Justification of Universal Iron Supplementation for Infants 6-12 months in Regions with a High Prevalence of Thalassemia

Phakatip Sinlapamongkolkul
Pacharapan Surapolchai
Vip Viprakasit (vip.vip@mahidol.edu)
Mahidol University

Research Article

Keywords: thalassemia minor, infants, prevalence, iron status, iron deficiency anemia, serum hepcidin

Posted Date: June 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-624967/v1

Abstract

Background

Many clinicians hesitate adopting a universal infant iron supplementation program due to the risk of increased iron absorption for those with thalassemia. We aimed to determine thalassemia prevalence in 6- to 12-month old infants, along with the iron status of those with and without thalassemia.

Procedures:

We performed a cross-sectional descriptive study of infants attending the Well Baby Clinic at Thammasat University Hospital for routine checkups. Complete blood count, hemoglobin electrophoresis, iron parameters, and molecular genetics for common α- and β-thalassemia were evaluated.

Results

Overall, 97 of 206 (47%) participants had thalassemia minor, the majority having Hb E traits. None had thalassemia intermedia or major. Familial history of anemia or thalassemia presented an increased risk of detecting thalassemia minor in offspring (OR 5.18; 95% CI 2.60-10.33, $p = 0.001$). There were no statistical differences in transferrin saturation, serum ferritin and hepcidin between iron-replete infants with thalassemia minor and those without. However, one-third of infants with thalassemia minor (31/97) also had iron deficiency anemia (IDA), with a similar risk of having iron deficiency to infants without thalassemia. There was no hepcidin suppression in our infants with thalassemia minor as compared to controls.

Conclusions

Both thalassemia and IDA are endemic to Southeast Asia. Infants with thalassemia minor, particularly with Hb E and α-thalassemia traits, are at risk of IDA. Our short-term universal iron supplementation program for 6 to 12-month old infants does not appear to increase the risk of those with thalassemia minor developing iron overload in the future.

Introduction

An estimated 300-million children worldwide had anemia in 2011[1], and iron deficiency anemia (IDA) remains the most common cause of this to date. The World Health Organization (WHO) publishes an international anemia control guideline that states all children and women living in settings where the prevalence of anemia exceeds 20% should receive supplemental iron.[2] In Thailand, this recommendation has been adopted by the Department of Health, Ministry of Public Health which recommends a universal

iron supplement for Thai infants over six months old to prevent IDA when these babies come for routine vaccination. Iron supplementation continues until 24 months of age with 12.5 mg of elemental iron weekly, according to WHO Guideline (2011).[3,4]

When compared to placebos or no intervention, intermittent iron supplementation is considered to be effective in reducing the risk of anemia or iron deficiency (ID) in children younger than 12 years old.[5] This is because infants older than six months have a high prevalence of IDA, which can impair physical, behavioral and cognitive functions and result in persistent neurocognitive defects, despite receiving iron therapy later.[6] However, local Thai practitioners, particularly pediatricians, have concerns about this policy since there is a high prevalence of thalassemia and hemoglobin disorders in the country.[7] It is widely accepted thalassemia disease could significantly increase risk of iron overload, leading to iron toxicity later in life.[8–9]

Thalassemia is characterized by inherited mutations of α and β globin genes causing decreased globin synthesis. At least of 5.2% of the world population carries one allele of globin gene variants (carrier or trait).[10] For α-thalassemia, there are two types based on molecular defects: α^0-thalalassemia caused by deletions of two linked α-globin genes *in cis* (–/αα) and α^+-thalassemia caused by deletions of one α-globin gene (-α/αα) or nucleotide mutations (α^Tα/αα or αα/αα^T). Coinheritance of two affected alleles in autosomal recessive mode leads to chronic hemolytic anemia and ineffective erythropoiesis known as thalassemia disease.[11] On the other hand, hemoglobinopathy is mainly caused by mutations of coding sequences and produces qualitative defects. Several hemoglobinopathies are innocuous and do not lead to any clinical consequences.[12] However, some mutations such as hemoglobin E (Hb E) at codon 26 of the β-globin genes (GAG > AAG) also have quantitative effects, and an interaction of Hb E with β-thalassemia mutations results in Hb E/β-thalassemia syndrome with heterogeneous clinical severity.

Around 30 to 40 percent of Thais are thalassemia carriers, including α-thalassemia, β-thalassemia, and Hb E. Due to a high prevalence of all genotypes, it is not uncommon to find individuals with combined α and β-globin abnormalities.[13–16] Collectively, these thalassemia traits, simple or in combination, are asymptomatic and do not require specific treatment; these are classified as “thalassemia minor”. Individuals with homozygous Hb E (Hb E/E) carrying two defective β-globin genes also present with milder forms of anemia without hepatosplenomegaly or blood transfusion being required.[17]

Several previous studies have examined iron status in patients with thalassemia[18–21], but little is known about thalassemia minor in comparison to normal populations[22], particularly in infants. Iron overload is one of the most common complications in those with thalassemia due to blood transfusions and increased iron absorption[8–9]. Intestinal iron intake in thalassemia is usually enhanced due to hepcidin suppression by the upregulation of erythropoietic markers such as GDF-11, GDF-15 and Erfe in response to chronic anemia and erythropoietin drive.[23–25] Hepcidin controls iron intake through duodenal enterocytes by limiting the expression of ferroportin: an intestinal iron gateway into circulation. Research consistently shows hepcidin suppression in thalassemia patients.[26,27] Recently, a Sri Lankan study

demonstrated that β-thalassemia carriers had mildly suppressed hepcidin concentrations out of proportion to their iron stores. It has been suggested that a widespread distribution of iron supplementation could possibly increase the risk of harmful iron overload in β-thalassemia carriers.[28] In Thailand, there has been no data on iron status and hepcidin levels in the pediatric population with our common thalassemia traits of α-thalassemia and Hb E and homozygous HbE, especially in infants who receive supplements through our national program.

