

RBC indices as an early screening tool for thalassemia disease among anemic 9-month-old children

Ornrumpha Tanago

Srinakarinwirot university

Patcharapun Sarisuta

Srinakarinwirot university

Orawin Luecha

orawinl@g.swu.ac.th

Srinakarinwirot university

Research Article

Keywords: Thalassemia disease, Anemia screening, RBC indices

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10955200/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Anemia is a major public health problem worldwide. Iron deficiency is the most common cause of anemia while thalassemia is also an important underlying etiology in Thailand. The American Academy of Pediatrics (AAP) recommends universal screening by measuring hemoglobin concentrations at 12 months of age. In routine practice at our institution, anemia screening is performed at 9 months of age. Several red blood cells discrimination indices have been proposed to distinguish iron deficiency anemia from beta thalassemia trait. This study aimed to evaluate red blood cell indices for identifying thalassemia among 9-month-old children with anemia detected through routine screening.

Methods

This prospective descriptive study was conducted at Panyananthaphikkhu Chonprathan Medical Center, Thailand, from August 2024 to July 2026. Children aged 9 months who diagnosed with anemia were included. All children received oral iron supplement for 1 month and then follow-up with CBC and hemoglobin analysis. The factors associated with thalassemia were compared with normal by univariable and multivariable binary logistic regression analysis. Diagnostic performance was assessed using sensitivity, specificity, the percentage correctly classified, positive predictive value (PPV), and negative predictive value (NPV), and the area under the ROC curve (AUC), with corresponding 95% CIs.

Results

A total 185 infants were included and 38% had normal hemoglobin typing. The beta-thalassemia Hb E group had significantly lower hemoglobin, MCV, and MCH levels. An $MCV \leq 50$ fL, an $MCH \leq 15$ pg, and the presence of both criteria were each significantly associated with higher odds of thalassemia. Combined with $MCV \leq 50$ fL and $MCH \leq 15$ pg, the sensitivity was 53.85%, specificity 98.51%, PPV 85.71%, NPV 97.06% and the area under the receiver operating characteristic curve (AUC) was 0.76 (95% CI, 0.62–0.90) with correctly classified of 91.25%.

Conclusion

Using RBC indices, $MCV \leq 50$ fL combined with $MCH \leq 15$ pg, may serve as a practical screening criterion for selecting children with anemia who should undergo hemoglobin typing in order to confirm diagnosis thalassemia disease.

Background

Anemia is a major public health problem worldwide. In 2019, the global prevalence of anemia among children aged 6–59 months was 39.8% (1). Data from the Southeast Asia Nutrition Survey II (SEANUT II) (2) conducted in 2020 showed that 14.5% of Thai children aged 6 months to 12 years had anemia. According to the World Health Organization (WHO), anemia in children aged 6–23 months is defined as a hemoglobin concentration below 11 g/dL (3). Clinical manifestations of anemia in children include pallor, sleepiness, irritability, tachypnea, and tachycardia. Iron deficiency is the most common cause of anemia in children worldwide. However, in Thailand, thalassemia is one

of the most prevalent inherited hematologic disorders. Approximately 30–40% of the Thai population are thalassemia carriers, and approximately 1.2% of infants are born with thalassemia each year (4). The clinical severity of thalassemia ranges from asymptomatic carrier status to non-transfusion-dependent thalassemia (NTDT) and transfusion-dependent thalassemia (TDT), contributing substantially to the burden of anemia in Thailand.

As a result of the high burden of anemia, many countries have established recommendations for anemia screening in children. The American Academy of Pediatrics (AAP) recommends universal screening by measuring hemoglobin concentrations at 12 months of age. These recommendations aim to facilitate early detection and timely treatment of anemia. If a child has anemia and risk factors for iron deficiency, iron replacement therapy should be initiated, followed by follow-up. In Thailand, the Royal College of Pediatricians of Thailand recommends screening for anemia by measuring hemoglobin concentration or hematocrit at four time points: 9 months, 4 years, 8 years, and during adolescence. However, in routine practice at our institution, anemia screening is performed only once, at 9 months of age, for children attending scheduled vaccination visits.

Despite these recommendations, the reported prevalence of anemia among Thai children undergoing routine screening at 9 months of age ranges from 11.1% to 33.3% (6, 7, 8). Iron deficiency anemia is the most common cause of anemia in this age group; however, thalassemia is also an important underlying etiology in Thailand. Although hemoglobin typing is the reference standard for diagnosing thalassemia, it is not routinely performed in all children because of its cost and limited availability. A previous Thai study was performed hemoglobin typing for only children with anemia who did not respond to iron therapy, the results showed abnormal hemoglobin typing, including beta thalassemia with Hb E, homozygous Hb E, and Hb H disease for 14.9% (8). Several red blood cell discrimination indices, including the red cell distribution width (RDW) index, Mentzer index, and Green and King index, have been proposed to distinguish iron deficiency anemia from beta thalassemia trait (9, 10). However, these indices have shown variable diagnostic performance across different populations, and data regarding their usefulness in 9-month-old Thai children are limited. Therefore, this study aimed to evaluate red blood cell indices for identifying thalassemia among 9-month-old children with anemia detected through routine screening.

