AcTion: Aceh Nutrition Journal
January – March, 2026
Original Article
DOI: http://dx.doi.org/10.30867/action.v11i1.2535
Pages: 193 – 204
p-issn 2527-3310; e-issn 2548-5741

Evaluating reticulocyte hemoglobin as a marker of iron depletion in pediatric patients in Banda Aceh

Evaluasi hemoglobin retikulosit sebagai penanda kekurangan besi pada pasien anak di Banda Aceh

Ayu Rezky Oktariany[1], Syafruddin Haris[2*], Heru Noviat[3], Sulaiman Yusuf[4], Eka Destianti Edward[5], Rusdi Andid[6]

[1] Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: dokterayuro86@gmail.com
[2] Division of Nephrology, Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: harissyafruddin@yahoo.com
[3] Division of Hematology Oncology, Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: herunoviat@gmail.com
[4] Division of Gastroenterology and Hepatology, Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: sly.aceh@gmail..com
[5] Division of Hematology Oncology, Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: ekadestiantie@gmail.com
[6] Division of Endocrinology, Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
E-mail: rusdi_andid@yahoo.com

***Correspondence Author:**
Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Jl. Teungku Tanoh Abee, Kopelma Darussalam, Kec. Syiah Kuala, Kota Banda Aceh, Aceh 24415, Indonesia.
E-mail: dokterayuro86@gmail.com

Article History:
Received: May 26, 2025; Revised: August 02, 2025; Accepted: December 27, 2025; Published: March 05, 2026.

Publisher:
Politeknik Kesehatan Aceh
Kementerian Kesehatan RI

Abstract

Anemia in children remains a major public health problem worldwide and in Indonesia, where national data report a prevalence of 21.7% among children aged ≥1 year. Iron deficiency is the leading cause, with iron depletion representing the earliest subclinical stagewhich is often difficult to detect using conventional parameters. Reticulocyte hemoglobin (Ret-He) has been proposed as a marker of functional iron availability during erythropoiesis. This study aimed to evaluate the diagnostic performance of Ret-He in identifying iron depletion in pediatric patients. A cross-sectional analytical study was conducted at Dr. Zainoel Abidin General Hospital in Banda Aceh between July and September 2024. A total of 87 pediatric patients aged 1 month to 18 years were initially enrolled; after excluding subjects with anemia to focus on the iron depletion stage, 54 children were included in the final analysis. Iron depletion was identified in 12.6% of the subjects. Receiver operating characteristic (ROC) analysis showed an optimal Ret-He cut-off value of 29.5 pg, with an area under the curve (AUC) of 0.609, sensitivity of 70%, specificity of 63.6%, and a non-significant p-value ($p = 0.398$), indicating a limited diagnostic accuracy. Ret-He levels were significantly positively correlated with mean corpuscular volume (MCV) ($r = 0.444$; $p = 0.001$) and mean corpuscular hemoglobin (MCH) ($r = 0.511$; $p < 0.001$), but were not significantly associated with hemoglobin, ferritin, or transferrin saturation. In conclusion, Ret-He cannot be used as a standalone diagnostic marker of iron depletion in pediatric patients. However, its association with erythrocyte indices suggests that Ret-He may serve as a complementary parameter when interpreted alongside conventional iron status markers during the early evaluation of iron-related disorders.

Keywords: Iron Depletion, Reticulocyte Hemoglobin (Ret-He), Pediatric Patients.

Abstrak

Anemia pada anak-anak tetap menjadi masalah kesehatan masyarakat yang serius di seluruh dunia dan di Indonesia, di mana data nasional melaporkan prevalensi sebesar 21,7% pada anak-anak berusia ≥1 tahun. Defisiensi besi merupakan penyebab utama, dengan deplesi besi mewakili tahap awal yang subklinis dan sering sulit dideteksi menggunakan parameter konvensional. Hemoglobin retikulosit (Ret-He) telah diusulkan sebagai penanda ketersediaan besi fungsional untuk eritropoiesis. Studi ini bertujuan untuk mengevaluasi kinerja diagnostik Ret-He dalam mengidentifikasi deplesi besi pada pasien pediatrik. Studi analitik potong lintang dilakukan di Rumah Sakit Umum Dr. Zainoel Abidin, Banda Aceh, antara Juli dan September 2024. Sebanyak 87 pasien pediatrik berusia 1 bulan hingga 18 tahun awalnya direkrut; setelah mengesampingkan subjek dengan anemia untuk fokus pada tahap kekurangan besi, 54 anak dimasukkan dalam analisis akhir. Kekurangan besi teridentifikasi pada 12,6% subjek. Analisis kurva karakteristik penerima (ROC) menunjukkan nilai ambang optimal Ret-He sebesar 29,5 pg, dengan luas di bawah kurva (AUC) 0,609, sensitivitas 70%, spesifisitas 63,6%, dan nilai p yang tidak signifikan (p = 0,398), menunjukkan akurasi diagnostik yang terbatas. Ret-He menunjukkan korelasi positif yang signifikan dengan volume korpuskular rata-rata (MCV) (r = 0,444; p = 0,001) dan hemoglobin korpuskular rata-rata (MCH) (r = 0,511; p < 0,001), tetapi tidak menunjukkan hubungan yang signifikan dengan hemoglobin, ferritin, atau saturasi transferrin. Kesimpulannya, Ret-He tidak dapat digunakan sebagai penanda diagnostik tunggal untuk defisiensi besi pada pasien pediatrik. Namun, hubungannya dengan indeks eritrosit menyarankan bahwa Ret-He dapat berfungsi sebagai parameter pelengkap ketika diinterpretasikan bersama dengan penanda status besi konvensional dalam evaluasi awal gangguan terkait besi.

