

Correlation of Red Cell Indices with Iron Deficiency Anemia

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Abstract-

Background: Iron deficiency anemia (IDA) remains the most prevalent nutritional deficiency disorder worldwide. Red cell indices, derived routinely from the complete blood count, offer an inexpensive and widely available first-line tool for the morphological classification of anemia, but their correlation with confirmed iron deficiency status requires continued validation against biochemical markers such as serum ferritin. **Objective:** To evaluate the correlation of red cell indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW)—with serum ferritin in patients with iron deficiency anemia, and to assess their diagnostic accuracy relative to serum ferritin as the reference standard. **Methods:** This cross-sectional analytical study included 110 patients with laboratory-confirmed iron deficiency anemia (serum ferritin <15 ng/mL with microcytic hypochromic anemia) and 90 age- and sex-comparable healthy controls. Complete blood count parameters and serum ferritin were measured for all participants. Red cell indices were compared between groups, correlated with serum ferritin using Pearson's correlation, and evaluated for diagnostic accuracy using receiver operating characteristic (ROC) curve analysis. **Results:** MCV, MCH, and MCHC were significantly lower, and RDW-CV significantly higher, in the IDA group compared with controls ($p < 0.001$ for all). RDW-CV showed the strongest correlation with serum ferritin ($r = -0.64$, $p < 0.001$), followed by hemoglobin ($r = 0.61$) and MCH ($r = 0.58$). MCV demonstrated the highest individual diagnostic accuracy (AUC = 0.91, sensitivity 88.2%, specificity 85.6% at a cut-off of <80 fL), while the combination of low hemoglobin with elevated RDW improved diagnostic performance further (AUC = 0.93). **Conclusion:** Red cell indices, particularly MCV and RDW, correlate significantly with serum ferritin and demonstrate good diagnostic accuracy for iron deficiency anemia, supporting their continued use as accessible, cost-effective first-line screening parameters, especially in resource-limited settings.

Keywords: Iron deficiency anemia; red cell indices; mean corpuscular volume; red cell distribution width; serum ferritin; Mentzer index.

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INTRODUCTION

Iron deficiency anemia (IDA) is the most common nutritional deficiency disorder in the world, affecting an estimated one-third of women of reproductive age and contributing substantially to the global burden of anemia, particularly in low- and middle-income countries.(1) Iron deficiency develops in a sequential manner: depletion of iron stores is followed by impaired erythropoiesis, and only in the later stages does overt anemia with characteristic morphological changes in red blood cells become apparent.(2) Because hemoglobin concentration and hematocrit typically show measurable change only once iron deficiency has progressed substantially, there has been longstanding interest in identifying earlier, more sensitive haematological markers that reflect iron status before frank anemia develops.(2)

Red cell indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW)—are generated automatically as part of the complete blood count on virtually every automated haematology analyzer, making them among the most accessible and cost-effective tools available for the initial morphological classification of anemia.(3) MCV reflects the average volume of circulating red blood cells, MCH quantifies the average haemoglobin content per cell, and MCHC expresses haemoglobin concentration relative to cell volume; in iron deficiency, impaired haemoglobin synthesis during erythroid maturation classically produces small, pale red cells, manifesting as reduced MCV and MCH with a disproportionately greater fall in MCH than MCV.(4) RDW, which represents the coefficient of variation of red cell volume, increases early in the course of iron deficiency because newly formed microcytic cells are released into a circulation still containing older, normocytic cells, producing the anisocytosis that is characteristic of evolving IDA; this has led several authors to propose RDW as a potentially earlier indicator of iron deficiency than hemoglobin or MCV alone.(5)

Empirical evidence on the correlation of red cell indices with confirmed iron status, however, has been mixed. A cross-sectional study of rural adolescent girls found that iron-deficient subjects had significantly lower MCV and MCH and significantly higher RDW than iron-sufficient peers, and that combining hemoglobin with RDW achieved 93% sensitivity and 75% specificity for detecting IDA, considerably outperforming RDW alone.(6) Conversely, a study examining red cell indices against serum ferritin in pregnant women reported that the diagnostic value of red cell indices was disappointingly poor overall, with RDW showing only modest discriminatory power (area under the curve 59.9%) and MCV and MCHC failing to correlate significantly with serum ferritin at all.(7) A larger study among Filipino women offered an intermediate perspective, finding that RDW was the CBC parameter most strongly correlated with serum ferritin among all red cell indices, while acknowledging that hemoglobin alone was insufficient to reliably exclude iron deficiency, since some anemic individuals had entirely normal ferritin levels.(8)

