Reticulocyte Hemoglobin Content as a Marker of Iron Deficiency in Chronic Kidney Disease: A Diagnostic Performance Study

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ABSTRACT

Background: Iron deficiency is a common and clinically significant contributor to anemia in patients with chronic kidney disease (CKD). Conventional iron status markers such as serum ferritin and transferrin saturation (TSAT) have limitations, particularly in the presence of chronic inflammation. Reticulocyte Hemoglobin Content (Ret-He) has emerged as a promising marker that reflects real-time iron availability for erythropoiesis.

Aim: To evaluate the diagnostic performance of Reticulocyte Hemoglobin Content (Ret-He) for detecting iron deficiency in patients with CKD stages 3–5D.

Materials and Methods: This hospital-based cross-sectional observational study included 100 patients with CKD stages 3–5D. Iron deficiency was defined as TSAT <20% and/or serum ferritin <100 ng/mL. Hematological and iron profile parameters, including Ret-He, serum ferritin, serum iron, TSAT, and total iron-binding capacity (TIBC), were evaluated. Correlation analysis and receiver operating characteristic (ROC) curve analysis were performed to assess the diagnostic utility of Ret-He.

Results: Among the 100 participants, 18% had iron deficiency. Mean Ret-He was significantly lower in iron-deficient patients compared to non-iron-deficient patients (26.04 ± 2.78 pg vs. 29.82 ± 2.17 pg; $p<0.001$). Ret-He showed a significant positive correlation with serum ferritin ($r=0.235$, $p=0.020$) and a strong positive correlation with TSAT ($r=0.664$, $p<0.001$). ROC analysis identified an optimal Ret-He cut-off value of <29.5 pg, with a sensitivity of 84%, specificity of 78%, positive predictive value of 72%, negative predictive value of 88%, and overall accuracy of 81%. The area under the curve was 0.85 (95% CI: 0.77–0.92; $p<0.001$).

Conclusion: Ret-He is a simple, rapid, and reliable biomarker for detecting iron deficiency in CKD stages 3–5D and may complement conventional iron status markers in the evaluation of CKD-associated anemia.

Keywords: Chronic kidney disease, Iron deficiency, Reticulocyte hemoglobin content, Ret-He, Anemia, Transferrin saturation, Serum ferritin, Diagnostic accuracy.

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INTRODUCTION

Chronic kidney disease (CKD) is a major global public health problem affecting millions of individuals worldwide and is associated with significant morbidity, mortality, and healthcare burden. As kidney function progressively declines, patients develop several metabolic and hematological complications, among which anemia is one of the most common and clinically important manifestations.[1] The prevalence and severity of anemia increase with advancing stages of CKD and contribute substantially to reduced quality of life, impaired cardiovascular function, increased hospitalization, and accelerated disease progression.[2]

Anemia in CKD is multifactorial in origin. Although inadequate erythropoietin production by the diseased kidneys remains the primary mechanism, iron deficiency is increasingly recognized as a major contributing factor.[3] Iron deficiency in CKD may occur as absolute iron deficiency due to depleted body iron stores or as functional iron deficiency, in which adequate iron stores are present but iron utilization for erythropoiesis is impaired because of chronic inflammation and elevated hepcidin levels.[4] Accurate assessment of iron status is

therefore essential for the appropriate management of anemia and optimization of iron replacement therapy.

Conventionally, serum ferritin and transferrin saturation (TSAT) are used to evaluate iron status in CKD patients. However, both parameters have important limitations. Serum ferritin is an acute-phase reactant and may be elevated in inflammatory states irrespective of iron stores, whereas TSAT demonstrates significant biological variability and may fluctuate with nutritional status and ongoing inflammation.[5] Consequently, reliance solely on these conventional markers can lead to underdiagnosis or overdiagnosis of iron deficiency, particularly in patients with advanced CKD.[6]

Reticulocyte Hemoglobin Content (Ret-He) has emerged as a promising hematological parameter for assessing iron availability for erythropoiesis. Reticulocytes are immature red blood cells that circulate in the peripheral blood for only one to two days. The hemoglobin content of these cells reflects the amount of iron that was available during recent erythropoiesis, thereby providing a real-time assessment of functional iron status.[7] Unlike ferritin and other biochemical markers, Ret-He is less influenced by inflammatory conditions and can be measured rapidly as part of automated hematology analyzer reports without requiring additional blood samples or specialized testing.[8]