Kata Kunci: Deplesi besi, reticulocyte hemoglobin (Ret-He), pasien anak.

Introduction

Anemia is a hematological condition characterized by reduced hemoglobin (Hb) concentration, hematocrit (Ht), or red blood cell count, resulting in impaired oxygen delivery to the tissues (Fitriany & Saputri, 2018; Jahangiri et al., 2020). Rather than a primary disease, anemia represents a clinical manifestation of underlying pathological processes and may present with symptoms ranging from mild fatigue to severe functional impairment in advanced cases (Safiri et al., 2021). Among the various anemia subtypes, microcytic anemia is frequently encountered in clinical practice, particularly in children and women of reproductive ages. Iron deficiency anemia (IDA) is the most common etiology, arising from an imbalance between iron requirements and availability due to inadequate intake, impaired absorption, or chronic blood loss (Pasricha et al., 2021; Yang et al., 2023).

Anemia continues to pose a major global public health challenge, especially in low- and middle-income countries (LMICs). The World Health Organization reported that more than 273 million children aged 6–59 months were affected by anemia worldwide in 2011, with approximately half of the cases attributed to iron deficiency (Safiri et al., 2021). In Indonesia, national data from the 2018 Basic Health Research (Riskesdas) revealed anemia prevalence of 21.7% among children aged ≥1 year and 28.1% among toddlers aged 12–59 months (Kementerian Kesehatan Republik Indonesia, 2019), underscoring the substantial local disease burden.

Iron is an essential micronutrient involved in oxygen transport, DNA synthesis, and cellular metabolism, particularly during childhood. Iron deficiency develops progressively in three stages: iron depletion, iron deficiency, and iron deficiency anemia (Natekar et al., 2022; Sun & Weaver, 2021). The earliest stage, iron depletion, is characterized by declining iron stores, primarily reflected by reduced ferritin levels, while hemoglobin concentration and conventional red cell indices often remain within normal ranges (Ganz & Nemeth, 2015; Andriastuti et al., 2019). This subclinical phase poses a diagnostic challenge, as early disturbances in iron availability may go undetected using routine hematological parameters alone.

Early identification of iron depletion is clinically important to prevent the progression to overt anemia and its associated developmental consequences. However, commonly used iron status markers, such as ferritin, are influenced by inflammatory conditions, limiting their reliability in pediatric populations with concurrent infections or chronic disease. Consequently, there is an unmet need for alternative parameters that reflect functional iron availability during the early stages of iron deficiency.

Reticulocyte hemoglobin (Ret-He) has been proposed as a marker of functional iron availability for erythropoiesis, as it reflects hemoglobin incorporation into newly produced reticulocytes and is less affected by inflammation than ferritin (Andriastuti et al., 2024). Previous studies have demonstrated significant associations between Ret-He and erythrocyte indices, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), as well as its utility in detecting iron deficiency and iron deficiency anemia in selected pediatric populations (Auerbach et al., 2021; Bó et al., 2023; Jahangiri et al., 2020; Kılıç et al., 2022; Neef et al., 2021). Nevertheless, evidence regarding the performance of Ret-He during the iron depletion phase, before anemia develops, remains limited and inconsistent.

To date, data evaluating Ret-He as a marker of iron depletion in Indonesian pediatric population are scarce. Banda Aceh represents a region with a high burden of childhood nutritional disorders and limited local evidence on early iron status assessment. Therefore, this study aimed to evaluate the diagnostic performance of Reticulocyte Hemoglobin (Ret-He) in detecting iron depletion among pediatric patients in Banda Aceh and to analyze its relationship with conventional hematological and iron status parameters. The findings are expected to contribute local evidence to clarify the potential role of Ret-He as a complementary marker in the early evaluation of iron-related disorders in children.

Methods

This study employed a descriptive observational design and a cross-sectional approach. Data were collected at Dr. Zainoel Abidin General Hospital in Banda Aceh, Indonesia, between July and September 2024. All eligible pediatric patients who visited the hospital during the study period were screened. Peripheral blood samples were obtained for complete blood count analysis, including Reticulocyte Hemoglobin (Ret-He), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum ferritin, serum iron, and total iron-binding capacity (TIBC). Ethical approval was granted by the Ethics Committee of Dr. Zainoel Abidin General Hospital (approval no. 169/ETIK-RSUDZA/2024, issued July 8, 2024).

The study population consisted of pediatric patients aged 1 month to 18 years from the Province of Aceh who attended the Dr. Zainoel Abidin General Hospital during the study period. The inclusion criteria were as follows: (1) age between 1 month and 18 years, (2) no history of blood transfusion within three months prior to enrollment, and (3) written informed consent obtained from parents or legal guardians. The exclusion criteria included chronic or systemic diseases (such as nephrotic syndrome, epilepsy, tuberculosis, diabetes mellitus, congenital heart disease, or other conditions requiring long-term medication), severe acute illness (e.g., sepsis), and severe malnutrition.

The sampling frame included all pediatric patients who met the eligibility criteria during the study period. Based on hospital registry data, the accessible population was estimated to be 100 children. The minimum sample size was calculated using Slovin's formula with a 5% margin of error, resulting in a target sample size of 80 participants. All eligible participants were recruited consecutively until the required sample size was reached.

Iron depletion was operationally defined as reduced iron stores, indicated by low transferrin saturation and/or low serum ferritin levels, while maintaining normal hemoglobin concentrations, consistent with the early stage of iron deficiency described in the literature. In accordance with the study objective to evaluate Ret-He during the iron depletion phase, participants with anemia (defined as hemoglobin levels below age-adjusted reference values) were excluded from the final analysis. This exclusion criterion was determined a priori to ensure that the analysis focused specifically on subclinical iron depletion before the development of overt anemia.