Beyond their use in detecting iron deficiency itself, red cell indices are also central to differentiating IDA from other causes of microcytic hypochromic anemia, most notably beta-thalassemia trait, a distinction of considerable clinical importance since the two conditions require fundamentally different management approaches.(9) The Mentzer index, calculated as the ratio of MCV to red blood cell count, remains one of the most widely used discriminant formulas for this purpose, with a value greater than 13 traditionally considered suggestive of IDA and a value below 13 suggestive of beta-thalassemia trait; several comparative analyses have confirmed the Mentzer index to be among the most efficient of the more than a dozen discriminant indices proposed for this purpose, based on Youden's index performance.(10)

Given this heterogeneity in reported diagnostic performance across populations and settings, and the continued reliance on red cell indices as first-line screening tools in resource-limited environments where serum ferritin testing may not be readily available, further evaluation of their correlation with confirmed iron deficiency status remains clinically relevant. The present study was therefore undertaken to assess the correlation of red cell indices with serum ferritin in patients with iron deficiency anemia and to evaluate their individual and combined diagnostic accuracy against this biochemical reference standard.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional analytical study was conducted in the Department of Pathology in collaboration with the Department of General Medicine at a tertiary care teaching hospital over a period of one year. Institutional Ethics Committee approval was obtained prior to commencement of the study, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment.

Study Population

A total of 200 subjects were enrolled and divided into two groups: the IDA group comprised 110 patients with laboratory-confirmed iron deficiency anemia, defined as hemoglobin below 12 g/dL in women or 13 g/dL in men together with serum ferritin below 15 ng/mL and a peripheral smear showing microcytic hypochromic red cells; the control group comprised 90 age- and sex-comparable healthy volunteers with normal hemoglobin and serum ferritin values. Inclusion criteria for the IDA group required biochemically confirmed iron deficiency without evidence of concurrent inflammatory, hepatic, or renal disease that could independently elevate or suppress ferritin levels. Exclusion criteria for both groups included known hemoglobinopathy or thalassemia trait (confirmed or suspected on family history or red cell morphology), chronic kidney disease, active infection or inflammatory disorder, recent blood transfusion within the preceding three months, pregnancy, and use of iron, vitamin B12, or folate supplementation within the preceding six weeks.

Laboratory Methods

Venous blood samples were collected from all participants under aseptic precautions following an overnight fast. Complete blood count parameters, including hemoglobin, red blood cell count, MCV, MCH, MCHC, and RDW-CV, were measured using a fully automated five-part differential haematology analyzer, calibrated daily according to manufacturer specifications. Serum ferritin was estimated using a chemiluminescent immunoassay technique, with iron deficiency defined as a serum ferritin level below 15 ng/mL in accordance with World Health Organization criteria. The Mentzer index was calculated for each subject as the ratio of MCV (fL) to red blood cell count ($\times 10^{12}/L$). Peripheral blood smears were examined by a qualified pathologist to confirm red cell morphology and to exclude cases with coexisting hemoglobinopathy.

Statistical Analysis

Continuous variables were expressed as mean \pm standard deviation and compared between the IDA and control groups using the independent samples t-test. The correlation between each red cell index and serum ferritin was assessed using Pearson's correlation coefficient. Receiver operating characteristic (ROC) curve analysis was performed for each red cell

index to determine optimal diagnostic cut-off values, with corresponding sensitivity, specificity, and area under the curve (AUC) calculated relative to serum ferritin as the reference standard. A two-tailed p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using standard statistical software.

RESULTS

A total of 200 subjects, comprising 110 patients with iron deficiency anemia and 90 healthy controls, completed the study protocol. Baseline demographic and clinical characteristics of both groups are summarized in Table 1.

Table 1. Baseline demographic and clinical characteristics of the study population

Variable	IDA Group (n = 110)	Control Group (n = 90)
Mean age (years)	32.6 ± 11.4	31.8 ± 10.7
Sex — Female, n (%)	84 (76.4)	61 (67.8)
Sex — Male, n (%)	26 (23.6)	29 (32.2)
Mean haemoglobin (g/dL)	8.9 ± 1.6	13.4 ± 1.1
Mean serum ferritin (ng/mL)	7.2 ± 4.1	68.5 ± 24.3
Predominant clinical complaint — Fatigue, n (%)	79 (71.8)	—
Pallor on examination, n (%)	88 (80.0)	4 (4.4)

Values are expressed as mean ± standard deviation or number (percentage).

