Evaluation of the Diagnostic Value of Ret-He among Sickle Cell Disease Children with Iron Deficiency Anaemia on Haematinics

David Ntiamoah Ofosu[1,2,3*], Clement Opoku-Okrah[2,3], Victoria Bam[4] and Selorm Philip Segbefia[1]

[1] *Department of Medical Laboratory, Garden City University College, Ghana.*
[2] *Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Ghana.*
[3] *Department of Haematology, Komfo Anokye Teaching Hospital, Ghana.*
[4] *Department of Nursing, Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Ghana.*

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ABSTRACT

Introduction: 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, the iron molecule found in reticulocytes, unlike most biochemical markers is not affected by inflammation. Hence its determination provides a better way of detecting the presence or absence of iron for erythropoiesis.
Objectives: The aim of the study was to evaluate the diagnostic value of Ret-He and to compare it with serum ferritin and Red Blood Cell (RBC) indices: haemoglobin (Hb), Mean Corpuscular

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*Corresponding author: E-mail: ntiamoah13@gmail.com, david.ntiamoah@uenr.edu.gh;

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. Iron deficiency anaemia (IDA) was diagnosed when subject's Hb, MCV, MCH, RDW, serum ferritin and Ret-He were below cut-off values.

Results: Receiver Operating Characteristic (ROC) analysis showed the following results: 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 red cell indices is the most accurate, sensitive and specific among the three diagnostics tools used in this study to detect IDA in SCD children on hematinics.

1. INTRODUCTION

Iron deficiency anaemia, (IDA) is the most common nutritional deficiency around the globe and affects mostly children and women [1-4]. IDA in sickle cell disease (SCD) children 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 IDA in SCD [6-8].

Iron deficiency anaemia 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 determination of 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]. The determination of IDA 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].

This study is relevant because the detection of IDA in children with SCD is crucial, as the lack of iron leads to worsening of anemia. This is likely to have detrimental long-term effects on their neuro-cognitive development and growth of the children [18].

The aim of the study was to evaluate the diagnostic relevance of Ret-Het among children with sickle cell disease having IDA and was on haematinics. Our main objectives were to compare the diagnostic value of three different parameters [RBC indices (Hb, MCV, MCH and RDW), Ret-He and serum ferritin] in diagnosing IDA among SCD children.

2. MATERIALS AND METHODS

2.1 Study Design/Site/Subjects

This was a cross sectional study carried out between February and April 2017 at the Sickle 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) children for the study. These participants were on hematinic and in a steady state.

2.2 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 1 year – 17 years. Sickle cell carriers were excluded from the study.

Parental/guardian consent was obtained for participants before they were recruited into the study.

2.3 Laboratory Methods and Sample Analysis

Three (3) mls of venous blood sample was taken from each subject into K_3EDTA 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) to establish the absence of inflammation among the children studied.

2.4 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.

2.5 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.

3. 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 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).
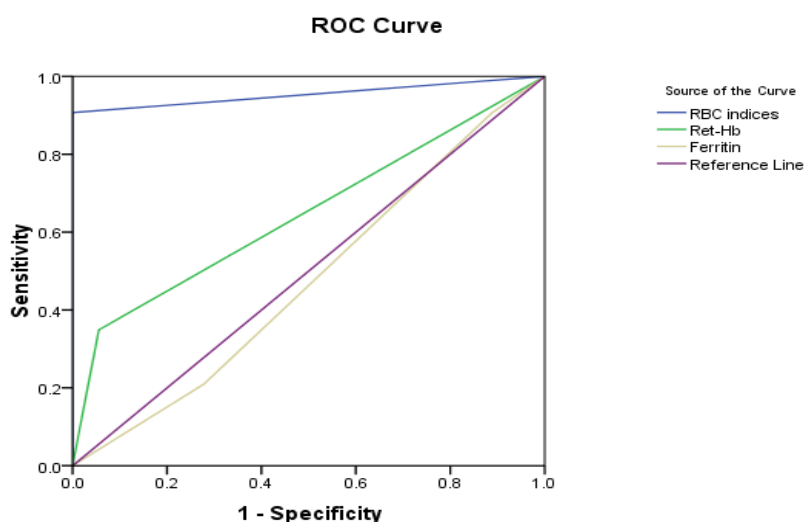
Fig. 1. ROC for RBC indices, Ret-He and Ferritin in the diagnosis of IDA among children with SCD

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

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. 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 (48.3%)
	Absent	39 (43.8 %)	70 (78.7%)	46 (51.6%)

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

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

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, RDW-CV, 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).

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].

4. 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 RBC indices (Hb, MCV, MCH and RDW) for the diagnosis of IDA in sickle cell disease children on iron supplementation.

The red blood cell indices (MCV, 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 RBC indices is a higher negative predicator for the absence of iron for haemopoiesis. This result 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 child 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 red cell indices relative to Ret-He and serum ferritin. This indicates that the red cell indices have a greater accuracy of diagnosing IDA 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 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 IDA. 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 (zincofer) 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 children. 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.

5. CONCLUSION

The findings of this study suggest that the 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 children. Monitoring of the responses to the therapy offers health professionals (physicians and nurses) working with SCD children valuable opportunity for objective assessment of the patients and health promotion to parents in addition to instituting other interventions.

CONSENT

Written informed consent was obtained from the parents or guardians of all subjects and explained to them in their own language.

ETHICAL APPROVAL

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).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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