Demographic and clinical data were obtained from medical records and recorded using standardized data collection forms. Blood samples were analyzed in the hospital laboratory according to routine clinical protocols. Hematological parameters, including complete blood count and Ret-H, were measured using an automated hematology analyzer, while serum ferritin, serum iron, and TIBC levels were analyzed using standard biochemical methods. All data were coded, anonymized, and entered into a computer database.

Statistical analyses were performed using SPSS version 26. Descriptive statistics were used to summarize the demographic and laboratory characteristics. The normality of continuous variables was assessed using the Shapiro–Wilk test for sample sizes <50 and the Kolmogorov–Smirnov test for sample sizes ≥50. Bivariate analyses were conducted using independent t-tests or nonparametric equivalents, as appropriate. The correlations between Ret-He and hematological or iron parameters were analyzed using Pearson or Spearman correlation tests based on the data distribution. The diagnostic performance of Ret-He in identifying iron depletion was evaluated using receiver operating characteristic (ROC) curve analysis. The optimal cutoff value was determined by maximizing the balance between sensitivity and specificity (Youden's index). A significance was set at $p < 0.05$.

A formal power analysis was not performed because of the exploratory nature of this study and the limited availability of prior local data. In addition, inflammatory markers such as C-reactive protein or hepcidin were not measured, which may have influenced the ferritin levels. These limitations were acknowledged and considered during the interpretation of the results.

Result and Discussion

Demographic characteristics

A total of 87 pediatric patients were recruited. After eligibility screening, 54 participants were included in the final analysis because participants diagnosed with anemia (37.9%) were excluded to align with the study objective of evaluating iron status changes during the iron depletion stage, when hemoglobin remains within the normal range. The analyzed cohort was categorized into three groups: normal iron status (11.5%), iron depletion (12.6%), and iron deficiency (37.9%). This grouping strategy was intended to capture early alterations in iron parameters prior to the development of overt anemia.

Table 1 summarizes the demographic and hematologic characteristics of the groups. The median age did not differ significantly among the normal, iron depletion, and iron deficiency groups (p=0.889), and the age-category distributions were also comparable (p=0.732), suggesting a broadly similar age structure at baseline. The sex distribution was not significantly different across the groups (p=0.504). Hemoglobin and hematocrit levels also did not differ significantly (Hb p=0.073; Ht p=0.422), consistent with the analytic focus on preanemic stages.

Among the erythrocyte indices, MCV showed no statistically significant difference across the groups (p=0.082). However, MCH differed significantly (p=0.020), indicating early changes in hemoglobin content per red cell that may precede the changes in Hb. Erythrocyte morphology distributions differed significantly (p=0.041), suggesting detectable shifts in red cell patterns even before the development of anemia. The iron biomarkers showed a mixed pattern. Ferritin did not differ significantly across groups (p=0.156), while serum iron and transferrin saturation differed significantly (both p<0.001). The TIBC levels were not significantly different (p=0.117). This pattern is compatible with early functional iron changes occurring alongside ferritin values, which may be influenced by inflammatory or clinical conditions.

Table 1. Characteristics of subjects

Characteristics	Iron Deficiency (n=33)	Iron Deplesion (n=11)	Normal (n=10)	p-value
Age (month), median (min-max)	52 (4-204)	64 (3-174)	71.5 (12-168)	0.889
Age Categories, n (%)				
1 – 12 Months	5 (15.2)	1 (9.1)	1 (10)	0.732
1 – 5 Months	15 (45.5)	4 (36.4)	4 (40)	
5 – 10 Months	5 (15.2)	1 (1.9)	3 (30)	
10 – 18 Months	8 (24.2)	5 (45.5)	2 (20)	
Sex, n (%)				
Male	20 (60.6)	5 (45.5)	7 (70)	0.504
Female	13 (39.4)	6 (54.5)	3 (30)	
Hb (gr/dL), median (min-max)	12.2 (11-14.4)	12.4 (11.9-13.9)	12.95 (11.6-20.2)	0.073
Ht (%), median (min-max)	36 (32-46)	37 (35-43)	36.5 (35-62)	0.422

MCV (fL), median (min-max)	77 (57-86)	81 (75-85)	80.5 (70-93)	0.082
MCH (pg), median (min-max)	26 (19-29)	27 (24-30)	27.5 (23-31)	0.020
MCHC (%), median (min-max)	33 (30-37)	34 (32-35)	33.5 (32-36)	0.376
Erythrocytes Cathegories, n (%)				
Normochromic normocytic	8 (24.2)	7 (63.6)	5 (50.0)	0.041
Microcytic hypochromic	5 (50.0)	4 (36.4)	5 (50.0)	
RDW (%), median (min-max)	13.6 ((11.9-18.1)	13.2 (12.3-13.7)	13 (11.7-18.7)	0.139
Ferritin (μg/dL), median (min-max)	50.1 (11.86-382.80)	57.61 (20.74-152.7)	90.3 (30.83-329.1)	0.156
Serum iron (μg/dL), mean ± SD	46.36 (22.03)	83.27 (13.68)	108.3 (27.07)	<0.001
TIBC (μg/dL), mean ± SD	307.97 (45.6)	324.91 (34.56)	287.3 (26.21)	0.117
Transferin Saturation, mean ± SD	15.5 (14.71)	25.71 (3.75)	37.59 (7.69)	<0.001

The distribution of clinical diagnoses across iron status groups suggests that pediatric iron disturbances may occur in heterogeneous clinical contexts rather than being confined to a single disease category. The predominance of endocrine and neurologic conditions among participants underscores that hospital-based pediatric populations often include children with chronic or recurrent health concerns that may indirectly affect dietary intake, growth demands and iron utilization. From a nutritional and clinical standpoint, these comorbidities can contribute to reduced appetite, restrictive diets, altered metabolism, and frequent healthcare encounters, factors that may influence iron balance even in the absence of overt anemia. This supports the importance of evaluating early iron impairment in real-world clinical populations, where iron depletion may coexist with non-hematologic diagnoses.

