CORRELATION BETWEEN RETICULOCYTE HEMOGLOBIN EQUIVALENT AND IRON STATUS IN PEDIATRIC CHRONIC KIDNEY DISEASE

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Abstract

Background: Anemia is a major complication of pediatric chronic kidney disease (CKD). Iron deficiency is one of the most common causes of anemia. Conventional markers of iron deficiency anemia, transferrin saturation (TSAT) and serum ferritin could be interfered with various factors. in CKD. Reticulocyte hemoglobin equivalent (Ret-He) is useful for assessing iron status among these patients.

Methods: A descriptive cross-sectional study enrolling children with CKD stage 3 and above was conducted between April and November 2021. Demographic information was also collected. Correlation of Ret-He, anemia indices and markers of iron status were analyzed.

Results: Among 50 participants, we found moderate positive correlations between Hb and Ret-He (r=0.518; p <0.001), Hct and Ret-He (r=0.403; p=0.004), and MCHC and Ret-He (r=0.667; p<0.001); a modest negative correlation between RDW and Ret-He (r=-0.616; p<0.001) and strong correlations between MCV and Ret-He (r=0.747; p<0.001) including MCH and Ret-He (r=0.865; p<0.001). No correlations between TSAT and Ret-He, serum ferritin and Ret-He, TSAT and Hb, or TSAT and Hct were observed. In addition, weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032) and between serum ferritin and Hct (r=-0.305; p=0.033) were detected. The median Ret-He was 28.42 ± 3.37 pg. Twenty-seven participants (54%) met the criteria for iron deficiency anemia (cut-off value <29 pg) of which 2 (4%) had absolute iron deficiency and 9 (18%) had functional iron deficiency defined by conventional markers.

Conclusion: Ret-He is a relevant marker of iron status among pediatric patients with CKD and correlates well with anemia indices which could help identify more patients with iron deficiency.

Keywords: Reticulocyte hemoglobin equivalent (Ret-He), Pediatric, Chronic kidney disease (CKD), Anemia, Iron deficiency

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Introduction

The worldwide prevalence of chronic kidney disease (CKD) is estimated to be 8 to 16% and continues to grow, driven by aging populations and increasing rates of obesity and type 2 diabetes mellitus.[1] Prevalence of 1.5 to 3.0 per 1,000,000 was found among children younger than 16 years old. The most common causes of CKD among children are urologic abnormalities (30-33%), glomerulopathies (25-27%), hereditary nephropathies (16%) and renal hypoplasia/ dysplasia (11%).[2] CKD affects children in many aspects such as growth, bone mineral density, hypertension, cardiovascular events and anemia. [3]

Anemia is one of the most common complications in pediatric CKD causing cognitive decline, decreased quality of life, cardiovascular risks, hospitalization and mortality. [4-7] Among adults, the National Kidney Foundation (NKF) has been working to improve patient outcomes by developing, disseminating and implementing the Dialysis Outcomes Quality Initiative (DOQI) Clinical Practice Guidelines (KDOQI), defining anemia as hemoglobin (Hb) <13.5 g/dL among males and <12 g/dL among females.[8] The most recent NKF-KDOQI clinical practice guidelines employed reference data from the National Health and Nutrition Examination Survey (NHANES) III to cite normative values among children and recommended initiating an evaluation for anemia when Hb levels fall below the age- and sex-specific 5th percentile value, although Hb differences by race are still not considered. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) have demonstrated that the prevalence of anemia among children was 73% at stage 3 CKD, 87% at stage 4 and higher than 93% at stage 5.[9] Anemia in CKD can be caused by erythropoietin deficiency, iron deficiency, increased hepcidin and iron-restricted erythropoiesis.[1, 3, 10, 11] Therefore, to evaluate iron status in CKD is important to manage properly.

Kidney Disease Improving Global Outcomes (KDIGO) recommended measuring serum ferritin and transferrin saturation (TSAT) to evaluate iron status and commencing oral iron or intravenous iron among patients undergoing hemodialysis (HD) when TSAT is ≤20% and ferritin is ≤100 ng/ml (≤100 µg/l).[12] However, interpreting serum ferritin and TSAT among patients with CKD as both measurements are confounded by inflammation and several other factors such as malnutrition, liver disease, and malignancy can give rise to high serum ferritin. TSAT is not actually measured but derived from measurement of serum iron and total iron binding capacity (TIBC). TIBC concentration in plasma is reduced by inflammation as it constitutes a negative acute-phase reactant. These implicate poor reliability of TSAT and serum ferritin in assessing iron status in CKD and end stage kidney disease (ESKD) exhibiting numerous confounding factors.[11, 13] Therefore, alternative measures were proposed to estimate available iron to incorporate in emerging erythrocytes. Reticulocyte hemoglobin content (CHr) is a measure using the Siemen blood count analyzer (Siemen ADVIA, Siemens Bayer Diagnostics, Tarrytown, NY, USA). As reticulocytes develop into erythrocytes within a few days, and hemoglobin production depends on available iron, the hemoglobin content of reticulocytes would represent short term changes in available iron for erythropoiesis. Another measure to assess iron bioavailability for emerging erythrocytes is reticulocyte hemoglobin equivalent (Ret-He), which is available using the Sysmex blood analyzers (Sysmex XN-series, Sysmex, Kobe, Japan). In this method, fluorescent markers were used to identify cellular RNA of red blood cell, reticulocytes and platelets. The reticulocyte hemoglobin equivalent (Ret-He) is calculated from a combination of fluorescence and forward scatter.[11] Both CHr and Ret-He are appropriate markers for assessing iron status among children with CKD as they measure a short half-life element that is dependent on availability of iron.

