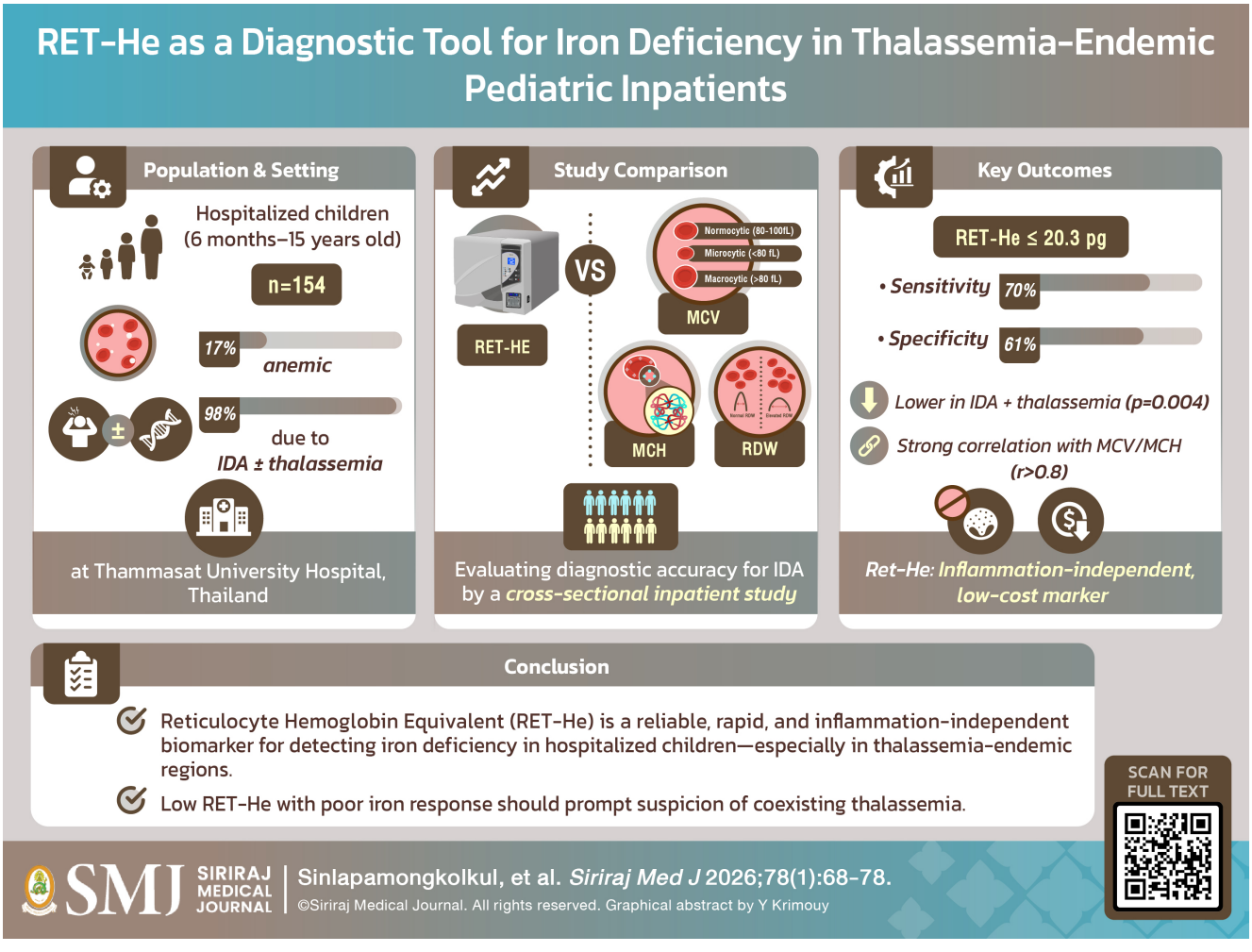
Diagnostic Utility of Reticulocyte Hemoglobin Equivalent for Identifying Iron Deficiency in Hospitalized Children in a Thalassemia-endemic Region: A Single-center Cross-sectional Study

Phakatip Sinlapamongkolkul, M.D.[1], Tasama Pusongchai, M.D.[1], Jassada Buaboonnum, M.D.[2], Wallee Satayasai, M.D.[1], Pacharapan Surapolchai, M.D.[1,*]

[1]Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Thammasat University, Pathumthani, Thailand, [2]Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.



**Corresponding author: Pacharapan Surapolchai*
E-mail: doctorning@hotmail.com
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ORCID ID:http://orcid.org/0000-0003-4180-8041
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ABSTRACT

Objective: Reticulocyte hemoglobin equivalent (RET-He) has been identified as a useful marker for diagnosing and monitoring iron deficiency anemia (IDA). This study evaluated anemia prevalence and assessed RET-He's effectiveness in detecting IDA in pediatric inpatients with high thalassemia burden.

Materials and Methods: A cross-sectional design was employed involving children aged 6 months to 15 years admitted with anemia. RET-He and red blood cell (RBC) indices were compared to explore diagnostic implications.

