*Original Article*

# Assessing Former Preterm Neonates for Iron Deficiency at Four Months of Age: Is Breastfeeding a Risk Factor?

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***Objective***: To determine the incidence and risk factors of iron deficiency (ID) among preterm infants when they reached four months postnatal age.

***Materials and Methods***: The present study was a prospective cohort study. Infants born at 34 weeks' gestation or earlier, weighing 2,000 grams or less, and treated at a university hospital in Bangkok, Thailand between January 2010 and June 2014 were enrolled. Study data collected included demographic and clinical information during hospitalization, breast milk or formula, and iron supplementation reported during follow-up visits, and studies of iron status at 4-months postnatal age. Univariate and multivariate analyses were performed to identify factors associated with ID.

***Results***: One hundred twenty-one infants completed the four months follow-up. At hospital discharge, all infants were exclusively or partially breastfed. Prophylactic ferrous sulfate was prescribed in 110 infants. At 4-month, 65% were exclusively or partially breastfed. Incidence of biochemical ID or ID anemia in exclusively breastfed, partially breastfed, and formula-fed infant were 19%, 6.9%, and 4.8%, respectively. After adjusting for birth weight and prophylactic ferrous sulfate supplementation, breastfeeding was not associated with increased risk of ID or ID anemia.

***Conclusion***: Incidence of ID at 4-month postnatal age of exclusively breastfed, partially breastfed, and formula-fed preterm infant were not statistically different. Prophylactic iron supplementation at 2 to 4 mg/kg/day should be prescribed to all exclusively or partially breastfed preterm infants of 34 weeks' gestation or less to prevent ID. The authors recommend checking iron status at the 4-month postnatal time point regardless of feeding type.

***Trial registration***: Thai Clinical Trials Registry, TCTR20201028002

***Keywords***: Breastfeeding; Cohort study; Infant iron status; Iron deficiency anemia; Iron supplements; Preterm infants

Received 31 March 2021 | Revised 22 April 2021 | Accepted 28 April 2021

**J Med Assoc Thai 2021;104(6):998-1004**

**Website:** http://www.jmatonline.com

Breastfeeding is widely recommended as the best nutrition for infants due to its beneficial effects on neurodevelopmental outcome[1-3]. However, an important unresolved concern is that exclusive breastfeeding (EBF) of prematurely born infants might place them at high risk for developing iron deficiency (ID). Adequate iron is essential to sustain a neonate's erythropoiesis, mitochondrial respiration, nucleic acid replication, immune function, and brain development. Neonates born prematurely are innately at risk for developing ID because 80% of a term infant's iron endowment is amassed during the third trimester of pregnancy. The relatively low iron content in human milk adds to this concern[4].

The incidence of ID among preterm infants varied from 15% to 40%, depended on type of milk ingested, iron supplementation protocol, and screening time point[5,6]. To avoid ID among breastfed preterm infants, the World Health Organization (WHO) and other health oversight groups recommend prophylactic oral iron supplementation at 2 to 4 mg/kg/day for 6 to 12 months or until growth and hematologic status are normalized[3,7-9]. In addition, if iron supplementation was administered before 1 to 2 months' postnatal age, oral vitamin E should be supplemented at 5 to 25 IU per day to prevent hemolysis secondary to vitamin E deficiency[10]. However, there was no recommendation on when to screen for ID in this population. Siriraj Hospital has successfully

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**How to cite this article:**

Ngerncham S, Sanpakit K, Kitsommart R, Chuanchai W, Kolatat T, Wongsiridej P. Assessing Former Preterm Neonates for Iron Deficiency at Four Months of Age: Is Breastfeeding a Risk Factor? J Med Assoc Thai 2021;104:998-1004.

doi.org/10.35755/jmedassocthai.2021.06.12800

encouraged breastfeeding in mothers of preterm infants during hospitalization and after discharge[11]. The hospital also encourages prophylactic oral iron and vitamin E supplementation of breastfed preterm infants. However, due to the importance of assuring iron sufficiency among growing preterm infants, the authors conducted the present study to evaluate the incidence and risk factors of ID. Specifically, the authors prospectively evaluated the iron status of a cohort of preterm infants at 4-month postnatal age. The hypothesis was that with the prophylactic iron supplementation recommended for this population, breastfeeding would not increase the risk of ID.