Methods

Study Design and Setting

This prospective descriptive study was conducted at the Well-Child Clinic of Panyananthaphikkhu Chonprathan Medical Center, Thailand, from August 2024 to July 2026. All children aged 9 months who attended the clinic for routine vaccination during the study period were screened for anemia as institutional protocol.

Study Population

Children diagnosed with anemia were eligible for inclusion in the study. Anemia was assessed using a venous blood sample for a complete blood count (CBC) and was defined as a hemoglobin (Hb) concentration of < 11 g/dL according to WHO criteria.

Exclusion Criteria

Children with an acute illness, a known chronic disease, or a history of iron supplementation 3 months before enrollment were excluded.

Withdrawal Criteria

Children who did not undergo blood sampling at the 1-month follow-up after initiation of iron supplementation were withdrawn from the study and excluded from analysis.

Study Procedures

All children diagnosed with anemia received oral iron supplementation at a dose of 4–6 mg/kg/day and were followed up by a hematologist for 1 month. At the 1-month follow-up visit, a CBC and hemoglobin analysis by high-performance liquid chromatography (HPLC) were performed.

Data collection and laboratory parameters

Demographic characteristics, growth parameters including weight and height, family history of thalassemia, prenatal history, and type of milk consumed were collected at enrollment. Hematological parameters obtained from the CBC at first and 1-month follow up visit included hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), mean corpuscular hemoglobin concentration (MCHC), white blood cells and platelet counts. The diagnosis of thalassemia was determined based on hemoglobin analysis using high-performance liquid chromatography (HPLC) performed at the 1-month follow-up. The study flow chart is presented in Fig. 1.

Outcomes

The primary outcome was to determine the predictive value of red blood cell (RBC) indices for screening thalassemia disease among 9-month-old children with anemia.

Secondary outcome was the prevalence of thalassemia among 9-month-old children with anemia.

Statistical analysis

Sample size was calculated based on the prevalence of thalassemia disease in 6–12 months old children in 2023, which was 47% (11). The type I error and the statistical power was set at 5%, 80% respectively. To account for potential data collection errors, and loss to follow-up, the sample size was increased by 10%, resulting in a final target sample size of 200 children. In total, 185 participants were included in the analysis.

Baseline demographic data were summarized using frequency and percentage for categorical data, and mean with standard deviation (SDs) for continuous data. The factors associated with the diagnosis thalassemia disease were compared with those in participants with normal hemoglobin typing. Univariable and multivariable binary logistic regression analyses were performed. The strengths of the associations were reported as unadjusted odds ratios (ORs) and adjusted odds ratios (aORs), respectively, with corresponding 95% confidence intervals (CIs). Covariates with a P-value < 0.15 in the univariable analyses were considered for inclusion in the multivariable logistic regression model to adjust for potential confounding. A two-sided P-value < 0.05 was considered statistically significant. Variables identified as being associated with the outcome were further evaluated to determine the optimal cut-off values for predicting thalassemia disease. Diagnostic performance was assessed using sensitivity, specificity, the percentage correctly classified, positive predictive value (PPV), and negative predictive value (NPV), and the area under the ROC curve (AUC), with corresponding 95% CIs.

Results

During the study period, from August 2024 to July 2026, a total of 192 infants who had anemia were eligible for the study, and 185 completed the study. Approximately 50% of the participants were male, and most were born at term. The mean weight was $8,379.72 \pm 1,146.81$ grams and mean height was 70.45 ± 2.87 cm. A family history of thalassemia status was reported in 53% of the participants. The baseline characteristics are presented in Table 1.

Among the 185 infants who completed the second blood test, 38% had a normal hemoglobin typing pattern, 3% had beta-thalassemia with Hb E, 9% had Hb H and Hb H constant spring, 12% had homozygous hemoglobin E, and 38% had other hemoglobin patterns, as shown in Table 1.

