Original Article

To Assess the Relationship between Reticulocyte Hemoglobin Content and Iron Status in Hemodialysis-Dependent Patients

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Abstract

Objective: This study aims to evaluate the diagnostic utility of reticulocyte hemoglobin content (RET-He) and its correlation with transferrin saturation (TSAT) and other iron markers for identifying iron deficiency anemia in hemodialysis patients.

Methodology: The study was conducted at Hematology Department of Shaikh Zayed Hospital in Lahore, from March 2022- April 2023. Total 120 patients with chronic kidney disease on hemodialysis were enrolled in this study. Ferritin was determined by chemiluminescence immunoassay. Flow cytometry and spectrophotometry was performed to determine RET-He and iron status including serum iron and Total iron binding capacity (TIBC). Patients of both genders, aged 18-80 and those on hemodialysis for 3 months or more were selected. Patients excluded were those with bleeding from any site in last three months, received oral or parenteral iron supplementation in one month.Results: With 1.5 mg/l as the recommended fluoride level in drinking water, the fluoride concentration ranged from 0.50 to 1.63 mg/l, with a mean of 0.58 ± 0.33 mg/l among individuals with dental fluorosis compared to those without. This difference was statistically significant (p = 0.013). Dental fluorosis was observed in 189 participants (47.3%), of whom 48.8% were male and 39.7% were female.

Results: In this study, 120 hemodialysis dependent chronic kidney disease patients were enrolled. Among them, 65 (54.2%) were males, while 55 (45.8%) were females; 18 years to 80 years was the age range, with 53.48 ± 13.782 years being mean age. Mean RET-He was 29.439 ± 3.51 pg/cell, while mean serum iron was 68.96 ± 38.63 µg/dl, mean TIBC was 224.41 ± 55.109 µg/dl, serum ferritin mean value being 386.91 ± 357.51 ng/ml and mean TSAT was 31.93 ± 18.52%. Sensitivity (Se) was 56.0%, specificity (Sp) was 74.6%, positive predictive value (PPV) 36.8%, negative predictive value (NPV) 86.6% and accuracy of RET-He in diagnosing Iron deficiency anemia was 70.8% respectively.

Conclusion: Reticulocyte hemoglobin content is highly effective in diagnosing iron deficiency anemia in chronic kidney disease patients. Its lower cost compared to traditional markers makes it a valuable tool for clinical practice.

Keywords: Chronic Kidney Disease, Hemodialysis, Iron Deficiency Anemia, Reticulocyte hemoglobin content, Transferrin Saturation.

Introduction

Chronic kidney disease and hemodialysis patients more frequently develop anemia, compromising their normal wellbeing and iron deficiency being the most common cause.[1] Anemia management in hemodialysis (HD) patients can be done with iron status monitoring and by treating iron deficiency.[2] Iron deficiency anemia (IDA) is main hindering factor for response of recombinant human erythropoietin in patients on hemodialysis.[3] Serum ferritin and transferrin saturation (TSAT) are commonly used to judge iron deficiency but they are affected by inflammatory or physiological conditions.[4] Serum ferritin is increased in inflammatory conditions, infectious diseases and in malignancies. TSAT has acute - phase reactivity as transferrin can be increased in inflammation. In chronic disease and nutritional deficiencies, low transferrin level are found due to decreased synthesis.[5]

Various factors lead to high prevalence of anemia in chronic kidney disease. Anemia increase progression of disease and affects survival of chronic kidney disease patients.[6] The importance of anemia avoidance, observance, and treatment in those with chronic kidney disease cannot be overemphasized, as balance must be kept between erythropoiesis stimulation and iron overload among those with chronic kidney disease.[7] In chronic kidney disease, serum erythropoietin is expected to be decreased and is not diagnostically that important. It also doesn't affect the starting dose or dose adjustment of erythropoietin stimulating agents. A low reticulocyte count shows lesser production of red blood cells whereas increased reticulocyte count depicts high destruction of red blood cells or their hemolysis as probable cause.[8] Less production of RBCs is the basic mechanism in anemia of chronic kidney disease, anemia of chronic illness, and in loss of blood.[9] So we should analyze reticulocyte count vigilantly.