Several recent studies have demonstrated significant correlations between Ret-He and established iron parameters such as serum ferritin and TSAT, suggesting its potential utility in diagnosing iron deficiency among CKD patients.[9] Furthermore, Ret-He has shown promising diagnostic accuracy for identifying both absolute and functional iron deficiency and may facilitate earlier therapeutic intervention before the development of overt hematological abnormalities.[10]

The present study aimed to evaluate the diagnostic performance of reticulocyte hemoglobin content (Ret-He) for detecting iron deficiency in patients with chronic kidney disease (CKD) stages 3–5D. The objectives were to compare Ret-He levels between iron-deficient and non-iron-deficient patients, assess its correlation with conventional iron status markers, determine the optimal diagnostic cut-off value using ROC analysis, and evaluate its sensitivity, specificity, predictive values, overall accuracy, and utility as a marker of functional iron availability.

MATERIALS AND METHODS

Study Design: Hospital-based cross-sectional observational study.

Study Place: Department of Pathology in collaboration with the Department of Nephrology at a tertiary care teaching hospital.

Study Duration: 18 months.

Study Population: Adult patients diagnosed with chronic kidney disease (CKD) stages 3–5D presenting to the Nephrology outpatient department, inpatient wards, and dialysis unit.

Sample Size: 100 patients with CKD stages 3–5D.

Sampling Method: Consecutive sampling of eligible patients fulfilling the inclusion criteria during the study period.

Ethical Approval: The study was conducted after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants prior to enrollment.

Inclusion Criteria:

- Age ≥18 years.
- Diagnosed cases of CKD stages 3–5D according to KDIGO guidelines.
- Patients with CKD-associated anemia.
- Patients willing to provide written informed consent.

Exclusion Criteria:

- History of blood transfusion within the preceding 3 months.
- Intravenous iron therapy within the preceding 4 weeks.
- Acute infection or active inflammatory disease.
- Hematological malignancies or primary hematological disorders.
- Active bleeding or recent major surgery.
- Pregnant women.

Definition of Iron Deficiency: Iron deficiency was defined according to conventional criteria for patients with chronic kidney disease (CKD), based on a transferrin saturation (TSAT) value of less than 20% and/or a serum ferritin level of less than 100 ng/mL. Patients fulfilling either or both of these criteria were classified as having iron deficiency. Based on their iron status, the study participants were categorized into two groups: the iron-deficient CKD group and the non-iron-deficient CKD group. This classification was used for comparative analysis of reticulocyte hemoglobin content (Ret-He) and other hematological and biochemical parameters.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software version 27.0. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Comparisons between iron-deficient and non-iron-deficient CKD patients were performed using the Mann–Whitney U test for continuous variables. Pearson's correlation coefficient was used to assess

the relationship between reticulocyte hemoglobin content (Ret-He) and conventional iron status markers, including serum ferritin and transferrin saturation (TSAT). Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of Ret-He in detecting iron deficiency and to determine the optimal cut-off value using the Youden Index. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall diagnostic accuracy, and area under the ROC curve (AUC) with 95% confidence intervals (CI) were calculated. A p-value of less than 0.05 was considered statistically significant.

RESULT

Table 1. Baseline Characteristics of the Study Population

Variable	Value
Age (years), Mean ± SD	58.29 ± 13.89
Male, n (%)	50 (50.0)
Female, n (%)	50 (50.0)
Hemoglobin (g/dL), Mean ± SD	8.10 ± 1.51
Total Leukocyte Count (×10³/cumm), Mean ± SD	10.04 ± 5.56
Mean Corpuscular Volume (MCV, fL), Mean ± SD	88.22 ± 7.43
Mean Corpuscular Hemoglobin (MCH, pg), Mean ± SD	28.43 ± 2.41
Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL), Mean ± SD	32.25 ± 1.49
Serum Creatinine (mg/dL), Mean ± SD	5.72 ± 3.31
Estimated Glomerular Filtration Rate (eGFR, mL/min/1.73 m²), Mean ± SD	14.22 ± 10.90
Iron Deficiency Present, n (%)	18 (18.0)
Iron Deficiency Absent, n (%)	82 (82.0)

Table 2. Comparison of Reticulocyte Hemoglobin Content and Conventional Iron Status Markers between Iron Deficient and Non-Iron Deficient CKD Patients