Among patients undergoing hemodialysis (HD), Brugnara et al. found that Ret-He is a reliable marker of cellular hemoglobin content and can be used to identify the presence of iron - deficient states. [14] Ret-He has been used as a marker of iron availability for erythropoiesis among adults on HD, although the cut-off values as well as specificity and sensitivity vary. [15]

Garzia et al. described an excellent diagnostic efficiency of Ret-He in evaluating patients needing iron support and demonstrated a strict correspondence between the classic CHr and the new Ret-He. [16] El-Halim et al. found that a statistically significant increase in Ret-He was observed after IV iron supplementation compared with baseline values. [17] The British Committee for Standards in Haematology guidelines for the laboratory diagnosis of functional iron deficiency 2013[18] and the National Institute for Health and Care Excellence (NICE) anemia guidelines 2015 update[19] recommended measuring CHr and Ret-He for diagnosis of iron deficiency. Analysis of CHr and Ret-He is performed on the same EDTA blood sample used for complete blood count; hence, this would require less blood sampling and reduce costs compared with traditional measures.[11] In CKD, absolute iron deficiency is likely to be present when TSAT is ≤20% and serum ferritin concentration is ≤100 ng/mL in predialysis and among patients on peritoneal dialysis (PD) or ≤200 ng/mL among patients undergoing hemodialysis. [20] Functional iron deficiency is usually characterized by TSAT ≤20% with elevated ferritin levels. [20]

Most studies of Ret-He were conducted among adults with CKD; however, studies in the pediatric population remain limited. Related studies of Ret-He among pediatric patients with CKD were conducted by Davidkova et al. [15] and Pinto et al. [21] In this study, we aimed to investigate the relationship between Ret-He and the currently-used traditional markers of iron and erythropoiesis among pediatric patients with CKD. We hypothesized that Ret-He could be a clinically useful tool to evaluate iron status in this population.

Methods

Study design and settings

A descriptive cross-sectional study of children with chronic kidney disease from stage 3 and above was conducted under the care of Phramongkutklao Hospital, Bangkok, Thailand. CKD classification was based on the KDIGO international guidelines.[22] This study was approved by the Ethics Committee of the Institutional Review Board, Royal Thai Army Medical Department; approval number R034h/64.

Participants

Between April and November 2021, children aged 6 months to 18 years with a diagnosis of CKD stages 3 to 5, as well as patients of post kidney transplant with impaired GFR (3T-5T) at the Division of Pediatric Nephrology, Phramongkutklao Hospital were enrolled in this study. Exclusion criteria included the presence of underlying diseases requiring regular blood transfusion, transfusion-dependent thalassemia, aplastic anemia, previous blood transfusion within three months, hemolytic anemia and chronic blood loss. Informed consent was obtained from all participants.

Data collection

Patients underwent history-taking, physical examination and laboratory tests. Data regarding age, sex, weight, height, causes of CKD, stage of CKD, mode of renal replacement therapy, history of kidney transplantation, thalassemia and hemoglobinopathy, erythropoietin use and iron supplement were collected. Anemia and iron status indices including Hb, hematocrit (Hct), serum iron, TIBC, serum ferritin, reticulocyte count, corrected reticulocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), c-reactive protein (CRP), serum albumin, serum creatinine (SCr), blood urea nitrogen (BUN) and Ret-He were measured. TSAT was calculated as TSAT = (serum iron divided by TIBC) × 100. [20] Glomerular filtration rate (eGFR) was calculated among all patients using Schwartz formula.[23]

Statistical analysis

Data were processed using SPSS Software, Version 21. Descriptive statistical analysis was performed; data with normal distribution was reported as mean ± standard deviation. Pearson's correlation coefficient was used to analyze relationships between Ret-He and Hb, Hct, serum iron, TIBC, serum ferritin, reticulocyte count, corrected reticulocyte count, MCV, MCHC,

MCH, RDW, CRP, SCr, BUN and TSAT, as well as correlations between TSAT and Hb, TSAT and Hct, serum ferritin and Hb, and serum ferritin and Hct. The prevalence of iron deficiency anemia (IDA) was calculated using the percentage of participants having Ret-He below 29 pg according to NICE anemia guidelines 2015 update.[19] Receiver operating characteristic (ROC) analysis was used to determine the performance of Ret-He in diagnosing iron deficiency. Results with p <0.05 were considered to be statistically significant.