## Materials and Methods

### Design

The present study was a prospective cohort study registered in the Thai Clinical Trials Registry, one of the primary registries of the WHO Registry Network. The study registration ID was TCTR20201028002.

### Setting

The present study was conducted at the Division of Neonatology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand between January 2010 and June 2014. Siriraj Hospital is a tertiary referral center with approximately 8,000 deliveries per year. Study infants were recruited while they were in the neonatal intensive care unit (NICU) or the intermediate care unit. The mother-infant dyads were followed-up at the Siriraj Preterm Clinic.

At Siriraj Hospital, the type of feeding is the mother's decision. Whenever the mother's own supply of milk is inadequate, premature formula is prescribed for preterm infants weighing 1,800 grams or less, whereas standard infant formula or premature formula is prescribed for preterm infants weighing more than 1,800 grams. Infants with birth weight less than 1,500 gram or very-low-birth-weight (VLBW), are usually started on enteral feeding within the first few days after birth. After full feeding is reached, VLBW infants are supplemented with a 2-week course of oral vitamin E (synthetic alpha-tocopherol) drop 25 mg/day to prevent vitamin E deficiency followed by oral ferrous sulfate ($FeSO_4$) drop at 2 to 4 mg/kg/day. In general, $FeSO_4$ is started around 1-month postnatal age, except in infants whose full feeding was delayed. For bigger preterm infants, oral vitamin E and $FeSO_4$ prescription are given according to the discretion of attending physicians. History of perinatal blood loss, frequent blood sampling during hospitalization, and EBF are common reasons for iron supplementation in these infants.

### Samples & outcome measurement

Participants were preterm infants of less than or equal to 34-weeks' gestation and birth weight of 2,000 grams or less, born in Siriraj Hospital, and admitted to the NICU or intermediate care unit after birth. The following infants were excluded, lethal congenital anomalies, mothers with a contraindication for breastfeeding or decided not to breastfeed, infants whose mothers do not understand Thai language, and infants who were still admitted in the hospital at four months of age.

The primary outcome was a composite of ID or iron deficiency anemia (ID/IDA).

Demographic data and clinical characteristics of the study infants during hospitalization were collected from medical records. Infants were followed-up at the preterm clinic following standard vaccination schedule or as indicated by the infant's condition. No extra study-related visits were scheduled. At each visit, type of feeding such as breast milk or formula, and dose of $FeSO_4$ drop prescribed were recorded.

At 4-month postnatal age, venous blood was obtained for complete blood count and iron studies. If the study infants were sick from any cause, the blood draw was postponed until recovered from that illness. For the study infants with abnormal results of blood analysis, the plan for treatment and follow-up were the responsibility of the physicians at the Preterm Clinic. For infants who were anemic, but had normal iron study, hemoglobin typing was performed if parental permission could be obtained.

Serum ferritin was measured by electrochemiluminescence immunoassay (Cobas Modular e 602; Roche Diagnostics, Basel, Switzerland); direct serum iron and unsaturated iron binding capacity (UIBC) were measured by a colorimetric study (Mindray DS 240; Mindray, Shenzhen, China). Transferrin saturation and total iron binding capacity were calculated from direct serum iron and UIBC.

### Operational definitions

Iron deficiency was defined as serum ferritin of less than 12 µg/L[5,6,12] or transferrin saturation of less than 17%[6]. Anemia was defined as hemoglobin concentration of less than 10.5 g/dL[12]. Iron deficiency anemia was defined as hemoglobin concentration of less than 10.5 g/dL, and either serum ferritin of less than 12 µg/L or transferrin saturation of less than 17%.

Exclusive breastfeeding was defined as receiving

only mother's own milk regardless of feeding method. Such infants may also receive drops or syrups of vitamins and minerals, medications, or oral rehydration solutions. Water taken with medicines was allowed. Partial breastfeeding (PBF) was defined as receiving mother's own milk plus any infant formula. These infants may have also received other food or water.

Feeding status was determined by interviewing the mother for type of milk such as breast milk or formula, and ratio of breastmilk to formula her infant received since the previous follow-up visit. The mother was asked to record the date when breastfeeding had stopped.

### Statistical analysis

Sample size calculation was based on an incidence of IDA in exclusively breastfed low-birth-weight infants of 40%[13]. The margin of error was estimated at plus or minus 10% of the prevalence. To compensate for a possible 35% loss to follow-up in the authors' preterm clinic setting, the authors aimed for an enrollment of 196 participants.