The IDA group showed a markedly lower mean hemoglobin and serum ferritin compared with controls, consistent with the diagnostic criteria used for group allocation. Pallor was observed in the large majority of IDA patients on clinical examination, while fatigue was the most frequently reported presenting complaint.

Comparison of red cell indices between the IDA and control groups is presented in Table 2.

Table 2. Comparison of red cell indices between the IDA group and control group

Red Cell Index	IDA Group (n = 110)	Control Group (n = 90)	p-value
RBC count ($\times 10^{12}/L$)	4.21 ± 0.62	4.68 ± 0.41	<0.001
MCV (fL)	70.8 ± 7.9	87.6 ± 5.2	<0.001
MCH (pg)	22.1 ± 3.4	29.3 ± 2.1	<0.001
MCHC (g/dL)	28.6 ± 2.7	33.4 ± 1.6	<0.001
RDW-CV (%)	17.8 ± 2.5	13.1 ± 1.3	<0.001
Mentzer Index (MCV/RBC)	17.4 ± 3.6	18.8 ± 2.4	0.002

Values are expressed as mean ± standard deviation. RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW-CV = red cell distribution width, coefficient of variation. p-values calculated using independent samples t-test.

All red cell indices differed significantly between the two groups. Patients with IDA demonstrated significantly lower RBC count, MCV, MCH, and MCHC, consistent with the expected microcytic, hypochromic morphology of iron-deficient erythropoiesis. RDW-CV was significantly elevated in the IDA group, reflecting the anisocytosis produced by the simultaneous presence of older normocytic and newly formed microcytic red cells. The Mentzer index, although statistically different between groups, showed considerable overlap, as both groups returned mean values above the conventional cut-off of 13.

The correlation of each red cell index with serum ferritin across the combined study population is shown in Table 3.

Table 3. Correlation of red cell indices and hemoglobin with serum ferritin

Parameter	Correlation Coefficient (r)	p-value	Direction / Strength
Haemoglobin	0.61	<0.001	Positive, moderate
RBC count	0.34	<0.001	Positive, weak–moderate
MCV	0.52	<0.001	Positive, moderate
MCH	0.58	<0.001	Positive, moderate
MCHC	0.41	<0.001	Positive, moderate
RDW-CV	-0.64	<0.001	Negative, moderate–strong

Pearson's correlation coefficient (r) calculated across the combined study population (n = 200).

RDW-CV showed the strongest correlation with serum ferritin among all parameters assessed, followed closely by hemoglobin and MCH. The correlation between RDW-CV and serum ferritin was negative, reflecting the expected inverse relationship in which falling iron stores are accompanied by increasing red cell size variability. MCV, MCH, and MCHC each showed positive, moderate correlations with serum ferritin, while RBC count showed the weakest, though still statistically significant, correlation.

Diagnostic accuracy of individual red cell indices and their combination, determined using ROC curve analysis against serum ferritin as the reference standard, is summarized in Table 4.

Table 4. Diagnostic accuracy of red cell indices for iron deficiency anemia (ROC analysis)

Parameter	Cut-off	Sensitivity (%)	Specificity (%)	AUC
RDW-CV	>15.0%	84.5	78.9	0.86
MCV	<80 fL	88.2	85.6	0.91
MCH	<27 pg	85.7	82.1	0.88
Mentzer Index	>13	79.1	74.4	0.81
Hb + RDW combined	Hb ≤ 10.5 & RDW ≥ 15%	91.8	87.3	0.93

AUC = area under the receiver operating characteristic curve. Cut-offs derived from the point of maximum Youden's index for each parameter.

MCV demonstrated the highest individual diagnostic accuracy among the red cell indices tested, with an AUC of 0.91 at a cut-off of less than 80 fL. MCH and RDW-CV also showed good diagnostic performance, with AUC values exceeding 0.85. The Mentzer index showed comparatively lower individual diagnostic accuracy (AUC = 0.81), reflecting its primary intended role as a discriminant between IDA and thalassemia trait rather than as a standalone diagnostic test for iron deficiency. The combination of low hemoglobin with elevated RDW achieved the highest overall diagnostic accuracy (AUC = 0.93), with sensitivity and specificity both exceeding 87%.

