ORIGINAL ARTICLE

Role of Reticulocyte Hemoglobin in Diagnosing Iron Deficiency in Anemia of Chronic Disease

Manoj Lakhotia[1], Pradeep Lalwani[2*], Yamini Verma[3]

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Abstract

Introduction: Serum ferritin, being an acute-phase reactant, is not useful in detecting iron deficiency in patients with anemia of chronic disease (ACD). Reticulocyte hemoglobin (RetHb) can be such a marker for detecting iron deficiency in ACD.

Objective: To study the role of RetHb in diagnosing iron deficiency in patients with ACD.

Method: A total of 130 patients were included. Bone marrow aspiration was performed to assess iron stores.

Results: Based on bone marrow iron stores, patients were classified into three groups: (1) iron deficiency anemia (IDA) ($n = 86$); (2) ACD with IDA ($n = 20$); and (3) ACD without IDA ($n = 24$). The AUC-ROC for RetHb was calculated as 0.96, with a cutoff of 27 pg/mL, sensitivity of 96%, and specificity of 93.4%. The AUC-ROC for serum ferritin was 0.82, with a cut-off of 50 ng/mL, sensitivity of 70.75%, and specificity of 95.83%. In IDA, RetHb correlated well with serum ferritin ($r = 0.331$, $p = 0.0005$), whereas in ACD with IDA, it did not ($r = -0.069$, $p = 0.774$).

Conclusion: RetHb is a cost-effective and easily available test for detecting IDA and is comparable with serum ferritin. It can even detect iron deficiency in ACD, where serum ferritin is not useful.

Keywords: Anemia of chronic disease, Iron deficiency anemia, Reticulocyte hemoglobin.

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Introduction

Iron deficiency anemia (IDA) is the most common cause of anemia, followed by anemia of chronic disease.[1] Diagnosis of IDA in a patient can be made by assessing serum ferritin level, and in uncomplicated cases it serves as an excellent marker of body iron stores.[2] However, anemia of chronic disease (ACD) is multifactorial, of which iron deficiency can be one reversible cause.[2] However, diagnosis of an iron deficiency state is difficult in this situation by an iron study profile, as ferritin, being a phase reactant, may be raised in chronic disease, in spite of iron deficiency.[3] Bone marrow staining is the gold standard in these situations but, being invasive, is not advocated.[4] This leads to the requirement of a noninvasive test to detect iron deficiency in ACD, which is noninvasive and cost-effective. Many newer tests have been formulated to detect iron deficiency. These are red cell protoporphyrin levels,[5] serum transferrin receptor protein,[6] but due to their higher cost and specialized technique, they are not routinely available.

A newer generation automated hematology analyzer provides another parameter: reticulocyte hemoglobin (RetHb), which measures the amount of hemoglobin in the reticulocytes.[7] RetHb is an early indicator of bone marrow iron store and can detect iron deficiency even in anemia of chronic disease with more sensitivity than other biochemical parameters.[8,9]

This study was undertaken to correlate RetHb with bone marrow iron store in patients of IDA and ACD and to compare its usefulness with serum ferritin in diagnosing iron deficiency.

Materials and Methods

This prospective study was approved by the Institutional Ethics Committee (letter Numbers SNMC/IEC/2019/561-563). Written consent was obtained from all patients. A total of 130 patients with anemia and hemoglobin less than 11 gm/dL were included in the study. Patients having hemoglobinopathy, B12 deficiency, folic acid deficiency, hematological malignancy, or chronic liver disease were excluded. Investigations, including hemogram and RetHb levels, were carried out by collecting blood by venipuncture in an EDTA vial. Analysis was done by an auto hematology Sysmex p-100 analyzer that uses optical flow cytometry techniques to do differential counting.[10] Samples were collected in plain vials for serum iron, Serum TIBC, and serum ferritin. Biochemical tests included plasma glucose, blood urea, serum creatinine, serum ALT, serum AST, serum B12, serum folic acid, serum FT4, and serum TSH. HIV, HBsAG, and HCV were done using card test. Complete urine analysis and stool examination, including stool for occult blood, were done in all patients. All patients underwent ultrasonography of abdomen–pelvis and chest X-ray PA view. Bone marrow aspiration was done after getting informed consent for the procedure. Aspiration was done from the posterior iliac crest using a Jamshidi bone marrow biopsy needle. Giemsa-stained bone marrow smears were evaluated using Prussian blue staining and graded by Gale's method and the intensive grading method.[11]