Final analysis included only study participants who completed a 4-month follow-up, and the primary outcome was ascertained. Data analysis was performed using PASW Statistics, version 18.0 (SPSS Inc., Chicago, IL, USA). Continuous data were presented as mean (standard deviation [SD]) or median (interquartile range [IQR]), as appropriated according to the normality of data. Categorical data were presented as number and percentage. Univariate analysis of continuous data was performed using independent samples t-test or Mann-Whitney U test for comparison between the two groups and analysis of variance (ANOVA) or Kruskal-Wallis test for comparison among three groups. Univariate analysis of categorical data was performed using chi-square or Fisher's exact test, as appropriate.

Multivariate analysis to determine risk factors for ID/IDA was performed using binary logistic regression analysis. A *p*-value of less than 0.05 was considered statistically significant. The relevant exposure in the present study was breastfeeding. Iron supplementation and other variables with a *p*-value less than or equal to 0.2 from univariate analysis were considered as possible confounding factors. Variables with missing data at random were ignored[14].

### Ethical approval

The protocol for the present study was approved by the Siriraj Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA 376/2009). Written informed consents were obtained from the mothers of all study infants.

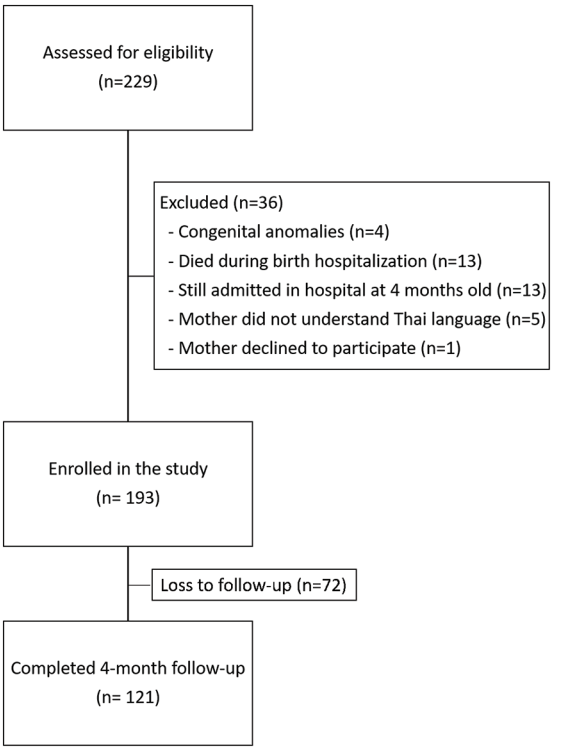


**Figure 1.** Diagram demonstrating the flow of participants in this study.

## Results

### Clinical characteristics during hospitalization

One hundred ninety-three infants were enrolled in the present study. A diagram demonstrating the flow of the study participants is shown in Figure 1. Seventy-two infants (37%) were lost to follow-up, so the remaining 121 infants with complete follow-up data were included in the final analysis. Fifty-two percent were female. Median gestational age was 31 weeks (IQR 29 to 33). The median birth weight was 1,440 grams (IQR 1,115 to 1,670), and 54.5% were VLBW infants. Fifty-three infants (44%) received blood transfusion during hospitalization, and the median number of blood transfusions was two times (IQR 1 to 6). Seventy-three percent of VLBW infants received blood transfusion compared to 9% among their larger preterm counterparts. No present study infants received blood transfusion during the follow-up period. Demographic and baseline clinical characteristics comparing between infants who did

**Table 1.** Demographic and baseline clinical characteristics comparing between infants who did and who did not have iron deficiency or iron deficiency anemia (n=121)

