

## ORIGINAL ARTICLE

# Reticulocyte Hemoglobin vis-a-vis Serum Ferritin as a Marker of Bone Marrow Iron Store in Iron Deficiency Anemia

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## Abstract

**Aim:** To evaluate reticulocyte hemoglobin (RET-He) vis-a-vis serum ferritin as a marker of bone marrow iron store in iron deficiency anemia (IDA).

**Material and Methods:** A hospital based analytic study was conducted among patients (age group 15-65 years) with newly diagnosed and untreated IDA admitted in medicine ward and not suffering from any inflammatory disorders (excluded by C-reactive protein). Patient having other forms of anemia/ hemoglobinopathies/ malignancy, MCV >80 fL and pregnant female were excluded. All patients were subjected to automated CBC, RET-He, iron studies and iron staining of bone marrow aspirates.

**Result:** Total 142 patients were included. Of these, 42 patients were excluded due to aparticle bone marrow aspirate. Remaining 102 patients were classified in to Group A (grade 0 and 1-depleted iron stores) and Group B (grade 2 and 3 - functional iron deficiency). There were significant difference in means of RET-He (Group A  $17.84 \pm 2.39$  vs. Group B  $25.08 \pm 4.42$ ;  $P < 0.0001$ ) and serum ferritin (Group A  $8.68 \pm 2.80$  vs. Group B  $15.61 \pm 4.68$ ;  $P < 0.0001$ ). We observed significant positive correlation of ferritin with RET-He in total patients ( $r = 0.7860$ ,  $p = 0.0000$ ), Group A ( $r = 0.7089$ ,  $p = 0.00$ ) and Group B ( $r = 0.4675$ ,  $p < 0.05$ ) patients. RET-He was the only significant predictor of bone marrow iron stores (at  $P < 0.05$ ). On ROC curve analysis, the AUC for RET-He was found to be 0.894 ( $P$  value  $< 0.01$ ) and best cut off value for predicting IDA was 22.4 pg (sensitivity 98.88%, specificity 84.21%). The AUC for serum ferritin was 0.891 ( $P$  value  $< 0.01$ ) and best cut off value for predicting IDA was 11.6 ng/ml (sensitivity 86.75%, specificity 89.47 %).

**Conclusion:** RET-He correlated significantly with serum ferritin and is also a better predictor of bone marrow iron stores than the latter.

## Editorial Viewpoint

- Serum ferritin being an acute phase reactant can be elevated due to inflammation.
- RET-He is a major of haemoglobin content of freshly produced red blood cells offering real-time information on iron supply for erythropoiesis.
- This study finds RET-He as a better predictor of bone marrow iron stores than serum ferritin.

However, it is an acute phase reactant and can be falsely elevated in the presence of inflammation. The traditional gold standard to diagnose iron deficiency is bone marrow iron store, assessed by Prussian blue staining of bone marrow aspiration. This again has its limitations of being an invasive procedure and having high inter-observer variation.<sup>1</sup>

Automated counters form an integral part of modern day hematology. Newer generation counters provide a new parameter, viz. reticulocyte hemoglobin (RET-He). It is a measure of hemoglobin content of the freshly

## Introduction

Iron deficiency is one of the most common nutrient deficiencies and a leading cause of anemia worldwide. Various laboratory investigations that are routinely used to diagnose iron deficiency anemia include hemoglobin (Hb), hematocrit (Hct), mean corpuscular

volume (MCV), mean corpuscular hemoglobin (MCH), serum ferritin and transferrin saturation (TSAT). Serum ferritin has been considered a good predictor of storage iron.