Initially, marrow fragments were assessed and were graded on a scale of grade 0 to grade 5. Grade 0 was defined as no iron stain and grade 1 as very slight iron stain. Then, an intensive histological grading method[11] was applied in which iron was assessed in three sites—the fragments, as in Gale's method, macrophages, and erythroblasts. Iron assessed in the fragments and macrophages represented iron stores, while iron in the erythroblasts represented utilizable iron. Presence of iron in macrophages present in twenty high-power fields around the fragments was looked for. Under high power magnification, sideroblasts (the percentage containing iron granules in their cytoplasm) were enumerated in hundred erythroblasts. If less than 30% of erythroblasts had visible iron granules, then iron deficiency was defined. Interpretation of histological grading was as follows:

- Both erythroblast and iron stores were normal, defined as normal iron status.
- If erythroblast iron is deficient but had normal iron stores, then it was defined as functional iron deficiency.
- If iron stores are deficient but erythroblast iron is normal, then defined as iron stores deficiency.
- If both iron stores and erythroblast iron were depleted, then defined as combined iron store and functional iron deficiency.
- According to bone marrow grading,[11] patients were classified into three groups, i.e., IDA, ACD with IDA, and ACD without IDA.

[1]Professor, Principal and Controller, Department of Clinical Immunology and Rheumatology, Mahatma Gandhi Hospital; [2]Assistant Professor, Department of Neurology; [3]Senior Demonstrator, Department of Pathology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India; *Corresponding Author

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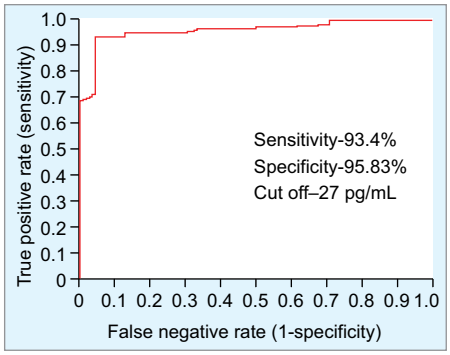

Fig. 1: ROC curve–RetHb

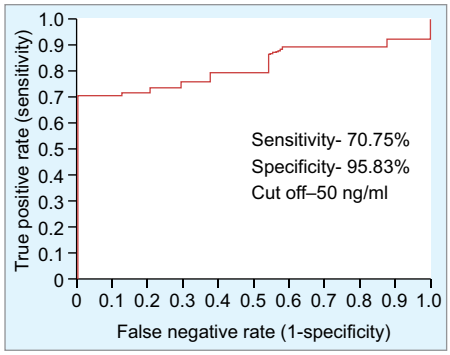

Fig. 2: ROC curve–serum ferritin

Table 1: AUC (ROC), sensitivity and specificity for iron indicators in IDA

Parameters	*Cut-off point*	*AUCROC*	*Sensitivity %*	*Specificity %*	*Positive predictive value%*	*Negative predictive value%*
RetHb (pg/mL)	≤27	0.96	93.4	95.83	99	76.67
serum ferritin (ng/mL)	≤50	0.82	70.75	95.83	98.68	42.59

RetHb, reticulocyte hemoglobin; AUC, area under curve

Table 2: Comparison of hematological and biochemical indices with respect to Bone marrow grade in IDA/ACD