| Characteristics | ID or IDA; n (%) | | *p*-value |
|---|---|---|---|
| | Yes (n=10) | No (n=111) | |
| Gestational age in weeks; median (IQR) | 31 (28 to 34) | 31 (29 to 33) | 0.90 |
| Birth weight in grams; median (IQR) | 1570 (1,198 to 1,885) | 1,440 (1,110 to 1,650) | 0.20 |
| Sex | | | 0.52 |
| Male | 6 (60.0) | 52 (46.8) | |
| Female | 4 (40.0) | 59 (53.2) | |
| Blood transfusion during hospitalization | | | 0.51 |
| Yes | 3 (30.0) | 50 (45.0) | |
| No | 7 (70.0) | 61 (55.0) | |
| Number of blood transfusions per infant (n=53); median (IQR) | 1 (1 to 1.5) | 2 (1 to 6) | 0.17 |
| Prophylactic $FeSO_4$ supplementation at hospital discharge | | | 0.23 |
| Yes | 8 (80.0) | 102 (91.9) | |
| No | 2 (20.0) | 9 (8.1) | |
| $FeSO_4$ was stopped before 4-months postnatal age (n=110) | | | 0.36 |
| Yes | 3 (37.5) | 20 (19.6) | |
| No | 5 (62.5) | 82 (80.4) | |
| Feeding status at 4-months postnatal age in all infants (n=121) | | | 0.16 |
| Exclusive BF | 4 (40.0) | 17 (15.3) | |
| Partial BF | 4 (40.0) | 54 (48.6) | |
| Formula | 2 (20.0) | 40 (36.0) | |
| Feeding status at 4-months postnatal age in VLBW infants (n=66) | | | 0.59 |
| Exclusive BF | 1 (20.0) | 10 (16.4) | |
| Partial BF | 3 (60.0) | 26 (42.6) | |
| Formula | 1 (20.0) | 25 (41.0) | |

BF=breastfeeding; $FeSO_4$=ferrous sulfate; IQR=interquartile range; ID=iron deficiency; IDA=iron deficiency anaemia; VLBW=very-low-birth-weight

and who did not have IDA or ID are shown in Table 1.

### Rate of breastfeeding

At hospital discharge, all 121 infants were on EBF or PBF. The number of infants on breastfeeding decreased from 1- to 4-months postnatal age. At 4-month postnatal age, the rates of EBF and PBF were 17.4% (21/121) and 47.9% (58/121), respectively. Infants on PBF at 4-month had a median proportion of mother's own milk to total intake during the four months period of 71.3% (IQR 56.6 to 87.5). For the 42 infants on formula feeding at 4-month, the median duration of breastfeeding was two months (IQR 1 to 3). None of the 121 infants included in the final analysis received supplementary foods before blood draw for iron study.

### Iron deficiency or iron deficiency anemia

At hospital discharge, prophylactic $FeSO_4$ was prescribed for 110 infants at a mean dose of 3.3±0.9 mg/kg/day. For those infants who were breastfed at 4-month postnatal age, $FeSO_4$ dose received was decreasing from 3.5±0.9 mg/kg/day at hospital discharge to 3.6±0.8 mg/kg/day at the first follow-up visit, 2.8±0.9 mg/kg/day at the second follow-up visit, and to 2.4±0.6 mg/kg/day at the third follow-up visit. Ferrous sulfate was continued throughout the study period in 87 of 110 infants (79.1%). The median postnatal age when $FeSO_4$ was discontinued unintentionally by the parents was 84 days (IQR 64 to 103). The percentage of infants taking $FeSO_4$ decreased from 91% and 90% at 1-month, to 84% and 86% at 2-month, 71% and 76% at 3-month, and to 70% and 71% at 4-month postnatal age in breastfed and formula-fed infants, respectively. On the day of venous blood draw for iron study, the median postnatal age and median corrected age of infants were 4.2 months (IQR 4.1 to 4.5) and 2.3 months (IQR 1.8

**Table 2.** Iron study results of breastfed and formula-fed infants at 4 months (n=121)

| Results | Type of feeding at 4 months; n (%) | | | *p*-value |
|---|---|---|---|---|
| | Exclusive BF (n=21) | Partial BF (n=58) | Formula (n=42) | |
| Iron deficiency anemia | 1 (4.8) | 2 (3.4) | 0 (0.0) | 0.41 |
| Iron deficiency | 3 (14.3) | 2 (3.4) | 2 (4.8) | 0.23 |
| Hemoglobin in g/dL; mean±SD | 11.1±1.6 | 11.4±1.4 | 11.4±0.9 | 0.60 |
| Hemoglobin <10.5 g/dL | 7 (33.3) | 14 (24.1) | 7 (16.7) | 0.34 |
| Serum ferritin in µg/L; median (IQR) | 149.7 (41.6 to 300.1) | 64.0 (40.8 to 124.6) | 70.2 (39.8 to 100.8) | 0.06 |
| Received blood transfusion (n=53) | 182.5 (67.4 to 576) | 85.0 (50.5 to 148.6) | 80.9 (35.8 to 144.4) | 0.06 |
| No blood transfusion (n=68) | 80.5 (34.5 to 186.4) | 51.3 (28.4 to 96.1) | 60.9 (40.1 to 89.8) | 0.51 |
| Serum ferritin <12 µg/L | 0 (0.0) | 1 (2.9) | 0 (0.0) | 1.0 |
| Serum transferrin saturation in % (n=113); median (IQR) | 33.1 (17.1 to 87.1) | 29.4 (23.1 to 38.7) | 25.8 (21.4 to 36.2) | 0.34 |
| Serum transferrin saturation <17% | 4 (21.1) | 3 (5.5) | 2 (5.1) | 0.09 |
| Serum iron in µg/dL (n=113); median (IQR) | 18 (11.4 to 24.3) | 18 (14.5 to 21.2) | 16.4 (11.8 to 21.4) | 0.61 |