Our main objective was to determine the iron status in infants aged six to 12 months from our Well Baby Clinic and identify the prevalence of ID and IDA among those with or without thalassemia. In addition, we evaluated clinical and laboratory characteristics of both groups to identify which factors, including hepcidin levels, would significantly influence iron status. We aimed to illustrate whether infants with thalassemia are at similar or lower risk of ID or IDA as compared to the similarly-aged general population and, thus, supply evidence regarding safe universal iron supplementation for Thai infants.

Methods

Study population

This is a cross-sectional descriptive study approved by the Human Ethics Committee of Thammasat University No. 1 (Faculty of Medicine). From June 2016 to June 2017, six- to 12-month old infants attending the Well Baby Clinic at Thammasat University Hospital for vaccinations and scheduled checkups were randomly recruited. Informed consent was given by their parents or legal guardians. Our inclusion criteria were term newborns (38–42 weeks gestation) with a birthweight between 2,500-4,000 grams having no prenatal and perinatal complications such as severe birth asphyxia, severe respiratory distress, or neonatal intensive care unit admission. Infants with chromosome abnormalities/syndromes, infectious/inflammatory diseases and any acute health problems were excluded. All clinical samples were collected before routine universal iron supplementation. We obtained demographic/clinical data through direct interviews with two investigators (PSi and PSu). Weight and length of participants were measured and evaluated by Z-score, according to WHO guidelines.[29] The Z-scores of weight-for-lengths below or above two standard deviations (SD) are categorized as underweight and overweight, respectively.

Hematological and biochemical evaluation

All participants underwent complete blood count evaluation using automated cell count (UniCel®DxH 800, Beckman Coulter, Brea, USA), hemoglobin typing by automated capillary electrophoresis analyzer (MINICAP, Sebia, Lisses, France), iron parameters including serum iron (SI), total iron binding capacity (TIBC) using a fully automated quantitative assay, and serum ferritin by electrochemiluminescence immunoassay (ECLIA or Elecsys® technology, Roche Diagnostics, Penzberg, Germany). SI, TIBC and serum ferritin assays were performed using a ROCHE COBAS BIO centrifugal analyzer according to manufacturer's instructions.[30]

Serum hepcidin was determined by a competitive inhibition enzyme-linked immunosorbent assay (cELISA)[31,32], with detection ranges of 2.47–200 ng/mL, according to manufacturer's instructions (Catalog No. CEb979Hu, Cloud-Clone Corp., Uscn Life Science Inc., Wuhan, China), with afternoon blood sampling to prevent diurnal variations.[31,33] In the assay, a monoclonal antibody specific to hepcidin was precoated onto a microplate. A competitive inhibition reaction was launched between biotin-labeled hepcidin and unlabeled hepcidin (standards or samples) with the antibody. After incubation, the unbound conjugate was washed off. Next, avidin conjugated to a horseradish peroxidase (HRP) was added to each microplate well and incubated. The amount of bound HRP conjugate was reversely proportional to the concentration of hepcidin in the sample. After addition of the substrate solution, the intensity of color developed was reversely proportional to the concentration of hepcidin in the sample.

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol of phenol-chloroform extraction. Alpha-globin genotyping was performed by a single-tube multiplex gap polymerase chain reaction (Gap-PCR) for detecting seven common α-globin deletions (–^{SEA}, –^{THAI}, -(α)$^{20.5}$, –^{FIL}, –^{MED}-α$^{3.7}$, -α$^{4.2}$) and a single-tube multiplex amplification refractory mutation system (ARMS-PCR) for screening six common non-deletional α-globin mutations in Thailand: initiation codon (ATG◊A-G), codon 30 (ΔGAG), codon 59 (GGC◊GAC), codon 125 (CTG◊CCG) or Hb QuangSze, termination codon (TAA◊CAA) or Hb Constant Spring, and termination codon (TAA◊TAT) or Hb Paksé.[34] Beta-globin genotyping was performed by ARMS-PCR for detecting 16 common beta-globin mutations (-28, CD8/9, CD17, CD19, CD26 (Hb E), CD26 G > T (stop codon), CD27/28, IVSI-I, IVSI-5, CD35, CD41, CD41/42, CD43, CD71/72, CD95 and IVSII-654).[35] A single-tube multiplex Gap-PCR and enzymatic amplification was used for common beta-globin gene deletions (3.48 kb, 619 bp, Filipino (β)°, SEA HPFH (β)°, Chinese ^{G}γ(^{A}γδβ)°, Thai (δβ)°, Hb Lepore, HPFH-6 ^{G}γ(^{A}γδβ)°, Siriraj-thal ^{G}γ(^{A}γδβ)°, Asian Indian type A, and Asian Indian type B).[36,37] Hemoglobin E testing was studied by restriction fragment length polymorphism (RFLP)-PCR utilizing *Mnl*I restriction enzyme.[38]

Definitions

Hemoglobin (Hb) < 11 g/dL was used to define anemia, in line with WHO criteria.[39] Participants were classified as having ID if their serum ferritin (SF) was < 30 ng/mL or transferrin saturation (TS) was < 16% (TS = SI/TIBC x100).[40] IDA was diagnosed if there was compatibility with either laboratory criteria or a therapeutic response to iron therapy of 4–6 mg/kg/day for 8–12 weeks as prescribed and followed up by hematologists. The diagnosis of β-thalassemia trait was based on Hb A2 level > 3.5%. Infants with Hb F > 10% were investigated for common beta-globin gene deletions to diagnose δβ-thalassemia traits and hereditary persistence of fetal hemoglobin (HPFH).[36,41]