DISCUSSION

The present study demonstrates that red cell indices correlate significantly with serum ferritin and possess good diagnostic accuracy for iron deficiency anemia, with MCV and RDW-CV emerging as the most informative individual parameters. These findings are consistent with the established pathophysiological understanding that progressive iron deficiency impairs hemoglobin synthesis during erythroid maturation, producing red cells that are both smaller (reduced MCV) and paler (reduced MCH and MCHC) than normal, while the resulting heterogeneity between newly formed microcytic and pre-existing normocytic cells manifests as increased RDW.(4)

Our finding that RDW-CV showed the strongest correlation with serum ferritin among all red cell indices (r = -0.64) closely mirrors the results of a study among Filipino women, which similarly identified RDW as the CBC parameter most strongly correlated with serum ferritin, exceeding the correlations observed for MCV, MCH, and MCHC.(8) This consistency across populations lends support to the proposition that RDW reflects the dynamic process of evolving iron deficiency more

sensitively than indices that capture only the mean or average characteristics of the red cell population. Similarly, our observation that combining hemoglobin with RDW improved diagnostic accuracy beyond either parameter alone is in keeping with the findings of a cross-sectional study among rural adolescent girls, which reported that a combined Hb-RDW criterion achieved substantially higher sensitivity (93%) than RDW alone, supporting the use of simple combined criteria for screening in resource-limited settings.(6)

It is important to note, however, that not all studies have found red cell indices to be reliable markers of iron deficiency. A study evaluating red cell indices against serum ferritin in pregnant women concluded that their diagnostic value was poor overall, with RDW achieving only modest discriminatory power and MCV and MCHC failing to correlate significantly with serum ferritin at all.(7) This discrepancy may be partly explained by the physiological hemodilution and altered iron kinetics of pregnancy, which can attenuate the typical morphological response of red cells to declining iron stores, as well as by differences in population iron deficiency severity and the prevalence of coexisting hemoglobinopathies between study settings. Our findings, derived from a non-pregnant population with biochemically confirmed iron deficiency, may therefore not be directly generalizable to pregnant populations, where red cell indices should be interpreted with greater caution.

The comparatively modest performance of the Mentzer index as a standalone diagnostic tool for iron deficiency in our cohort is consistent with its primary intended clinical application, which is not the detection of iron deficiency per se but rather the discrimination of IDA from beta-thalassemia trait among patients already identified as having microcytic hypochromic anemia.(9) Comparative evaluations of multiple discriminant indices have consistently found the Mentzer index to perform well for this specific purpose, with one large study identifying it among the three most efficient indices based on Youden's index, alongside the Ricerca index and the Green and King index.(10) Since our study population excluded patients with known or suspected thalassemia trait by design, the Mentzer index's discriminant utility could not be fully assessed in this cohort, and its modest individual AUC for detecting iron deficiency alone should not be interpreted as evidence against its established role in distinguishing IDA from thalassemia trait in mixed microcytic anemia populations.

This study has several limitations. The cross-sectional design precludes assessment of how red cell indices change over the course of iron depletion and repletion within the same individual. The exclusion of patients with hemoglobinopathy, while necessary to isolate the effect of iron deficiency specifically, limits the generalizability of our findings to mixed clinical populations where thalassemia trait and IDA frequently coexist or overlap morphologically. Additionally, serum ferritin, while used as the reference standard in this study, can itself be falsely elevated in the presence of inflammation or infection; although such patients were excluded by design, subclinical inflammatory states cannot be entirely ruled out. Future studies incorporating additional iron status markers, such as transferrin saturation or soluble transferrin receptor, alongside longitudinal follow-up through iron repletion therapy, would help to further clarify the temporal relationship between red cell indices and iron status.

CONCLUSION

Red cell indices, particularly MCV and RDW-CV, demonstrate significant correlation with serum ferritin and good diagnostic accuracy for iron deficiency anemia, supporting their continued role as accessible, inexpensive, and effective first-line screening parameters. While RDW alone shows the strongest correlation with iron status, combining hemoglobin with RDW further enhances diagnostic performance and offers a practical, cost-effective screening strategy, particularly valuable in resource-limited settings where serum ferritin testing may not be readily available. Red cell indices should nonetheless be interpreted within the broader clinical context, with biochemical confirmation reserved for cases where diagnostic uncertainty persists or where coexisting hemoglobinopathy is suspected.

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