Results: Among the 881 pediatric inpatients included during the study period, 17% (154 patients) were identified as having anemia. IDA was the major cause of anemia (98%), including IDA (70.1%) and IDA coexisting with thalassemia (27.9%). Median RET-He (IQR) of all anemic patients was 21.05 (18.70, 24) pg. Notably, RET-He values were lower in patients with combined IDA and thalassemia than in those with IDA alone ($p = 0.004$). Significant correlations were observed between RET-He and RBC indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). With a cut-off of ≤20.3 pg, RET-He showed moderate sensitivity (70.3%) and specificity (60.5%) for diagnosing IDA.

Conclusion: These findings advocate for RET-He's use as an iron status marker in hospitalized children, especially in areas endemic for thalassemia. Low RET-He in non-responders to iron therapy should raise suspicion of underlying thalassemia.

Keywords: Anemia; children; diagnostic accuracy; hospitalization; iron deficiency; receiver operating characteristic; reticulocyte hemoglobin equivalent; thalassemia (Siriraj Med J 2026;78(1):68-78)

INTRODUCTION

Iron deficiency anemia (IDA) remains the leading cause of pediatric anemia worldwide, with the highest burden in resource-limited countries.[1] The incidence of IDA ranges from 20-40%, depending on age and socioeconomic status.[2] IDA can cause delayed growth and increased susceptibility to infection.[3] Additionally, IDA can impair neurophysiological functioning, which in turn affects school performance and academic achievement.[4] Diagnosing IDA relies on evaluating hemoglobin levels, serum ferritin, and iron studies.[5] However, these diagnostic methods have notable limitations: ferritin measurements may yield misleadingly high values when infection or inflammatory processes are present, and iron studies are not universally accessible.[6] Given the aforementioned disadvantages of such methods, further investigations are warranted. Reticulocytes are the immature form of erythroid cells, which subsequently differentiates into mature erythrocytes. Therefore, hemoglobin content in reticulocytes appears to reflect the availability of iron for heme production accurately. Furthermore, the reticulocyte hemoglobin equivalent (RET-He) is not affected by the inflammatory process and diurnal variations. Due to these advantages, RET-He is one of the diagnostic tools increasingly used in IDA.[7,8]

Another crucial cause of microcytic anemia in Thailand and Southeast Asia is thalassemia and hemoglobinopathy, a group of genetic disorders affecting hemoglobin synthesis.[9,10] Differentiating between IDA, thalassemia, or the co-existence of both conditions is paramount for accurate diagnosis and appropriate management. Both IDA and thalassemia can manifest as microcytic hypochromic anemia, making their differential diagnosis challenging. Although Ret-He is valuable in diagnosing IDA, some studies indicate that Ret-He can also be reduced in thalassemia patients, particularly in those with thalassemia trait or combined IDA and thalassemia. However, RET-He tends to be reduced the most in subjects with concomitant IDA and thalassemia.[11,12]

Hospitalized pediatric patients often present with complex health conditions, including co-existing inflammation, thereby complicating the assessment of iron status using traditional methods.[13,14] The present study intends to evaluate the anemia prevalence and determine RET-He's utility in detecting IDA among hospitalized pediatric patients at Thammasat University Hospital, in the central region of Thailand. The benefits of our study include measuring the iron status of hospitalized pediatric patients and emphasizing the importance of IDA coexisting with thalassemia in this endemic area, which is applicable in inpatient settings. A thorough understanding of Ret-He values in hospitalized children with IDA, thalassemia, and combined conditions will enable clinicians to achieve more rapid and accurate diagnoses, leading to appropriate treatment and minimizing long-term health consequences for the pediatric population.

MATERIALS AND METHODS

Demography

This descriptive cross-sectional study complied with ethical standards and was approved by the Human Research Ethics Committee of Thammasat University (Medicine), Thailand (MTU-EC-PE-1-344/64). Patients aged above 7 years, as well as their parents or authorized guardians, provided the necessary assent and/or informed consent. The clinical characteristics of participants who were diagnosed with anemia and received treatment at the inpatient pediatric department of our institute, aged 6 months to 15 years old, were obtained through direct interviews and inpatient records of the electronic hospital database at Thammasat University Hospital, Thailand between January and December 2022, utilizing Research Electronic Data Capture (REDCap)[15], a secure, web-based electronic data capture system managed by the Faculty of Medicine at Thammasat University, Thailand. Patients who were excluded: pediatric patients with incomplete records, known hematological problems, or bone marrow diseases. We obtained clinical and laboratory data using direct patient interviews combined with data extracted from medical files.

Anthropometric measurements

Weight, height and body mass index (BMI) were measured. Body mass index (BMI)-for-age Z-scores or standard deviation (SD) scores were computed using the WHO Anthro and AnthroPlus software programs.[16,17] In this study, BMI Z-scores (BMIZ) less than -2 SD, greater than 1 SD, and greater than 2 SD were classified as underweight, overweight, and obese, respectively.