Results

Patient characteristics

Fifty patients were enrolled in the study, and their characteristics are summarized in **Table 1**. The majority of participants were male (64%) with a mean age of 12.52 ± 5.18 years. The most

Table 1: Demographic and clinical characteristics of study population

Characteristics	N	%
Gender		
- Male	32	64
- Female	18	36
Age (yrs)		
- Mean ± SD	12.52 ± 5.18	
Weight (kg)		
- Mean ± SD	35.61 ± 20.07	
Height (cm)		
- Mean ± SD	130.71 ± 28.46	
Cause of CKD		
- CAKUT	17	34
- Glomerular disease	11	22
- Unknown	11	22
- Ischemia	3	6
- Other	8	16
CKD stage		
- 3	9	18
- 3T	16	32
- 4	8	16
- 4T	4	8
- 5	11	22
- 5T	2	4
Dialysis		
- Yes	12	24
- Hemodialysis	2	4
- Peritoneal dialysis	10	20
Transplantation		
- Yes	22	44

Table 1: Demographic and clinical characteristics of study population (Ext.)

Characteristics	N	%
Thalassemia		
- Hb E trait	3	6
- HbH disease	1	2
- No thalassemia	10	20
- Not tested	36	72
Erythropoietin		
- Receiving treatment	13	26
- Dose of erythropoietin (u/kg/week)	207.23 ± 142.74	
Iron supplement		
- Receiving treatment	26	52
- Dose of oral iron supplement (mg/kg/day)	3.09 ± 1.70	
- Route of administration		
o Oral	25	50
o IV	1	2

SD=standard deviation, yrs=years, kg=kilogram, cm=centimeter, CKD=chronic kidney disease, CAKUT= congenital anomalies of urinary tract and kidney, Hb= hemoglobin, u= unit, mg= milligram, IV= intravenous

common cause of CKD was CAKUT (34%). Most participants were in CKD stage 3T (32%). Twelve participants had undergone renal replacement therapy, two had hemodialysis, and ten had peritoneal dialysis. Twenty-two participants had undergone kidney transplantation (44%). Most patients were not tested for thalassemia (72%) and most were not receiving erythropoietin (74%). More than one-half of the patients received iron replacement therapy (52%), mostly via the oral route.

Anemia and iron status

Table 2 illustrated laboratory parameters of the participants. Mean Hb was 11.37 ± 2.12 g/dL; mean Hct was 35.24 ± 6.22 %.

Median Ret-He was 28.42 ± 3.37 pg. When applying a Ret-He threshold of 29 according to NICE anemia guidelines 2015 update [19], 27 participants (54%) met the criteria of IDA. On the other hand, when using conventional markers, two participants (4%) had absolute iron deficiency and nine (18%) had functional iron deficiency. One participant had TSAT <20% but lacked the data of serum ferritin. Thereby, a higher number of patients with iron deficiency could be identified with the use of Ret-He compared with conventional measures.

Linear regression analysis revealed moderate positive correlations between Hb and Ret-He (r=0.518; p <0.001) **(Figure 1)**, Hct and Ret-He (r=0.403; p=0.004) (**Figure 2)**, and MCHC and Ret-He (r=0.667; p<0.001) **(Figure 3)**. A modest negative correlation between RDW and Ret-He (r=-0.616; p<0.001) was depicted (**Figure 4)**. We also found strong correlations between MCV and Ret-He (r=0.747; p<0.001) **(Figure 5)** and between MCH and Ret-He (r=0.865; p<0.001) (**Figure 6)**. On the other hand, no correlations were found between TSAT and Ret-He (r=0.170; p=0.239) or serum ferritin and Ret-He (r=-0.021; p=0.887). Weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032), and serum ferritin and Hct (r=-0.305; p=0.033) were observed. However, no correlations between TSAT and Hb (r=-0.011; p=0.940) or TSAT and Hct (r=-0.030; p=0.838) were detected.

ROC analysis was performed to determine

Table 2. Laboratory parameters

Parameters	n	Mean ± SD
Hemoglobin (g/dL)	50	11.37 ± 2.12
Hematocrit (%)	50	35.24 ± 6.22
Serum iron (μg/dL)	50	78.50 ± 42.84
TIBC (μg/dL)	50	235.16 ± 54.35
Serum ferritin (ng/mL)	49	377.18 ± 347.59
Reticulocyte count (%)	50	1.79 ± 0.81
Correct reticulocyte count (%)	50	1.73 ± 0.75
TSAT (%)	50	33.93 ± 20.12
MCV (fl)	50	80.60 ± 8.08
MCHC (g/dL)	50	32.22 ± 1.39
MCH (pg)	50	26 ± 3.08
RDW (%)	50	14.54 ± 2.96
CRP (mg/L)	45	4.47 ± 8.19
Albumin (g/dL)	43	4.22 ± 0.69
SCr (mg/dL)	50	3.00 ± 2.84
eGFR (mL/min/1.73m^2)	50	31.74 ± 18.54
BUN (mg/dL)	50	36.07 ± 24.06
Ret-He (pg)	50	28.42 ± 3.37
- Ret-He below 29 pg		27 (54%)

TIBC = total iron binding capacity, TSAT = transferrin saturation, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, RDW = red cell distribution width, CRP = c-reactive protein, SCr =serum creatinine, eGFR= estimated glomerular filtration rate, BUN = blood urea nitrogen, Ret-He = reticulocyte hemoglobin equivalent

