iron deficiency anemia in children aged 9 months.

Materials and Methods

Study Population

Healthy 4-month-old infants attending the Bhumibol Adulyadej Hospital (BAH) during October 2020-December 2022 were considered for enrollment in a randomized, controlled trial. Only infants who met the following criteria were considered eligible: (1) gestational age 37-42 weeks, (2) birthweight more than 2,500 gm. Exclusion criteria for this study included the following: (1) congenital disease and genetic disease, (2) gastrointestinal disease, (3) renal disease,(4) thalassemia. The purpose of the study was explained and consent was obtained from parents ofeligible infants.

In this study, anemia is defined as serum hemoglobin level < 11 g/dL. Iron deficiency refers to serum ferritin level < 30 ng/ml. Iron deficiency anemia signifies serum hemoglobin level < 11 g/dL and serum ferritin level < 30 ng/ml.

Randomized and Blinded patients

Patients were randomized into an intervention group and a placebo group by using the computer-generated numeric code. At the initial appointment (4 months of age), infants were examined and blood samples taken for complete blood count (CBC), serum ferritin, and hemoglobin typing. Iron supplement or placebo were given to infants for 5 months' supply (4-9 months of age). The follow-up was done by telephone every month after medications to inquire adherence and side effects from iron supplementation and placebo. The 9-month infants returned to the hospital for blood sample collection and CBC, serum ferritin testing.

This study was a double blinded randomized control trial. Names of each patient was replaced by a numeric code. The medications were also objectively blinded, namely iron supplement (15 mg/0.6 ml Ferdek drops, average dose 1.5-2 mg/kg/day) and placebo equal to iron supplement (All iron supplement and placebo manufactured by OLIC(Thailand) Limited Bangpa-In, Ayutthaya, Thailand, under license of RANBAXY(Thailand) Co., Ltd. Bangkok, Thailand, approved by Thai Food and Drug Administration). The medications were labeled with a numeric code and six bottles assigned to one patient. The code was not revealed until final data analysis. Ferdek drops and placebo had similar bottle features, color, and odor.

Statistical Analysis

This study used of IBM SPSS statistics 23 software for data analysis. Shapiro-Wilk test was used for assessing the normal distribution of data. Chi-square test was used for categorical data, while independent t-test and Mann-Whitney tests were used for continuous data. The data was reported using various statistical measures such as mean, standard deviation, median, range,frequency, and percentage. The results are considered statistically significant if the *p*-value were less than 0.05.

The trial was registered at Thaiclinicaltrials.org with the Trial Identifier number: TCTR20230112002.

Result

There were 42 patients eligible for the study. However, two patients were excluded due to thalassemia trait. Thus, the patients were divided into a study group (n=20) and a control group (n=20). There was no significant difference gender, gestational age, gravida, route of delivery, body weight atbirth, length at birth, and maternal factor (Age, hematocrit, MCV) between two groups as shown in Table 1.

Table 1 Basic demographics data among the randomly assign iron supplement (study) andplacebo (control) groups

	Study (n=20)	Control (n=20)	*p*-value
Male*	12 (52.5)	11 (47.8)	0.75
GA (days)**	269 (9.0)	271 (11)	0.24
Gravida**	2 (1.0)	2 (1.0)	0.92
Vaginal delivery***	10 (52.6)	9 (47.4)	0.75
BW (kg)***	3.1±0.2	3.1±0.2	0.68
Maternal			
Age (years)***	27.9 ± 3.7	30.0 ± 3.7	0.08
Hct (%)***	34.2 ± 2.8	33.8 ± 2.7	0.62
MCV (fl)***	81.7 ± 6.7	81.9 ± 7.4	0.95

*N (%),**Median (Interquartile range),***Mean ± Standard deviation (SD), GA: gestational age,
BW: body weight at birth, Length: Length at birth, HC: newborn head circumference, Hct:hematocrit, MCV: mean corpuscular volume

There was no significant difference of anthropometrics and laboratory measurements at age 4 months between both groups (Table 2). Nonetheless, infants at age 9 months in study group had body weight more than control group (9.5 (0.7), 9.3 (0.9), *p*-value=0.004). as also higher in the study group than the control groups at 9 months of age (58.0 (13.3), 37.5 (16.0), respectively *p*-value <0.001). Overall, at age 4 months, 2 patients (10%) had ID in study group comparing with 1 patient (5%) had ID compared to 1 patient (5%) in the control group. At age 9 months, only 1 patient (5%) in the study group had ID compared to 5 patients (25%) in the control group. Besides, 1 patient (5%) in the study group was diagnosed IDA when compared with 4 patients (20%) in the control group, with no statistical difference (*p*-value = 0.15).