Laboratory investigations

EDTA blood samples were tested for a complete blood count, including RET-He, using automated hematology analyzers (DxH 900, Beckman Coulter, Brea, USA, for CBC and XN-1000, Sysmex, Kobe, Japan, for RET-He). Hemoglobin typing was performed on EDTA-anticoagulated blood samples using capillary electrophoresis with the Capillarys 2 Flex Piercing system (Sebia, Lisses, France). Serum ferritin was analyzed using clot blood samples by DxI 800, Beckman Coulter, Brea, USA. Genomic DNA was isolated from leukocytes present in peripheral blood by employing a conventional phenol-chloroform extraction method. For alpha-globin gene analysis, a single-tube multiplex gap-PCR was utilized to identify four prevalent deletions ($^{--SEA}$, $^{--THAI}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$). Additionally, a single-tube multiplex amplification refractory mutation system (ARMS-PCR) was conducted to screen for two common non-deletion alpha-globin mutations in the Thai population: the termination codon mutations leading to Hb Constant Spring (TAA→CAA) and Hb Paksé (TAA→TAT).[18] Beta-globin genotyping involved ARMS-PCR targeting 16 frequent mutations including -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.[19]

Definition of anemia, iron deficiency, and thalassemia

In this investigation, anemia was defined based on WHO guidelines for age-specific hemoglobin thresholds regardless of gender: children aged 6 to 59 months were considered anemic if their hemoglobin (Hb) was below 11.0 g/dL; those aged 5 to 11 years if Hb was under 11.5 g/dL; and children aged 12 to 14 years if Hb was less than 12.0 g/dL. For individuals aged 15 years and older, anemia was classified as Hb below 12.0 g/dL in females and below 13.0 g/dL in males.[20] Iron deficiency (ID) was determined by either a RET-He less than 28 pg or serum ferritin levels below 30 ng/mL.[21-23] IDA was diagnosed based on either meeting laboratory criteria or showing a positive therapeutic response (either RET-He or serum ferritin) to iron supplementation administered at 3–6 mg/kg/day for a duration of 8 to 12 weeks, with follow-up by hematology specialists. Thalassemia genotypes were determined through hemoglobin analysis and molecular DNA testing. Diagnosis of beta-thalassemia trait was established when Hb A2 levels exceeded 3.5%. Children presenting with fetal hemoglobin (Hb F) levels above 10% underwent further screening for common beta-globin gene deletions to identify beta-thalassemia traits and hereditary persistence of fetal hemoglobin (HPFH).[24,25]

Statistical data analysis

The sample size was calculated using Cochran's formula: $N = Z^2pq/d^2$, $Z = 1.96$, $p = 0.18$, $q = 0.82$, $d = 0.065$; based on an expected anemia prevalence of approximately 18% in hospitalized pediatric patients to estimate prevalence with adequate precision. A minimum of 150 participants was required, accounting for a 10% dropout rate. While the primary aim included assessing RET-He diagnostic performance for ID, formal sample size calculation for diagnostic accuracy parameters was not conducted. Diagnostic metrics were evaluated exploratively and internally validated using Receiver operating characteristic (ROC) curve analysis, Cohen's d effect size, and confidence intervals. This limitation is acknowledged, and diagnostic findings should be interpreted as preliminary. Continuous clinical and laboratory data were summarized as medians with interquartile ranges

(IQR), while categorical data were presented as counts and percentages. Differences between groups were assessed using appropriate tests according to data type and distribution: t-tests, Mann-Whitney U tests, ANOVA, or Kruskal-Wallis tests for continuous variables, and Chi-square or Fisher's exact tests for categorical variables. The strength of correlations was evaluated using Pearson's or Spearman's rank correlation coefficients, depending on data characteristics. ROC curve analysis was performed to determine the diagnostic accuracy of variables related to ID. Internal validation of the cut-off value was performed using multiple statistical approaches to assess threshold stability and discriminatory performance. Descriptive statistics, including mean, SD, 95% confidence interval (CI), and coefficient of variation (CV) were calculated for the entire dataset and stratified by threshold groups. The threshold validation was considered satisfactory if Cohen's d ≥ 0.8, CV ≤ 30%, and minimal overlap existed in the critical threshold zone. Statistical analyses were carried out using Microsoft Excel 2019 and STATA version 14 (StataCorp, College Station, Texas, USA). A *p*-value less than 0.05 was considered statistically significant in two-tailed tests.

RESULTS

Clinical characteristics

A total of 881 pediatric inpatients were included during the study period. Of these, 154 patients (17%) were diagnosed with anemia, including 72 males (46.8%) with a mean age of 2.40 years (IQR 1–5.88). Of these 154 patients, 151 patients (98%) had IDA (with or without coexisting thalassemia), and three patients had other causes of anemia, including two with autoimmune hemolytic anemia, and one with anemia of inflammation. Besides gender, other clinical characteristics (age, nutritional status, and underlying diseases/conditions), including treatment of anemia, did not show significant differences (Table 1). Interestingly, 43 patients (27.9%) were diagnosed with IDA alongside some form of thalassemia or hemoglobinopathy. Among them, 18 individuals (11.7% of the total cohort) had alpha-thalassemia, 21 (13.6%) carried beta-globin mutations—primarily Hb E accounting for 11%—and 4 patients (2.6%) exhibited combined alpha- and beta-globin abnormalities. None of the patients with IDA who also had thalassemia exhibited clinical features typical of transfusion-dependent thalassemia, and none had ever received red blood cell transfusions. Therefore, all were categorized as non-transfusion-dependent and included in further analyses.

Laboratory characteristics

Comprehensive hematological data are detailed in Table 1. No significant differences were observed in hemoglobin (Hb), hematocrit (Hct), or mean corpuscular hemoglobin concentration (MCHC) between patients with IDA alone and those with IDA coexisting with thalassemia. However, patients presenting with both conditions showed significantly lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values ($p < 0.001$ for both) compared to other groups. Conversely, RBC count and red cell distribution width (RDW) were markedly elevated in the combined IDA and thalassemia group ($p = 0.014$ and 0.030, respectively). Notably, patients diagnosed with both IDA and thalassemia exhibited the greatest reduction in RET-He values ($p = 0.004$) (Fig 1).