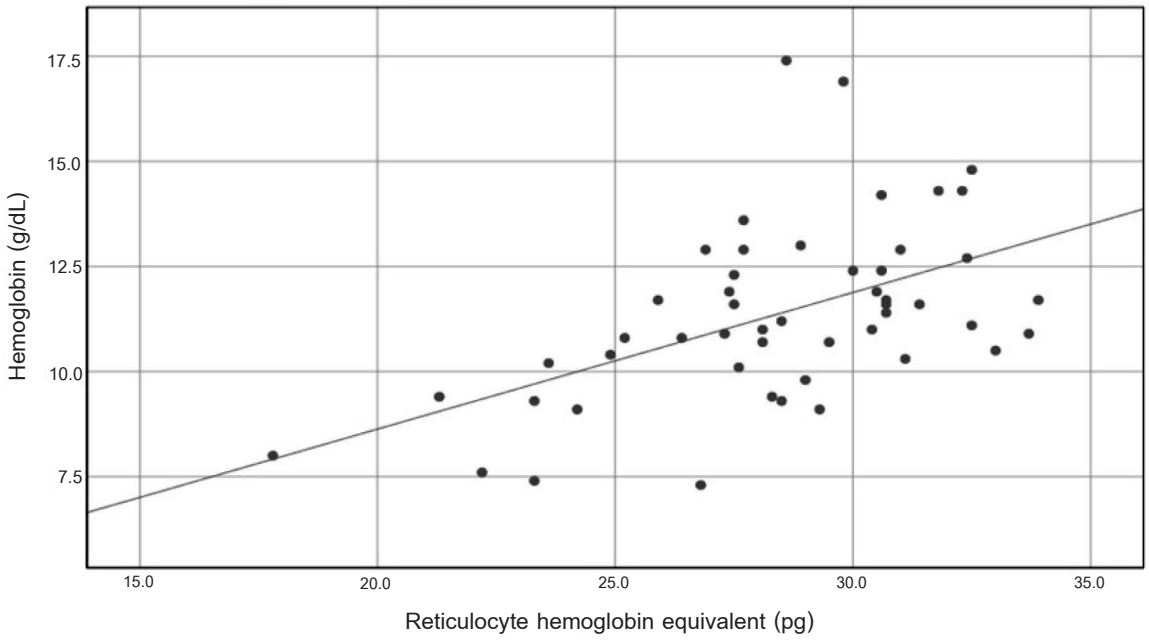

Figure 1. Correlation between hemoglobin and Ret-He

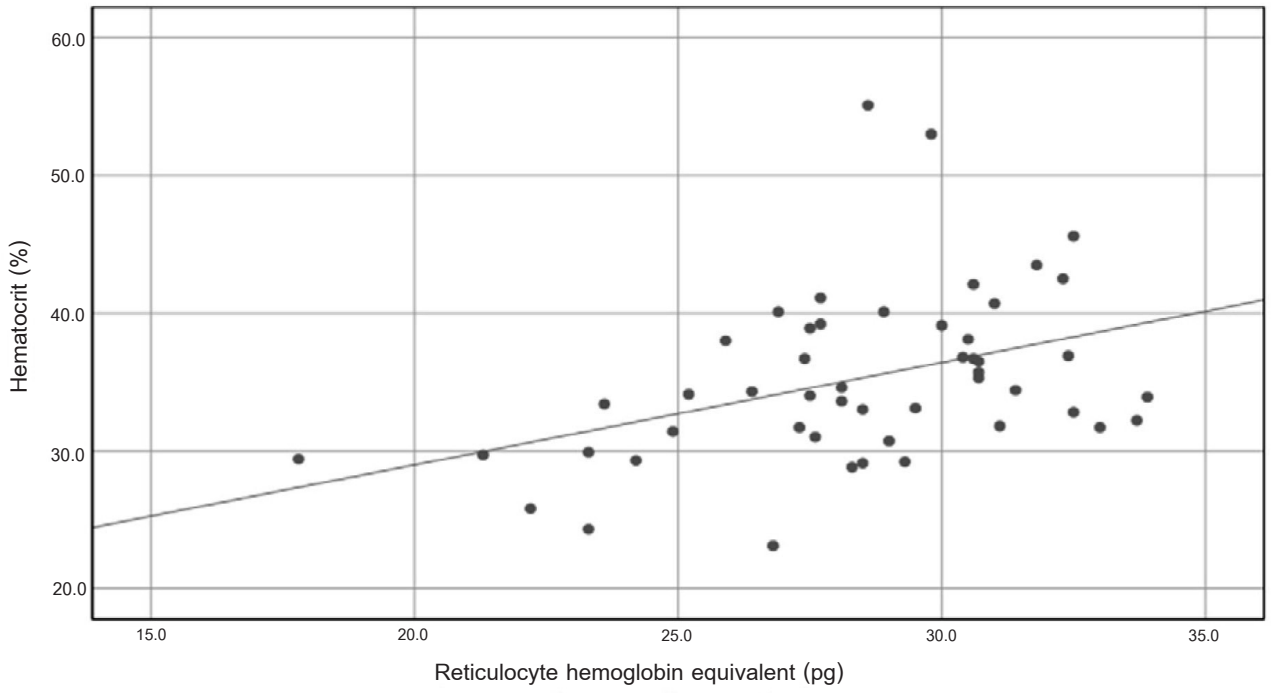