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**Table 1: Comparison of various parameters between storage iron deplete (Group A) and storage iron replete (Group B) patients**

	Total	Group A (N = 83)	Group B (N = 19)	P value
Age (years)	35.63 ± 15.96	34.82 ± 15.93	39.16 ± 16.07	0.297
Hb(g/dL)	4.26 ± 1.16	4.28 ± 1.15	4.14 ± 1.24	0.651
MCV(fL)	72.17 ± 3.55	71.78 ± 3.51	73.85 ± 3.32	0.022 <sup>†</sup>
MCH(pg)	19.38 ± 1.23	19.38 ± 1.23	19.40 ± 1.27	0.944
RDW-CV(%)	18.96 ± 1.11	19.12 ± 1.11	18.29 ± 0.81	0.001 <sup>†</sup>
RET-He(pg)	19.19 ± 4.02	17.84 ± 2.39	25.08 ± 4.42	0.0001 <sup>†</sup>
Ferritin (ng/ml)	9.97 ± 4.19	8.68 ± 2.80	15.61 ± 4.68	<0.0001 <sup>†</sup>
Iron (ug/dl)	19.22 ± 5.59	19.45 ± 5.59	18.21 ± 5.64	0.396
TIBC(ug/dl)	429.32 ± 36.07	428.66 ± 36.75	432.21 ± 33.71	0.687
TSAT(%)	4.51 ± 1.39	4.58 ± 1.40	4.21 ± 1.33	0.291

<sup>†</sup>Significant

produced red blood cells and thus offers real-time information on iron supply for erythropoiesis. The determination of RET-He can be performed on the haematology analyser together with the routine parameters from peripheral blood. RET-He is not an acute phase reactant and has been found to be more sensitive than serum ferritin in diagnosing iron deficiency.<sup>2-5</sup>

This study was an effort to evaluate reticulocyte hemoglobin visa-vis serum ferritin as a marker of bone marrow iron store in iron deficiency anemia (IDA).

## Materials and Methods

This hospital based observational, analytic study was conducted at a tertiary care center in Rajasthan, during September 2013 to December 2014, after obtaining due permission from Research Review board/ Institutional Ethics committee and informed consent of the study participants. Patients (age group 15-65 years) with newly diagnosed and untreated iron deficiency anemia admitted in medicine wards and not suffering from any inflammatory disorders (excluded by C-reactive protein) were included in this study. Patients having other forms of anemia/ hemoglobinopathies/ any malignancy, having MCV > 80 fL and pregnant females were excluded from this study.

Blood samples from patients were drawn in EDTA vials for CBC including RET-He and

peripheral blood smear and in plain vials for serum ferritin, serum iron and TIBC. Patients having microcytic hypochromic anemia, serum ferritin below 20 ng/ml and transferrin saturation < 20% were selected. Patient's clinical history, findings of physical examination and other relevant data, including lab test results, were recorded in structured forms. CBC and RET-He were tested on Sysmex XT 4000i automated analyzer. Serum ferritin was measured on IMMULITE 2000 Systems analyzer using a solid-phase, two-site chemiluminescent immunometric assay. Serum iron was measured using colorimetric assay. TIBC was measured using saturation - precipitation method. Transferrin saturation (TSAT) was calculated as TSAT = (serum iron/TIBC) × 100 and expressed as percentage.

Under strict aseptic precaution bone marrow aspirates were obtained from posterior iliac crest and sent for Wright - Giemsa staining along with Prussian blue staining for estimation of iron store.<sup>6</sup>

## Statistical Analysis

Microsoft Excel® and SPSS® 20 for Windows® were used for data storage and analysis. The qualitative data were expressed in percentages and quantitative data were expressed as mean ± standard deviation. Student's t test and Chi-Square test were used to determine statistical difference

between variables. Correlation analysis was done by using Pearson correlation coefficient and linear regression was performed. Logistic regression was done to find out the best predictor of bone marrow iron stores. ROC curves were made to determine usefulness of various parameters to predict iron deficiency anemia and optimal cut off values (best possible combination of sensitivity and specificity) were determined. The critical  $\alpha$  level used was 1% and results were considered significant if  $P < 0.05$ .