Parameters	*IDA (n = 86)*	*ACD with IDA (n = 20)*	*ACD without IDA (n = 24)*	*p-value*
MCV (fL)	63.80 ± 10.35	78.65 ± 15.17	77.74 ± 9.68	0.0001
RetHb (pg/mL)	19.03 ± 3.49	24.825 ± 4.09	29.58 ± 2.16	<0.0001
serum ferritin (ng/mL)	21.09 ± 28.76	154.44 ± 15.09	698.34 ± 759.92	<0.0001
serum iron (µg/dL)	35.20 ± 23.97	50.30 ± 12.32	113.61 ± 47.24	0.0004
TIBC (µg/dL)	460.76 ± 99.71	182.39 ± 30.07	162.17 ± 43.12	<0.0001
TS (%)	7.5 ± 5.18	18.83 ± 7.51	35.03 ± 25.01	<0.0001

MCV, mean corpuscular volume; RetHb, reticulocyte hemoglobin; TIBC, total iron binding capacity; TS, transferrin saturation

Table 3: Correlation of RetHb with serum ferritin in reference to bone marrow iron status

Correlation	*IDA (n = 86)*		*ACD with IDA (n = 20)*	
	R	*P*	*R*	*P*
Ferritin with RetHb	0.331	0.0005	−0.069	0.774

- IDA when bone marrow had depleted iron stores with no erythroblast iron.[11]
- ACD with IDA when bone marrow had normal iron store but no erythroblast iron i.e., functional iron deficiency[11] with clinical evidence of chronic diseases.
- ACD without IDA when bone marrow had normal iron store with normal erythroblast iron[11] in the presence of evidence of chronic diseases.

Statistical Analysis

Data were stored in SPSS 20 and analyzed in Microsoft Excel. ROC curve was made to determine the sensitivity and specificity of RetHb and other parameters. The optimal cutoff value of RetHb and serum ferritin was determined by the ROC curve. Student's *t*-test, ANOVA test, and chi-square test were used to determine statistical differences between variables. A *p*-value less than 0.05 was considered statistically significant, and the critical α level used was 1%.

Results

A total of 130 patients were included in the study, with a mean age of 41.35 ± 17.54 years. Out of which 93 (71.5%) were females, and 37 (28.5%) were males.

Based on bone marrow iron and erythroblast iron, the patients were classified into three groups: (1) IDA, (2) ACD with IDA, and (3) ACD without IDA.

Out of 130 patients, 86 (58.46%) had no iron in erythroblasts with depleted iron stores, 20 (7.69%) had deficient erythroblast iron but normal iron stores, and 24 (18.6%) had normal erythroblast iron with normal iron stores in bone marrow.

To calculate the sensitivity and specificity of serum ferritin and RetHb, an ROC curve was constructed as per bone marrow iron grading, which is shown in Figs 1 and 2, respectively. From the ROC curve, the cut-off values of RetHb and serum ferritin were derived (Table 1). It was seen that RetHb levels of 27 pg/mL were most suited, with a sensitivity of 93.4% and a specificity of 95.83%, which is close to the highest sensitivity for distinguishing IDA. Similarly, the cut-off for serum ferritin was 50 ng/mL with 70.75% sensitivity and 95.83% specificity.

A comparison was made between the three groups. It was seen that patients with IDA without evidence of chronic disease had low RetHb, low serum iron, low Transferrin Saturation, and low serum ferritin, whereas patients with ACD and iron deficiency anemia had normal serum iron, normal TIBC, high serum ferritin but low RetHb. In patients with ACD where iron deficiency was not present, serum iron was normal, TIBC was high, and RetHb was high (Table 2).

There was a significant positive correlation of serum ferritin with RetHb in patients with IDA ($r = 0.331$, $p = 0.0005$), but a negative correlation was found in patients with ACD with IDA (r = −0.069, *p* 0.774) (Table 3).