Figure 2. Correlation between hematocrit and Ret-He

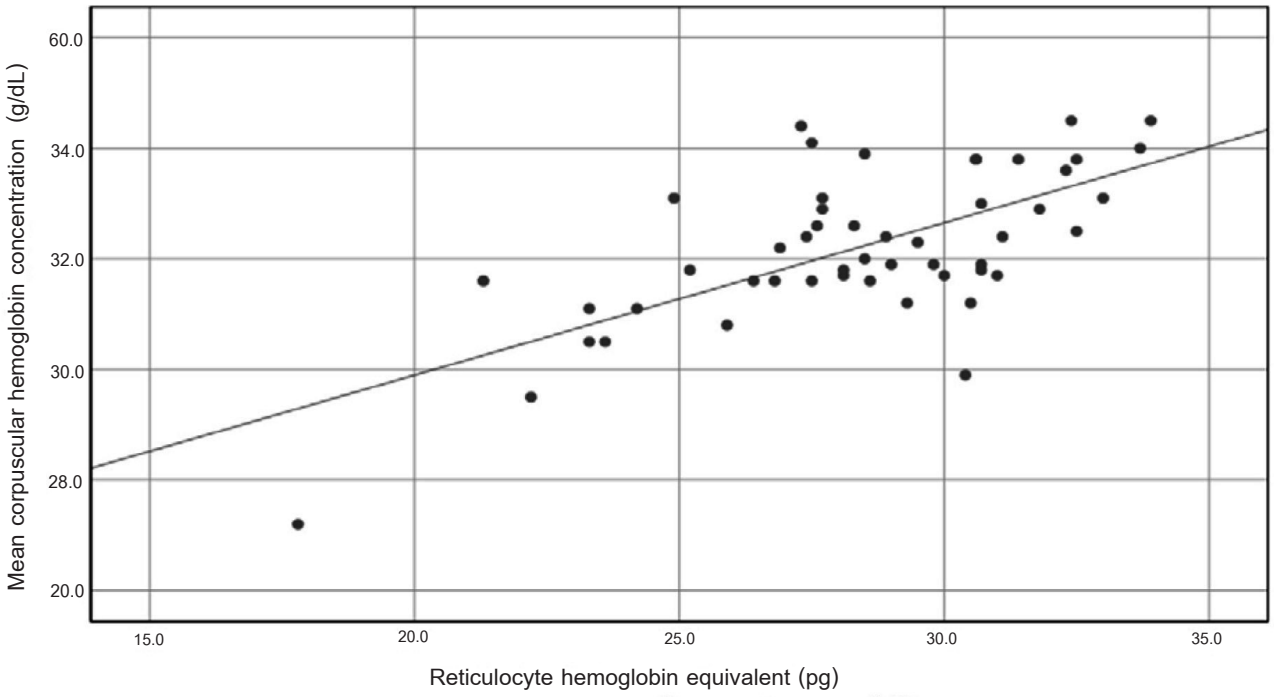

Figure 3. Correlation between mean corpuscular hemoglobin concentration and Ret-He