Parameter	Iron Deficient (n = 18) Mean ± SD	Non-Iron Deficient (n = 82) Mean ± SD	p-value
Reticulocyte Hemoglobin (Ret-He), pg	26.04 ± 2.78	29.82 ± 2.17	<0.001
Serum Ferritin (ng/mL)	135.58 ± 44.47	743.19 ± 579.32	<0.001
Serum Iron (µg/dL)	31.98 ± 17.75	56.05 ± 23.38	0.002
Transferrin Saturation (TSAT), %	12.41 ± 5.67	31.65 ± 14.18	<0.001
Total Iron Binding Capacity (TIBC), µg/dL	259.45 ± 74.34	186.21 ± 72.67	<0.001

Table 3. Correlation of Reticulocyte Hemoglobin Content with Conventional Iron Status Markers in CKD Patients

Correlation Pair	Pearson Correlation Coefficient (r)	p-value	Interpretation
Ret-He vs Serum Ferritin	0.235	0.020	Significant positive correlation
Ret-He vs TSAT	0.664	<0.001	Strong positive correlation

Table 4. Diagnostic Performance of Reticulocyte Hemoglobin Content for Detection of Iron Deficiency in CKD Patients

Diagnostic Parameter	Value
Optimal Ret-He Cut-off	<29.5 pg
Sensitivity (%)	84
Specificity (%)	78
Positive Predictive Value (PPV), %	72
Negative Predictive Value (NPV), %	88
Overall Accuracy (%)	81
Area Under the ROC Curve (AUC)	0.85
95% Confidence Interval (CI)	0.77–0.92
p-value	<0.001

Figure: 1. Comparison of Ret-He and Iron Status Markers in CKD Patients

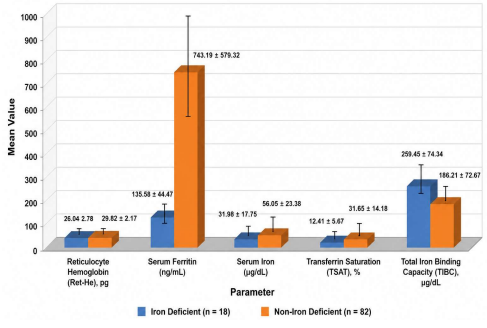

Figure: 2. Correlation of Ret-He with Iron Status Markers

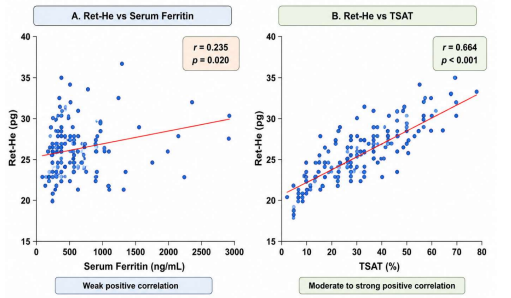

Figure: 3. Diagnostic Accuracy of Ret-He for Iron Deficiency in CKD Patients

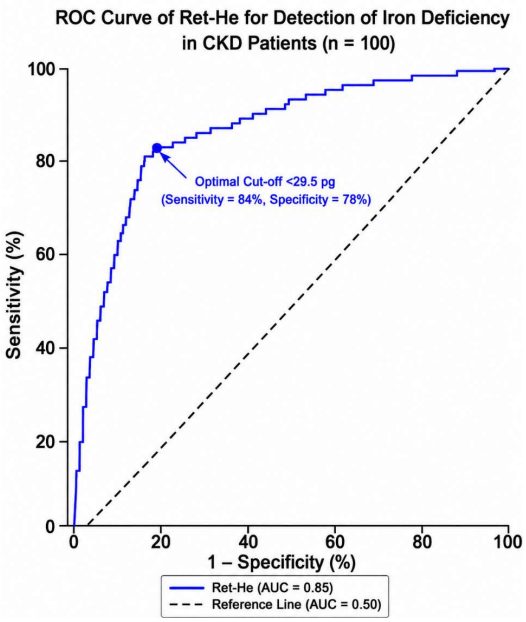

Table 1. A total of 100 patients with chronic kidney disease (CKD) stages 3–5D were included in the study. The mean age of the study population was 58.29 ± 13.89 years. The gender distribution was equal, with 50 (50.0%) males and 50 (50.0%) females. The mean hemoglobin level was 8.10 ± 1.51 g/dL, indicating a high prevalence of anemia among the study participants. The mean total leukocyte count was 10.04 ± 5.56 ×10³/cumm. The mean red blood cell indices included MCV of 88.22 ± 7.43 fL, MCH of 28.43 ± 2.41 pg, and MCHC of 32.25 ± 1.49 g/dL. The mean serum creatinine level was 5.72 ± 3.31 mg/dL, while the mean estimated glomerular filtration rate (eGFR) was 14.22 ± 10.90 mL/min/1.73 m², reflecting advanced renal dysfunction in the study population. Based on conventional iron status criteria, iron deficiency was identified in 18 (18.0%) patients, whereas 82 (82.0%) patients were categorized as non-iron deficient.