Age did not appear to meaningfully differentiate iron status in this cohort, which may reflect the relatively uniform dietary and healthcare environments among participants in this single-center setting. Although infants and toddlers are generally considered to be at a higher risk for early iron depletion due to rapid growth and limited dietary iron density, this risk is not universal and can vary substantially depending on feeding practices, dietary diversity, and background nutritional patterns (Osei Bonsu et al., 2024; Pasricha et al., 2021). Importantly, several studies have also noted that age alone may not predict iron status when exposure to dietary patterns and access to nutrition are relatively homogeneous (Osei Bonsu et al., 2024; Mantadakis et al., 2020; Kessy et al., 2019). In this context, age neutrality may indicate that the risk of iron depletion extends across pediatric age ranges in clinical care, reinforcing the need for broader awareness beyond traditional "high-risk" age windows.

Similarly, the absence of a clear sex pattern is plausible in pediatric cohorts that include multiple developmental stages. Sex-related vulnerability to iron deficiency is often context-dependent. Male infants may have higher iron requirements influenced by growth velocity and metabolic factors (Sundararajan & Rabe, 2021), whereas adolescent girls may experience an increased risk related to menstrual blood loss (Abbas et al., 2023; Zia et al., 2022). When a study population spans infancy through adolescence, these opposing trends can offset one another, yielding no dominant sex signals. This emphasizes that sex effects may be best interpreted within narrower age strata, particularly when the sample sizes in each subgroup are limited.

The hematologic profile in early iron disturbance is also biologically consistent with the concept that iron depletion precedes clinically apparent anemia. In the depletion stage, the body can mobilize iron stores to sustain hemoglobin synthesis, maintaining hemoglobin levels within reference limits despite declining reserves (Camaschella, 2015). Simultaneously, subtle changes in erythrocyte indices may emerge before hemoglobin levels decrease, reflecting early constraints on hemoglobinization and red cell maturation. This pattern aligns with established physiology: microcytosis and hypochromia typically arise as iron availability becomes increasingly limited for erythropoiesis, with MCV and MCH providing early signals before the development of iron deficiency anemia (Mohammed et al., 2020). In particular, reductions in MCH are frequently described as early markers of hypochromia, potentially preceding more overt alterations in red blood cell size and hemoglobin concentration (Mohammed et al., 2020) (Hwang et al., 2021). Meanwhile, MCHC often remains relatively stable in the early stages, as measurable reductions in hemoglobin concentration per cell may become more pronounced only as iron-restricted erythropoiesis advances (Archer & Brugnara, 2015). Together, these concepts support the clinical logic of focusing on iron depletion as an early, subclinical stage where anemia may not yet be detectable, but erythrocyte indices and morphology can begin to reflect iron-limited hematopoiesis (Jahangiri et al., 2020).

With respect to RDW, the literature frequently describes it as a sensitive marker of early iron deficiency because anisocytosis can develop as the erythrocyte population becomes more heterogeneous during iron restriction (Al-Numan & Al-Obeidi, 2021). However, RDW performance can be context-specific, particularly in clinical cohorts with mixed diagnoses and potential concurrent nutritional or inflammatory influences that may affect erythropoiesis. Therefore, the role of RDW should be interpreted cautiously as supportive rather than definitive evidence of early iron disturbance, especially when other indices and iron biomarkers provide a more direct assessment of iron physiology.

Iron biomarkers further illustrate a key interpretive challenge in pediatric clinical populations: serum ferritin is widely used to reflect iron stores; howeverit is also an acute-phase reactant and may increase in inflammatory states or chronic diseases, potentially masking depleted stores (Ganz & Nemeth, 2015). This is particularly relevant in hospital-based cohorts, where subclinical inflammation may be more common than in community-based samples. In contrast, transferrin saturation reflects the circulating availability of iron for erythropoiesis and may more directly reflect functional iron limitation in the early stages. Evidence suggests that low transferrin saturation is a meaningful indicator of functional iron depletion, particularly when ferritin levels may be confounded (Mathew et al., 2024). Taken together, these mechanisms highlight the need for a panel-based approach to interpret early iron depletion, integrating hematologic indices (e.g., MCV and MCH), iron transport measures, and clinical context rather than relying on a single marker.

Reticulocyte Hemoglobin in Iron Depletion and It Association

Reticulocyte hemoglobin (Ret-He) levels did not differ significantly between children with iron depletion and those with normal iron status (p = 0.426). Although the median Ret-He value was numerically lower in the iron depletion group, the substantial overlap in value ranges between the groups indicates that Ret-He alone does not reliably discriminate early iron depletion from normal iron status in this cohort.

