

EVALUATION OF THE DIAGNOSTIC VALUE OF Ret-He AMONG SICKLE CELL DISEASE PATIENTS WITH IRON DEFICIENCY ANAEMIA ON HAEMATINICS

ABSTRACT

The determination of the amount of hemoglobin in reticulocytes provides a more real-time assessment of the iron status of the bone marrow. Ret-He unlike most biochemical markers is not affected by inflammation. Hence, it provides a better way of detecting the presence/absence of iron for erythropoiesis. The aim of the study was to evaluate the diagnostic value of Ret-He and to compare it with serum ferritin and combined Red Blood Cell (RBC) indices: haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Red Cell Distribution Width (RDW) in detecting iron deficiency among Sickle Cell Disease (SCD) children. **Materials and Methods:** 89 SCD children attending KATH sickle cell clinic were enrolled in the study. Complete Blood Count (CBC), Reticulocyte haemoglobin content (Ret-He) and biochemical tests [ferritin and C - reactive protein (CRP)] were performed from venous blood samples. **Results:** Iron deficiency (ID) was diagnosed when subject's Hb, MCV, MCH, RDW, serum ferritin and Ret-He were below cut-off values. Receiver Operating Characteristic (ROC) analysis showed the following results: combined RBC indices (AUC=0.953, sensitivity=90.7%, specificity=100%, $p < 0.05$), Ret-He (AUC=0.647, sensitivity=34.9%, specificity=94.4%, $p > 0.05$) and serum ferritin (AUC=0.476, sensitivity=90.2%, specificity=11.1%, $p > 0.05$). **Conclusion:** The findings of this study suggest that the combined red cell indices is the most accurate, sensitive and specific among the three diagnostics tools used in this study to detect ID in SCD children on hematinics.

Key words: Sick cell disease, ferritin, iron-deficiency, red blood cell indices; reticulocytes, anaemia.

BACKGROUND

Iron deficiency, (ID) is the most common nutritional deficiency around the globe and affects mostly children and women [1-4]. ID in sickle cell disease (SCD) patients may be considered rare because of the increased iron bioavailability from RBCs during hemolysis and increased gut absorption of iron during the hemolysis [5]. Moreover, there is also a high load of iron derived from multiple blood transfusions [5]. However, other studies in different parts of the world have indicated the prevalence of ID in SCD [6-8].

Iron deficiency diagnosis is based primarily on laboratory measurements. The gold standard test for diagnosing storage iron depletion is the absence of stainable iron in the bone marrow. This can be detected using the Perl's Prussian Blue stain. However, this procedure is invasive and costly, hence it cannot be used to screen patients [9, 10]. Conventional measurements involve red cell indices like MCV, MCH, MCHC, RDW and other reticulocyte indices (Ret-He, retic index, etc). However, RBC indices reflect iron status for a longer period of time, since the lifespan of mature RBC is about 120 days [9, 10]. This is unlike reticulocytes which take 1-2 days to mature in peripheral circulation [11, 12], thus, allowing a reticulocyte index like reticulocyte hemoglobin equivalent (Ret-He) to provide a more real-time assessment of marrow iron than the red cell indices [12-16]. It also involves a panel of biochemical measurements like serum ferritin, serum transferrin saturation, serum iron, soluble transferrin receptor, soluble transferrin receptor index. However, most of these tests are expensive and comorbidities like liver disease,

inflammation and chronic infections may affect the accurate measurements of these biochemical parameters [17].

The detection of IDA in children with SCD is crucial, as IDA leads to worsening of anemia. This is likely to have detrimental long-term effects on their neuro-cognitive development and growth [18]. In this study, we sought to compare the diagnostic value of three different parameters [combined RBC indices (Hb, MCV, MCH and RDW), Ret-He and serum ferritin] in diagnosing ID among SCD children.

MATERIALS AND METHODS

Study design/site/subjects: This was a cross sectional study carried out between February and April 2017 at the Sickie Cell Clinic of the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. Simple random sampling technique was used to select eighty-nine (89) SCD (HbSS/HbSC) patients for the study. These patients were on hematinic and in a steady state.

Inclusion and exclusion criteria: The subjects were sickle cell children in a steady state with genotypes Hb SS and Hb SC with an age range of 1year – 17years. Sickle cell carriers were excluded from the study. Parental/guardian consent was obtained for participants before they were recruited into the study.