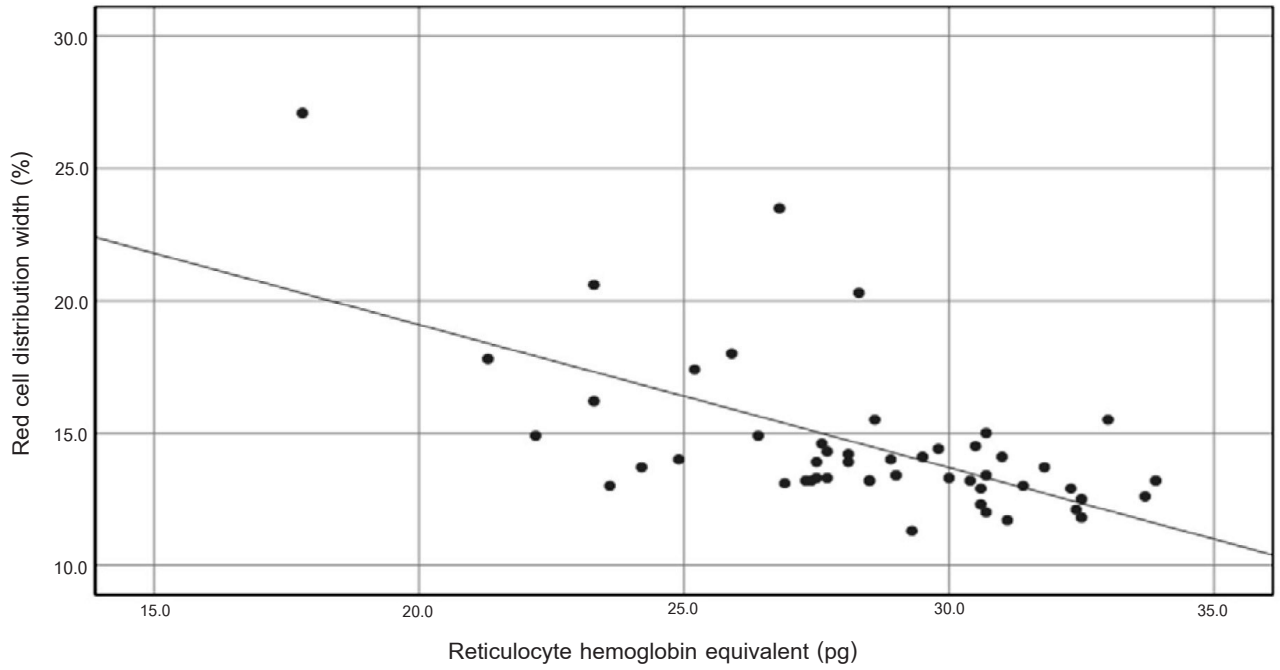

Figure 4. Correlation between red cell distribution width and Ret-He

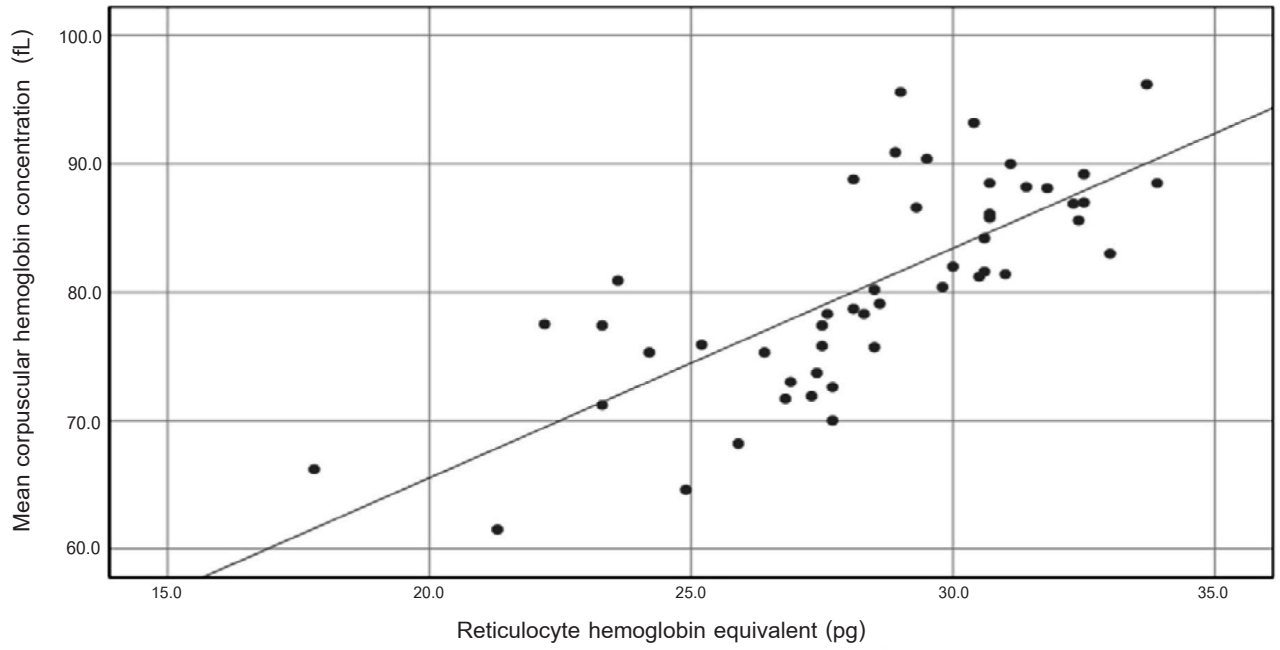

Figure 5. Correlation between mean corpuscular volume and Ret-He

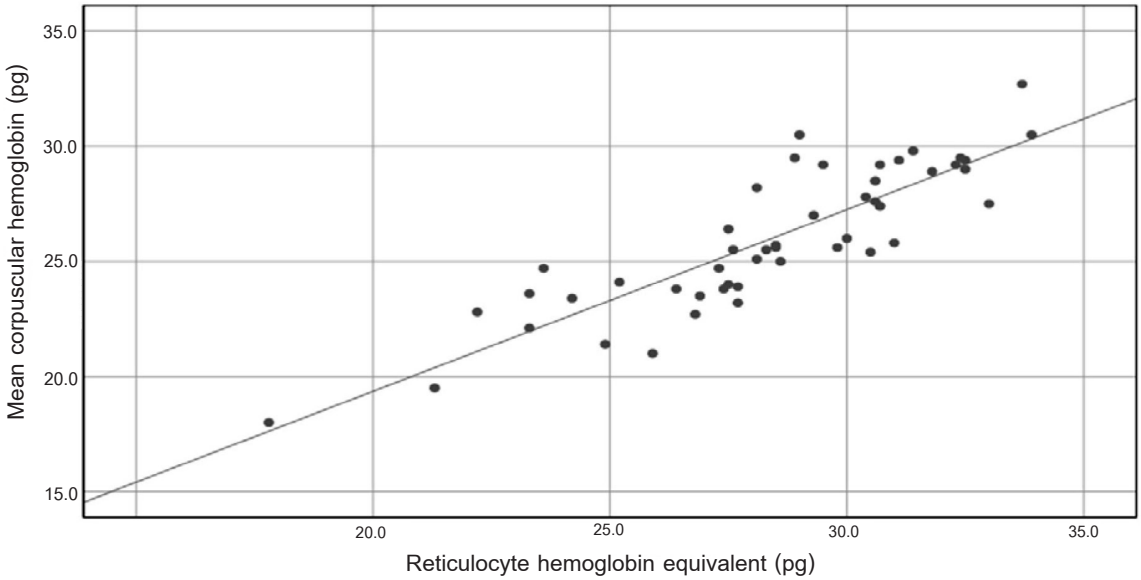

Figure 6. Correlation between mean corpuscular hemoglobin and Ret-He

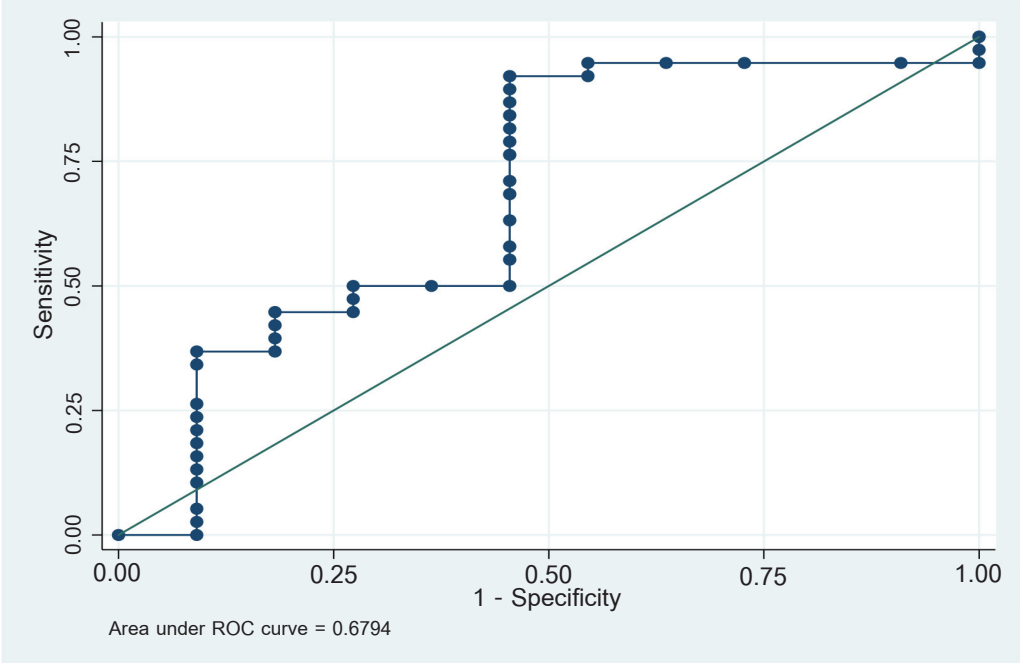

Figure 7. ROC curve of reticulocyte hemoglobin equivalent (Ret-He) to detect absolute and functional iron deficiency

the diagnostic performance of Ret-He against traditional diagnostic markers of iron deficiency, both functional and absolute iron deficiency. The area under the curve (AUC) was 0.68 (**Figure 7**). The ROC analysis demonstrated that a cut-off value of Ret-He was ≤25.9 pg detected iron deficiency with 92.11% sensitivity and 54.44% specificity.

Discussion

Anemia is an important problem among children with CKD. Lower glomerular filtration rates (GFR) are associated with lower levels of hemoglobin and become more pronounced when GFR falls below 60 mL/min per 1.73 m^2 among adults. [24] This study investigated the use of Ret-He as an indicator of iron deficiency in pediatric CKD, particularly in the low clearance group, namely CKD 3 and above. We identified