Table 2 Anthropometrics measurement and laboratory measured in iron supplement (study) andplacebo (control) groups

	Study (n=20)	Control (n=20)	*p*-value
4 months			
BW4 (kg)***	7.37 ± 0.14	7.43 ± 0.13	0.127
Hb4 (g/dL)***	10.8 ± 0.9	11.4 ± 2.4	0.36
Hct4 (%)***	33.9 ± 2.6	34.4 ± 2.5	0.51
MCV4 (fl)***	72.2 ± 3.25	72.8 ± 3.90	0.28
Ferritin4 (ng/ml)**	47.0 (16.3)	47.0 (25.8)	0.75
EBF*	6 (30)	5 (25)	0.72
Fe def4*	2 (10)	1 (5)	0.56
9 months			
BW9 (kg) ***	9.29 ± 0.4	9.00 ± 0.4	0.004
Hb9 (g/dL)***	11.6 ± 0.8	11.3 ± 0.6	0.14
Hct9 (%)***	34.7 ± 2.2	33.8 ± 1.9	0.17
MCV9 (fl)***	74.4 ± 5.0	72.9 ± 3.9	0.32
Ferritin9 (ng/ml)**	58.0 (13.3)	37.5 (16.0)	<0.001
Fe def9*	1 (5)	5 (25)	0.18
IDA9*	1 (5)	4 (20)	0.15

*N (%),**Median (Interquartile range),***Mean ± standard deviation (SD), BW4: body weight at age 4 months, Length4: length at age 4 months, HC4: head circumference at age 4 month, Hb4: hemoglobin at age 4 months, Hct4: hematocrit at age 4 months, MCV4: mean corpus vascular volume at age 9 months, Ferritin4: ferritin at age 4 months,, EBF: exclusive breast feeding, Fe def4:number of patients had iron deficiency at age 4 months, BW9: body weight at age 9 months, Length9: length at age 9 months, HC4: head circumference at age 9 month, Hb9: hemoglobin at age 9 months, Hct9: hematocrit at age 9 months, MCV9: mean corpus vascular volume at age 9 months,Ferritin9: ferritin at age 9 months, Fe def9: number of patients had iron deficiency at age 9 month,
IDA9: number of patients had iron deficiency anemia at age 9 months.

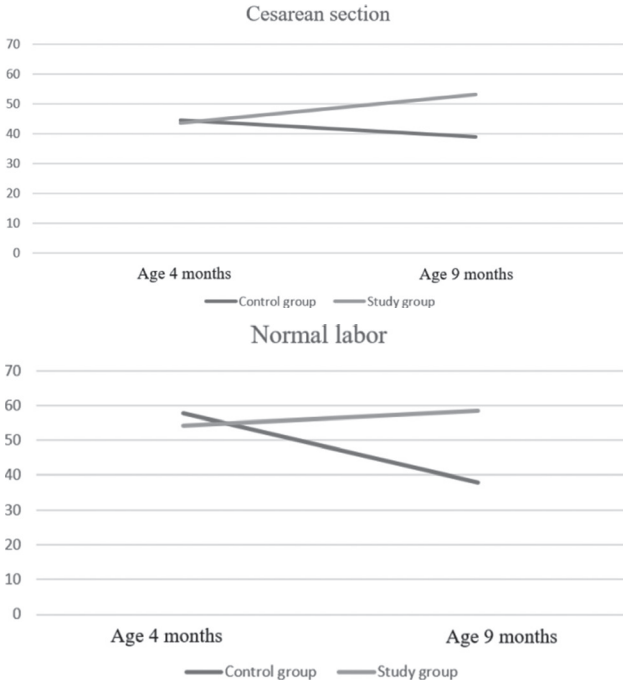
A comparison of different values at 4 months and 9 months of age yielded a higher change in weight, hemoglobin, and serum ferritin levels during 5 months' trial (Table 3). There was more significant difference of change in weight between 4 months and 9 months of age in the study group (2.05 ± 0.50) than the control group (1.697 ± 0.89) (p-value=0.005). Whereas,

the change in hemoglobin of the study group (0.4 ± 0.6) was higher than the control group (-0.22 ± 1.0) (p-value 0.02). Also, the change in serum ferritin level was higher in the study group (6.9 ± 11.8) than the control group (-12.1 ± 13.6) (p-value 0.02, table 3). There was an increase of average serum ferritin (6.9 ng/dl) in the study group. Meanwhile, the placebo group had a decrease of average serum ferritin (5.1 ng/dl). Moreover, a statistically significant change of hemoglobin level in infants age 9 months was observed in the study group (p-value 0.02, in Table 3). The highest increase in serum ferritin of infants was up to 73 ng/dl. All infants in this study had no serious adverse effects from iron supplementation or placebo such as vomiting, and abdominal pain.