Iron deficiency anemia treatment is basic part of chronic kidney disease patient care, and has various advantages like physical activity endurance, better life quality, and lower mortality.[10] Parameters that can directly assess bone marrow iron availability, such as reticulocyte hemoglobin content, have been identified.[11] Hemoglobin in the reticulocyte is assessed by reticulocyte hemoglobin content and it gives idea of iron in bone marrow during erythropoiesis. Reticulocyte hemoglobin is estimated by blood analyzer with software in upgraded form.[12] Inflammatory conditions don't affect reticulocyte hemoglobin content. It has earlier response to iron treatment and is cost effective.[13] Previous researches indicate we can use reticulocyte hemoglobin content as an alternative test for iron deficiency anemia cases on hemodialysis.[9,10,11] However, there is no local published study on this topic. Owing to lack of local research and the scarcity among existing international literature, current study is performed to study association of reticulocyte hemoglobin with Transferrin Saturation and serum ferritin in those on hemodialysis. These parameters are used to determine iron deficiency anemia. Reticulocyte hemoglobin content is latest parameter, by studying its association with iron status (Transferrin Saturation and Serum Ferritin) we will be able to advise only reticulocyte hemoglobin content to hemodialysis patients. The results of this study will help in better management of such patients in future practices. The aim of the current study is to evaluate the association between RET-He and iron status in hemodialysis patients. To the best of our knowledge, the comprehensive comparison of RET-He with a panel of iron status markers, including serum iron, TIBC, serum ferritin, and calculated TSAT, has not been performed previously.

Methodology

This was a cross-sectional observational study, conducted at Hematology Department of Sheikh Zayed Hospital, Lahore, and its duration was from March 2022 to April 2023. It was approved by Advanced Scientific and Research Board and Ethical Review Committee of University of Health Sciences Lahore, No. UHS/Education/126-20/272. Convenient sampling technique was used to collect samples. The study included patients of both genders with ages 18-80 years and on hemodialysis for three months or more. Patients with significant bleeding from any site in the last three months, including from the gastro intestinal tract caused by uremic environment, concurrent medication or systemic anticoagulation with heparin during dialysis were excluded, those with blood transfusion in last three months, or having inflammatory, infectious disease or any malignancy were excluded from the study. Total 120 patients on regular hemodialysis fulfilling the inclusion criteria were enrolled. Before study, each patient gave informed consent. A sample of 3.5 ml sample was taken in serum separating tube gel vial. Serum ferritin was determined by Chemi luminescence immunoassay while serum iron and Total iron binding capacity was determined by spectrophotometry.

A sample of 3 ml was taken in Ethylene Diamine Tetra acetic Acid and flow cytometry was performed to determine reticulocyte hemoglobin content. Results were analyzed by SPSS v23.0. Quantitative variables like age, hemoglobin, transferrin saturation, serum iron and ferritin were presented as mean and standard deviations. Qualitative variables like gender and iron deficiency anemia were presented as frequency and percentages. Pearson's Correlation was applied to find correlation of reticulocyte hemoglobin content with serum ferritin and transferrin saturation. A 2x2 contingency was generated to calculate sensitivity, specificity, Positive predictive value, Negative predictive value and accuracy.

Data Collection Procedure

Total 120 patients on regular hemodialysis fulfilling the inclusion criteria were enrolled. Before study, each patient gave informed consent. Demographic data was collected by filling out the relevant proforma. Blood samples were taken and RET-He and iron status were measured and their association was made. All the data was collected through a pre designed proforma.