Table 2. Hemoglobin reticulocyte values in depletion and normal groups

	Depletion (n=11)	Normal (n=10)	p-value
Median (min-max)	28.8 (21.5-33.5)	30.4 (20.3-34.6)	0.426

Table 3. Logistic regression analysis of the relationship between Ret Hb and iron depletion in children

Variable	OR (95%CI)	p-value
Ret-He	1.07 (0.85:1.33)	0.546

Logistic regression analysis demonstrated no statistically significant association between reticulocyte hemoglobin (Ret-He) levels and the likelihood of iron depletion in children (p > 0.05). The confidence interval for the odds ratio crossed unity, indicating substantial uncertainty in the direction and magnitude of the association. This finding suggests that variations in Ret-He do not independently predict iron depletion in this cohort. From an interpretive standpoint, the lack of significance reinforces that Ret-He, when considered in isolation, has limited discriminatory value at the iron depletion stage, where functional iron supply for erythropoiesis may still be preserved. Consequently, Ret-He should not be regarded as a stand-alone predictor of iron depletion in pediatric populations but rather as a complementary parameter whose clinical relevance depends on integration with other hematologic and iron status indicators.

Evidence from previous pediatric studies indicates that reticulocyte hemoglobin (Ret-He) has limited sensitivity in identifying early iron depletion, particularly in populations where iron stores have not yet reached critically low levels. More recent pediatric investigations have confirmed that Ret-He declines more consistently in established iron deficiency or iron deficiency anemia than during the initial depletion phase. For example, contemporary diagnostic accuracy studies have demonstrated that Ret-He performs substantially better when iron deficiency is defined by clear biochemical or hematologic thresholds, whereas its ability to detect subtle iron depletion remains constrained (Neef et al., 2021).

Age-related variability further complicates the interpretation of Ret-Hemoglobin. Studies in early infancy have shown that Ret-He demonstrates low sensitivity despite high specificity, reflecting developmental differences in erythropoiesis and iron metabolism during the first few months of life (Ringoringo et al., 2023). In contrast, Ret-He appears more informative in older infants and children when age-specific reference values are applied, supporting the view that uniform cut-off points are inappropriate across pediatric age groups (Mouzaki et al. 2018). These findings underscore that the diagnostic performance of Ret-He is population-dependent and must be interpreted within a clearly defined developmental and clinical context.

Recent literature has also highlighted that Ret-He correlates more strongly with parameters reflecting functional iron utilization, such as erythrocyte indices, than with markers of iron storage. Large pediatric screening studies have reported moderate-to-strong associations between Ret-He and indices of erythrocyte hemoglobinization, whereas correlations with ferritin or transferrin saturation are often inconsistent (Neef et al., 2021; Auerbach et al., 2021). This supports the biological premise that Ret-He reflects iron incorporation into newly produced erythrocytes, rather than total body iron reserves.

The limited predictive value of Ret-He observed in this study may also be explained by the confounding effects of inflammation on iron biomarkers, particularly ferritin. Contemporary reviews emphasize that ferritin remains an acute-phase reactant and may be elevated independent of iron status in the presence of infection, inflammation, or chronic diseaseconditions commonly encountered in hospital-based pediatric populations (Carman et al. 2019). In recognition of this limitation, the current WHO guidelines recommend interpreting ferritin levels alongside inflammatory markers and caution against its isolated use for diagnosing early iron deficiency in populations with a high inflammatory burden (WHO, 2020).

Importantly, Ret-He primarily reflects the current iron availability for erythropoiesis, not iron stores. During the early depletion stage, iron reserves may decline, whereas the functional iron supply remains sufficient to sustain hemoglobin synthesis, resulting in minimal changes in Ret-He. This physiological dissociation explains why Ret-He may fail to demonstrate meaningful discrimination at the depletion stage, despite its established utility in the later phases of iron deficiency (Neef et al., 2021; Mouzaki et al., 2018).

Taken together, these findings reinforce that Ret-He should not be considered a standalone diagnostic marker of iron depletion in pediatric patients. Instead, its clinical value lies in its role as a complementary indicator of iron-restricted erythropoiesis, which is best interpreted in combination with conventional iron indices, inflammatory context, and age-specific considerations. This integrative approach is increasingly advocated in pediatric nutrition and hematology to improve the early identification of iron-related disorders before the progression to overt anemia.

Diagnostic Performance of Reticulocyte Hemoglobin for Iron Depletion

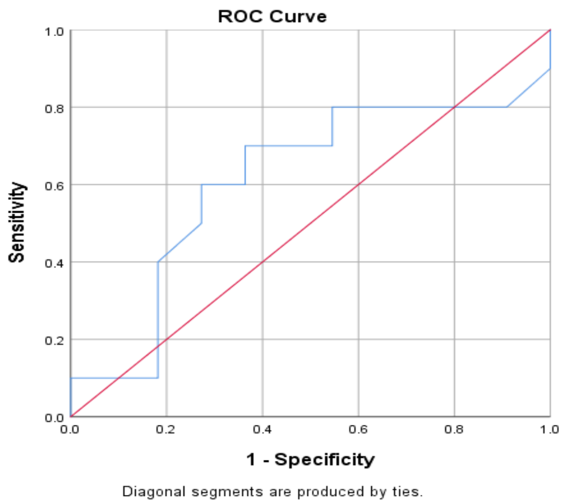


Figure 1. ROC curve of Ret He against iron depletion

Receiver operating characteristic (ROC) analysis demonstrated that reticulocyte hemoglobin (Ret-He) showed limited discriminatory ability for identifying iron depletion in pediatric patients. The area under the curve (AUC) was 0.609, indicating a weak diagnostic performance. The proposed cutoff value of 29.5 pg yielded a sensitivity of 70% and specificity of 63.6%, with no statistically significant association between Ret-He and iron depletion (p = 0.398) (Table 4, Figure 1).