## Results

A total of 144 patients were included in the study. Of these, 42 (29.2%) patients were excluded from final statistical analysis as their bone marrow aspirates were aparticulate and therefore, their iron stores could not be assessed. From the bone marrow aspirates of remaining 102 patients, Prussian blue stained films were examined and graded.<sup>6</sup>

These 102 study participants had mean age 35.63 ± 15.96 years (range 15 to 65 years) with male: female ratio 1:1.83. Of the 102 patients, 73 (71.6%) had no stainable iron in bone marrow (grade 0), 10 (9.8%) had grade 1 stainable iron, 8 (7.8%) had grade 2 stainable iron and 11 (10.8) had grade 3 stainable iron. Based on the grading of bone marrow iron store, the patients were classified into Group A (grade 0 and 1- depleted iron stores) and Group B (grade 2 and 3 -functional iron deficiency).

In comparison to Group B, patients of Group A had significantly higher RDW-CV (Group A 19.119 ± 1.1145 vs. Group B 18.289 ± .8123;  $P = 0.001$ ), lower RET-He (Group A 17.84 ± 2.39 vs. Group B 25.08 ± 4.42;  $P < 0.0001$ ) and lower serum ferritin (Group A 8.68 ± 2.80 vs. Group B 15.61 ± 4.68;  $P < 0.0001$ ) (Table 1).

We observed significant positive correlation of ferritin with RET-He

**Table 2: Correlation of various parameters among study groups**

	Total		Group A		Group B	
	R	P	R	P	R	P
Ferritin with RET-He	0.7860	0.0000 <sup>†</sup>	0.7089	0.0000 <sup>†</sup>	0.4675	0.0435 <sup>†</sup>
Ferritin with RDW-CV	0.0228	0.8197	-0.1239	0.2646	0.1597	0.5138
Ferritin with TS AT	0.0013	0.9900	0.0950	0.3932	0.0862	0.7258
RET-He with RDW-CV	0.0144	0.8855	-0.0099	0.9290	-0.3011	0.2103
RET-He with TSAT	-0.0602	0.5478	-0.0487	0.6620	0.1950	0.4236

<sup>†</sup>Significant

**Table 3: Predictor for Bone marrow iron store by using logistic regression**

	B	S.E.	Wald	df	Sig. (P)	Exp(B)
Hb	5.371	3.260	2.715	1	0.099	215.075
MCV	-0.534	0.387	1.905	1	0.167	0.586
MCH	0.368	0.695	0.280	1	0.596	1.445
RDW-CV	0.753	0.729	1.066	1	0.302	2.123
RET-He	-0.823	0.353	5.427	1	0.020 <sup>†</sup>	0.439
Ferritin	-0.942	0.710	1.764	1	0.184	0.390
TSAT	0.281	0.611	0.211	1	0.646	1.324
Constant	22.178	33.209	0.446	1	0.504	428311054 4.378

<sup>†</sup>Significant

in total patients ( $r=0.7860$ ,  $p$  0.0000), Group A ( $r = 0.7089$ ,  $p$  0.00) and Group B ( $r = 0.4675$ ,  $p < 0.05$ ) patients (Table 2).

Logistic regression analysis was done to predict bone marrow iron store (deplete vs. replete) using Hb, MCV, MCH, RDW-CV, RET-He, Ferritin and TSAT as predictors. A test of full model was statistically significant indicating that the predictors reliably distinguished between bone marrow iron store (Chi square 81.579,  $df$  7,  $P < 0.001$ ). The Wald tests showed RET-He was the only significant predictor of bone marrow iron stores (at  $P < 0.05$ ) (Table 3).

The ROC curves of RET-He, serum ferritin and transferrin saturation for predicting iron deficiency anemia was constructed using presence or absence of bone marrow iron, defined by grade 0 and 1 iron stain of bone marrow aspirate, as the dependent variable. The AUC for RET-He was found to be 0.894 ( $P$  value  $< 0.01$ ). The best cut off value of RET-He for predicting iron deficiency anemia was 22.4 pg (sensitivity 98.88%, specificity 84.21%). The AUC for serum ferritin was 0.891 ( $P$  value  $< 0.01$ ). The best cut off value of serum ferritin for predicting iron