Table 1
Baseline characteristics

Baseline characteristics	Overall (N = 185)
Sex, N (%)	
Male	93 (50)
Female	92 (50)
Gestational Age (weeks), mean $\pm$ s.d.	37.94 ± 1.16
Birth weight (grams), mean $\pm$ s.d.	$2,999.72 \pm 392.57$
Weight (grams), mean $\pm$ s.d.	$8,379.72 \pm 1,146.81$
Height (centimeters), mean $\pm$ s.d.	70.45 ± 2.87
Type of milk feeding, N (%)	
Exclusive breast feeding	53 (29)
Breast feeding + formula feeding	63 (34)
Formula feeding	69 (37)
Family history of thalassemia, N (%)	
Yes	98 (53)
No	87 (47)
Age at first time blood sampling (months), mean $\pm$ s.d.	9.49 ± 0.77
Age at follow-up (months), mean $\pm$ s.d.	10.59 ± 0.88
Hemoglobin typing, N (%)	
Normal hemoglobin typing	70 (38)
Beta thalassemia with Hb E	5 (3)
Hemoglobin H and hemoglobin H CS	16 (9)
Homozygous Hb E	23 (12)
Others abnormal hemoglobin typing	71 (38)
Notes: N, frequency; %, percentage; s.d., standard deviation.	

The red blood cell indices of the anemic infants are presented in Table 2. At baseline, the mean hemoglobin levels ranged from 8.70–10.18 g/dL in the first blood test and increased to 9.23–11.49 g/dL after one month of iron supplementation, as measured in the second blood test. The baseline mean hematocrit levels ranged from 27.89% – 32.45% and increased to 29.24% – 35.82% at the second assessment. The other baseline red blood cell indices were as follows: mean corpuscular volume (MCV), 52.10–68.66 fL; mean corpuscular hemoglobin (MCH), 16.15–22.29 pg; mean corpuscular hemoglobin concentration (MCHC), 29.93–33.47 g/dL; and red cell distribution width (RDW), 14.44% – 22.64%. Further details are provided in Table 2.

Table 2
Laboratory findings

Laboratory findings	Unit	First time (Pre-iron treatment), mean ± s.d.	Second time (Post-iron treatment), mean ± s.d.
Hemoglobin (Hb)	g/dL	9.44 ± 0.74	10.36 ± 1.13
Hematocrit (HCT)	%	30.17 ± 2.28	32.53 ± 3.29
MCV	fL	60.38 ± 8.28	61.88 ± 8.25
MCH	pg	19.22 ± 3.07	19.80 ± 3.02
MCHC	g/dL	31.70 ± 1.77	31.82 ± 1.68
RDW	%	18.54 ± 4.10	19.30 ± 4.25
White blood cells (WBC)	cells/uL	10,320.22 ± 2,707.60	10,359.14 ± 2,685.86
Platelet counts	cells/uL	487,205.41 ± 270,151.05	423,427.03 ± 113,703.02
Notes: s.d., standard deviation; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; g/dL, grams (g) per deciliter (dL); fL, femtoliters (fL); pg, picogram (pg); cells/uL, cells per microliters (uL)			

Table 3 compares the red cell indices from the first blood test among infants with normal hemoglobin typing, beta-thalassemia Hb E, Hb H and Hb H CS, homozygous hemoglobin E, and other hemoglobinopathies. The beta-thalassemia Hb E group had significantly lower hemoglobin, MCV, and MCH levels than the other groups. RDW levels were also high in both the normal hemoglobin typing group, and the Hb H and Hb H CS and beta-thalassemia Hb E groups.

Table 3
Laboratory findings – At First Time (Pre-iron treatment)

Laboratory findings	Laboratory Finding at first time (Pre-iron treatment) (mean $\pm$ s.d.)						P-value
	Overall, (N = 185)	Normal Hb typing, (N = 70)	Beta thalassemia with Hb E, (N = 5)	HbH and HbHCS, (N = 16)	HomoE, (N = 23)	Others Hb patterns, (N = 71)	
Hemoglobin (g/dL)	9.49 $\pm$ 0.74	9.00 $\pm$ 0.71	8.74 $\pm$ 0.57	9.35 $\pm$ 0.78	9.62 $\pm$ 0.66	9.44 $\pm$ 0.74	< 0.001*
Hematocrit (%)	31.01 $\pm$ 2.01	26.60 $\pm$ 2.61	29.00 $\pm$ 2.42	28.13 $\pm$ 1.77	30.51 $\pm$ 1.92	30.17 $\pm$ 2.28	< 0.001*
MCV (fL)	64.21 $\pm$ 8.49	56.00 $\pm$ 7.65	50.58 $\pm$ 5.19	53.22 $\pm$ 3.67	61.43 $\pm$ 6.32	60.38 $\pm$ 8.28	< 0.001*
MCH (pg)	20.14 $\pm$ 3.35	18.80 $\pm$ 2.39	15.09 $\pm$ 0.86	17.78 $\pm$ 1.65	19.74 $\pm$ 2.58	19.22 $\pm$ 3.07	< 0.001*
MCHC (g/dL)	31.06 $\pm$ 1.46	34.00 $\pm$ 1.22	30.01 $\pm$ 1.98	33.43 $\pm$ 1.38	31.99 $\pm$ 1.45	31.70 $\pm$ 1.77	< 0.001*
RDW (%)	17.10 $\pm$ 3.66	24.32 $\pm$ 3.85	25.01 $\pm$ 1.26	21.41 $\pm$ 2.11	17.17 $\pm$ 3.13	18.54 $\pm$ 4.10	< 0.001*
WBC (cells/uL)	10,368.00 $\pm$ 2,851.24	9,290.00 $\pm$ 2,584.04	9,573.75 $\pm$ 2,279.04	11,223.04 $\pm$ 2,870.86	10,221.41 $\pm$ 2,586.27	10,320.22 $\pm$ 2,707.60	0.331
Platelet counts (cells/uL)	457,028.57 $\pm$ 115,236.49	509,800.00 $\pm$ 153,097.03	510,187.50 $\pm$ 133,616.48	490,956.52 $\pm$ 122,968.02	508,971.83 $\pm$ 408,995.28	487,205.41 $\pm$ 270,151.05	0.832
Notes: N, frequency; %, percentage; s.d., standard deviation; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; g/dL, grams (g) per deciliter (dL); fL, femtoliters (fL); pg, picogram (pg); cells/uL, cells per microliters (uL); *, Statistically significant at P-value < 0.05							