Results

In this study, 120 patients with chronic kidney disease on hemodialysis were enrolled. Among these patients, 65 (54.2%) were males, while 55 (45.8%) were females. Age range in this study was from 18 years to 80 years with mean age of 53.48 ± 13.782 years. Majority of the patients 81(67.5%) had age > 50 years, while 39 (32.5%) patients had age ≤50 years. Among 120 patients, 38 (31.7%) patients showed IDA on RET-He level, while 25(20.8%) showed Iron deficiency anemia on Transferrin saturation level. Mean reticulocyte hemoglobin content was 29.439 ± 3.51 pg, while mean serum iron was 68.96 ± 38.63 µg/dl, mean Total iron binding capacity was 224.41 ± 55.109 µg/dl, mean serum ferritin level was 386.91 ± 357.51 ng/ml and mean TSAT was 31.93 ± 18.52% (Table 1).

There was a positive correlation between Reticulocyte hemoglobin content levels & serum ferritin with Pearson's correlation coefficient of 0.170 with p-value 0.034. Positive correlation was also observed between Reticulocyte hemoglobin content (RET-He) and Transferrin saturation with Pearson's correlation coefficient of 0.302 with p-value 0.001 (Table2). Table 3 shows the comparison of iron deficiency anemia (IDA) detection using RET-He and TSAT, presenting the number of positive and negative cases for each diagnostic method. Table 4 displays the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of RET-He in diagnosing iron deficiency anemia.

Table 1: Comparison of Iron Deficiency Anemia Diagnosis Using RET-He and TSAT Based on Gender and Age

	Gender		Age		Iron deficiency anemia on RET-He		Iron deficiency anemia on TSAT	
Frequency	Male	65	≤ 50 years	39	Yes	38	Yes	25
	Female	55	>50 years	81	No	82	No	95
Percentage	Male	54.2	≤ 50 years	32.5	Yes	31.7	Yes	20.8
	Female	45.8	>50 years	67.5	No	68.3	No	79.2

Table 2: Correlation between RET-He and serum iron and TSAT levels

	Sample size(n)	Correlation coefficient	P-value
Correlation between RET-He level and serum iron level	120	0.170	0.034
Correlation between RET-He and TSAT levels	120	0.302	0.001

Table 3: Identification of iron deficiency anemia on RET-He vs. TSAT

IDA on RET-He	IDA on TSAT		Total
	Positive	Negative	
Yes	14	24	38
No	06	76	82
Total	20	100	120

Table 4: Sensitivity, specificity, PPV, NPV and accuracy of RET-He

Sensitivity	56%
Specificity	74.6%
Positive Predictive Value	36.8%
Negative Predictive Value	93%
Accuracy	70.8%

Discussion

In this study, we explored the usefulness of reticulocyte hemoglobin content as a tool for diagnosing iron deficiency anemia in patients with chronic kidney disease undergoing hemodialysis. Our findings suggest that RET-He is a valuable marker for assessing iron levels, particularly for identifying patients who are not iron deficient, due to its ability to rule out anemia effectively. Compared to conventional markers of iron deficiency, RET-He is simpler, more cost-effective, and provides a real-time reflection of the body's iron status. This highlights its potential to improve the routine management of anemia in hemodialysis patients, offering a practical alternative for clinical practice.

Our findings demonstrated that RET-He had a sensitivity of 56.0%, specificity of 74.6%, positive predictive value (PPV) of 36.8%, and a notably high NPV of 86.6%, with an overall accuracy of 70.8%. While the moderate sensitivity suggests that RET-He may not identify all cases of iron deficiency anemia, its high specificity and NPV indicate that it is particularly reliable in ruling out IDA in this patient population. Additionally, the mean RET-He value (29.44 ± 3.51 pg/cell), when analyzed alongside conventional markers such as mean serum iron (68.96 ± 38.63 µg/dl), total iron-binding capacity (224.41 ± 55.11 µg/dl), serum ferritin (386.91 ± 357.51 ng/ml), and transferrin saturation (31.93 ± 18.52%), provided a comprehensive overview of iron status.