Table 3 Measurement of anthropometrics and change in laboratory results over 5 months in the iron supplement (study) group and the placebo (control) group

	Study (n=20)	Control (n=20)	*p*-value
BW change (kg)***	1.93 ± 0.38	1.57 ± 0.17	0.005
Hb change (g/dL)***	0.4 ± 0.6	-0.22 ± 1.0	0.02
Hct change (%)***	0.8 ± 0.8	-0.6 ± 3.0	0.06
MCV change (fl)***	2.2 ± 3.4	0.4 ± 6.0	0.2
Ferritin change (ng/dL) **	5.0 (18.5)	-11.5 (21.8)	<0.001

**Median (Interquartile range), ***Mean ± standard deviation (SD), BW change: the change in body weight change during 5 months, Length change: the change in length change during 5 months, Hb change: the change in hemoglobin change during 5 months, Hct change: the change in hematocrit during 5 months, MCV change: the change in mean corpus vascular volume during in 5 months, Ferritin change: the change in ferritin during 5 months.



Route of delivery and serum ferritin

Figure 1 The relationship between route of delivery and serum ferritin

Children who underwent cesarean section at birth had lower SF levels at 4 months old than those born through normal delivery. Nevertheless, no difference in SF levels was noted at 9 months old. Infants with iron supplementation had higher iron levels than those without iron supplementation. Thus, factors affecting SF levels at 9 months old were likely related to iron intake, not the route of delivery (Figure 1)

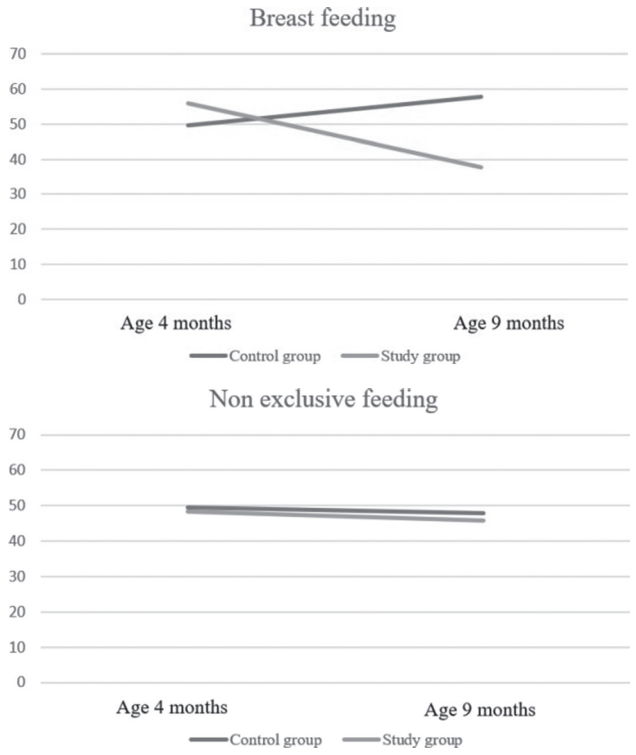


Figure 2 The relationship between breast feeding and serum ferritin

Infants fed with iron-fortified formula, regardless of whether or not they received iron supplements, had similar serum ferritin levels at 9 months, with no difference from the levels at 4 months. However, this was not the case for exclusively breastfed infants. Those with iron supplements had significantly higher serum ferritin levels at 9 months than those at 4 months. Whilst, infants without iron supplements had significantly lower serum ferritin levels at 9 months than those at 4 months. Thus, iron supplementation was crucial for iron improvement in the exclusively breastfed infants (Figure 2).

Discussion

In this study, iron supplementation could decrease the prevalence of iron deficiency and iron deficiency anemia in infants at age 9 months. There was more statistically significant increase of weight gain, hemoglobin level, MCV, and serum ferritin in the study group than the control group at age 9 months. Thus, iron supplementation may be more effective to reduce the incidence of iron deficiency in the study group rather than the placebo group.

Correspondingly, Magnus Domellöf, et al. (2000) [8] reported that there was a low proportion of IDA (<3%) in all groups of 4-month infants in Sweden but in Hondurus. In Honduras, iron supplementation significantly affected Hb (increase of 8.6 g/L, both groups combined). In Sweden, iron supplementation during 4-9 months significantly had an effect on Hb (increase of 4.5 g/L, $p = .002$). In Honduras, the iron supplementation after 5 months resulted in a decrease prevalence of iron deficiency anemia at 9 months (5%) when compared with the placebo (29%). This was compatible to our study for the same prevalence of IDA after iron and placebo supplementation.

Likewise, Pasricha Sant Rayn, et al (2013) [9]. showed that the daily iron supplementation in infants aged 4-23 months could effectively reduce anemia. However, the adverse effect profile of iron supplements and the impacts on child development and growth remained uncertain. This was compatible to our study that iron supplementation reduce iron deficiency.