Laboratory methods and sample analysis

Three (3) mls of venous blood sample was taken from each subject into K₃EDTA anticoagulant tubes. Complete blood count (including Hb, MCV, MCH, RDW and Ret-He) was performed on the samples within 4 hours of collection using the SYSMEX XT-4000i automated hematology analyzer (Sysmex Corporation, Kobe, Japan). The serum was separated by centrifugation and

aliquotted into eppendorf tubes and frozen at -20°C. Repetitive freezing and thawing was avoided. Serum ferritin estimation was done within 30 days of storage using the enzyme immunoassay (PISHTAZTEB Diagnostics, Germany) for quantitative determination of ferritin concentration in human serum/plasma. The reading was done using the Mindray MR-96A [(Microplate Reader) (Mindray, China)]. Estimation of C- reactive protein (CRP) was performed using the HUMATEX CRP latex agglutination slide test (Human GmbH, Wiesbaden, Germany).

Table 1: Cut off criteria for iron deficiency diagnosis.

Age(yrs)	Hb (g/dL)	MCV(fL)	MCH(pg)	RDW-CV	Ret-He (pg)	Ferritin(ug/L)	CRP(mg/ml)
1-5	< 11.0	< 72.0	< 25.0	> 15.0			
6-17	< 12.0	< 78.0	< 26.0	> 14.0	< 26.0	< 273.0	<6.0

STATISTICAL DATA ANALYSIS

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) (version 21.0; IBM Corporation, New York, USA). Categorical variables were compared using Fisher's exact tests as indicated. P-values <0.05 were considered statistically significant. To compare Ret-He, serum ferritin and the red cell indices for diagnosing iron deficiency anemia, receiver operating characteristic (ROC) curves with areas under the curve (AU-CROC) were calculated.

ETHICAL CONSIDERATIONS

The study was approved by Committee on Human Research, Publications and Ethics (CHRPE) for Kwame Nkrumah University of Science and Technology, School of Medical Sciences and

Komfo Anokye Teaching Hospital (KATH). Permission was also sought from the study site (KATH Research/Development unit). Written informed consent was obtained from the parents or guardians of all subjects and explained to them in their own language.

RESULTS

Table 2 shows the demographic characteristic of participants. The study comprised 89 children, 45 (50.6%) males and 44 (49.4%) females; 72 with HbSS (80.9%) and 17 with HbSC (19.1%). The mean age of children was 6.573 years.

Table 2: Demographic characteristics of study participants

Variables	Frequency	Percentage
Age(years) (Mean±SEM)	6.573 ± 0.4240	
Gender		
Male	45	50.60%
Female	44	49.40%
Sickle cell disease		
HbSS	72	80.90%
HbSC	17	19.10%

SEM = standard error of mean

Table 3 shows the diagnosis of IDA based on RBC indices, Ret-He and Ferritin. A significantly greater percentage of iron deficiency was recorded when RBC indices 56.2% was used as the criteria for diagnosing IDA followed by Ferritin (46.7%) then Ret-He 21.3% (p=0.012)

Table 3: Diagnosis of IDA (based on RBC indices, Ret-He and Ferritin) *

		†RBC indices	Ret-He	Ferritin
IDA	Present	50 (56.2%)	19 (21.3%)	43 (46.7%)
	Absent	39 (43.8 %)	70 (78.7%)	6 (6.5%)