BF=breastfeeding; IQR=interquartile range; SD=standard deviation

**Table 3.** Logistic regression analysis of factors associated with iron deficiency or iron deficiency anemia

| | Adjusted odds ratio | 95% confidence interval | | *p*-value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Birth weight (100 gram) | 1.11 | 0.89 | 1.39 | 0.36 |
| Prophylactic ferrous sulfate supplementation at hospital discharge | 0.63 | 0.09 | 4.53 | 0.64 |
| Feeding status at 4 months of age | | | | |
| Infant formula | Reference | | | |
| Partial breastfeeding | 1.47 | 0.25 | 8.52 | 0.67 |
| Exclusive breastfeeding | 4.54 | 0.74 | 27.77 | 0.10 |

to 2.8), respectively.

Direct serum iron and UIBC could not be run due to technical errors in eight specimens. Hemoglobin and iron results of breastfed versus formula-fed infants at 4-month are demonstrated in Table 2. The serum ferritin level of the infants received blood transfusion during hospitalization was significantly higher than those who did not [85.3 µg/L (IQR 48.4 to 178.3) versus 57.1 µg/L (IQR 32.3 to 97.3); $p$=0.001].

The overall incidence of ID/IDA at 4-month postnatal age was 8.3%. The incidence of ID/IDA was not statistically significant different among exclusively breastfed, partially breastfed, and formula-fed infants at 19%, 6.9%, and 4.8%, respectively, ($p$=0.16), nor was it statistically significant different among exclusively breastfed, partially breastfed, and formula-fed in the VLBW subgroup at 9.1%, 10.3%, and 3.8%, respectively ($p$=0.59) (Table 1). Binary logistic regression analysis of ID/IDA with adjustment for possible confounding factors such as birth weight, prophylactic $FeSO_4$ supplementation at hospital discharge, and breastfeeding status at 4-month, revealed that none of these factors was significantly associated with ID/IDA (Table 3). Twenty-five infants were anemic with a median hemoglobin level of 10.1 g/dL (IQR 9.5 to 10.25) and had normal iron study. Of those, 19 infants had additional hematological studies. The results were normal in 13 infants, alpha thalassemia-2 trait in four infants, alpha thalassemia-1 trait in one infant, and homozygous alpha thalassemia-2 in one infant.

## Discussion

The present study demonstrated that at 4-month postnatal age, exclusively breastfed infants had a higher incidence of ID/IDA than partially breastfed and formula-fed infants in both VLBW infants and larger preterm infants. However, it was not statistically significant. After adjusting for birth weight and prophylactic $FeSO_4$ supplementation at hospital discharge, breastfeeding status at 4-month was not significantly associated with ID/IDA.

The incidence of ID/IDA among breastfed preterm infants in the present study was lower than the rates reported in the previous studies[5,6,13]. In a randomized controlled trial of early versus late iron supplementation of VLBW infants, the incidence of ID at 60 days in the early iron supplementation group was 15% compared to 40% in the late iron supplementation group[6]. Another observational study reported that when breastfed VLBW infants received late iron supplementation, 24% had ID and 4% had anemia[5]. This variation in incidence among studies may be due to differences in iron supplementation protocol, study population, or screening time point. One similar finding across the studies is that iron supplementation before two-months postnatal age was associated with a lower incidence of ID/IDA in breastfed preterm infants. It has been speculated that early iron supplementation of infants with low vitamin E levels might increase free radicals and cause hemolysis. Iron plus vitamin E supplementation started at two weeks of life resulted in a significantly higher hemoglobin level and non-significantly higher iron and ferritin concentrations[6,15]. The present study practice of vitamin E supplementation before starting iron supplementation may have played a role in lower incidence of ID/IDA.