S. Jain et al (2000) [10] revealed that the highly significant impact of early iron supplementation was also observed on the prevalence of anemia ($p < 0.001$) for 23.8% in infants with regular iron supplementation from the age of 6 months and 68.4% in those with either occasional or irregular iron supplementation, or no iron supplementation as elicited by history.

These results are similar to the findings of Nicolai Petry, et al. (2016) [11], which demonstrated that providing up to 15 mg iron per day during infancy led to a 4 g/L increased mean hemoglobin ($p < 0.001$), a 17.6 µg/L increase in mean serum ferritin concentration ($p < 0.001$) and a reduced the risk for anemia by 41% ($p < 0.001$), iron deficiency by 78% (ID; $p < 0.001$) and iron deficiency anemia by 80%.

In contrast, Kathryn G. Dewey, et al. (2002) [12]. Only at 4–6 months among those withinitial hemoglobin (Hb) <11.0 g/dL. There was no

significant main effect of iron supplementation on morbidity, nor any significant interaction between iron supplementation andsite. There was an interaction between iron supplementation and initial Hb.

This differ from the findings of Chenxi Cai, et al. (2017) [13]. Iron supplementation had no significant effect on iron deficiency or iron deficiency anemia, serum ferritin level, or hemoglobin level.

Study limitation

This study has limitations. First, the follow-up should be longer than 5 months on developmental outcomes. Second, this was a single center study with a relatively small number of patients.

Conclusion

After undergoing 5 months of iron supplementation, there was a statistically significant increase in hemoglobin levels and serum ferritin within the study group, compared to the control group, at the age of 9 months. The prevalence of iron deficiency anemia in the control group was 20%. However, the study group exhibited a lower occurrence of iron deficiency anemia, specifically at 5%. Consequently, iron supplementation appears to effectively reduce the prevalence of iron deficiency and iron deficiency anemia among infants at the age of 9 months.

However, further study is recommended in different sites with higher number of populations. Also, the advantage of iron supplementation should be evaluated to ratify and elucidate on child health improvement.

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การให้ยาเสริมธาตุเหล็กในเด็กอายุ 4 เดือน เพื่อป้องกันการเกิดภาวะโลหิตจาง จากการขาดธาตุเหล็กในเด็กเล็ก

ภูริชญา วราชิต, สุรกานต์ เจนสัจวรรณ์, ณัฐพรทิรา ผลากรกุล

วัตถุประสงค์: การศึกษาผลการเสริมยาธาตุเหล็กต่อระดับฮีโมโกลบินและระดับธาตุเหล็กในเลือดในเด็กอายุ 9 เดือน

วิธีการ: ในการทดลองควบคุมแบบสุ่มนี้ มีกลุ่มศึกษา (n=20) และกลุ่มควบคุม (n=20) เด็กอายุ 4 เดือน ในกลุ่มศึกษาจะได้รับยาเสริมธาตุเหล็ก (1.5-2 มิลลิกรัมต่อกิโลกรัมต่อวัน) และกลุ่มควบคุมจะได้รับยาหลอกในปริมาณที่เท่ากัน เมื่อวัดระดับฮีโมโกลบินและระดับธาตุเหล็กในเลือดที่อายุ 4 และ 9 เดือน (หลังรับประทานยา 5 เดือน) โดยผลการศึกษาหลักเพื่อเปรียบเทียบระดับฮีโมโกลบินและระดับธาตุเหล็กในเลือดระหว่างกลุ่มศึกษาและกลุ่มควบคุม และผลการศึกษารองเพื่อศึกษาความชุกของการเกิดภาวะขาดธาตุเหล็กและภาวะโลหิตจางจากการขาดธาตุเหล็กที่อายุ 9 เดือน

ผลการศึกษา: ผลของการให้ยาเสริมธาตุเหล็กสามารถเพิ่มน้ำหนัก, เพิ่มระดับธาตุเหล็กในเลือด, เพิ่มระดับฮีโมโกลบินในเลือด, และการเปลี่ยนแปลงของระดับฮีโมโกลบินและระดับธาตุเหล็กในช่วง 5 เดือน ในกลุ่มศึกษามีแนวโน้มเพิ่มขึ้น เมื่อเปรียบเทียบกับกลุ่มควบคุม และยังลดความชุกของการเกิดภาวะโลหิตจางจากการขาดธาตุเหล็กในเด็กอายุ 9 เดือนได้

สรุป: การให้ยาเสริมธาตุเหล็กเป็นเวลา 5 เดือนสามารถช่วยลดการเกิดภาวะโลหิตจางจากการขาดธาตุเหล็กในเด็กอายุ 4-9 เดือนได้ และการให้ยาเสริมธาตุเหล็กยังสามารถเพิ่มระดับฮีโมโกลบินและระดับธาตุเหล็กในเลือดได้