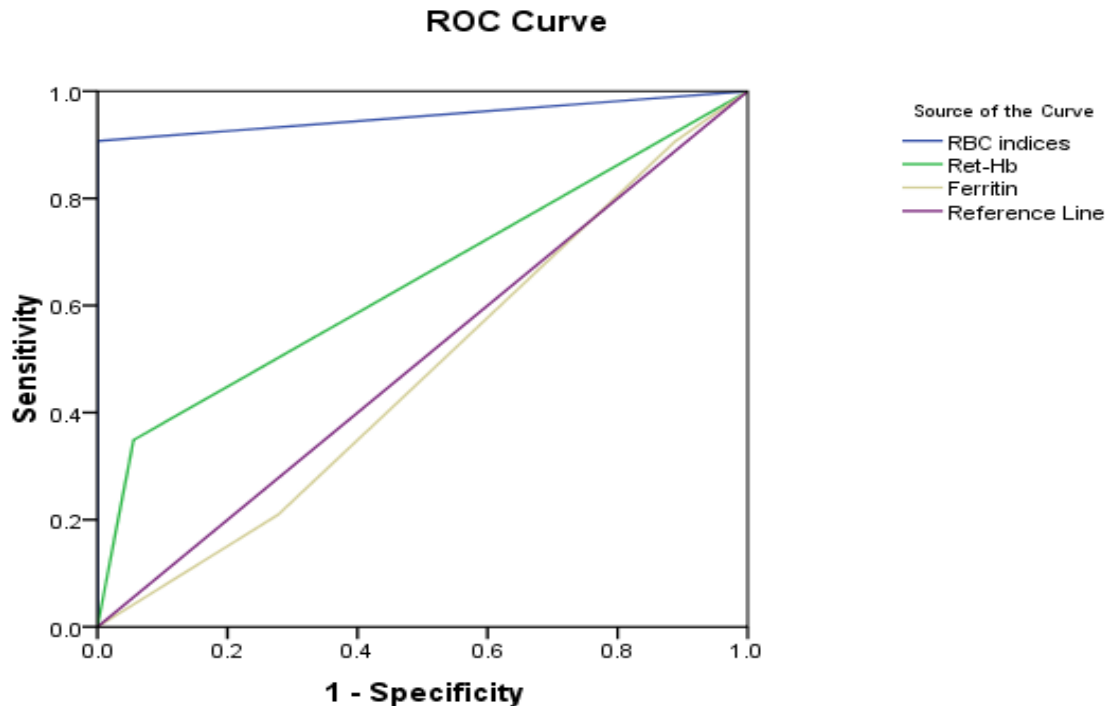
* p = 0.012. Fischer exact test † RBC indices = MCV, Hb, MCH, RDW

Comparison between laboratory tests of children who did not receive blood transfusion is shown in Table 4. Regarding the hematological tests, SC children presented with higher Hb, not shown in the table. There was a significant difference in Hb, MCV, MCH, RDWCV, absolute reticulocyte and Ret-He between the two groups. Ferritin level though higher in SC than SS showed no statistical significant difference (p = 0.6097).

Table 4: Comparison between hematological and biochemical tests of children who did not receive blood transfusion (n = 64), according to the type of sickle cell disease (SC or SS).

Parameters	SS children (n=50)	SC children(n=14)	p-value
Hb (g/dL)	8.444 ± 0.2019	9.971 ± 0.3109	0.0005
MCV (fL)	73.07 ± 1.249	63.71 ± 2.171	0.0007
MCH (pg)	25.60 ± 0.4186	23.39 ± 0.7776	0.0157
RDW-CV	21.74 ± 0.5601	18.63 ± 0.7925	0.0083
Ret-He (pg)	29.97 ± 0.5954	26.90 ± 1.147	0.0194
Ferritin (ug/L)	131.7 ± 14.11	148.1 ± 34.95	0.6097

Hb = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean cell hemoglobin concentration; RDWSD = red cell distribution width - standard deviation; RDWCV = red cell distribution width - coefficient of variation; ARC = Absolute reticulocyte count; Ret-He = reticulocyte hemoglobin equivalent; †Mean values and unpaired t test.



Diagonal segments are produced by ties.

Figure 1: ROC for RBC indices, Ret-He and Ferritin in the diagnosis of IDA among children with SCD.

Comparatively, RBC indices [Area= 0.953 (95% CI 8.940-1.009), sensitivity = 90.7 % and specificity =100%, p value = <0.001] was more accurate for diagnosing IDA in SCD children than Ret-He [Area= 0.647 (95% CI 0.506-0.787), sensitivity = 34.9 % and specificity =94.4%, p value = 0.073] and serum Ferritin [Area= 0.476 (95% CI 0.312-0.640); Sensitivity= 90.2%; Specificity= 11.1%, p value = 0.770]