Table 2. Comparison of iron-related parameters between iron-deficient and non-iron-deficient CKD patients demonstrated statistically significant differences across all measured variables. The mean Reticulocyte Hemoglobin Content (Ret-He) was significantly lower in iron-deficient patients (26.04 ± 2.78 pg) compared with non-iron-deficient patients (29.82 ± 2.17 pg), and this difference was highly significant ($p < 0.001$). Similarly, serum ferritin levels were markedly lower in the iron-deficient group (135.58 ± 44.47 ng/mL) than in the non-iron-deficient group (743.19 ± 579.32 ng/mL) ($p < 0.001$). Serum iron concentration was also significantly reduced among iron-deficient patients (31.98 ± 17.75 µg/dL) compared to those without iron deficiency (56.05 ± 23.38 µg/dL) ($p = 0.002$). Transferrin saturation (TSAT), an important indicator of iron availability, was substantially lower in the iron-deficient group (12.41 ± 5.67%) than in the non-iron-deficient group (31.65 ± 14.18%) ($p < 0.001$). In contrast, total iron-binding capacity (TIBC) was significantly higher in iron-deficient patients (259.45 ± 74.34 µg/dL) compared with non-iron-deficient patients (186.21 ± 72.67 µg/dL) ($p < 0.001$). These findings demonstrate that Ret-He closely reflects iron status and effectively differentiates iron-deficient from non-iron-deficient CKD patients.

Table 3. Correlation analysis was performed to evaluate the relationship between Reticulocyte Hemoglobin Content (Ret-He) and established iron status markers. Ret-He showed a statistically significant positive correlation with serum ferritin levels ($r = 0.235$, $p = 0.020$), indicating that higher Ret-He values were associated with increased iron stores. A much stronger positive correlation was observed between Ret-He and transferrin saturation (TSAT) ($r = 0.664$, $p < 0.001$). The strength of this association suggests that Ret-He reflects functional iron availability more accurately than stored iron reserves. The significant correlations observed with both ferritin and TSAT support the utility of Ret-He as a reliable marker for assessing iron status in CKD patients and reinforce its potential role in the early detection of iron deficiency.

Table 4. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the diagnostic performance of Reticulocyte Hemoglobin Content (Ret-He) for identifying iron deficiency among CKD patients. The optimal cut-off value for Ret-He was determined to be <29.5 pg. At this threshold, Ret-He demonstrated a sensitivity of 84%, indicating that it correctly identified 84% of iron-deficient patients. The specificity was 78%, reflecting a good ability to correctly classify non-iron-deficient individuals. The positive predictive value (PPV) was 72%, while the negative predictive value (NPV) was 88%, suggesting that patients with Ret-He values above the cut-off were unlikely to have iron deficiency. The overall diagnostic accuracy of Ret-He was 81%. Furthermore, the area under the ROC curve (AUC) was 0.85 (95% CI: 0.77–0.92), indicating good discriminatory ability and excellent diagnostic performance. The association was statistically significant ($p < 0.001$), confirming that Ret-He is an effective and clinically

useful biomarker for the detection of iron deficiency in patients with CKD stages 3–5D.

DISCUSSION

The present study evaluated the diagnostic performance of Reticulocyte Hemoglobin Content (Ret-He) for detecting iron deficiency in patients with CKD stages 3–5D. Iron deficiency was identified in 18% of the study population, highlighting its continued importance as a contributor to CKD-associated anemia. The mean age of the participants was 58.29 ± 13.89 years, with an equal distribution of males and females. Similar demographic characteristics have been reported by Sany et al., who observed that anemia and iron deficiency are predominantly encountered among middle-aged and elderly CKD patients, reflecting the progressive nature of renal dysfunction with advancing age.[11]

In the present study, iron-deficient patients demonstrated significantly lower Ret-He values (26.04 ± 2.78 pg) compared with non-iron-deficient patients (29.82 ± 2.17 pg; $p<0.001$). Significant differences were also observed in serum ferritin, serum iron, TSAT, and TIBC. These findings are in agreement with the observations of Dinh et al., who reported significantly reduced reticulocyte hemoglobin values among iron-deficient end-stage renal disease patients and concluded that Ret-He accurately reflects iron-restricted erythropoiesis.[12] Similarly, Chinudomwong et al. demonstrated that RET-He values were significantly lower in iron-deficient individuals than in those without iron deficiency, supporting its role as a sensitive marker of iron status even in the presence of chronic inflammatory conditions.[13] The lower Ret-He values observed in our study can be explained by reduced iron availability during hemoglobin synthesis within newly produced erythrocytes.

