Nephro-Urol Mon. 2018 May; 10(3):e65629. doi: 10.5812/numonthly.65629.

Published online 2018 April 1. **Research Article**

The Role of Reticulocyte Hemoglobin Content in the Management of Iron Deficiency Anemia in Patients on Hemodialysis

Abdul Halim Abdul Gafor,[1,*] Ravih Subramaniam,[1] Firdaus Hadi,[1] Rizna Cader,[1] Kong Wei Yen,[1] Rozita Mohd,[1] and Shamsul Azhar Shah[1]

[1]Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

[*]*Corresponding author*: Abdul Halim Abdul Gafor, Department of Medicine, Universiti Kebangsaan, Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +60-391456392, Fax: +60-391735316, E-mail: halimgafor@gmail.com

Received 2018 January 17; **Accepted** 2018 March 07.

Abstract

Background: The objectives of this study were to correlate reticulocyte Hemoglobin Content (CHr) with Hemoglobin (Hb), ferritin, and Transferrin Saturation (TSAT) in Patients on Hemodialysis (HD) and to determine the Hb response with intravenous iron supplementation in anemic HD patients with adequate ferritin and TSAT yet low CHr.
Methods: This was a prospective, randomized controlled trial involving patients on HD. Patients with baseline results that fulfilled the randomization criteria (CHr < 29 pg, Hb < 9.5 g/dL, ferritin > 400 ng/mL and/or TSAT > 20%) were then randomized to group A and B. Group A patients received a total of 1 g of intravenous iron and group B served as the control.
Results: One hundred and twenty - one patients were enrolled in this study. There was a significant correlation between CHr and TSAT, ferritin, and Hb. A total of 12 patients were randomized to group A and B. There was a significant increase in Hb level observed in group A with median change from baseline of 1g/dL (IQR: - 0.5 to 2.8) at the end of the trial compared to the control group.
Conclusions: There was a significant correlation between CHr, Hb, ferritin, and TSAT. Intravenous iron supplement improved Hb level in patients on HD with adequate serum ferritin and/or TSAT levels yet low CHr.

Keywords: Ferritin, Reticulocyte Hemoglobin Content, Hemodialysis, Hemoglobin, Iron Deficiency Anemia, Transferrin Saturation

1. Background

Management of anemia in patients on hemodialysis (HD) could be achieved with close monitoring of iron status and effective treatment of iron deficiency (1). Iron Deficiency Anemia (IDA) is one of the major inhibiting factors for recombinant Human Erythropoietin (rHuEPO) response in chronic HD patients (2). The common tests used to assess iron deficiency are serum ferritin and Transferrin Saturation (TSAT) (3). However, they are indirect markers that are influenced by physiological or inflammatory conditions (4). Serum ferritin is less than ideal for determining iron deficiency because it is an acute phase reactant and there are gender differences (normally lower in females). It is raised in inflammatory, infectious diseases, and malignancies. Furthermore, TSAT has acute - phase reactivity as transferrin may be elevated in the setting of inflammation, which would lower the TSAT if circulating iron is constant. Malnutrition and chronic disease will cause low transferrin level due to decreased synthesis. There is also significant (17 to 70%) diurnal fluctuation in TSAT that results in difficulty in its interpretation (5). Thus, the best investigation for iron status in this setting would be a test that directly measures iron at the level of erythrocyte or one of its precursors (6). This study investigated the role of reticulocyte Hemoglobin Content (CHr) in the management of IDA in HD patients.

Reticulocytes are the youngest erythrocytes released from the bone marrow to circulating blood. They mature in 1 to 3 days within the bone marrow and circulate for 1 to 2 days before maturing to erythrocytes. Hemoglobin Content provides an indirect measure of the functional iron available for new red blood cell production over the previous 3 to 4 days. Measurement of CHr in peripheral blood samples is useful for diagnosis of iron deficiency in adults (7). It provides an early measure of response to the iron therapy, increasing within 2 to 4 days of the initiation of intravenous iron (8). A study from Turkey on the clinical significance of CHr in diagnosis of IDA demonstrated that CHr is a useful parameter that can be confidently used in the diagnosis of IDA, and the CHr cut - off value of 29 pg predicts IDA (9). The diagnostic performance of CHr in identifying the iron - deficient state was compared with traditional pa-

rameters for iron deficiency (serum iron < 40 g/dL, TSAT < 20%, ferritin < 100 ng/mL, and hemoglobin < 11 g/dL). By using a CHr cut - off level of 27.2 pg, iron deficiency could be diagnosed with a sensitivity of 93.3% and a specificity of 83.2% (10).