DISCUSSION

Ret-He is relatively new parameter that some manufacturers of haematology analysers are beginning to add to the test parameters. We sought to evaluate the diagnostic value of the

parameter Ret-He in comparison to serum ferritin and the combined RBC indices (Hb, MCV, MCH and RDW) for the diagnosis of ID in sickle cell disease patients on iron supplementation. The combined red cell indices (MCV, Hb, MCH, MCHC and RDW) were able to significantly ($p < 0.05$) exclude most of subjects with iron deficiency followed by ferritin and Ret-He (Table 3). However, the mean Ret-He for the HbSS and HbSC subjects was above the cutoff value of 26pg used in this investigation (Table 4.). This is quite different from the red cell indices which were below the normal values (Table 1.) despite the iron supplementation therapy. It can therefore be said that the combined RBC indices is a higher negative predictor for the absence of iron for haemopoiesis. This also suggests that Ret-He is a better and useful tool in monitoring response to iron supplementation because they are early respondents to iron therapy [19, 20]. In cases where a patient is responding to treatment with hematinics, the value of Ret-He will increase first, indicating response to the therapy.

ROC curve analysis showed a greater AUC, sensitivity and specificity of the combined red cell indices relative to Ret-He and serum ferritin. This indicates that the combined red cell indices have a greater accuracy of diagnosing ID in sickle cell children in steady state and on haematinics. Our observation contrasts the work of other investigators who indicated that Ret-He was more accurate than a singled-out red cell indices, ie., MCV [18, 21, 22]. However, this study suggests a greater accuracy of the MCV when combined with other red cell indices (Hb, MCH and RDW) than Ret-He in diagnosing iron deficiency in SCD children on haematinics.

Previous studies indicated a greater accuracy of Ret-He in diagnosing IDA compared to serum ferritin [13, 18, 21-23]. This is consistent with our results where Ret-He proved superior to serum ferritin in the diagnosis of ID. However, we reported a significantly lower sensitivity of

Ret-He (34.9%) compared to previous studies. These significantly lower values could be as result of the difference in diagnostic criteria for IDA. The subjects were also on haematinics (zincifer) which may have had influence on our result. Moreover, most of these authors reported these findings in hemodialysis patients and other non-SCD subjects. whereas our current study focused on SCD patients. Different sensitivities and specificities were obtained by the various authors in those studies probably because of variation in the criteria for diagnosing IDA and also because of the different cut off values for the different parameters or variation in the study population. Some researchers [24] however reported a different finding in 6-24month old children in Kaunas, Lithuania, where they indicated a greater accuracy of ferritin than Ret-He in diagnosing IDA. This was probably due to the different cut off value of serum ferritin (<12ng/ml) used by this investigator in the diagnosis of IDA.

CONCLUSION

The findings of this study suggest that the combined red cell indices is a very valuable tool in the diagnosis of IDA in SCD children on hematinic and might even be useful in diagnosing IDA in resource-limited facilities.

Ret-He is a trustworthy marker of cellular hemoglobin content and might be used to identify the presence of iron-deficient states and to aid in the monitoring of response to iron supplementation therapy in IDA patients. Monitoring of the responses to the therapy offers health professionals (physicians and nurses) working with SCD patients valuable opportunity for objective assessment of the patients and health promotion to parents in addition to instituting other interventions.

REFERENCES

1. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *The Lancet*. 2016;387(10021):907-16.
2. WHO, 2015. The global prevalence of anaemia in 2011..
3. Patel S, Dhuppar P, Bhattar A. Nutritional Anemia Status in Adolescent Girls in Rural Schools of Raipur, India. *Med Chem (Los Angeles)*. 2017;7:853-6.
4. Thomas D, Chandra J, Sharma S, Jain A, Pemde HK. Determinants of nutritional anemia in adolescents. *Indian pediatrics*. 2015;52(10):867-9.
5. Porter JB, de Witte T, Cappellini MD, Gattermann N. New insights into transfusion-related iron toxicity: Implications for the oncologist. *Critical reviews in oncology/hematology*. 2016;99:261-71.
6. Mohanty D, Mukherjee M, Colah R, Wadia M, Ghosh K, Chottray G, et al. Iron deficiency anaemia in sickle cell disorders in India. *Indian Journal of Medical Research*. 2008;127(4):366.
7. Kassim A, Thabet S, Al-Kabban M, Al-Nihari K. Iron deficiency in Yemeni patients with sickle-cell disease. *Eastern Mediterranean Health Journal*. 2012;18(3):241.
8. Mohanty P, Jena RK, Sethy S. Variability of Iron Load in Patients of Sickle Cell Anaemia (HbSS): A study from Eastern India. *Journal of clinical and diagnostic research: JCDR*. 2017;11(3):EC19.
9. Tkaczyszyn M, Comín-Colet J, Voors AA, van Veldhuisen DJ, Enjuanes C, Moliner-Borja P, Rozentryt P, Poloński L, Banasiak W, Ponikowski P, van der Meer P. Iron deficiency and red cell indices in patients with heart failure. *European journal of heart failure*. 2018 Jan; 20(1):114-22.
10. Ervasti M. Evaluation of Iron Status Using Methods Based on the Features of Red Blood Cells and Reticulocytes: Doctoral dissertation, University of Kuopio, 2008, 1-106,[[www. uku. fi/vaitokset/2008/isbn978-951-27-0956-4](http://www.uku.fi/vaitokset/2008/isbn978-951-27-0956-4). pdf, con accesso 1-12-2009] Vai; 2008.
11. Koepke JA. Update on reticulocyte counting. *Laboratory Medicine*. 2015;30(5):339-43.
12. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. *American journal of hematology*. 2008;83(4):307-10.