Statistical analysis

The sample size was calculated from this formula: $N = Z^2pq/d^2$, Z = 1.96, p = 0.4, q = 0.6, d = 0.07. The study population, according to prevalence of thalassemia in Thailand (30–40%),[13,15] was 188; we

approximated needing 200 participants, reflecting a 10% dropout. Demographic data was summarized as frequency and percentage for qualitative data and as mean and SD for quantitative data. Student's t-test or Mann-Whitney U test was used to compare continuous variables; the chi-square or Fisher's exact test was used for categorical variables, as appropriate. Univariate and multivariate logistic regression analyses were performed to identify risk factors: p of < 0.05 was considered statistically significant.

Results

Clinical characteristics

A total 206 infants, with 114 males (55.3%) and a mean age of 8.2 months (SD 2.0, range 6–12 months), were randomly enrolled. Interestingly, we found 97 individuals (47%) with some form of thalassemia minor, 39 with α-thalassemia (18.9% of total population), 45 with β-globin mutation mainly Hb E (21.8%), and 13 with combined α and β globin abnormalities (6.3%). None of these individuals had genotypes found in thalassemia diseases such as HbH disease or Hb E/β-thalassemia; therefore, they were classified as thalassemia minor and subsequently used for further analysis. Details of all comprehensive genotype data are shown in Table 1. Infants with thalassemia minor had no history of blood transfusion and no hepatosplenomegaly.

Table 1
Summary of globin genotypes found in 97 infants with thalassemia minor

Classification (%)	Type of thalassemia minor	Genotypes		Number (%)
		α-globin	**β-globin**	
α-globin mutations n = 39 (40.1)	- α^+-thalassemia trait	- $\alpha^{3.7}/\alpha\alpha$ or - $\alpha^{4.2}/\alpha\alpha$	β/β	24 (24.7)
	- Hb CS trait	$\alpha^{CS}\alpha/\alpha\alpha$	β/β	10 (10.3)
	- α^0-thalassemia trait	- - $^{SEA}/\alpha\alpha$	β/β	5 (5.1)
β-globin mutations n = 45 (46.3)	- Hb E trait	αα/αα	β^E/β	40 (41.1)
	- homozygous Hb E	αα/αα	β^E/β^E	4 (4.1)
	- β-thalassemia trait	αα/αα	β^T/β	1 (1.1)
Combined α- and β-globin mutations n = 13 (13.6)	- α^+-thalassemia trait with Hb E trait	- $\alpha^{3.7}/\alpha\alpha$	β^E/β	10 (10.3)
	- α^0-thalassemia trait with Hb E trait	- - $^{SEA}/\alpha\alpha$	β^E/β	1 (1.1)
	- α^+-thalassemia trait with β thalassemia trait	- $\alpha^{3.7}/\alpha\alpha$	β^T/β	1 (1.1)
	- homozygous Hb E with Hb CS trait	$\alpha^{CS}\alpha/\alpha\alpha$	β^E/β^E	1 (1.1)

Note: Hb CS, Hb Constant Spring is due to a termination codon mutation, TAA > CAA in α2 gene

We found no significant differences in all clinical characteristics: age, gender, growth and nutrition parameters, and iron markers such as SF, TS, and hepcidin levels. The exception was having a family history of anemia or thalassemia being more common in infants with thalassemia minor (43.3%) versus those without thalassemia (12.8%): Table 2. In our logistic regression analysis, having a history of anemia or thalassemia showed an increased risk of thalassemia minor in infants with an odds ratio of 5.18 (95% CI: 2.60-10.33), $p = 0.001$. Notably, the number of infants with both thalassemia minor and IDA at first diagnosis was higher than those with normal globin genotypes (32.0% *vs.* 20.2%); moreover, the number of infants with normal iron status and ID was significantly different among infants with and without thalassemia minor ($p = 0.037$): Table 2.

Table 2
Clinical and laboratory characteristics of 206 infants with and without thalassemia minor

Characteristics	Infants without thalassemia minor (n = 109, 53%)	Infants with thalassemia minor (n = 97, 47%)	*p*
Clinical characteristics			
Age in months	8.2 (1.9)	8.3 (2.0)	0.736
- mean (SD)	9 (6–12)	9 (6–12)	
- median (range)			
Male [n, (%)]	62 (56.9)	52 (53.6)	0.675
Weight in kg [mean, (SD)]	8.5 (1.1)	8.4 (1.2)	0.807
Length in cm [mean, (SD)]	70.2 (3.8)	70.7 (3.7)	0.356
Weight-for-length z-score (SD)	0.10 (1.11)	-0.11(1.27)	0.213
- normal [n, (%)]	104 (95.4%)	86 (88.7%)	0.130
- underweight [n, (%)]	1 (0.9%)	5 (5.2%)	
- overweight [n, (%)]	4 (3.7%)	6 (6.2%)	
History of anemia and/or thalassemia in family	14(12.8%)	42(43.3%)	< 0.001*
Laboratory characteristics			
Ferritin, ng/mL	49.3 (37.0)	51.3 (39.9)	0.698
- mean (SD)	42.5 (3.5-170.6)	35.7 (6.9-204.6)	
- median (range)			
Transferrin saturation, %	18.2 (13.2)	18.3 (7.6)	0.965
- mean (SD)	17.5 (0.5-131.9)	17.8 (2.7–39.1)	
- median (range)			
Hepcidin, ng/mL	5.2 (4.2)	4.9 (3.6)	0.586
- mean (SD)	3.8 (2.0-23.5)	3.9 (1.4–19.3)	
- median (range)			
Iron status	42 (38.5)	41 (42.3)	0.037*
- normal [n, (%)]	45 (41.3)	25 (25.7)	
- ID [n, (%)]	22 (20.2)	31 (32.0)	
- IDA [n, (%)]			