Following a one-month therapeutic trial of iron supplementation, the patients returned for repeat complete blood count (CBC) assessment. As shown in Table 4, mean hemoglobin levels at follow-up ranged from 9.85–11.79 g/dL. An increase in hemoglobin was most evident in the Hb H and Hb H CS, homozygous hemoglobin E, and other hemoglobin pattern groups. However, MCV and MCH remained largely unchanged compared with the initial assessment.

Table 4
Laboratory findings – At Second Time (Post-iron treatment)

Laboratory findings	Laboratory Finding at second time (Post-iron treatment) (mean $\pm$ s.d.)						P-value
	Overall, (N = 185)	Normal Hb typing, (N = 70)	Beta thalassemia with Hb E, (N = 5)	HbH and HbHCS, (N = 16)	HomoE, (N = 23)	Others Hb patterns, (N = 71)	
Hemoglobin (g/dL)	10.82 $\pm$ 0.97	8.60 $\pm$ 1.14	8.90 $\pm$ 0.62	10.13 $\pm$ 0.97	10.44 $\pm$ 1.02	10.36 $\pm$ 1.13	< 0.001*
Hematocrit (%)	34.58 $\pm$ 2.65	26.00 $\pm$ 2.92	29.83 $\pm$ 2.69	29.87 $\pm$ 2.58	32.43 $\pm$ 2.49	32.53 $\pm$ 3.29	< 0.001*
MCV (fL)	66.50 $\pm$ 7.56	57.00 $\pm$ 7.42	51.42 $\pm$ 5.86	53.65 $\pm$ 2.76	62.68 $\pm$ 6.23	61.88 $\pm$ 8.25	< 0.001*
MCH (pg)	21.03 $\pm$ 3.07	19.00 $\pm$ 2.35	15.16 $\pm$ 1.07	18.09 $\pm$ 1.08	20.25 $\pm$ 2.46	19.80 $\pm$ 3.02	< 0.001*
MCHC (g/dL)	31.31 $\pm$ 1.43	33.60 $\pm$ 0.89	29.64 $\pm$ 1.72	33.52 $\pm$ 0.90	32.14 $\pm$ 1.35	31.82 $\pm$ 1.68	< 0.001*
RDW (%)	18.47 $\pm$ 4.14	24.64 $\pm$ 3.93	25.52 $\pm$ 1.76	20.41 $\pm$ 1.28	17.99 $\pm$ 3.85	19.30 $\pm$ 4.25	< 0.001*
WBC (cells/uL)	10,466.43 $\pm$ 2,877.30	10,518.00 $\pm$ 873.22	10,403.12 $\pm$ 2,984.14	10,297.83 $\pm$ 2,819.87	10,252.11 $\pm$ 2,514.76	10,359.14 $\pm$ 2,685.86	0.993
Platelet counts (cells/uL)	408,442.86 $\pm$ 119,439.95	393,200.00 $\pm$ 91,854.23	501,500.00 $\pm$ 114,278.61	432,782.61 $\pm$ 103,416.17	419,704.23 $\pm$ 107,486.53	423,427.03 $\pm$ 113,703.02	0.051
Notes: N, frequency; %, percentage; s.d., standard deviation; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; g/dL, grams (g) per deciliter (dL); fL, femtoliters (fL); pg, picogram (pg); cells/uL, cells per microliters (uL); *, Statistically significant at P-value < 0.0							

Because the number of infants with beta-thalassemia Hb E was too small, the beta- thalassemia Hb E, Hb H and Hb H CS groups were combined into a thalassemia disease group for analysis. Red cell indices obtained from the second blood test were evaluated to identify potential screening criteria. MCV of ≤ 50 fL combined with MCH of ≤ 15 pg were used to identify infants suspected of having thalassemia disease and need further investigation.