The primary objective of this study was to correlate CHr with Hemoglobin (Hb), serum ferritin, and TSAT in patients on HD. The secondary objective was to determine the Hb response with intravenous iron supplementations in patients on HD with adequate serum ferritin and/or TSAT level yet low CHr.

2. Methods

2.1. Study Population and Design

This was a prospective, randomized controlled study conducted at University Kebangsaan Malaysia Medical Centre from October 2014 to May 2015. The study was approved by the Ethics and Research Committee (Code Project FF - 2014 - 347). Patients on HD for more than 3 months and on stable oral/intravenous iron supplement and/or erythropoietin stimulating agent were eligible for this study. The researchers excluded patients with recent gastrointestinal bleeding within the previous 6 weeks, pregnant/lactating females, patients, who received blood transfusion within the last 3 months, and patients with clinical evidence of inflammatory/infectious disease or malignancy and history of hemoglobinopathy.

Informed consent was obtained prior to any study related procedures. Full history and basic data were taken from the patients, including age, gender, race, duration of dialysis and pre - morbid illness. Full blood count, CHr, serum ferritin, and TSAT were measured in all patients. Once the results were obtained, patients with Hb < 9.5 g/dL and CHr < 29 pg with serum ferritin $\geq$ 400 ng/mL and/or TSAT > 20% and on stable erythropoietin therapy were randomization to 2 groups, group A and B. Patients were randomized using a block of 4 randomization methods. Group A patients were treated with a total of 1 gm of intravenous iron (100 mg per HD session for 10 sessions) and group B patients served as the control group. The baseline dose of oral iron and erythropoiesis stimulating agents were maintained in both groups. Blood investigations were repeated at the end of 6 weeks in both groups.

2.2. Statistical Analysis

Sample size was calculated using Everard's calculation and 112 patients were needed for a power of 80% and confidence interval of 95%. To provide a slight margin of error given the possibility of subject attrition, or target recruitment was 120 patients. The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. Qualitative and quantitative demographic data were summarized and data were tabulated. Results are presented in tabulated and graphic format. Data are expressed as mean $\pm$ Standard Deviation (SD), median (interquartile range of 25th centile to 75th centile), and percentage. Discrete variables were analyzed using a chi - square test and continuous variables, which were normally distributed, were analyzed using independent student t - test. The non - normally distributed data were analyzed using the Mann - Whitney U test. Data correlation testing was achieved by Spearman or Pearson correlation test. Any test with P value of < 0.05 was statistically significant.

3. Results

A total of 180 patients were screened and 121 patients, who met the inclusion criteria, were recruited in this study. All patients were given detailed explanation of the study prior to recruitment and informed consent were obtained prior to study-related procedures.

The study patients consisted of 79 (65.3%) Malays, 33 (27.3%) Chinese, and 9 (7.4%) Indians. Seventy - two were males and 49 females. Mean age for the study population was 54.2 $\pm$ 14.5 years and median duration of HD in the study population was 3 (2 to 6) years. Among the patients, 71 (58.7%) had diabetes, 102 (84.3%) hypertension, and 12 (10.1%) glomerulonephritis. The baseline laboratory parameters are tabulated in Table 1. The CHr showed a significant correlation with Hb (p = 0.0001, r = 0.3), TSAT (p = 0.001, r = 0.3), and ferritin levels (p = 0.001, r = 0.3).