Characteristics	Infants without thalassemia minor (n = 109, 53%)	Infants with thalassemia minor (n = 97, 47%)	*p*

Note: cm, centimeters; ID, iron deficiency; IDA, iron deficiency anemia; kg, kilograms

Data is expressed as mean and standard deviation (SD) or no. (%), according to the nature of variables. Statistical methods used: chi-square, one-way analysis of variance, Mann-Whitney U or Student's t-test, as appropriate. *$p < 0.05$ was considered statistically significant.

The primary diagnosis of infants with IDA using Hb levels with SF and TS values was further confirmed when the majority responded to iron therapy. Thirty-four out of 53 IDA infants with (n = 31) and without thalassemia minor (n = 22) have received iron therapy, and all showed therapeutic response displayed as a significant increase across all red blood cell (RBC) parameters as compared to the baseline within their groups ($p < 0.05$). Of note, IDA infants without thalassemia minor (n = 18) had slightly greater increments in Hb and mean corpuscular volume (MCV) after iron therapy than IDA infants with some type of thalassemia minor (n = 16); however, there were no statistically significant differences between groups: **Supplementary Table 1**.

Iron status in infants with or without thalassemia minor

We performed subgrouping analysis within the groups of infants with and without thalassemia minor to determine the effects of normal iron status and IDA on hematological parameters (Table 3). There were significant differences in Hb, hematocrit (Hct), MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW) within both groups suggesting iron played a significant role in determining Hb, Hct, and red blood cell indices. With the exception of increased RBC counts and RDW, the other RBC parameters were lower in those with IDA versus normal iron. Interestingly, infants with thalassemia minor who had IDA (n = 31) displayed statistically lower values in MCV, MCH, with the higher RDW: Table 3. This suggests that the coinheritance of globin mutations can have epistatic hematological effects on top of any primary effects on iron status.

Table 3
Laboratory parameters of infants with and without thalassemia minor who had either normal iron status or iron deficiency anemia

Parameters	Infants without thalassemia minor		*p*-1[£]	Infants with thalassemia minor		*p*-2[£]
	Normal iron status (n = 39)	With IDA (n = 22)		Normal iron status (n = 41)	With IDA (n = 31)	
Hb, g/dL	12.3 (0.7)	10.1 (0.8)	< 0.001*	11.7 (0.7)	10.3 (0.7)	< 0.001*
Hct, %	37.3 (2.3)	32.3 (1.7)	< 0.001*	36.1 (2.0)	32.5 (1.9)	< 0.001*
RBC, $x10^6$/cu mm	4.78 (0.36)	4.82 (0.48)	0.996	5.21 (0.39)	5.25 (0.66)	0.749
MCV, fL	78.1 (3.4)	67.5 (7.1)	< 0.001*	69.6 (4.3)	63.0 (7.1)	< 0.001*
MCH, pg	25.8 (1.4)	21.2 (2.8)	< 0.001*	22.5 (1.7)	19.8 (2.6)	< 0.001*
MCHC, %	33.0 (0.8)	31.3 (1.1)	< 0.001*	32.6 (1.8)	31.4 (1.4)	0.003*
RDW, %	13.8 (0.9)	16.4 (1.6)	< 0.001*	14.9 (1.8)	17.2 (2.6)	< 0.001*
Hb A, %	93.6 (2.4)	94.3(3.2)	0.337	76.8 (18.4)	68.5 (29.8)	0.150
Hb A_2, %	2.7 (0.3)	2.5 (0.4)	< 0.001*	3.0 (0.6)	3.1 (0.7)	0.517
Hb F, %	3.7 (2.5)	2.9 (2.9)	0.262	6.4 (5.4)	7.1 (5.6)	0.594

Note: Hb, hemoglobin; Hct, hematocrit; IDA, iron deficiency anemia; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell count; RDW, red blood cell distribution width. Hb A, Hb A2, and Hb F data from infants with thalassemia minor include those with α-thalassemia trait, β-thalassemia trait, Hb E trait or homozygous Hb E.

Data is expressed as mean and standard deviation (SD) or no (%), according to the nature of variables. Statistical methods used: Mann-Whitney U or Student's t-test, as appropriate. [£]*p*-1 and *p*-2 compared laboratory parameters between three different iron status (normal iron status and IDA) among infants without and with thalassemia minor, respectively *$p < 0.05$ considered statistically significant.

Effects of thalassemia minor on hematologic and iron parameters

We further compared those infants with normal iron and those with IDA to see the effects of thalassemia minor. Coinheritance of thalassemia had significant effects on Hb, Hct, RBC, MCV, MCH, RDW, but not MCHC, solely in those with normal iron status: **Supplementary Table 2.** However, we found only RBC and MCV to be significantly different in infants who already had IDA, suggesting this epistatic effect took place only within those two parameters. In addition, we found an effect of iron on significantly decreased levels of Hb A2 in infants without thalassemia minor but not in those with thalassemia. On the other hand, the basal Hb F in infants with thalassemia minor was generally higher than those without, suggesting a delay in globin switching, one of the consequences of globin abnormality.[42,43] Iron status does not appear to be significantly associated with the levels of persistent Hb F expression within the groups of both infants with and without thalassemia minor (Table 3).