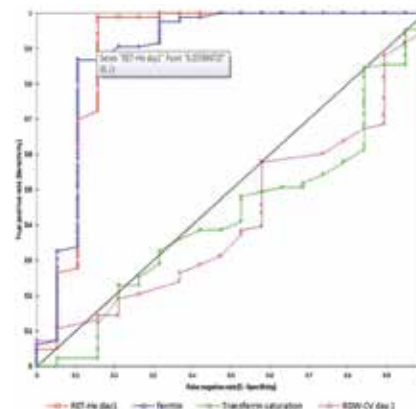
deficiency anemia was 11.6 ng/ml (sensitivity 86.75%, specificity 89.47%). The AUC for RDW-CV and transferrin saturation were 0.420 and 0.423 respectively, and they were not significantly different from 0.5 ( $P$  value  $> 0.05$ ). RDW-CV and transferrin saturation were therefore not good tests to predict iron deficiency anemia (Figure 1).

## Discussion

All the patients included in our study had microcytic hypochromic anemia with an increased RDW-CV, low serum ferritin and low transferrin saturation. Those with grade 0 and 1 were considered to have depleted iron stores and, therefore, represented absolute iron deficiency. Those with grade 2 and 3 in our study had functional iron deficiency.<sup>7</sup>

### Correlation of Serum ferritin and transferrin saturation with RET-He

In our study, Ret-He was found to have significant positive correlation with serum ferritin ( $r= 0.7860$ ;  $P < 0.0001$ ). None of the reticulocyte parameters showed significant correlation with transferrin saturation. Similar finding was observed in children (aged 1-6 years) ( $r = 0.464$ ,  $P < 0.01$ ).<sup>8</sup> Mittman et al<sup>9</sup> also observed



**Fig. 1: The ROC curves of RET-He, RDW-CV, serum ferritin and transferrin saturation for predicting IDA. For RET-He AUC was 0.894 ( $P < 0.01$ ) and best cut-off value for predicting IDA was 22.4 (sensitivity 98.88%, specificity 84.21%). For serum ferritin AUC was 0.891 ( $P < 0.01$ ) and best cut off value for predicting IDA was 11.6 ng/ml (sensitivity 86.75%, specificity 89.47%). The AUC for RDW-CV and transferrin saturation were 0.420 and 0.423 respectively ( $P > 0.05$  for each)**

weak but consistent correlation of RET-He with serum ferritin similar to our study but their finding of correlation of RET-He with transferrin saturation was not in echo with our study. Few other studies have shown significant positive correlation of RET-He with transferrin saturation which is also contrary to our results.<sup>10-11</sup>

### Predictor for bone marrow iron store

RET-He was the only significant predictor of bone marrow iron stores. In children, Mateoset al<sup>12</sup> also found RET-He as the most accurate marker independently associated with iron deficiency. Similarly another study among 210 children, using TSAT  $< 20\%$  to define iron deficiency, also found that RET-He was a significant predictor of iron deficiency.<sup>13</sup>

Reticulocytes are non-nucleated immature red blood cells (RBCs) in peripheral blood. Reticulocytes

may be more sensitive in detecting erythropoietic activity as they have a more rapid turnover in circulation than mature red cells (1-2 vs. 120 days). Reticulocyte indices provide a realtime evaluation of the bone marrow activity, reflecting the balance between iron and erythropoiesis of the preceding 48 hours.<sup>14,15</sup> Iron deficiency could be detected at an earlier stage, when RBC indicators are still normal but the iron stores are depleted to the point of affecting hematopoiesis and inducing production of a certain percentage of reticulocytes with reduced Hb content, resulting in a progressive reduction of RET-He.<sup>13-16</sup>

With the advent of newer automated hematology analyzer, both red blood cells and reticulocyte parameters can be measured in the same blood sample; this can be helpful in early diagnosis of RBC disorders.