a higher prevalence of iron deficiency anemia (54%) based on Ret-He criteria compared with those of 4 and 18% for absolute and functional iron deficiency, respectively, diagnosed using traditional markers, namely, serum ferritin and TSAT. The result was comparable with that of the related study by Pinto et al. among children on hemodialysis, reporting the incidence of absolute iron deficiency being 13% when using traditional markers and 44% when using Ret-he with a cut-off point 29 pg.[21]

Before correcting anemia, accurate measurement of iron status is required to avoid unnecessary supplement. The widely-used markers in CKD are TSAT and serum ferritin, although they are known to have limited reliability in assessing iron availability among patients with CKD. Our present study demonstrated that Ret-He had moderate positive correlations with Hb (r=0.518; p <0.001), Hct (r=0.403; p=0.004) and MCHC (r=0.667; p<0.001), which was consistent with related studies. Davikova et al. conducted a study among children on chronic dialysis, exhibiting a modest positive correlation between Ret-He and Hb (r=0.22, p<0.001).[15] A study among children on hemodialysis from Pinto et al. also found a weak positive correlation between Hb and Ret-He (r=0.35, p<0.001).[21] Moreover, a moderate negative correlation between RDW and Ret-He (r=-0.616; p<0.001) and strong correlations between Ret-Hb and MCV (r=0.747; p<0.001) and MCH (r=0.865; p<0.001) were demonstrated. Therefore, this could imply that Ret-He had strong correlations with RBC indices.