Table 1. Baseline Laboratory Parameters

Parameters	Baseline Results
Hemoglobin (g/dl)	10.4 $\pm$ 1.9[a]
CHr (pg)	32.9 $\pm$ 4.1[a]
MCV (fl)	89.3 $\pm$ 6.9[a]
MCH (pg)	29.5 $\pm$ 3.1[a]
RDW (%)	14.7 $\pm$ 1.5[a]
Platelet (10^9/L)	237.0 (195.5 - 291.5)[b]
WBC (10^9/L)	7.4 (6.0 - 9.35)[b]
Ferritin (ng/mL)	428.0 (309.8 - 623.0)[b]
TSAT	26.4 (20 - 36)[b]
Erythropoeitin (IU)/week	5090.9 $\pm$ 2408.3[a]

Abbreviations: MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; WBC, white blood count.
[a]Mean $\pm$ SD.
[b]Median (25th - 75th centile).

Only 12 patients fulfilled the criteria for randomization

(Hb < 9.5 g/dL and CHr < 29 pg with serum ferritin ≥ 400 ng/mL and/or TSAT > 20% and stable erythropoietin therapy). Their clinical and laboratory parameters at baseline and end of the study are tabulated in Table 2. The researchers also analyzed the laboratory parameter changes from baseline (CFM) in each group (Table 3).

4. Discussion

Anemia in End Stage Renal Disease (ESRD) is a chronic disorder and related to severe complications in patients on HD. In Malaysia it has been reported that up to 88% of the dialysis population receive either oral iron or parenteral iron therapy for treatment of IDA (11). From this report 66% of patients on HD from Malaysia achieved the ferritin target of > 200ng/mL (median ferritin of hemodialysis patient was 500 ng/mL) and 74% had a mean hemoglobin of 11.4 g/dL (11). The commonly used indicators for detecting iron deficiency anemia in HD patient are TSAT and ferritin (12). However, it is well established that specificity and sensitivity of serum ferritin level may be reduced due to inflammation, infection, malnutrition or malignancy (13). One of the potentially more accurate tests is reticulocyte Hemoglobin Content (CHr) and was found to be a good test to guide in iron management. In the current study, the researchers evaluated the role of CHr in patients on HD for determining iron deficiency and demonstrating the response of the rise in hemoglobin, when patients with adequate serum ferritin and/or TSAT yet low CHr are given intravenous iron.

Reticulocyte Hemoglobin Content was proven to be related to changes of iron and ferritin level, especially in patients on HD. In the current study, CHr was shown to be correlated with Hb, TSAT, and ferritin. Brugnara and Saito in their studies revealed similar correlations of CHr with iron, ferritin, and TSAT (8, 14). Many studies revealed that low CHr in IDA was associated with low level of iron, ferritin, and TSAT (15). With a good correlation of CHr with IDA indices, CHr will be an important marker to identify iron deficiency, especially in patients on HD with malnutrition and infection. It is well - known that serum ferritin and TSAT are acute phase reactants and will be raised in patients with infection or inflammation (5). Thus, by utilizing CHr in detection of IDA, management and treatment of anemia in patients on HD would be more accurate and effective.

In this study, the researchers analyzed the effect of intravenous iron supplementation given to patients on HD with low CHr yet adequate ferritin and TSAT. There were no significant differences in patients, who received intravenous iron and the control group at baseline and end of the study. Nevertheless, by analyzing changes from baseline, the researchers noticed that patients, who received intravenous iron had increment of 1.0 g/dL (IQR: - 0.5 to 2.8) (p = 0.025) in Hb.

Adhikary demonstrated the efficacy of intravenous iron compared to oral iron, proven to be effective in increment of hemoglobin level in patients with low TSAT and ferritin level (16). Another trial by Chaim Charytan found that when 1 gm of intravenous iron sucrose was administered in 10 divided doses to hemodialysis patient with TSAT less than 20% and ferritin less than 300 ng/mL, there was increment in hemoglobin (17).