Correlation analysis

As shown in Fig 2, RET-He exhibited strong positive correlations with MCV, MCH, and MCHC, all with *p*-values less than 0.001 and correlation coefficients (*r*) of 0.825, 0.853, and 0.713 respectively. Conversely, RET-He demonstrated moderate negative correlations with RBC count and RDW, with both *p*-values below 0.001 and correlation coefficients of -0.439 and -0.491 respectively.

Receiver operating characteristic curve and internal validation analysis

All the parameters examined exhibited an area under the curve (AUC) exceeding 0.4 in the ROC analysis, including RET-He, Hb, MCV, MCH, MCHC, and RDW. Among the parameters studied, RET-He exhibited a moderate diagnostic performance for detecting IDA in patients with concurrent thalassemia. At a diagnostic cut-off of ≤ 20.3 pg, the AUC was 0.649 (95% CI: 0.550–0.748), with a sensitivity of 70.3% and specificity of 60.5%. The mean RET-He value was 21.45 pg with an SD of 4.40 pg, 95% CI of 20.63 – 22.27 pg, resulting in a CV of 20.5% and a Cohen's d effect size of 1.55. Of the RBC parameters, MCV and MCH had higher AUCs of 0.708 and 0.680, respectively, at cut-off values ≤ 66.1 fL and ≤ 20.9 pg, but lower sensitivity and higher specificity. Lower AUCs of 0.543, 0.539, and 0.610, respectively, were observed for Hb, MCHC, and RDW. (Fig 3, Table 2)

DISCUSSION

Anemia remains a major public health concern among pediatric populations, especially in regions burdened by nutritional deficiencies and a high frequency

TABLE 1. Clinical and laboratory characteristics of study participants.

Characteristics	All patients (n=154)	IDA patients (n=108)	IDA coexisting with thalassemia patients (n=43)	*p*-values
Clinical characteristics				
Median age (IQR), years	2.40 (1, 5.88)	2.10 (1, 6.90)	2.90 (1.30, 5.40)	0.221
Male [n, (%)]	72 (46.8)	43 (39.8)	26 (60.5)	0.029*
BMI z-score (IQR)	-0.36 (-1.44, 0.81)	-0.39 (-1.55, 0.84)	-0.28 (-0.86, 0.66)	0.554
Nutritional status				0.432
Underweight [n, (%)]	22 (14.3)	18 (16.7)	4 (9.3)	
Normal [n, (%)]	100 (64.9)	66 (61.1)	32 (74.4)	
Overweight [n, (%)]	16 (10.4)	13 (12.0)	3 (7.0)	
Obese [n, (%)]	16 (10.4)	11 (10.2)	4 (9.3)	
Underlying diseases/conditions [n, (%)]	46 (30)	34 (31.5)	13 (30.2)	0.881
Treatment of anemia				0.555
Oral iron therapy [n, (%)]	135 (87.7)	95 (88.0)	40 (93.0)	
RBC transfusion [n, (%)]	2 (1.3)	2 (1.9)	0 (0)	
Oral iron therapy + RBC transfusion [n, (%)]	11 (7.1)	8 (7.4)	3 (7.0)	
Laboratory characteristics				
Hb (IQR), g/dL	9.90 (8.93, 10.68)	10.10 (9.10, 10.70)	9.80 (8.85, 10.55)	0.407
Hct, (IQR), %	31.20 (28.60, 33.05)	31.65 (29.13, 33.10)	30.90 (28.30, 32.90)	0.667
RBC count (IQR), x10^6/cu.mm.	4.80 (4.21, 5.32)	4.63 (4.20, 5.25)	5.07 (4.55, 5.38)	0.014*
MCV (IQR), fL	65.20 (59.23, 72.38)	67.55 (60.4, 73.4)	60.40 (54.55, 65.25)	< 0.001*
MCH (IQR), pg	20.7 (18.33, 23.6)	21.6 (18.9, 23.8)	18.9 (17.8, 20.75)	< 0.001*
MCHC (IQR), g/dL	31.90 (31.03, 32.60)	31.90 (31.03, 31.10)	31.80 (30.70, 32.45)	0.452
RDW (IQR), %	16.25 (14.63, 19.55)	15.95 (14.38, 19.30)	16.70 (15.75, 21.70)	0.030*
Platelet (IQR), x10^3/cu.mm.	360.50 (273.25, 474.50)	364.00 (282.50, 454)	351.00 (256, 530)	0.998
RET-He (IQR), pg	21.05 (18.70, 24)	21.80 (19.30, 24.30)	19.20 (17.50, 22.85)	0.004*

Data is expressed as median and IQR or N (%), according to the nature of the variables. Statistical method used: Mann-Whitney U or Student's t test, as appropriate.

* $p < 0.05$ was considered statistically significant.