Davikova et al. showed a modest relationship between Ret-He and TSAT (r=0.34, p<0001) and a poor correlation between Ret-He and ferritin (r=0.09, p=0.04).[15] However, Pinto et al. reported a significant positive correlation between TSAT and Ret-He (r=0.52, p<0.001).[21] In adult studies, Rovani et al. observed significant correlations between Ret-He and TSAT (r=0.416, p=0.019) among adult patients with CKD.[25] Dalimunthe et al. also found significant correlations between Ret-He and serum ferritin (r = 0.499, p< 0.0001) and TSAT (r = 0.592, p<0.0001) among adult patients on hemodialysis.[26] We were unable to find correlations between Ret-He and TSAT (r=0.170; p=0.239) or serum ferritin (r=-0.021; p=0.887); this could have been due to the small sample size.

Our study was concordant with the study from Pinto et al.[21], in which neither study found correlations between TSAT and Hb (r=-0.011; p=0.940), or Hct (r=-0.030; p=0.838, in contrast to the study from Davidkova et al. that demonstrated a weak positive correlation between Ret-He and Hb (r=0.12, p=0.007).[15] This could have resulted from inflammation that confounded the measurements. In the context of systemic inflammation, reduction in TIBC leads to a higher level of TSAT independent on the patient's iron status. Therefore, inflammation is implicated in the poor reliability of TSAT as a measure of iron status in CKD. [11]

Weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032), and Hct (r=-0.305; p=0.033) were demonstrated in this study, which was consistent with findings from related studies.[15,21] Ferritin is an intracellular iron storage protein, and its concentration is influenced by several factors, such as intracellular iron stores. Serum ferritin is also an inflammatory marker and plays a key role in diagnosing systemic inflammatory processes such as macrophage activation syndrome[11] and also affects the patient's nutritional status, as observed among patients on adult hemodialysis.[27] Given various confounding influences, the use of ferritin as a marker of iron status among patients with kidney disease has been questioned.

From ROC analysis, we found that a cut-off value of Ret-He was ≤ 25.9 pg with 92.11% sensitivity and 54.44% specificity. The cut-off value was lower than that of the study of Davikova et al., conducted among children undergoing chronic dialysis demonstrating a cut-off value of 28.9 pg to detect absolute iron deficiency anemia with 90% sensitivity and 75% specificity, and a cut-off value of 27.7 pg to detect functional iron deficiency anemia with 55% sensitivity and 83% specificity.[15] In addition, our cut-off value was lower than the standard guidelines, and and the NICE anemia guidelines 2015 update, recommending

using Ret-He below 29 pg in chronic kidney disease in diagnosing iron deficiency. [19] These could be due to the interference of baseline hemoglobinopathies among our patients due to the high prevalence of thalassemia in Thai populations. [28]

The major limitation encountered in this study was the small sample size due to the COVID-19 pandemic. Most patients avoided traveling and preferred to have blood work done at nearby hospitals, affecting the Ret-He investigation. We did not achieve the calculated sample size of 62 that could have affected the results of this study. Another challenge was that we could not check thalassemia among all participants. In Southeast Asia, hemoglobinopathies such as α-thalassemia, β-thalassemia, hemoglobin (Hb) E and Hb Constant Spring (CS) are highly prevalent.[28] Therefore, interpreting Ret-He in this population should be done with caution. Kadegasem et al. conducted a school-based study among Thai children to determine Ret-He in various groups: Ret-He of patients in the thalassemia trait group (26.7 ± 2.4 pg), iron deficiency group (29.0 ± 2.9 pg), iron deficiency anemia group (25.4 ± 2.7 pg), iron deficiency+ thalassemia trait group (26.6 ± 2.8 pg), and iron deficiency anemia + thalassemia trait group (24.6 ± 2.3 pg) were significantly lower than those in the normal control group (30.8 ± 1.7 pg; p<0.001, 0.01, 0.006, 0.002 and < 0.001, respectively). [29] Chaipokam et al. conducted a study among adults over 18 years of age having MCV <80 fl and treated at King Chulalongkorn Memorial Hospital, Thailand. They found that CHr among patients with IDA and thalassemia disease were significantly less than those among patients with anemia of inflammation and thalassemia trait (p<0.001), who in turn had lower CHr compared with normal controls (p<0.001).[30] This emphasized that hemoglobinopathies could interfere with Ret-He level, especially in Thai populations which are known to be highly prevalent with thalassemia.

In this study, we were able to display a moderate positive correlation between Hb and Ret-He among pediatric patients with CKD. This could give confidence to the use of Ret-He in evaluating iron status that would not be inferior to conventional markers. In fact, we could possibly diagnose a greater number of patients and provide prompt treatment. Furthermore, additional advantages included less blood sampling and reduced costs by 78% when compared with conventional measures.

Conclusion

Ret-He had significant moderate correlations with Hb, Hct and MCHC, a moderate negative correlation with RDW and strong positive correlations with MCV and MCH among pediatric patients with CKD. Thus, Ret-He is a relevant marker to assess iron status among pediatric patients with CKD and correlates well with anemia indices. Ret-He could identify more patients with iron deficiency than those of conventional markers. Ret-He value ≤25.9 pg had 92.11% sensitivity and 54.44% specificity in pediatric chronic kidney disease. Further studies with a larger sample size are still needed.

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