Table 4. Analysis of the role of reticulocyte hemoglobin as a predictor of iron depletion in pediatric patients

Variable	AUC	Cut Off	Sensitivity	Specification	p Value
Ret-He	0.609	29.5	70%	63.6%	0.398

** Receive operating curve analysis*

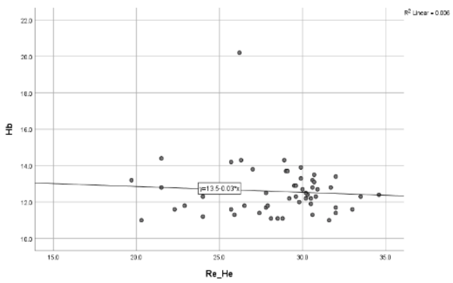
An AUC close to 0.5 suggests that the predictive capacity of Ret-He for iron depletion approaches random classification, indicating that Ret-He alone is insufficient as a screening tool in the early stages of iron deficiency. Although the sensitivity was moderate, the relatively low specificity limits its clinical utility, as a substantial proportion of non-depleted children may be misclassified. These findings align with contemporary pediatric studies showing that Ret-He performs better in detecting established iron deficiency or iron deficiency anemia than in identifying early iron depletion, where functional iron supply to erythropoiesis may still be preserved (Neef et al., 2021; Barr et al., 2023). The non-significant p-value further reinforces that Ret-He should not be used as a standalone diagnostic marker for iron depletion in heterogeneous pediatric populations.

Furthermore, correlation analysis revealed that Ret-He was significantly associated with mean corpuscular volume (MCV) (p = 0.001) and mean corpuscular hemoglobin (MCH) (p < 0.001). In contrast, no significant correlations were observed between Ret-He and hemoglobin concentration, ferritin, or transferrin saturation (all p > 0.05) (Table 5, Figure 2).

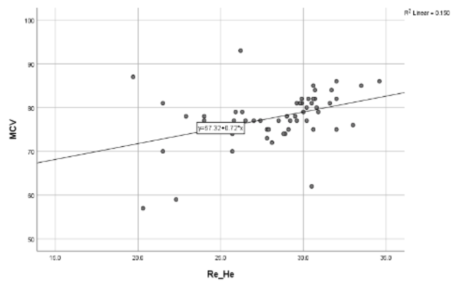
Table 5. Relationship between Reticulocyte Hemoglobin to Hb, MCV, MCH, Ferritin and Transferrin Saturation

Variables	Correlation	P value
Hemoglobin	-0.008	0.957
MCV	0.444	0.001
Ferritin	-0.046	0.740
MCH	0.511	<0.001
Transferrin Saturation	0.110	0.429

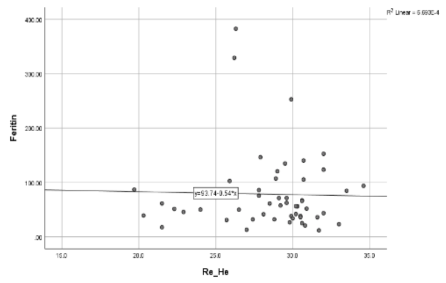
a. Hemoglobin



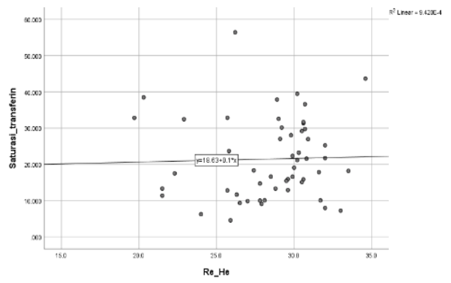
b. MCV



c. Ferritin



d. TSAT



e. MCH

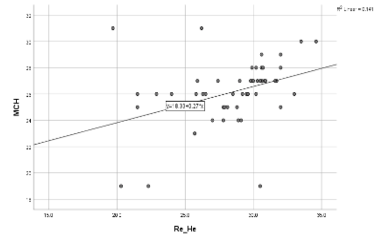


Figure 2. Scatter diagram of the relationship between Ret-He and Hb, MCV, Ferritin, Transferrin Saturation and MCH

The significant associations between Ret-He and MCV and MCH suggest that Ret-He reflects early changes in erythrocyte morphology and hemoglobinization in patients with diabetes. Ret-He represents the hemoglobin content of newly formed reticulocytes and mirrors functional iron availability during erythropoiesis. As iron supply becomes limited, hemoglobin incorporation into erythroid precursors decreases, resulting in smaller and less hemoglobinized blood cells. Similar patterns have been reported in pediatric cohorts, where Ret-He declines in parallel with MCV and MCH before measurable reductions in hemoglobin levels occur (Mouzaki et al., 2018; Neef et al., 2021).

In contrast, the lack of correlation between Ret-He and ferritin or transferrin saturation highlights the important physiological and methodological considerations. Ferritin reflects iron storage but is also an acute-phase reactant, and its concentration may be elevated in inflammatory or infectious states, potentially obscuring depleted iron stores (Carman et al. 2019). Transferrin saturation, although indicative of circulating iron availability, can fluctuate with short-term metabolic changes. These discrepancies support the interpretation that Ret-He primarily reflects functional iron utilization rather than total body iron reserves or iron transport.

Taken together, these findings indicate that while Ret-He is closely linked to erythrocyte indices associated with iron-restricted erythropoiesis, its ability to discriminate between iron depletion and normal iron status is limited. The weak ROC performance and absence of significant associations with storage and transport markers suggest that Ret-He should be interpreted cautiously when assessing early iron depletion. In pediatric nutrition and health practice, Ret-He may provide complementary information regarding functional iron status but should be integrated with conventional iron biomarkers and clinical context rather than being used as a single diagnostic indicator.