Binary logistic regression was performed to assess factors associated with thalassemia. In the univariable analysis, higher RDW and lower MCH levels in the first blood test were significantly associated with thalassemia. In the second blood test, higher RDW and lower hematocrit, MCV, and MCH levels were also significantly associated with thalassemia. In the multivariable analysis, RDW and MCHC in the first blood test, as well as RDW and hematocrit in the second blood test, remained significantly associated with thalassemia, as shown in Table 5.

Table 5

Binary logistics regression for assessing the factors associated with Thalassemia disease vs Normal Hb Typing

Covariates	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P-value	aOR (95%CI)	P-value
First time (Pre-iron treatment)				
RDW (%)	1.80 (1.38–2.34)	< 0.001*	1.70 (1.26–2.30)	< 0.001*
MCHC (g/dL)	0.97 (0.73–1.28)	0.825	1.95 (1.08–3.50)	0.026*
MCH (pg)	0.56 (0.42–0.75)	< 0.001*	0.63 (0.39–1.04)	0.069
Second time (Post-iron treatment)				
RDW (%)	1.62 (1.30–2.02)	< 0.001*	1.67 (1.05–2.64)	0.030*
Hematocrit (%)	0.43 (0.29–0.65)	< 0.001*	0.38 (0.17–0.84)	0.017*
MCV (fL)	0.78 (0.70–0.87)	< 0.001*	2.15 (0.82–5.59)	0.118
MCH (pg)	0.52 (0.39–0.70)	< 0.001*	0.06 (0.00–1.72)	0.099
MCHC (g/dL)	0.78 (0.58–1.04)	0.089	4.43 (0.76–25.65)	0.097
Notes: n, frequency; %, percentage; s.d., standard deviation; OR and aOR, unadjusted and adjusted odds ratios; 95% CI, 95% confidence interval; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; fL, femtoliters (fL); *, Statistically significant at P-value < 0.05.				

Binary logistic regression was used to assess factors associated with thalassemia based on the results of the first blood test. As shown in Table 6, RDW was significantly associated with thalassemia status. Infants with an RDW of $\leq 20\%$ had substantially lower odds of thalassemia than those with an RDW of $> 20\%$, which was used as the reference category (OR, 0.01; 95% CI, 0.00–0.09; $p < 0.001$). In contrast, an MCV of ≤ 50 fL, an MCH of ≤ 15 pg, and the presence of both criteria were each significantly associated with higher odds of thalassemia. The corresponding ORs were 42.46 (95% CI, 4.89–368.80), 33.00 (95% CI, 8.49–128.20), and 77.00 (95% CI, 8.07–734.81), respectively. After one month of iron supplementation, the findings in Table 7 showed a similar pattern to those observed in the first blood test.

Table 6

Binary logistics regression for assessing the factors associated with Thalassemia disease vs Normal Hb Typing

Covariates	Normal Hb typing, (N = 70)	Thalassemia disease, (N = 21)	OR (95%CI)	P-value
First time (Pre-iron treatment)				
RDW (%)				
RDW ≤ 20	57 (81)	1 (5)	0.01 (0.00-0.09)	< 0.001*
RDW > 20	13 (19)	20 (95)	1	
MCV (fL)				
MCV ≤ 50	1 (1)	8 (38)	42.46 (4.89– 368.80)	< 0.001*
MCV > 50	69 (99)	13 (62)	1	
MCH (pg)				
MCH ≤ 15	4 (6)	14 (67)	33.00 (8.49– 128.20)	< 0.001*
MCH > 15	66 (94)	7 (33)	1	
HCT (%)				
HCT ≤ 40	0 (0)	0 (0)	-	-
HCT > 40	70 (100)	21 (100)	-	-
MCHC (g/dL)				
MCHC ≤ 30	24 (34)	9 (43)	1.44 (0.53–3.89)	0.475
MCHC > 30	46 (66)	12 (57)	1	
MCV (fL) + MCH (pg)				
MCV ≤ 50 + MCH ≤ 15	1 (1)	7 (54)	77.00 (8.07- 734.81)	< 0.001*
MCV > 50 + MCH > 15	66 (99)	6 (46)	1	
Notes: n, frequency; %, percentage; s.d., standard deviation; OR and aOR, unadjusted and adjusted odds ratios; RDW, red cell distribution width; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; g/dL, grams (g) per deciliter (dL); fL, femtoliters (fL); pg, picogram (pg); cells/uL, cells per microliters (uL); n/a, not applicable; *, Statistically significant at P-value < 0.05.				