The formula used for the present study participants after hospital discharge was infant formula or post-discharge formula, which had iron content of 1.22 mg per 100 mL and 1.34 mg per 100 mL, respectively. Iron intake from these formulas would likely be adequate if infants consume at least 170 mL/kg/day. All the participants were started with breastfeeding at hospital discharge, so prophylactic iron supplementation was prescribed at the recommended dose of 2 to 4 mg/kg/day. Prescription of iron supplementation during the follow-up was at the discretion of the attending physician. There was a trend toward decreasing $FeSO_4$ doses over time in 4-month breast-fed infants. This practice may have reflected the unawareness of the physician of the possible suboptimal dose of $FeSO_4$ supplementation according to the increasing body weight of the growing infants. The suboptimal dose of $FeSO_4$ may have contributed to the higher incidence of ID/IDA in 4-month breastfed infants compared to formula-fed infant, though the difference was not statistically significant. The importance of iron supplementation should be emphasized with mothers to improve compliance, especially among mothers who continue breastfeeding.

It is difficult to recommend a uniform dose of iron supplementation for all preterm infants in all circumstances and settings. Iron requirements at hospital discharge are affected by the number of red blood cell transfusions received, the type of feeding, and possibly the growth rate[16]. In the present study, 73% of VLBW infants and 9% of larger preterm infants received red blood cell transfusion during their hospitalization, and their serum ferritin levels were higher than those who did not. Multiple blood transfusions during hospitalization can render extremely-low-birth-weight infants iron-replete for up to 16 weeks[17]. Agostoni and Buonocore et al[9] recommended that the dose of $FeSO_4$ should not exceed 5 mg/kg/day due to the possible risk of retinopathy of prematurity. Taking all these issues into account, it may be beneficial for preterm infants who received blood transfusion during hospitalization to have iron studies performed before hospital discharge to help determine appropriate iron supplementation.

There is no recommended specific time point for ID screening in preterm infants. In infants born at 32 to 34 weeks gestation, Schiza et al[12] observed that serum ferritin levels decreased from the second week to the third month, and that they remained stable thereafter. Iron deficiency can occur within the first three months, even with iron supplementation[5,6,18]. Based on the present study cohort analysis, the authors reaffirm that it is prudent to screen for iron status in preterm infants when they are four months old[19]. Both hemoglobin and iron study should be used to screen for ID since most ID infants are not anemic[20].

Studies of long-term outcomes, including growth and neurodevelopmental outcomes, are necessary next steps in assuring iron sufficiency for all preterm infants.

## Limitation

The incidence of ID/IDA in the present study cohort was lower than predicted for sample size calculation, and this might have reduced the discriminative power of the present study. The low follow-up rate is another important limitation of the study; however, the sample size was increased by 35% to compensate for study infants lost to follow-up. Moreover, recall bias of the mother about type and amount of milk feeding may be inevitable.

## Conclusion

Breastfeeding combined with prophylactic iron supplementation did not increase risk of ID/IDA in preterm infant at 4-month postnatal age. All infants born at or before 34 weeks' gestation weighing 2,000 grams or less should receive recommended dose of

prophylactic iron supplementation as long as they are breastfed to prevent ID. The authors recommend screening all prematurely born infants for ID and anemia at 4-month postnatal age regardless of feeding type, so that if ID was identified, therapeutic treatment with iron can be timely started.


## Acknowledgement

The authors gratefully acknowledge Professor Vip Viprakasit of the Department of Pediatrics and Thalassemia Centre, Faculty of Medicine Siriraj Hospital, Mahidol University for performing hemoglobin typing. The authors would also like to graciously thank Professor Robert D. Christensen of the Division of Neonatology, Department of Pediatrics, University of Utah School of Medicine, United State of America for providing valuable suggestions. Lastly, the authors would like to thank Dr. Chulaluk Komoltri of the Division of Clinical Epidemiology, Department of Health Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University for assistance with statistical analysis, and Kevin P. Jones for assistance with English language editing.

## Funding disclosure

The present study was supported by the Vejdusit Foundation, which is a charitable organization that was patronized by the late Her Royal Highness Princess Galyani Vadhana Krom Luang Naradhiwas Rajanagarinda.


## Conflicts of interest

The authors declare no conflict of interest.

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