Conclusion

This study evaluated the usefulness of reticulocyte hemoglobin (Ret-He) in identifying iron depletion in pediatric patients in Banda Aceh. The findings indicate that Ret-He is not appropriate as a stand-alone screening tool for iron depletion, as its diagnostic performance was limited and not statistically significant, despite an identified cutoff value of 29.5 pg. However, the observed associations between Ret-He and erythrocyte indices suggest that Ret-He reflects functional iron availability during erythropoiesis rather than iron storage.

Accordingly, Ret-He may serve as a complementary parameter when interpreted together with conventional iron markers, such as ferritin and transferrin saturation, within the appropriate clinical and inflammatory context. Clinically, a multimarker approach is recommended for the early assessment of pediatric iron status before anemia develops. Future studies should involve larger prospective cohorts, include inflammatory markers, and evaluate combined diagnostic models to better define the role of Ret-He in early iron deficiency screening.

Acknowledgments

The authors would like to acknowledge and thank the respondents who participated in this study, the reviewers whose insightful comments have improved the paper significantly, and Editor-in-Chief, who has eased the publishing process of this paper

References

Abbas, M., Gandy, K., Salas, R., Devaraj, S., & Calarge, C. A. (2023). Iron deficiency and internalizing symptom severity in unmedicated adolescents: A pilot study. *Psychological Medicine, 53*(6), 2274–2284. https://doi.org/10.1017/S0033291721004098

Al-Numan, A. H., & Al-Obeidi, R. A. (2021). The value of the red cell distribution width index versus other parameters in the differentiation between iron deficiency anemia and beta thalassemia trait among children in Mosul, Iraq. *Expert Review of Hematology, 14*(4), 401–406. https://doi.org/10.1080/17474086.2021.1905514

Andriastuti, M., Adiwidjaja, M., & Satari, H. I. (2019). Diagnosis of iron deficiency and iron deficiency anemia with reticulocyte hemoglobin content among children aged 6–18 years. *Iranian Journal of Blood and Cancer, 11*(4), 127–132.

Andriastuti, M., Fathinasari, A. D., Fahriyani, A., Widiatmika, K. S., Salsabila, K., & Primacakti, F. (2024). The role of reticulocyte hemoglobin content in diagnosing iron deficiency in childhood cancer. *Indian Journal of Medical and Paediatric Oncology, 45*(5), 396–401. https://doi.org/10.1055/s-0044-1779047

Auerbach, M., Staffa, S. J., & Brugnara, C. (2021). Using reticulocyte hemoglobin equivalent as a marker for iron deficiency and responsiveness to iron therapy. *Mayo Clinic Proceedings, 96*(6), 1510–1519. https://doi.org/10.1016/j.mayocp.2020.10.042

Baker, R. D., Greer, F. R., Bhatia, J. J. S., Abrams, S. A., Daniels, S. R., Schneider, M. B., Silverstein, J., Stettler, N., Thomas, D. W., Grummer-Strawn, L., Hubbard, V. S., Marchand, V., Silverman, B. M., Soto, V., & Burrowes, D. L. (2010). Clinical report: Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics, 126*(5), 1040–1050. https://doi.org/10.1542/peds.2010-2576

Barr, H., Kulkarni, K., Singh, B., Sandila, N., Morrison, L., Beach, L., & Ghotra, S. (2023). Accuracy of reticulocyte hemoglobin for diagnosing iron deficiency in former very preterm infants: A population-based cohort study. *Frontiers in Pediatrics, 11*, 1281513. https://doi.org/10.3389/fped.2023.1281513

Bó, S. D., Fragoso, A. L. R., Farias, M. G., Hubner, D. P. G., & de Castro, S. M. (2023). Evaluation of RET-He values as an early indicator of iron deficiency anemia in pregnant women. *Hematology, Transfusion and Cell Therapy, 45*(1), 52–57. https://doi.org/10.1016/j.htct.2021.05.006

Camaschella, C. (2015). Iron-deficiency anemia. *New England Journal of Medicine, 372*(19), 1832–1843. https://doi.org/10.55735/qybbh562

Carman, N., Muir, R., & Lewindon, P. (2019). Ferric carboxymaltose in the treatment of iron deficiency in pediatric inflammatory bowel disease. *Translational Pediatrics, 8*(1), 28–34. https://doi.org/10.21037/tp.2019.01.01

Fitriany, J., & Saputri, A. I. (2018). Anemia defisiensi besi. *Averrous: Jurnal Kedokteran dan Kesehatan Malikussaleh, 4*(2), 1. https://doi.org/10.29103/averrous.v4i2.1033

Ganz, T., & Nemeth, E. (2015). Iron homeostasis in host defence and inflammation. *Nature Reviews Immunology, 15*(8), 500–510. https://doi.org/10.1038/nri3863

Hwang, I., Cheong, J., & Subramonia, S. (2021). Hypochromia is an early marker of iron deficiency anaemia. *British Journal of Surgery, 108*(Suppl. 7). https://doi.org/10.1093/bjs/znab362.057

Jahangiri, M., Rahim, F., Saki Malehi, A., Pezeshki, S. M. S., & Ebrahimi, M. (2020). Differential diagnosis of microcytic anemia, thalassemia or iron deficiency anemia: A diagnostic test accuracy meta-analysis. *Modern Medical Laboratory Journal, 3*(1), 16–29. https://doi.org/10.30699/mmlj17.3.1.16

Kementerian Kesehatan Republik Indonesia. (2019). *Prevalensi anemia menurut karakteristik: Riskesdas 2018.* Kementerian Kesehatan Republik Indonesia, Jakarta.