Abbreviations: Hb, hemoglobin; Hct, hematocrit; IDA, iron deficiency anemia; IQR, interquartile range; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells; RDW, red blood cell distribution width; RET-He, reticulocyte hemoglobin equivalent

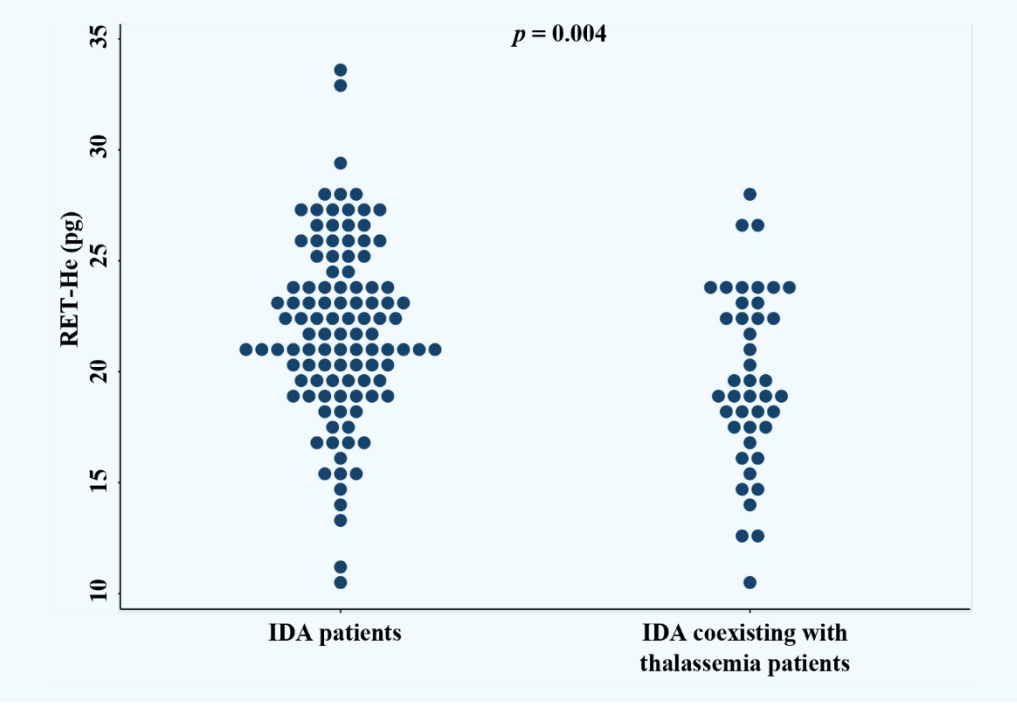


Fig 1. Differences in the RET-He among patients with IDA and IDA coexisting with thalassemia.
Statistical method used: Mann-Whitney U or Student's t test, as appropriate.
* $p < 0.05$ was considered statistically significant.
Abbreviations: IDA, iron deficiency anemia; RET-He, reticulocyte hemoglobin equivalent