The findings of our study align with previously published research on the role of RET-He in diagnosing IDA among hemodialysis patients. In our cohort of 120 patients, the average age was 53.48 ± 13.78 years, with an age range of 18–80 years. Among these, 38 patients (31.7%) were identified as having IDA based on RET-He (cutoff <28 pg), while 25 patients (20.8%) were diagnosed with IDA using transferrin saturation (TSAT) levels (cutoff <20%) (Figure 1). A positive correlation was observed between RET-He and both serum ferritin and TSAT levels, further validating its role in assessing iron status. The mean values of iron parameters in our study were: RET-He 29.44 ± 3.51 pg, serum iron 68.96 ± 38.6 µg/dL, total iron-binding capacity (TIBC) 224.41 ± 55.11 µg/dL, serum ferritin 386.91 ± 357.51 ng/mL, and TSAT 31.93 ± 18.52%.

Our findings align with those of Sany et al. (2020), who evaluated RET-He in comparison with traditional iron deficiency markers in 50 dialysis patients.[14] Their study reported strong diagnostic performance for RET-He, with an AUC of 0.84 and a cutoff value of 27 pg, yielding a sensitivity of 90.4% and specificity of 80.8%. These results highlight RET-He as a reliable marker for monitoring iron status in hemodialysis patients. Similarly, Dalimunthe (2016) analyzed 72 patients and assessed RET-He's diagnostic ability for iron deficiency using ROC curve analysis. The study reported an AUC of 0.818, a cutoff value of 31.65 pg, and a mean RET-He of 32.96 pg. Using transferrin saturation (cutoff <20%) as a reference, 16 patients (29.6%) were diagnosed with iron deficiency anemia, with RET-He showing a sensitivity of 81.5% and specificity of 61.6%.[15]

Miwa et al. (2010) conducted a study to assess the utility of measuring reticulocyte hemoglobin content (RET-He) in managing iron deficiency among hemodialysis patients.[16]

The study included 217 hemodialysis patients, with RET-He measured using the XE-2100 automated blood cell counter. The mean RET-He value was reported as 32.40 pg. ROC curve analysis revealed an AUC of 0.776, with a cutoff value of 33 pg. The sensitivity and specificity of RET-He for predicting iron deficiency anemia were 74.3% and 64.9%, respectively. Rudiansyah (2020) conducted study in which 181 patients of chronic kidney disease undergoing hemodialysis were selected. Serum iron was correlated to RET-He and transferrin saturation. Mean RET-He was 27 pg and cut off value was 29 pg. Positive correlation of RET-He was found with serum iron ($r=0.348$, $p<0.006$) and TSAT($r=0.454$, $p<0.01$).[17]

Elareny (2023) conducted a study involving 200 patients undergoing regular hemodialysis and compared RET-He with traditional markers of iron deficiency.[18] In this study, the mean RET-He was 33.18 pg, serum iron was 84.65 µg/dL, TSAT was 47.53%, and TIBC was 12.76 µg/dL. The area under the curve (AUC) for RET-He was found to be 0.724, with a cut-off value of 31.4 pg. Sensitivity and specificity for RET-He were reported as 71.43% and 71.51%, respectively. These findings are somewhat consistent with those of our study, where RET-He had a moderate ability to predict iron deficiency anemia, with a sensitivity of 56.0% and specificity of 74.6%. The differences in sensitivity between the two studies could be attributed to variations in sample size, patient demographics, or study methodology.

Mehta (2016) conducted a study involving 102 patients and reported significant differences in RET-He levels between two groups: Group A, representing iron depletion, and Group B, reflecting functional iron deficiency.[19] A strong positive correlation was observed between RET-He and serum ferritin levels, highlighting RET-He as a significant predictor of bone marrow iron stores. These findings support the role of RET-He as a reliable marker for diagnosing iron deficiency, consistent with previous studies.