#### ROC curve analysis

The ROC curve analysis revealed that AUC for RET-He (0.894,  $P < 0.01$ ) was more than serum ferritin (0.891,  $P < 0.01$ ) and it also had better sensitivity (98.88 %,) compared to serum ferritin (86.75%) for predicting iron deficiency anemia. But the RET-He had lower specificity (84.21%) compared to serum ferritin (89.47%) for predicting iron deficiency anemia. The best cutoff value of RET-Hb for diagnosis of IDA was 22.4 pg. These findings proved that RET-Hb is superior to serum ferritin for predicting IDA and supports the utility of RET-Hb as a proxy to stainable iron in bone marrow aspirate in diagnosis of IDA

Similar to our study, in a group of elderly anemic patients for diagnosis of iron deficiency anemia, T. Karlsson<sup>3</sup> reported that although RET-He had a higher sensitivity than ferritin (93% vs. 87%), it had a lower specificity (69% vs. 95%) in IDA.

Brugnara et al<sup>17</sup> revealed Ret-He cutoff level 27.2 pg to diagnose

iron deficiency with sensitivity of 93.3%, and a specificity of 83.2% with AUC 0.913 ( $P < 0.0001$ ). A study in children with mean age of 2.9 years, showed a higher AUC for RET-He than serum ferritin (0.78 vs. 0.57). RET-He (cut off 26 pg) had 83% sensitivity and 75% specificity for IDA.<sup>13</sup> In another study also RET-Hb found to have a sensitivity of 90.7% and specificity of 80.1% for IDA among children.<sup>12</sup>

Mast et al.<sup>4</sup> reported that RET-He had a higher AUCROC (0.735 ± 0.14) than ferritin (0.690 ± 0.14), TSAT (0.637 ± 0.16) and MCV (0.570 ± 0.15). RET-Hb was also more sensitive (73.9%) for diagnosis of IDA than ferritin (52.4%), TSAT (65%) and MCV (31.8%). Among chronic hemodialysis patients, RET-He (<26pg) had 73% sensitivity and 100 % specificity in diagnosing iron deficient erythropoiesis.<sup>8</sup>

Markovic et al<sup>5</sup> also showed a better overall sensitivity and specificity of RET-He than MCV and ferritin in the diagnosis of iron deficiency on ROC curve analysis. In pregnant women also RET-He had higher sensitivity (80.7%) and higher AUC (0.79) than serum ferritin (0.77).<sup>19</sup>

Fishbane et al<sup>20</sup> had reported a sensitivity of 100% and specificity of 80% for RET-He 26pg to diagnose iron deficiency. It was better than serum ferritin < 100 ng/ml (sensitivity 71.4% and specificity 60%) and TSAT (sensitivity 57.1% and specificity 80%). Lorenz et al<sup>10</sup> also reported an optimal cut off of 29 pg for RET-He (sensitivity 85%, specificity 73%) for detecting iron deficiency. AUC-ROC tended to be higher for RET-He compared with ferritin (0.92 vs 0.75), TSAT (0.90 vs. 0.82) and MCV (0.81 vs. 0.72).

Deng et al<sup>8</sup> reported an optimal cutoff value of 27.8 pg for RET-He with 88% sensitivity and 90% specificity. Canals et al<sup>21</sup> reported an optimal cutoff of 25 pg for RET-He with a sensitivity of 76% and a specificity of 81%.

All these studies support utility

of RET-He as a marker of IDA. RET-He had the advantage of being measured on routine haemogram at a little increment of cost and the results can be obtained readily. The RET-He also indicate bone marrow iron store in a real time manner. So RET-He can be used as a marker of IDA.

#### Limitations

Our study had some limitations. We included patients with severe anemia in our study. Whether our findings can be extrapolated to those with mild to moderate degree of anemia or with iron deficiency state is debatable. This study was done at a tertiary care centre, and recruited admitted patients only resulting in a limited sample size, thus the nature of the investigation and the results do not imply a general case, and further studies with a larger sample size are needed.

#### Conclusion

This study showed that RET-He correlated significantly with serum ferritin and is also a better predictor of bone marrow iron stores than the latter. An invasive procedure like bone marrow aspiration can thus be avoided in IDA. Thus RET-He is a sensitive marker of bone marrow iron store in IDA.

#### Conflict of Interests

None of the authors have a conflict of interest.

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