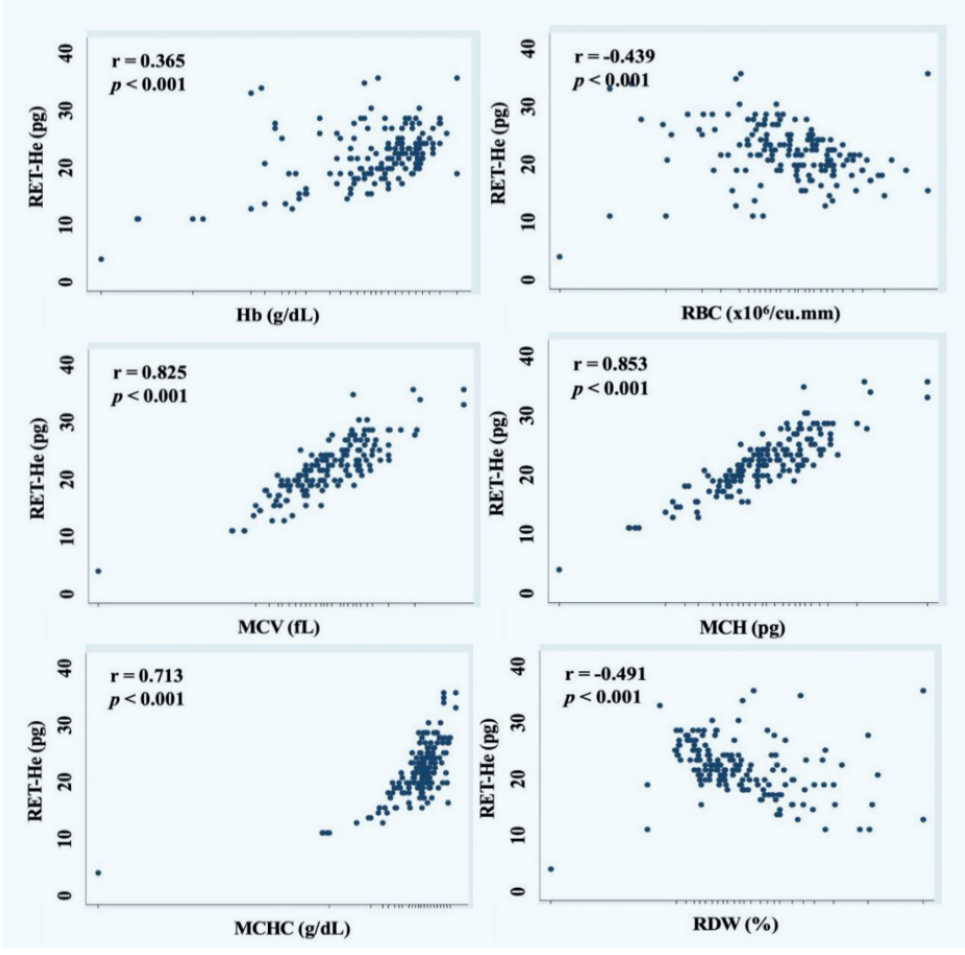


Fig 2. Relationship of RET-He and Hb, MCV, MCH, MCHC, and RDW. Data is expressed as a correlation coefficient (*r*).
Statistical method used: Pearson or Spearman rank correlation, as appropriate.
* $p < 0.05$ was considered statistically significant.
Abbreviations: Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells; RDW, red blood cell distribution width; RET-He, reticulocyte hemoglobin equivalent

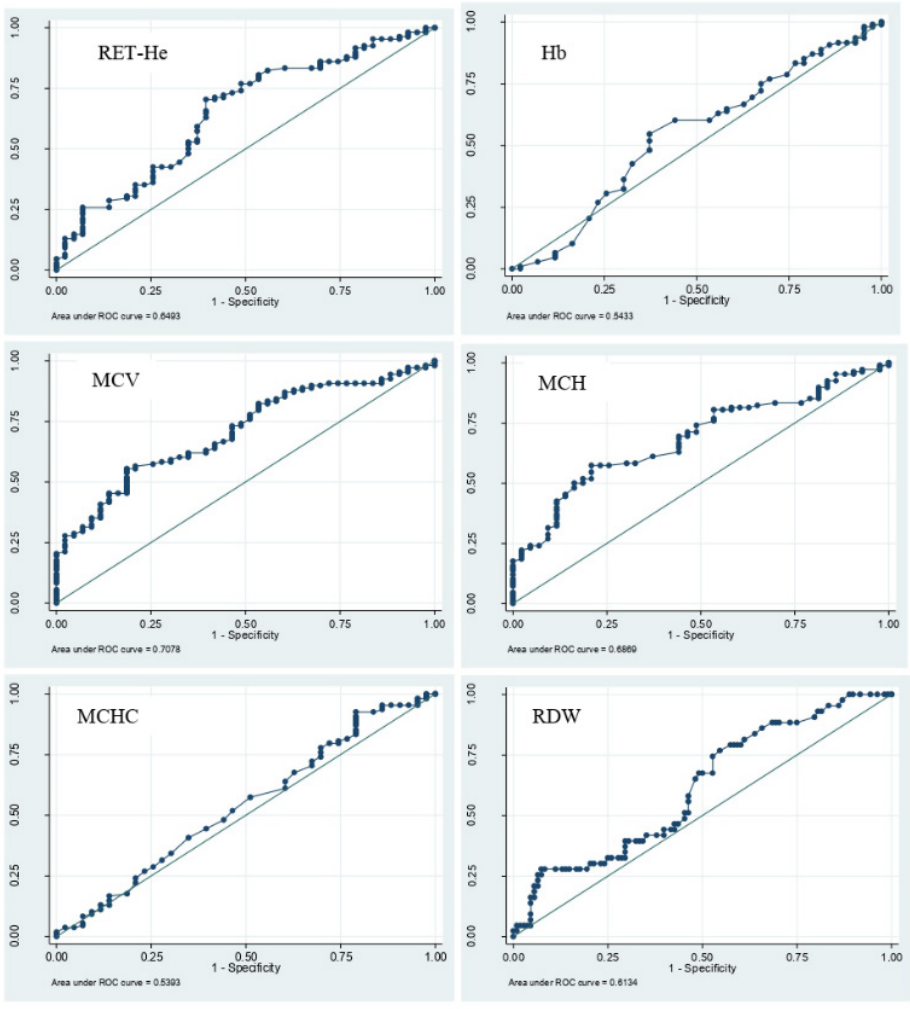


Fig 3. Receiver operating characteristic curve analysis of RET-He and Hb, MCV, MCH, MCHC, and RDW.
Data is expressed as AUC, sensitivity, and specificity.
Statistical method used: receiver operating characteristic (ROC) curves for diagnostic performance of RET-He and RBC parameters.
Abbreviations: AUC, area under the curve; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET-He, reticulocyte hemoglobin equivalent

TABLE 2. Sensitivity and specificity of RET-He and RBC parameters for diagnosis of iron deficiency anemia.

Parameters	AUC	Cut-off	Sensitivity (%)	Specificity (%)
RET-He, pg	0.649	20.3	70.3	60.5
Hb, g/dL	0.543	9.9	54.6	62.8
MCV, fL	0.708	66.1	55.6	81.4
MCH, pg	0.680	20.9	57.4	79.1
MCHC, g/dL	0.539	31.8	57.4	48.4
RDW, %	0.610	15.8	74.2	47.2

Data is expressed as AUC, cut-off, sensitivity, and specificity.
Statistical method used: receiver operating characteristic (ROC) curves for diagnostic performance of RET-He and RBC parameters.
Abbreviations: AUC, area under the curve; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; RET-He, reticulocyte hemoglobin equivalent

of thalassemia. This study provides new insights into the prevalence, etiology, and diagnostic approach to anemia in hospitalized children at Thammasat University Hospital, with particular emphasis on the utility of RET-He as a marker for IDA, especially in the context of coexisting thalassemia. Among pediatric inpatients in this study, anemia prevalence was 17%, aligning with previous reports from similar settings in Southeast Asia. The overwhelming majority (98%) of anemic cases were attributable to IDA, either alone or in combination with thalassemia, underscoring the dominance of nutritional and genetic causes in this population. Notably, 27.9% of anemic children had coexisting thalassemia, a figure that reflects the widespread occurrence of thalassemia traits in Thailand and neighboring countries. This dual pathology complicates both diagnosis and management, as clinical and laboratory features of IDA and thalassemia often overlap.