Kessy, J., Philemon, R., Lukambagire, A., Abdulrahmani, M., Urio, G., Beyanga, G., Musyoka, A., Ndaro, A., Mwitalemi, R., Maro, M., Majaliwa, E., Kinabo, G., & Mmbaga, B. (2019). Iron depletion, iron deficiency, and iron deficiency anaemia among children under 5 years old in Kilimanjaro, Northern Tanzania: A hospital-based cross-sectional study. *East African Health Research Journal, 3*(1), 42–47. https://doi.org/10.24248/eahrj.v3i1.597

Kılıç, M., Özpınar, A., Serteser, M., Kilercik, M., & Serdar, M. (2022). The effect of reticulocyte hemoglobin content on the diagnosis of iron deficiency anemia: A meta-analysis study. *Journal of Medical Biochemistry, 41*(1), 1–13. https://doi.org/10.5937/JOMB0-31435

Mantadakis, E., Chatzimichael, E., & Zikidou, P. (2020). Iron deficiency anemia in children residing in high and low-income countries: Risk factors, prevention, diagnosis and therapy. *Mediterranean Journal of Hematology and Infectious Diseases, 12*(1), e2020041. https://doi.org/10.4084/MJHID.2020.041

Mathew, B. M., Santhakumar, R., Innah, S. J., Cherian, V. J., & Rafi, A. M. (2024). Evaluating RET-Hb vs Hb in predicting early response in oral iron therapy: A prospective interventional study. *Journal of Clinical and Diagnostic Research, 18*(11), 01–05. https://doi.org/10.7860/jcdr/2024/70908.20223

Mohammed, S., Mousa, A., & Hashim, A. (2020). Role of hypochromia and microcytosis in the prediction of iron deficiency anemia. *Minia Journal of Medical Research, 31*(3), 262–268. https://doi.org/10.21608/mjmr.2022.220289

Mouzaki, M., Bramlage, K., Arce-Clachar, A. C., & Xanthakos, S. A. (2018). Serum immunoglobulin A levels do not correlate with liver disease severity in pediatric nonalcoholic fatty liver disease. *Journal of Pediatric Gastroenterology and Nutrition, 67*(5), 631–634. https://doi.org/10.1097/MPG.0000000000002104

Natekar, P., Deshmukh, C., Limaye, D., Ramanathan, V., & Pawar, A. (2022). A micro review of a nutritional public health challenge: Iron deficiency anemia in India. *Clinical Epidemiology and Global Health, 14*, 100992. https://doi.org/10.1016/j.cegh.2022.100992

Neef, V., Schmitt, E., Bader, P., Zierfuß, F., Hintereder, G., Steinbicker, A. U., Zacharowski, K., & Piekarski, F. (2021). The reticulocyte hemoglobin equivalent as a screening marker for iron deficiency and iron deficiency anemia in children. *Journal of Clinical Medicine, 10*(16), 3506. https://doi.org/10.3390/jcm10163506

Osei Bonsu, E., Addo, I. Y., Boadi, C., Boadu, E. F., & Okeke, S. R. (2024). Determinants of iron-rich food deficiency among children under 5 years in sub-Saharan Africa: A comprehensive analysis of Demographic and Health Surveys. *BMJ Open, 14*(3), e079856. https://doi.org/10.1136/bmjopen-2023-079856

Pasricha, S.-R., Tye-Din, J., Muckenthaler, M. U., & Swinkels, D. W. (2021). Iron deficiency. *The Lancet, 397*(10270), 233–248. https://doi.org/10.1016/S0140-6736(20)32594-0

Ringoringo, H. P., Purnamasari, L., Yunanto, A., Syahadatina, M., & Hidayah, N. (2023). Reference range of complete blood count, Ret-He, immature reticulocyte fraction, reticulocyte production index in healthy babies aged 1–4 months. *Scientific Reports, 13*(1), 423. https://doi.org/10.1038/s41598-023-27579-3

Safiri, S., Kolahi, A. A., Noori, M., Nejadghaderi, S. A., Karamzad, N., Bragazzi, N. L., Sullman, M. J. M., Abdollahi, M., Collins, G. S., Kaufman, J. S., & Grieger, J. A. (2021). Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: Results from the Global Burden of Disease Study 2019. *Journal of Hematology & Oncology, 14*(1), 185. https://doi.org/10.1186/s13045-021-01202-2

Sun, H., & Weaver, C. M. (2021). Decreased iron intake parallels rising iron deficiency anemia and related mortality rates in the US population. *The Journal of Nutrition, 151*(7), 1947–1955. https://doi.org/10.1093/jn/nxab064

Sundararajan, S., & Rabe, H. (2021). Prevention of iron deficiency anemia in infants and toddlers. *Pediatric Research, 89*(1), 63–73. https://doi.org/10.1038/s41390-020-0907-5

Thane, C. W., Walmsley, C. M., Bates, C. J., Prentice, A., & Cole, T. J. (2000). Risk factors for poor iron status in British toddlers: Further analysis of data from the National Diet and Nutrition Survey of children aged 1.5–4.5 years. *Public Health Nutrition, 3*(4), 433–440. https://doi.org/10.1017/S1368980000000501

Yang, J., Li, Q., Feng, Y., & Zeng, Y. (2023). Iron deficiency and iron deficiency anemia: Potential risk factors in bone loss. *International Journal of Molecular Sciences, 24*(8), Article 6891. https://doi.org/10.3390/ijms24086891

Zia, A., Stanek, J., Christian-Rancy, M., Savelli, S., & O'Brien, S. H. (2022). Iron deficiency and fatigue among adolescents with bleeding disorders. *American Journal of Hematology, 97*(1), 60–67. https://doi.org/10.1002/ajh.26389