190 13. Igartua EU, Hoffmann JJ, Izquierdo-Álvarez S, Escanero JF. Reticulocyte hemoglobin
191 content (MCHr) in the detection of iron deficiency. *Journal of Trace Elements in Medicine and*
192 *Biology*. 2017;43:29-32.

193 14. Lorenz L, Arand J, Büchner K, Wacker-Gussmann A, Peter A, Poets CF, et al. Reticulocyte
194 haemoglobin content as a marker of iron deficiency. *Archives of Disease in Childhood-Fetal and*
195 *Neonatal Edition*. 2015;100(3):F198-F202.

196 15. Scherer PS, Moraes D, Munhoz TP, Sgnaolin V. New red blood cell and reticulocyte
197 parameters and reference values for healthy individuals and in chronic kidney disease. *Jornal*
198 *Brasileiro de Patologia e Medicina Laboratorial*. 2015;51(2):77-84.

199 16. Clark SF. Iron deficiency anemia: diagnosis and management. *Current opinion in*
200 *gastroenterology*. 2009;25(2):122-8.

201 17. Urrechaga E, Borque L, Escanero JF. Assessing iron status in CKD patients: new Laboratory
202 parameters. *Chronic Kidney Disease: InTech*; 2012.

203 18. Cai J, Wu M, Ren J, Du Y, Long Z, Li G, et al. Evaluation of the Efficiency of the
204 Reticulocyte Hemoglobin Content on Diagnosis for Iron Deficiency Anemia in Chinese Adults.
205 *Nutrients*. 2017;9(5):450.

206 19. Garzia M, Di Mario A, Ferraro E, Tazza L, Rossi E, Luciani G, et al. Reticulocyte
207 hemoglobin equivalent: an indicator of reduced iron availability in chronic kidney diseases
208 during erythropoietin therapy. *Laboratory hematology: official publication of the International*
209 *Society for Laboratory Hematology*. 2007;13(1):6-11.

210 20. Kim J, Ihm C, Kim H. Evaluation of reticulocyte haemoglobin content as marker of iron
211 deficiency and predictor of response to intravenous iron in haemodialysis patients. *International*
212 *journal of laboratory hematology*. 2008;30(1):46-52.

213 21. Marković M, Majkić-Singh N, Subota V, Mijusković Z. Reticulocyte hemoglobin content in
214 the diagnosis of iron deficiency anemia. *Clinical laboratory*. 2004;50(7-8):431-6.

215 22. Parodi E, Giraudo MT, Ricceri F, Aurucci ML, Mazzone R, Ramenghi U. Absolute
216 reticulocyte count and reticulocyte hemoglobin content as predictors of early response to
217 exclusive oral iron in children with iron deficiency anemia. *Anemia*. 2016;2016.

- 218 23. Miwa N, Akiba T, Kimata N, Hamaguchi Y, Arakawa Y, Tamura T, et al. Usefulness of
219 measuring reticulocyte hemoglobin equivalent in the management of haemodialysis patients with
220 iron deficiency. *International Journal of Laboratory Hematology*. 2010;32(2):248-55.
- 221 24. Kiudeliienė R, Griniūtė R, Labanauskas L. Prognostic value of reticulocyte hemoglobin
222 content to diagnose iron deficiency in 6–24-month-old children. *Medicina*. 2008;44(9):673-7.

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