RET-He has emerged as a promising biomarker for assessing iron status, given its ability to reflect the hemoglobin content of newly produced reticulocytes and, by extension, the immediate availability of iron for erythropoiesis. In the context of hospitalized pediatric patients, RET-He is particularly advantageous. Traditional iron studies are not always feasible in acutely ill children, especially in those with infections or chronic inflammatory states. RET-He, being part of the automated CBC panel in modern hematology analyzers, offers a rapid, low-cost, and accessible alternative with the potential for point-of-care screening. Despite its promise, RET-He is not without limitations. Sedick *et al.* emphasized that while RET-He could reliably distinguish between IDA and thalassemia, its performance varied by patient age, analyzer platform, and the presence of coexisting disorders.[11] In our study, although RET-He showed a moderate diagnostic accuracy, other parameters like MCV and MCH had higher AUC values (0.708 and 0.680, respectively). However, the higher sensitivity of RET-He highlights its utility as an initial screening tool in clinical practice. In this study, the median RET-He among all anemic patients was 21.05 pg, with significantly lower values observed in those with both IDA and thalassemia compared to those with IDA alone (p = 0.004). Our observations align with the report by Kadegasem *et al.*, showing that RET-He decreases in ID and thalassemia, with the most marked reduction when both disorders are present.[12] This additive effect of ID on hemoglobin production in thalassemic patients can exacerbate anemia and complicate diagnosis.

There were strong positive associations observed between RET-He and MCV, MCH, and MCHC (r = 0.825, 0.853, and 0.713, respectively; all $p < 0.001$) reinforce the close relationship between iron supply and erythrocyte indices. Conversely, the moderate negative correlations with RBC count and RDW (r = -0.439 and -0.491, respectively; both $p < 0.001$) reflect the compensatory erythropoietic response and anisocytosis characteristic of iron-restricted erythropoiesis and thalassemia. The diagnostic utility of RET-He was further supported by ROC analysis, which demonstrated that using a cut-off value of ≤ 20.3 pg yielded a sensitivity of 70.3% and a specificity of 60.5% for diagnosing IDA with coexisting thalassemia. The mean (range) RET-He value was 21.45 pg (10.6 to 34.3 pg), resulting in a CV of 20.5%, which indicates moderate variability of RET-He measurements around the mean. The internal validation of the RET-He threshold included calculation of Cohen's d effect size, which was 1.55, indicating a large standardized difference between groups defined by the 20.3 pg cut-off. This large effect size demonstrates that the threshold reliably separates patients with lower RET-He levels from those with higher levels, supporting its stability and clinical utility. These metrics support the stability and precision of the chosen cut-off value of 20.3 pg for the diagnosis within the study population. While these values indicate moderate diagnostic accuracy, they suggest that RET-He can serve as a useful screening tool, particularly when used in conjunction with other RBC indices such as MCV, MCH, and RDW. Lian *et al.* demonstrated that combining RET-He with RDW enhances diagnostic accuracy, particularly in identifying coexisting cases.[26] In our study, the combination of RET-He with MCV and RDW did show significant correlations, suggesting that a multiparametric approach could enhance the diagnostic utility beyond RET-He alone. Although not highly specific, this sensitivity makes RET-He a valuable tool for ruling out IDA in our hospitalized pediatric patients. RET-He can be considered a useful adjunct marker, but should be interpreted alongside other hematological indices for accurate diagnosis. This aligns with the findings from Jamnok *et al.*, who demonstrated that RET-He can be an effective early screening tool for ID in areas with high thalassemia prevalence, supporting its integration into diagnostic algorithms.[27] Complete blood count (CBC) parameters, including MCV, MCH, and RDW, offer some discriminatory value but lack specificity when thalassemia traits are prevalent. In this study, MCV and MCH also demonstrated reasonable diagnostic performance (AUCs of 0.708 and 0.680, respectively), but RET-He provided a more direct assessment of functional iron availability. Khorwanichakij *et al.* proposed a novel thalassemia–iron deficiency discrimination predictive score derived from

routine red blood cell indices, including MCH, RDW, RBC, and platelet count.[28] This score demonstrated high sensitivity (90.4%) and specificity (78.7%) for differentiating all thalassemia subtypes from IDA in a Thai cohort. Integrating such predictive formulas with RET-He values may enhance early and accurate discrimination between IDA and thalassemia, thereby guiding targeted management in settings with limited access to specialized confirmatory testing.