Table 7

Binary logistics regression for assessing the factors associated with Thalassemia disease vs Normal Hb Typing

Covariates	Normal Hb typing, (N = 70)	Thalassemia disease, (N = 21)	OR (95%CI)	P-value
Second time (Post iron treatment)				
RDW (%)				
RDW ≤ 20	48 (69)	1 (5)	0.02 (0.00-0.18)	< 0.001*
RDW > 20	22 (31)	20 (95)	1	
MCV (fL)				
MCV ≤ 50	0 (0)	7 (33)	-	-
MCV > 50	70 (100)	14 (67)	-	-
MCH (pg)				
MCH ≤ 15	2 (3)	13 (62)	55.25 (10.51- 290.34)	< 0.001*
MCH > 15	68 (97)	8 (38)	1	
HCT (%)				
HCT ≤ 40	68 (97)	21 (100)	-	-
HCT > 40	2 (3)	0 (0)	-	-
MCHC (g/dL)				
MCHC ≤ 30	17 (24)	10 (48)	2.83 (1.03–7.83)	0.044*
MCHC > 30	53 (76)	11 (52)	1	
MCV (fL) + MCH (pg)				
MCV ≤ 50 + MCH ≤ 15	0 (0)	6 (46)	-	-
MCV > 50 + MCH > 15	67 (100)	7 (54)	-	-
Notes: n, frequency; %, percentage; s.d., standard deviation; OR and aOR, unadjusted and adjusted odds ratios; RDW, red cell distribution width; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; g/dL, grams (g) per deciliter (dL); fL, femtoliters (fL); pg, picogram (pg); cells/uL, cells per microliters (uL); *, Statistically significant at P-value < 0.05.				

Using an MCV cutoff of ≤ 50 fL in the first blood test, the sensitivity for detecting thalassemia was 38.1%, while the specificity was 98.57%. The positive predictive value (PPV) was 87.50%, the negative predictive value (NPV) was 88.46%, and the area under the receiver operating characteristic curve (AUC) was 0.68 (95% CI, 0.58–0.79). An MCH cutoff of ≤ 15 pg showed a higher overall correct classification rate of 87.91 and the highest AUC, at 0.80 (95% CI, 0.70–0.91) with a sensitivity of 66.67, specificity 94.29, PPV 76.47 and NPV 95.65. moreover, if we combine the 2 criteria, the sensitivity was 53.85, specificity 98.51, PPV 85.71, NPV 97.06 and the area under the receiver operating characteristic curve (AUC) was 0.76 (95% CI, 0.62–0.90) with correctly classified of 91.25%. For the second blood

test, $MCV \leq 50$ fL and an $MCH \leq 15$ pg, the sensitivity was 46.15, specificity 100, PPV 73.33, NPV 90.67 and the area under the receiver operating characteristic curve (AUC) was 0.73 (95% CI, 0.59–0.87) with correctly classified of 91.36% as shown in Table 8.

Table 8

Sensitivity, specificity, correctly classification, positive predictive value (PPV), negative predictive value (NPV) and area under ROC curve. Thalassemia vs Normal Hb Typing

Predictors	Sensitivity (%)	Specificity (%)	Correctly Classified (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)	Area under ROC curve (AUC) (95% CI)
First time (Pre-iron treatment)						
$MCV \leq 50$ (fL)	38.10	98.57	84.62	87.50	88.46	0.68 (0.58–0.79)
$MCH \leq 15$ (pg)	66.67	94.29	87.91	76.47	95.65	0.80 (0.70–0.91)
$MCHC \leq 30$	42.86	65.71	60.44	79.08	81.40	0.54 (0.42–0.66)
$MCV \leq 50 + MCH \leq 15$	53.85	98.51	91.25	85.71	97.06	0.76 (0.62–0.90)
Second time (Post-iron treatment)						
$MCV \leq 50$ (fL)	33.33	100.00	84.62	70.65	83.33	0.67 (0.56–0.77)
$MCH \leq 15$ (pg)	61.90	97.14	89.01	86.67	89.47	0.79 (0.69–0.90)
$MCHC \leq 30$	47.62	75.71	69.23	75.06	76.92	0.62 (0.50–0.73)
$MCV \leq 50 + MCH \leq 15$	46.15	100.00	91.36	73.33	90.67	0.73 (0.59–0.87)
Notes: %, percentage; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.						

Discussion

Anemia is a common public health concern among children aged 9–12 months, with iron deficiency being the most frequent cause (12). However, thalassemia should also be considered in this age group, particularly in children with persistent anemia or an inadequate response to iron supplementation. Further diagnostic evaluation is therefore essential to confirm thalassemia disease. Early detection of thalassemia and appropriate follow-up care lead to better outcomes (13).