Effects of thalassemia minor on hepcidin expression

We compared serum hepcidin, serum ferritin, and TS in infants without thalassemia having normal iron status to those having different types of thalassemia minor: Fig. 1. Measurements for each group are in **Supplementary Table 3A** (iron replete) and **3B** (iron deplete). Levels of hepcidin appeared to be slightly lower in those with thalassemia minor and lowest in those with combined α and β globin mutations. This was consistent with the slightly higher serum ferritin and TS seen in those with combined thalassemia minors, although without statistically significant differences. No differences within these parameters were found between groups and by gender) (data not shown).

Discussion

We found the prevalence of thalassemia minor (carrier) in nearly half of the infants, at 47%. It was higher than previously reports of 30–40%.[13,15] One of the main reasons was the DNA testing used in our current study was far more comprehensive than the approaches such as cord blood hemoglobin studies, hemoglobin typing, etc. used 20 years ago. This is consistent with a report on thalassemia prevalence by Viprakasit V *et al*. in 2009[44], stating that Hb E trait was the most common type of thalassemia minor[14,15,44] in Thailand, with a frequency up to 50–60% in Southeast Asia.[14] We found no individuals with thalassemia disease. This may point to the effectiveness of Thailand's prevention and control program that screens for thalassemia carriers in pregnant women and their partners in order to identify the genetic risk of severe thalassemia syndromes.[16] Therefore, our studied population is likely to represent relatively "healthy" infants receiving routine health care and are a primary target for the iron supplementation program.

A previous study of β-thalassemia traits noted the presence of mildly increased erythropoiesis, as seen through elevated erythropoietin levels.[45] It has also been observed that adults with α- or β-thalassemia traits have shown increases in soluble transferrin receptors or erythropoietin concentrations, indicating ineffective erythropoiesis and increased erythropoietic drive leading to hepcidin suppression and upregulated iron absorption.[20] Prior studies in India and Iran examining the iron status of adults with β-thalassemia traits concluded that β-thalassemia traits had higher serum ferritin than the controls,

representing an advantage in iron balance.[46,47] These particular findings did not concur with others, which had stated that ID might commonly coexist with thalassemia traits.[20,48,49] These conflicting results have caused uncertainty in iron supplementation strategies for areas with a high prevalence of hemoglobinopathy. With a potential increase in risk of iron overload for individuals with thalassemia minors, universal iron supplementation programs remain a point of contention.

A recent community study of 1821 Sri Lankan schoolchildren aged 8–18 years (48.3% males) from the Oxford group has shown that this might be the case for those with β-thalassemia traits.[28] Eighty-two β-thalassemia carriers with iron-replete had evidence of increased erythropoiesis, a slight but significant reduction in hepcidin, and suppression of hepcidin out of proportion to their iron stores: lower hepcidin-ferritin ratio compared with non-carrier controls (n = 176 with normal MCV and MCH). Another Sri Lankan cross-sectional study of 2273 children (aged 12–19 years) from a total of 7526 students, reported the same effect in iron-replete α-thalassemia carriers as compared to the non-iron deficient controls without thalassemia minor (4.8 ng/mL vs 5.3 ng/mL, $p = 0.02$).[50] However, this was not observed in those with Hb E traits from both cohorts.[28,50] Based on these results, it has been proposed that a hepcidin cutoff of < 3.2 ng/mL could be used to select cases for iron supplementation in countries with high rates of thalassemia carriers.[50] Both studies were conducted in primary and secondary school students as this is the age group at which iron supplementation is given in Sri Lanka. However, the effects of being a thalassemia carrier on hepcidin suppression, as well as the risk of iron accumulation in younger cases with thalassemia minor, remain unclear.

Our study determined this iron supplement issue in infants with thalassemia minor. While we could not find significant hepcidin suppression in our infants with thalassemia minors as compared to previous studies, our results were somewhat in line with such findings. Most of our thalassemia minors were Hb E traits, and this condition did not show a significant enough globin imbalance leading to ineffective erythropoiesis and subsequent hepcidin suppression. Moreover, even for individuals with homozygous Hb E, we found no evidence of this effect. Our infants with α-thalassemia carriers also demonstrated no effects of hepcidin suppression, differing from the previous study.[50] This may be because our population was younger with remaining Hb F expression (Tables 1 and 3) and had less globin imbalance and ineffective erythropoiesis *per se*. It is, therefore, possible the erythropoietic drive that suppresses hepcidin was not fully operative yet.

In addition, the normal physiology of hepcidin expression, especially within the first year of life, might be more dynamic. A recent study in late preterm infants (32–36 weeks gestation) described a physiologic decrease of hepcidin levels during the first four months of life to increase iron availability.[51] Another longitudinal study that followed 140 Spanish healthy and full-term infants found hepcidin levels increased from six to 12 months of age with hepcidin levels positively correlated with iron status.[52] These results suggested that, in normal babies, a regulation of hepcidin production is under development during the first year of life; this may also be true for infants with thalassemia. Therefore, the effects of ineffective erythropoiesis on hepcidin suppression in thalassemia traits are likely not fully apparent

during the first year of their life. This warrants further study to define at what age this effect would first be identified.

We still found our infants with thalassemia minor having a high proportion of iron depletion (57.7%), similar to infants without thalassemia (61.5%); the number of infants with both thalassemia and IDA was even significantly higher than infants without thalassemia minor (32 vs 20.2%) (Table 2). Thus, the likely causes and possible risk factors of ID need to be further identified. Nevertheless, infants with thalassemia minor who have IDA or ID would benefit from proper iron supplementation. Interestingly, infants with a coexisting thalassemia minor and IDA had significantly reduced Hb, MCV, MCH, and MCHC with increased RDW versus those having thalassemia minor with normal iron (Table 3). These findings were consistent with previous studies in India where MCV and MCH were significantly lower in adults with combined thalassemia traits and IDA than with either of these conditions.[48] We believe our RBC indices to present a comprehensive analysis of thalassemia carriers at this age group. Our findings could be useful as references.