A major finding of the present study was the significant positive correlation between Ret-He and serum ferritin ($r=0.235$, $p=0.020$) and an even stronger correlation with TSAT ($r=0.664$, $p<0.001$). The stronger association with TSAT suggests that Ret-He reflects functional iron availability rather than merely iron storage. Comparable findings were reported by El-Shahawi and colleagues, who demonstrated significant correlations between reticulocyte hemoglobin indices and established iron parameters in hemodialysis patients.[14] Likewise, Lundgren, in a systematic review and meta-analysis, concluded that reticulocyte hemoglobin parameters consistently correlate with biochemical markers of iron status and may provide earlier detection of iron deficiency than conventional laboratory tests.[15]

The diagnostic performance of Ret-He observed in the present study was particularly noteworthy. ROC curve analysis identified an optimal cut-off value of <29.5 pg, yielding a sensitivity of 84%, specificity of 78%, PPV of 72%, NPV of 88%, and overall accuracy of 81%, with an AUC of 0.85. These findings indicate good discriminatory ability for identifying iron deficiency in CKD patients. Similar diagnostic performance has been reported by Auerbach et al., who found that reticulocyte hemoglobin equivalent demonstrated good sensitivity and specificity for identifying iron deficiency and monitoring response to iron therapy.[16] Their study emphasized the practical advantages of Ret-He because it can be obtained simultaneously with routine complete blood count analysis without additional cost or blood sampling.

The AUC value of 0.85 obtained in our study indicates excellent diagnostic accuracy. Comparable ROC performance was reported by Rana et al. from Eastern India, who demonstrated that reticulocyte hemoglobin equivalent possesses strong diagnostic capability for iron deficiency and may serve as an effective screening tool in routine clinical practice.[17] Similar conclusions were reached by Almashjary et al., who evaluated patients with end-stage kidney disease undergoing hemodialysis and reported that reticulocyte hemoglobin parameters effectively identified iron-restricted erythropoiesis with clinically useful diagnostic accuracy.[18]

The superior performance of Ret-He may be attributed to its ability to reflect real-time iron incorporation into newly formed erythrocytes. Unlike ferritin, which may be elevated during inflammation, infection, or liver disease, Ret-He directly reflects iron availability during erythropoiesis. Ogawa et al. highlighted that reticulocyte hemoglobin content is a dynamic indicator of iron-restricted erythropoiesis and often detects iron deficiency earlier than conventional biochemical markers.[19] Furthermore, recent studies have suggested incorporating Ret-He into routine anemia evaluation algorithms for CKD patients to facilitate timely diagnosis and treatment decisions.[20]

Overall, the findings of the present study support the growing body of evidence that Ret-He is a reliable, rapid, and clinically useful biomarker for detecting iron deficiency in CKD stages 3–5D. Its strong correlation with established iron markers and favorable diagnostic performance suggest that Ret-He may complement or, in certain clinical situations, outperform traditional iron indices in the evaluation of iron deficiency among CKD patients.

CONCLUSION

The present study demonstrates that Reticulocyte Hemoglobin Content (Ret-He) is a valuable and reliable marker for detecting iron deficiency in patients with chronic kidney disease (CKD) stages 3–5D. Ret-He levels were significantly lower in iron-deficient patients and showed significant positive correlations with conventional iron status markers, particularly transferrin saturation (TSAT).

The diagnostic performance of Ret-He was excellent, with an optimal cut-off value of <29.5 pg yielding high sensitivity, specificity, and overall accuracy. Unlike conventional biochemical markers that may be influenced by inflammation and chronic disease states, Ret-He provides a real-time assessment of iron availability for erythropoiesis and can be measured rapidly as part of routine hematological analysis. Therefore, Ret-He may serve as an effective adjunctive tool for the early identification of iron deficiency in CKD patients. Incorporation of Ret-He into routine anemia evaluation protocols could facilitate timely diagnosis, optimize iron therapy, and improve the management of CKD-associated anemia.

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