Thalassemia is an inherited disorder caused by reduced or abnormal synthesis of globin chains, which are the major protein components of hemoglobin within red blood cells. The imbalance between globin-chain production and ineffective erythropoiesis results in the characteristic hematological pattern of microcytic and hypochromic anemia. Mean corpuscular volume (MCV) reflects the average volume of red blood cells, whereas mean corpuscular hemoglobin (MCH) indicates the average amount of hemoglobin per red blood cell. Accordingly, thalassemia is typically characterized by microcytic, hypochromic anemia, manifested by reduced MCV and MCH values. Previous study showed mean values of RDW was markedly raised in both beta thalassemia major and beta thalassemia hemoglobin E with low mean values of RBC count, MCV and MCH in accordance with the result in our study (14, 15). MCV 71.3 fL and MCH 22.5 pg was proposed as cut off values for detect beta thalassemia trait (14) but no studies have established criteria for red blood cell indices cutoffs for identifying individuals suspected of having thalassemia. Therefore, in this study, children with an $MCV \leq 50$ fL and an $MCH \leq 15$ pg should undergo hemoglobin typing to confirm the diagnosis thalassemia disease. The area under the ROC curve was 0.76, and the overall correct classification rate was 91.25%, indicating that these red blood cell indices are appropriate for use as screening tools. Hemoglobin typing can therefore provide clinically relevant information not only for diagnosis but also for genetic counseling, family screening, and prevention of severe thalassemia disease. Early diagnosis is particularly important in children with thalassemia disease because it allows timely referral for appropriate follow-up, monitoring of complications, transfusion planning when indicated, and assessment of iron overload risk.

The proportion of thalassemia disease in this study was 11.3%, while thalassemia trait and other abnormal hemoglobin patterns were observed in 12.4% and 38.0% of participants, respectively. In this study, the prevalence of thalassemia could not be estimated because hemoglobin typing was performed only in patients who presented with anemia. Consequently, the study population was not representative of the general population, and the findings cannot be directly compared with prevalence estimates from other studies.

Follow-up complete blood count (CBC) testing after iron therapy demonstrated an increase in hemoglobin levels in both children without detectable thalassemia and those with thalassemia. This finding is consistent with previous evidence showing that iron deficiency may coexist of individuals with hemoglobinopathies (16, 17). Therefore, iron supplementation in children identified as anemic through screening appears to be safe, including those with thalassemia, provided that treatment is appropriately monitored.

Several discrimination indices, including the Mentzer index, Shine and Lal, England and Fraser, red blood cell distribution width index, and Green and King indices, have previously been developed to differentiate beta thalassemia trait from iron deficiency anemia (18, 19, 20). Although these indices can be calculated from routinely available complete blood count parameters, their clinical application is not always practical. Different indices use different formulas and cut-off values, and their diagnostic performance varies across populations. In addition, most indices were developed primarily for distinguishing thalassemia trait from iron deficiency anemia rather than for identifying children with clinically significant thalassemia disease. Thus, an MCV of ≤ 50 fL and an MCH of ≤ 15 pg had high correctly accuracy and may be used as RBC indices screening criteria for identifying anemic children aged 9 months who should undergo further hemoglobin typing for definitive diagnosis.

Strengths and limitations

A major strength of this study is its prospective design. All participants received iron supplementation and were followed longitudinally. In addition, hemoglobin typing was performed in all participants to identify underlying hemoglobinopathies.

This study has several limitations. First, it was conducted at a single center, which may limit the generalizability of the findings. Second, iron deficiency anemia was not confirmed using iron studies. Instead, participants with normal hemoglobin typing were presumed to have anemia attributable to iron deficiency. Third, adherence to iron supplementation was not recorded; therefore, differences in treatment compliance may have influenced the red blood cell indices observed after treatment. Further prospective multicenter studies are warranted to validate these cutoff values in diverse pediatric populations and to compare their performance with other diagnostic indices.

Conclusions

RBC indices, $MCV \leq 50$ fL combined with $MCH \leq 15$ pg, may serve as a practical screening criterion for selecting children with anemia who should undergo hemoglobin typing. However, these hematological parameters cannot replace confirmatory testing. Hemoglobin typing remains essential for establishing the diagnosis thalassemia disease.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Panyananthaphikkhu Chonprathan Medical Center, Srinakharinwirot University (EC 009/67), in accordance with the Declaration of Helsinki and relevant local regulations. Written informed consent was obtained from all participants prior to enrollment. For participants under 16 years of age, consent was obtained from their parents or legal guardians.

Consent for publication

Not applicable, as this study not contain any individual person's data in any form that could be used to identify them

Competing interests

The authors declare no conflicts of interest. The funder had no role in the study design; data collection, analysis, or interpretation; manuscript writing; or the decision to submit for publication.