Among 36 thalassemia minor infants with anemia, we found five cases who did not have coexisting IDA, including infants with two α-thalassemia traits ($-\alpha^{3.7}/\alpha\alpha$ and $--^{SEA}/\alpha\alpha$), one β-thalassemia trait, one Hb E trait and one homozygous Hb E. This suggested that α- and β-thalassemia traits may be the cause of mild anemia in some infants. Accordingly, anemic infants unresponsive to oral iron therapy should be investigated for thalassemia, rather than continuously undergoing long-term iron therapy by default, as toxicity or other side effects may develop. Familial history of anemia or thalassemia as shown herein was found to be strongly associated with thalassemia minor in offspring and could be used to diagnose future cases early.

In conclusion, our study showed that infants in Thailand, from six to 12 months old, with thalassemia minor, in which the majority had Hb E and α-thalassemia traits, are at similar risk of developing IDA as the general population. This may partially be due to a lack of hepcidin suppression at this age or the type of mutations we found. Therefore, a universal short-term period of iron supplementation in infants would likely not be harmful. More than half of this population could benefit from this strategy. Beyond this age group, however, particularly for school children, a proper measurement of serum hepcidin along with using a cutoff as described earlier would be an alternative approach to select those who should genuinely receive iron supplementation. This would minimize the chance of overtreating individuals with thalassemia minor in areas of high prevalence of thalassemia and hemoglobinopathies.[50]

Declarations

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to express our gratitude to all infants and their parents/guardians for their participation, Miss Pornpen Gamnarai (Faculty of Medicine, Thammasat University) for her technical assistance, and Prof Somdet Srichairatanakool (Faculty of Medicine, Chiang Mai University) for his contribution on the analysis of iron-related parameters. The English language editing was done by Debra Kim Liwiski, international instructor, Clinical Research Center, Faculty of Medicine, Thammasat University. The authors gratefully acknowledge the financial support provided by Thammasat University Research Fund under TU Research Scholar, contract no 2/67/2560. VV was supported by a Chalermprakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Author contributions

PSi and PSu were the co-principal investigators of the project, evaluated all study participants, collected data, performed analysis, and drafted the manuscript. VV, as senior author, developed the concept, analysis plan, overall interpretation of results, and revised the manuscript. All authors read and approved the final version of the manuscript.

References

1. Guideline: Daily iron supplementation in infants and children. Geneva: World Health Organization; 2016.
2. Essential Nutrition Actions: Improving maternal, newborn, infant and young child health and nutrition. Geneva: World Health Organization; 2013.
3. Department of Health: Ministry of Public Health of Thailand. Guidelines for controlling and preventing anemia from iron deficiency. Available at: URL: http://hpc.go.th/director/data/mch/IDAControl.pdf. Accessed Mar 27, 2019.
4. Guideline: Intermittent iron supplementation in preschool and school-age children Geneva: World Health Organization; 2011.
5. De-Regil LM, Jefferds MED, Sylvetsky AC, Dowswell T. Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. Cochrane Database of Systematic Reviews 2011, Issue 12. Art. No.: CD009085.
6. Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA, Kaciroti N,et al. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. Nutritional Neuroscience. 2010;13:54-70.
7. Surapolchai P, Sinlapamongkolkul P, Thaweekul P, Viprakasit V. Prevalence of iron deficiency in 6-12 month-old infants at the Well Baby Clinic. In: Navarawong W, editor. Proceedings of the 52th TSH Annual meeting; 2018 Mar 4-7; Bangkok, Thailand. Bangkok: TSH; 2018. p. 212.
8. Porter J, Viprakasit V. Iron overload and chelation. In: Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, eds. Guidelines for the management of transfusion dependent thalassaemia, 3rd ed. Thalassaemia international federation; 2014. p. 42-97.

9. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. Iron overload and chelation. In: Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V, editors. Guidelines for the management of non transfusion dependent thalassaemia, 1st ed. Thalassaemia international federation; 2013. p. 35-50.
10. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86:480–487.
11. Weatherall DJ, Clegg JB, eds. The Thalassaemia Syndromes. 4th ed. Oxford: Blackwell Science; 2001.
12. Globin gene server home page. Available at: URL: http://globin.cse.psu.edu/. Accessed Mar 1, 2019.
13. Panich V, Pornpatkul M, Sriroongrueng W. The problem of thalassemia in Thailand. Southeast Asian J Trop Med Public Health 1992;23 Suppl 2:1-6.
14. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia: molecular biology and clinical medicine. Hemoglobin. 1997;21:299-319.
15. Fucharoen S, Winichagoon P, Wisedpanichkij R, Sae-Ngow B, Sriphanich R, Oncoung W, et al. Prenatal and postnatal diagnoses of thalassemias and hemoglobinopathies by HPLC. Clin Chem. 1998;44:740–748.
16. Viprakasit V, Limwongse C, Sukpanichnant S, Ruangvutilert P, Kanjanakorn C, Glomglao W, et al. Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med. 2013; 51:1605–1614.
17. Tachavanich K, Viprakasit V, Chinchang W, Glomglao W, Pung-Amritt P, Tanphaichitr VS. Clinical and hematological phenotype of homozygous hemoglobin E: revisit of a benign condition with hidden reproductive risk. Southeast Asian J Trop Med Public Health. 2009;40:306-316.
18. Taher A, Hershko C, Cappellini MD. Iron overload in thalassaemia intermedia: reassessment of iron chelation strategies. Br J Haematol. 2009;147:634-640.
19. Winichakoon P, Tantiworawit A, Rattanathammethee T, Hantrakool S, Chai-adisaksopha C, Rattarittamrong E, et al. Prevalence and risk factors for complications in patients with nontransfusion dependent alpha- and beta-thalassemia. Anemia. 2015;2015:1-7.
20. Aydinok Y, Porter JB, Piga A, Elalfy M, Beshlawy AE, Kilinc Y, et al. Prevalence and distribution of iron overload in patients with transfusion-dependent anemias differs across geographic regions: results from the CORDELIA study. Eur J Haematol. 2015;95:244-253.
21. Krittayaphonga R, Viprakasit V, Saiviroonpornc P, Siritanaratkuld N, Siripornpitake S, Meekaewkunchornf A, et al. Prevalence and predictors of cardiac and liver iron overload in patients with thalassemia: A multicenter study based on real-world data. Blood Cells Mol Dis. 2017;66:24–30.
22. Zimmermann MB, Fucharoen S, Winichagoon P, Sirankapracha P, Zeder C, Gowachirapant S, et al. Iron metabolism in heterozygotes for hemoglobin E (HbE), α-thalassemia 1, or β-thalassemia and in compound heterozygotes for HbE/ β –thalassemia. Am J Clin Nutr. 2008;88:1026–1031.

23. Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, et al .High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med. 2007;13:1096–1101.
24. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. Curr Opin Hematol. 2015;22:199–205.
25. Gupta R, Musallam KM, Taher AT, Rivella S. Ineffective erythropoiesis: anemia and iron overload. Hematol Oncol Clin N Am. 2018;32:213–21.
26. Gardenghi S, Marongiu MF, Ramos P, Guy E, Breda L, Chadburn A, et al. Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood. 2007;109:5027-5035.
27. Camberlein E, Zanninelli G, Détivaud L, Lizzi AR, Sorrentino F, Vacquer S, et al. Anemia in β-thalassemia patients targets hepatic hepcidin transcript levels independently of iron metabolism genes controlling hepcidin expression. Haematologica. 2008;93:111-115.
28. Jones E, Pasricha SR, Allen A, Evans P, Fisher CA, Wray K. Hepcidin is suppressed by erythropoiesis in hemoglobin E β-thalassemia and β-thalassemia trait. Blood. 2015;125:873-880.
29. WHO Anthro for personal computers, version 3.2.2, 2011: Software for assessing growth and development of the world's children. Geneva: WHO, 2010. (http://www.who.int/childgrowth/software/en/)
30. Schlosnagle DC, Hutton PS, Conn RB. Ferrozine assay of serum iron and total iron-binding capacity adapted to the COBAS BIO centrifugal analyzer. Clin Chem. 1982;28:1730-1732.
31. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112:4292-4297.
32. Zipperer E, Post JG, Herkert M, Kündgen A, Fox F, Haas R, et al. Serum hepcidin measured with an improved ELISA correlates with parameters of iron metabolism in patients with myelodysplastic syndrome. Ann Hematol. 2013;92:1617–1623.
33. Troutt JS, Rudling M, Persson L, Ståhle L, Angelin B, Butterfield AM, et al. Circulating human hepcidin-25 concentrations display a diurnal rhythm, increase with prolonged fasting, and are reduced by growth hormone administration. Clin Chem. 2012;58:1225-1232.
34. Eng B PM, Walker L, Chui DHK, Waye JS. Detection of severe nondeletional α-thalassemia mutations using a single-tube multiplex ARMS assay. Genet Test. 2001;5:327-329.
35. Newton CR, Graham A, Heptinstall LE, Powell J, Summers C, Kalsheker N, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research. 1989;17:2503–2516.
36. Tritipsombut J, Phylipsen M, Viprakasit V, Chalaow N, Fucharoen S, Harteveld CL, et al. A single-tube multiplex gap-polymerase chain reaction for the detection of eight beta-globin gene cluster deletions common in Southeast Asia. Hemoglobin. 2012;3:571-580.
37. Craig JE, Barnetson RA, Prior J, Raven JL, Thein SL. Rapid detection of deletions caused β-thalassemia and hereditary persistence of fetal hemoglobin by enzymatic amplification. Blood. 1994;83:1673-1682.