The commonness of thalassemia traits in Thailand and Southeast Asia necessitates careful interpretation of anemia workups. Both IDA and thalassemia present as microcytic, hypochromic anemia, and their coexistence can exacerbate anemia severity and complicate treatment decisions. The significantly lower RET-He values in patients with combined IDA and thalassemia highlight the need for clinicians to consider thalassemia in children who fail to respond to iron therapy despite low RET-He. This is critical to avoid unnecessary and potentially harmful iron supplementation in children with underlying thalassemia traits. Interestingly, reticulocyte parameters have also been proposed for therapeutic monitoring. Almashjary et al. showed that increases in RET-He following iron supplementation precede changes in hemoglobin, suggesting that RET-He can also be used to assess response to iron therapy within days.[29] Although our study did not longitudinally assess RET-He post-treatment, this represents an important future direction, especially in hospitalized patients requiring close monitoring. Genetic testing for alpha- and beta-thalassemia mutations, as performed in this study, remains the gold standard for definitive diagnosis. However, such testing may not be universally available, making RET-He and other hematological indices valuable adjuncts in the diagnostic algorithm. This supports earlier recommendations by Yuan *et al.* and Saboor *et al.*, who advocated for integrating RET-He, HbA2, and RDW to guide further genotyping and targeted management.[30,31]

The high prevalence of thalassemia necessitates the proposed diagnostic protocols that address dual pathology: an initial assessment for children with microcytic anemia (MCV < 75 fL) and the measurement of RET-He and RBC indices. RET-He interpretation with clinically relevant thresholds is as follows: 1) a higher cut-off of >28 pg can be used to rule out IDA and prompt investigation of alternative etiologies; 2) values between 20 and 28 pg are ambiguous, warranting serum ferritin measurement if inflammation is absent; 3) a lower cut-off of ≤20 pg indicates high suspicion for IDA with or without coexisting thalassemia. For thalassemia risk stratification, a low RET-He combined with a high RBC count ($>5 \times 10^{12}$/L) or a Mentzer index (MCV/RBC ratio) less than 13 should trigger further Hb electrophoresis and genetic testing. Therapeutic trials of iron supplementation at 3–6 mg/kg/day for 8–12 weeks, followed by subsequent RET-He reassessment, are recommended. Non-responders may require additional genetic evaluation. This approach mitigates the risks of inappropriate iron therapy in thalassemia carriers, which can accelerate oxidative organ damage.

Several limitations of this study should be acknowledged. First, the cross-sectional design limits the ability to assess changes in RET-He over time or evaluate treatment response longitudinally. Second, as a single-center study in a tertiary care hospital, the findings may not be generalizable to other settings or broader populations, particularly given the complexity of hospitalized pediatric patients. Third, although RET-He demonstrated moderate diagnostic accuracy and was internally validated using multiple statistical methods, the sample size was initially calculated based on anemia prevalence (using Cochran's formula) rather than formal sample size estimation for diagnostic accuracy measures such as sensitivity, specificity, or AUC. This may limit the precision and generalizability of the diagnostic performance estimates. Fourth, inflammatory markers like C-reactive protein (CRP) were not assessed, which could have helped clarify confounding effects of inflammation on iron status biomarkers. Fifth, while molecular genotyping for common alpha- and beta-thalassemia mutations was performed, rarer variants may have been missed. Finally, incorporation bias may be present, as RET-He was part of the composite reference standard for ID diagnosis, potentially inflating diagnostic accuracy estimates. Future studies should include prospective designs with sample size calculations specifically tailored for diagnostic accuracy endpoints to validate and build upon these preliminary findings. Nonetheless, this study underscores the need for practical and accessible tools for evaluating anemia in children, particularly in regions with high thalassemia prevalence. RET-He fulfills many of these criteria and should be considered a frontline screening tool alongside traditional red cell indices. It provides a rapid and cost-effective means of differentiating types of microcytic anemia and identifying patients who warrant further diagnostic evaluation, such as hemoglobin electrophoresis or genetic testing. Future research should focus on prospective studies evaluating the utility of RET-He as a screening tool for ID and for monitoring treatment response after iron therapy, and in differentiating between IDA, thalassemia, and other causes of anemia in diverse pediatric populations.

Integration of RET-He into standardized anemia workups, particularly in resource-limited and thalassemia-endemic areas, may improve early detection and management of ID and reduce the burden of anemia-related morbidity.

CONCLUSION

This study highlights RET-He as an effective and sensitive biomarker for detecting IDA among hospitalized pediatric participants, particularly in regions where thalassemia is prevalent. RET-He provides a rapid, reliable, and inflammation-independent assessment of iron status, and its use in combination with other hematological indices can facilitate more accurate diagnosis and appropriate management. Because of the frequent coexistence of thalassemia, physicians should be vigilant in interpreting low RET-He values and consider genetic testing or alternative diagnoses in children who do not respond to iron therapy. Broader implementation of RET-He in clinical practice may help to address the persistent challenge of anemia in pediatric populations and improve long-term health outcomes.

Data Availability Statement

De-identified data were available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare no conflict of Interest.

Author Contributions

Conceptualization and methodology, P.Si., P.Su. ; Investigation, P.Si., T.P., P.Su. ; Formal analysis, P.Si., T.P., P.Su. ; Visualization and writing – original draft, P.Su. ; Writing – review and editing, P.Si., J.B., P.Su. ; Funding acquisition, P.Su. ; Supervision, W.S. All authors have read and agreed to the final version of the manuscript.

Use of Artificial Intelligence

AI-based tools (ChatGPT, Perplexity AI) were used for language editing under author supervision.

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