Nassim (2022) reported a significant positive correlation between RET-He and serum ferritin in children with chronic liver disease. The study also demonstrated the diagnostic utility of RET-He in identifying iron deficiency anemia with high sensitivity and specificity.[20] While our study had a lower sensitivity (56.0%) for RET-He, it had a higher specificity (74.6%) (Table 4). The higher sensitivity reported in Nassim's study could reflect differences in patient populations (children with chronic liver disease versus adult hemodialysis patients) and the use of different diagnostic criteria or methods.

Given its lower cost, ease of use, and ability to reflect real-time iron availability compared to traditional markers, RET-He shows significant potential as a practical and efficient tool for the routine diagnosis and management of anemia in hemodialysis patients.

Limitations

It was a single centered study, focusing on a specific patient population, which may not be representative of broader populations. The sample size was relatively small in relation to the larger population affected by iron deficiency anemia. Additionally, other tests such as unsaturated iron binding capacity and soluble transferrin receptors could have been included to enhance the study's scope. As the study was conducted only at Sheikh Zayed Hospital, the findings cannot be generalized to other healthcare settings.

Conclusion

Reticulocyte hemoglobin content is a reliable marker for diagnosing iron deficiency anemia. In the context of the Pakistani population, where iron deficiency is prevalent, it offers a cost-effective and precise method for diagnosing this condition, making it a convenient tool for clinical practice.

Future directions

In the future, we could recommend using reticulocyte hemoglobin content alone to diagnose iron deficiency anemia. This approach would reduce the need for multiple tests, improve the accuracy of identifying iron deficiency, and offer a more cost-effective solution.

Conflict of interest: Authors declare no conflict of interest.

Authors' contribution: SH; Contributed to data collection, flow cytometry and spectrophotometry analysis for determining RET-He and iron status, and the initial drafting of the manuscript; AL; Supervised the data analysis and statistical evaluation of sensitivity, specificity, and accuracy of RET-He in diagnosing iron deficiency anemia. Provided critical revisions to the manuscript for intellectual content; RAS; Assisted in the design and methodology of the study, ensured the validity of laboratory procedures, and contributed to interpreting the findings; MAG; Provided overall supervision and guidance throughout the study. Reviewed and approved the final manuscript for publication.

References

1. Iseki K, Kohagura K. Anemia as a risk factor for chronic kidney disease. Kidney International. 2007; 1;72-90. Doi:https://www.sciencedirect.com/science/article/pii/S0085253815525568

2. Palaka E, Grandy S, van Haalen H, McEwan P, Darlington O. The impact of CKD anaemia on patients: incidence, risk factors, and clinical outcomes—a systematic literature reviews. International Journal of nephrol.2020;2020(1):1-21. doi: https://onlinelibrary.wiley.com/doi/full/10.1155/2020/7692376

3. Edriss A. Failure of response to erythropoietin in end stage renal disease patients undergoing haemodialysis. Sudan Medical Journal 2018:1-7.doi:https://platform.almanhal.com/Files/Articles/117097

4. Mikhail A, Brown C, Williams JA, Mathrani V, Shrivastava R, Evans Jet, al. Renal association clinical practice guideline on Anaemia of Chronic Kidney Disease. BMC nephrology 2017 ; 18:1-29. doi: https://link.springer.com/article/10.1186/s12882-017-0688-1

5. Cullis JO, Fitzsimons EJ, Griffiths WJ, Tsochatzis E, Thomas DW, British Society for Haematology. Investigation and management of a raised serum ferritin. British Journal of Haematology 2018;181(3):331-40.doi:https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15166

6. Hazin MA. Anemia in chronic kidney disease. Revista da Associação Médica Brasileira. 2020 : 66 : 8-25.doi: https://www.mdpi.com/1422-0067/21/3/725

7. Hamdan M, Alwassouf S. The Diagnostic Value of Serum Ferritin in Assessing Iron Status in Syrian Hemodialysis Patients.

Bulletin of Pharmaceutical Sciences Assiut University 2022 1;45(1):351-7.doi: https://journals.ekb.eg/article_239527.html.