38. Ekwattanakit S MY, Riolueang S, Tachavanich K, Viprakasit V. Association of XmnI polymorphism and hemoglobin E haplotypes on postnatal gamma globin gene expression in homozygous hemoglobin E. Adv Hematol. 2012;2012:1-5.
39. Iron deficiency anemia: assessment, prevention and control. A guide for programme managers. Geneva, World Health Organization, 2001 (WHO/NHD/01.3).
40. Camaschella C. Iron deficiency: new insights into diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2015;2015:8-13.
41. Galanello R. Screening and diagnosis for haemoglobin disorders. In: Old J, editor. Prevention of thalassaemias and other haemoglobin disorders: volume 1, 2nd ed. Nicosia, Cyprus: Thalassaemia international federation; 2013.
42. Tachavanich K, Viprakasit V, Chinchang W, Glomglao W, Pung-Amritt P, Tanphaichitr VS. Clinical and hematological phenotype of homozygous hemoglobin E: revisit of a benign condition with hidden reproductive risk. Southeast Asian J Trop Med Public Health. 2009;40:306-316.
43. Vrettou C, Kanavakis E, Traeger-Synodinos J, Metaxotou-Mavrommati A, Basiakos I, Maragoudaki E, et al. Molecular studies of beta-thalassemia heterozygotes with raised Hb F levels. Hemoglobin. 2000;24:203-220.
44. Viprakasit V, Lee-Lee C, Chong QT, Lin KH, Khuhapinant A. Iron chelation therapy in the management of thalassemia: the Asian perspectives. Int J Hematol. 2009;90:435-445.
45. Tassiopoulos T, Konstantopoulos K, Tassiopoulos S, Rombos Y, Alevizou-Terzaki V, Kyriaki P, et al. Erythropoietin levels and microcytosis in heterozygous beta-thalassaemia. Acta Haematol. 1997;98:147-149.
46. Mehta BC, Pandya BG. Iron status of beta thalassemia carriers. Am J Hematol. 1987;24:137-141.
47. Hoorfar H, Sadrarhami S, Keshteli AH, Ardestani SK, Ataei M, Moafi A. Evaluation of iron status by serum ferritin level in Iranian carriers of beta thalassemia minor. Int J Vitam Nutr Res. 2008;78:204-207.
48. Dolai TK, Nataraj KS, Sinha N, Mishra S, Bhattacharya M, Ghosh MK. Prevalance of iron deficiency in thalassemia minor: a study from tertiary hospital. Indian J Hematol Blood Transfus. 2012;28:7–9.
49. Hinchliffe RF, Lilleyman JS. Frequency of coincident iron deficiency and beta-thalassaemia trait in British Asian children. J Clin Pathol. 1995;48:594-595.
50. Wray K, Allen A, Evans E, Fisher C, Premawardhena A, Perera L, et al. Hepcidin detects iron deficiency in Sri Lankan adolescents witha high burden of hemoglobinopathy: A diagnostic test accuracy study. Am J Hematol. 2017;92:196–203.
51. Uijterschout L, Domellöf M, Berglund SK, Abbink M, Vos P, Rövekamp L, et al. Serum hepcidin in infants born after 32 to 37 wk of gestational age. Pediatr Res. 2016;79:608-613.
52. Aranda N, Bedmar C, Arija V, Jardí C, Jimenez-Feijoo R, Ferré N, et al. Serum hepcidin levels, iron status, and HFE gene alterations during the first year of life in healthy Spanish infants. Ann Hematol. 2018;97:1071-1080.

Figures

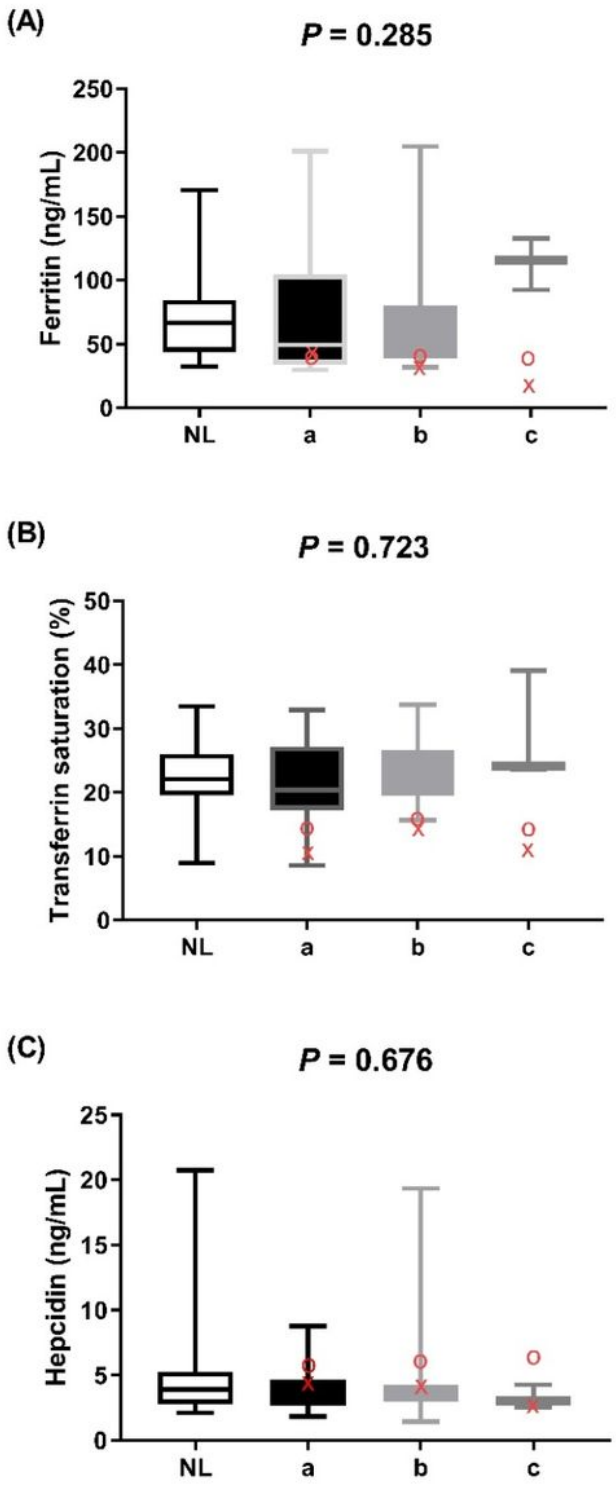

Figure 1

Comparison of serum ferritin (A), transferrin saturation (B) and serum hepcidin (C) between normal infants (NL) and infants with 3 subgroups of thalassemia minor; α-thalassemia trait, β-thalassemia trait or hemoglobin (Hb) E trait, and combined α- and β-globin mutations

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SinlapamongkolkulBMCPedTables.docx
- SinlapamongkolkulBMCPedsuppldata.docx
- SinlapamongkolkulBMCPedsuppldata.docx