Discussion

RetHb, a newer parameter, measures the amount of hemoglobin in reticulocytes, which exist for 1–2 days in circulation before becoming mature RBCs.[7] It is an early indicator of bone marrow iron stores and can detect iron deficiency even in ACD with more sensitivity than serum ferritin.[10]

In the present study, the diagnostic efficiency of RetHb and serum ferritin was compared in reference to bone marrow iron stores in patients with iron deficiency. The ROC curve analysis shows the AUC_{ROC} for RetHb as 0.96, and this suggests the higher accuracy of RetHb in diagnosing iron deficiency. The RetHb values were between 12.5 pg/mL and 36.2 pg/mL. Based on AUC_{ROC}, it was found that the cut-off point of 27 pg/mL has a positive predictive value of 99%, a negative predictive value of 76.67%, a sensitivity of 93.4%, and a specificity of 95.83%, which is close to the highest sensitivity for diagnosing IDA. AUC_{ROC} for serum ferritin was calculated as 0.82. The serum ferritin values were between 2 and 2000 ng/mL. Based on AUC_{ROC}, it was found that the cut-off point of 50 ng/mL has a positive predictive value of 98.68%, a negative predictive value of 42.59%, a sensitivity of 70.75%, and a specificity of 95.83%. Hence, RetHb has a better diagnostic efficiency, indicating its superior ability to diagnose IDA over serum ferritin. The standard cutoff value for diagnosing IDA for serum ferritin is less than 15 µg/L,2 but there is no standard cut-off defined for RetHb. In previous studies, RetHb values have been calculated as 28, 31.8, and 30.5 pg/mL using bone marrow grading as the gold standard for diagnosing IDA.[9,12,13] In the Indian scenario, one study found the cut-off for RetHb as 22.3 pg/mL and AUC for RetHb (0.894, $p < 0.01$), which was more than serum ferritin (0.891, $p < 0.01$).[14] Other studies used traditional biochemical parameters, i.e., serum iron and transferrin saturation, in place of bone marrow examination for diagnosing IDA and found cutoffs of RetHb as 27.2, 29, 27.8, and 25 pg/mL.[8,15–18]

In patients of IDA, both serum ferritin and RetHb correlated well in diagnosing iron deficiency ($r = 0.331$, $p = 0.0005$) (Table 3). A similar correlation of RetHb with serum ferritin has been observed in previous studies.[14,19] However, in patients of ACD having iron deficiency diagnosed with bone marrow iron stain, it was seen that mean serum ferritin (154 ± 15 ng/mL) was higher than the cutoff value, whereas mean RetHb (24.89 ± 4.09 pg/mL) was lower than the cutoff. This suggests that in uncomplicated IDA, both serum ferritin and RetHb are excellent markers for iron deficiency, but in ACD, iron deficiency can be diagnosed by RetHb but not serum ferritin.

Other markers of iron deficiency not affected by inflammation includes red cell protoporphyrin levels which has been found to be a sensitive and specific measure of iron deficiency in a previous study,[20] but hematfluorometer is required for testing levels of red cell protoporphyrin[5] which is not easily available and is costly test, whereas RetHb is an extended CBC parameter which is obtained using automated hematology analyser[8] and being cheap with readily available result it becomes an automatic first choice for diagnosing iron deficiency in any situation.

Thus, when evaluating IDA in ACD, RetHb is a better predictor of body iron store than serum ferritin.

Limitations

In this study, we only included patients who were admitted to the hospital; hence, mild cases of anemia were not included. The number of patients included in the study is small, especially for ACD.

Conclusion

Assessing bone marrow iron stores, to date, is considered the gold standard for the iron deficiency state. Serum ferritin can diagnose iron deficiency in uncomplicated IDA, and RetHb correlates well with it, making it an alternative choice. However, in situations of iron deficiency in chronic disease, Serum Ferritin falters as it is a phase reactant and is raised despite iron deficiency. In comparison, RetHb is not affected by chronic disease and can be used to diagnose iron deficiency in situations of chronic disease. With the present study, it can be concluded that by assessing RetHb, iron deficiency can be diagnosed in both uncomplicated IDA and iron deficiency in chronic disease. Being noninvasive, cheap, and readily available as an extended parameter in an advanced hematology analyzer, it will become an automatic choice for the diagnosis of iron deficiency anemia.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Orcid

Pradeep Lalwani https://orcid.org/0009-0006-9544-7164

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