8. Ogawa C, Tsuchiya K, Maeda K. Reticulocyte hemoglobin content. Clinica Chimica Acta. 2020; 504:138-45.doi: https://www.sciencedirect.com/science/article/abs/pii/S0009898120300528

9. Gaur M, Sehgal T. Reticulocyte count: a simple test but tricky interpretation!. Pan African Medical Journal 2;40(1). doi:https://www.ajol.info/index.php/pamj/article/view/229089

10. Gutiérrez OM. Treatment of iron deficiency anemia in CKD and end-stage kidney disease. Kidney International Reports 2021; 6(9) :2261-9. https://doi.org/10.1016/j.ekir.2021.05.020

11. Hayes W. Measurement of iron status in chronic kidney disease. Pediatric nephrology 2019 1;34(4): 605-13. doi:https://link.springer.com/article/10.1007/s00467-018-3955-x

12. Aedh AI, Khalil MS, Abd-Elkader AS, El-Khawanky MM, Alshehri HM, Hussein A, et,al. Reticulocyte hemoglobin as a screening test for iron deficiency anemia: A new cut-off. Hematology Reports. 2023 14;15(1):11-201.doi:https://www.mdpi.com/2038-8330/15/1/21

13. Abd El-Halim AF, Soliman JS, Abdelhamid MG. Reticulocyte hemoglobin equivalent (RET-He) as a predictor of response to intravenous iron in hemodialysis patients: A hospital based analytical study. Life Sciences Journal 2018; 15(8). doi:https://www.researchgate.net/profile/Ayman-Arafa-4/publication/338397254

14. Sany D, El Shahawi Y, Taha J. Diagnosis of iron deficiency in hemodialysis patients: Usefulness of measuring reticulocyte hemoglobin equivalent. Saudi Journal of Kidney diseases and transplantation 2020:1;31(6):1263-72. DOI: 10.4103/1319-2442.308335

15. Dalimunthe NN, Lubis AR. Usefulness of reticulocyte hemoglobin equivalent in management of regular hemodialysis patients with iron deficiency anemia. Romanian Journal of Internal Medicine. 2016 ;54(1):31-6.doi: https://intapi.sciendo.com/pdf/10.1515/rjim-2016-0003

16. Miwa N, Akiba T, Kimata N, Hamaguchi Y, Arakawa Y, Tamura T, et, al. Usefulness of measuring reticulocyte hemoglobin equivalent in the management of haemodialysis patients with iron deficiency. International Journal of Laboratory Hematology 2010 ;32(2):248-55. doi.org/10.1111/j.1751-553X.2009.01179.x

17. Rudiansyah M, Supriyadi R, Lubis L, BANDIARA R, Martakusumah AH, ROESLI R, et,al. The Correlation between Serum Iron Level and Transferrin Saturation with Reticulocyte Hemoglobin Equivalent (RET-He) in Routine Hemodialysis. Int J Pharm Res. (09752366). 2021:13(1).doi: .org/10.31838/ijpr/2021.13.01.318.

18. Hasibuan HG, Nasution S, Tarigan RR. Correlation of reticulocyte hemoglobin equivalent (Ret-He) levels and iron deficiency anemia in CKD patients treating regular hemodialysis. International Journal of Research and Review. 2021;8(11):10-6. DOI: https://doi.org/10.52403/ijrr.20211102

19. Mehta S, Goyal LK, Kaushik D, Gulati S, Sharma N, Harshvardhan L, et,al. Reticulocyte hemoglobin vis-a-vis serum ferritin as a marker of bone marrow iron store in iron deficiency anemia. Journal of the Association of Physicians of India 2016 ;64(11):38-42.

20. Nassim MS, El Raziky MS, Baiomy K, Abd El Salam M. Reticulocyte hemoglobin content: a simple parameter for detection of iron deficiency anemia in children with chronic liver disease. Egyptian Liver Journal. 2022 ;12(1):60-70. Doi:https://link.springer.com/article/10.1186/s43066-022-00222-0