Funding

This study was funded by Panyananthaphikkhu Chonprathan Medical Center, Srinakharinwirot University.

Author Contribution

Conceptualization: Ornrumpha Tanago

Methodology: Orawin Luecha, Patcharapun Sarisuta

Data curation: Orawin Luecha, Patcharapun Sarisuta

Formal analysis: all authors

Writing-original draft: Ornrumpha Tanago, Orawin Luecha

Writing-review and editing: all authors

Supervision: Ornrumpa Tanago

All authors have read and approved the final manuscript.

Acknowledgement

The author would like to special thanks to all families of children who participated in the study.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to the presence of potentially identifiable patient information and ethical and privacy considerations, but are available from the corresponding author on reasonable request.

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Figures

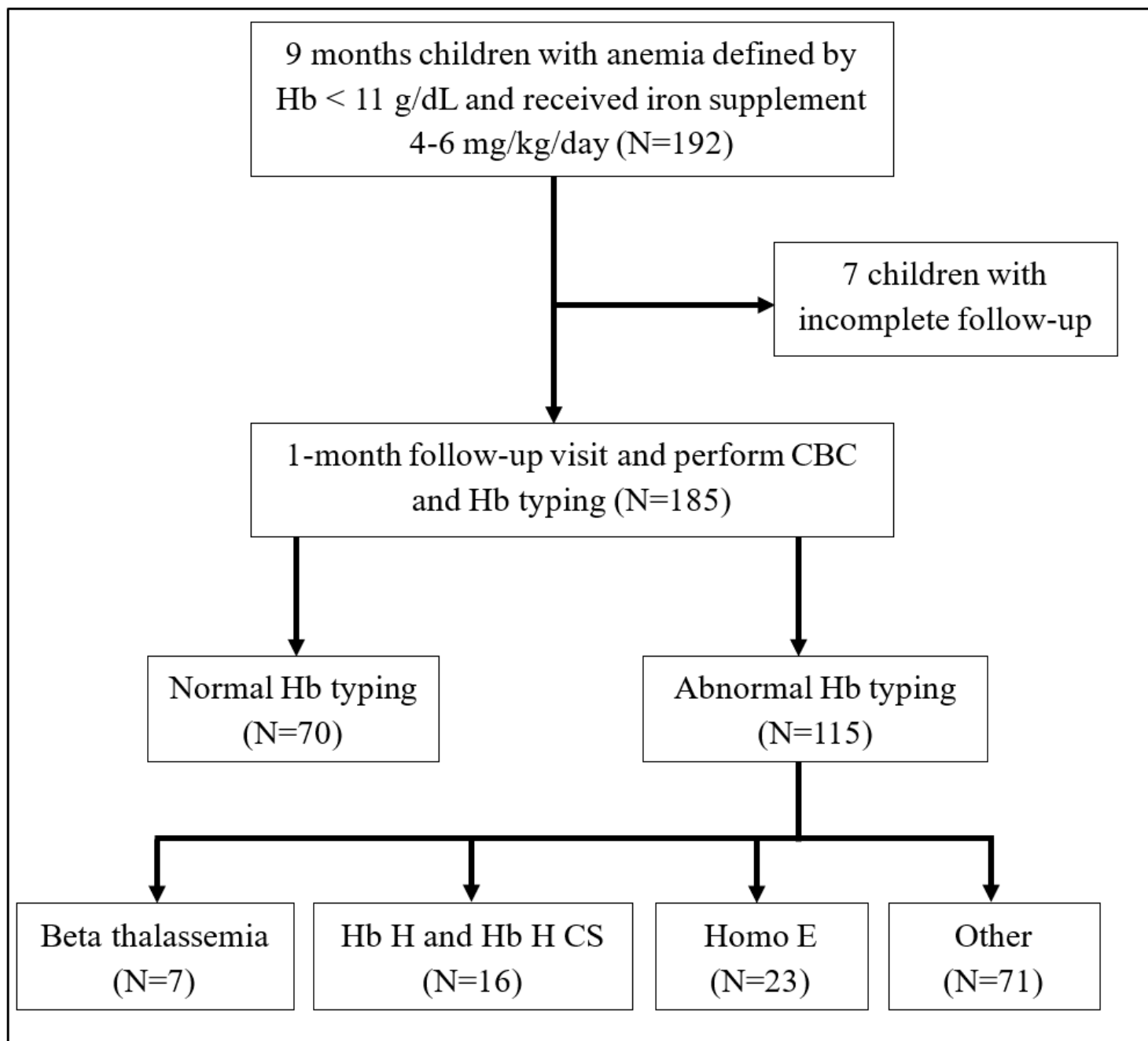


Figure 1

Legend not included with this version.