The current study was different from previous studies, as the patients were treated with apparently adequate iron status (serum ferritin ≥ 400 ng/mL and/ or TSAT > 20%) with intravenous iron. Although these patients had apparently adequate iron status, they demonstrated an increment of 1 g/dl in Hb with intravenous iron treatment in 6 weeks. As widely accepted, 1 g/dL Hb increment has an important clinical significance in patients with HD. This finding established the role of CHr in the management of iron deficiency anemia in patients with HD. Furthermore, CHr indirectly helps in reducing the cost of the erythopoeitin stimulating agent treatment in HD patients by detecting accurately the patient who had IDA even with adequate TSAT and ferritin level. This is supported by a publication which showed that CHr proven to be economical favourable by reducing the need of erythropoietin therapy. Sunder - Plassmann found that high - dose intravenous iron therapy guided by CHr level dramatically reduced the weekly rHuEpo requirement by 70% of the initial dose (18).

The authors strongly suggest that CHr should be a routine investigation for IDA in patients on HD. The use of CHr can not only improve the clinical outcome but also potentially reduce the cost of erythropoietin treatment.

4.1. Conclusion

This study showed the significant correlation of CHr with Hb, serum ferritin, and TSAT. Furthermore, improvement in Hb level was indicated in anemic HD patients, who had adequate serum ferritin and TSAT, yet low CHr was seen in patients, who received intravenous iron therapy.

Acknowledgments

The authors would like to thank the Dean of the Faculty of Medicine, University Kebangsaan Malaysia, for allowing these data to be published. The authors also like to thank the members of nephrology unit, UKMMC, for their support in this study.

Table 2. Clinical and Laboratory Parameters: Comparison Between Intravenous Iron Group and Control Group at Baseline and Post Treatment[a, b]

Parameter	Baseline			Post Treatment		
	Group A (IV Iron)	Group B (Control)	P Value	Group A (IV Iron)	Group B (Control)	P Value
Age (year)	58.0 (55.5 - 63.5)	61.0 (42.5 - 70.5)	0.40	58.0 (55.5 - 63.5)	61.0 (42.5 - 70.5)	0.40
Duration of Dialysis (year)	2 (1.0 - 6.5)	3 (2 - 6.5)	0.39	2 (1.0 - 6.5)	3 (2 - 6.5)	0.39
Erythropoeitin (IU/week)	6000 (4000 - 10 000)	6000 (5000 - 10 000)	0.59	6000 (4000 - 10 000)	6000 (5000 - 10 000)	0.59
Hb (g/dL)	8.0 (7.1 - 8.9)	9.2 (8.1 - 9.5)	0.17	9.4 (7.95 - 10.5)	9.7 (8.5 - 11.15)	0.60
Platelet (10^9/L)	260.0 (180 - 313.5)	249.0(179 - 274.5)	0.46	244 (199 - 260.5)	253(191 - 261.5)	0.75
WCC (10^9/L)	7.6 (4.8 - 8.6)	7.2 (5.9 - 10.1)	0.75	8.2(6.1 - 11.4)	8.4(6.1 - 11.2)	0.92
MCV (fl)	92 (79.4 - 95)	81.8 (72 - 84)	0.09	94.1(84.3 - 99)	81.6(73.4 - 86)	0.05
MCH (pg)	29 (24.4 - 30.5)	26 (23.5 - 28.5)	0.24	30.3 (26.7 - 33.9)	26.2 (23 - 27.7)	0.05
Ferritin (ng/ml)	435 (252.8 - 663.0)	578 (327.7 - 998.5)	0.46	1029(603 - 1427)	517 (228 - 1404)	0.46
TSAT (%)	25 (22.2 - 65.5)	33 (21.9 - 45.5)	0.83	45.4 (28 - 58.1)	25 (17.5 - 52)	0.25
CHr (pg)	27.8 (21.8 - 28.9)	27.0 (25.5 - 28.3)	0.92	34.8 (27.5 - 38)	31.4 (26.6 - 33.2)	0.17

Abbreviations: MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; RDW: Red cell distribution width; WBC: white blood count.
[a]Values are in median (25th - 75th centile).
[b]All tests performed using Mann Whitney U test.

Table 3. Hematological, Chemical and Iron Indices and Rate of Responders with in Group[a, b]

Parameter	Iv Iron Group				Control Group			
	Baseline	Post Treatment	CFB	P Value	Baseline	Post Treatment	CFB	P Value
Hb (g/dL)	8 (7.1 - 8.9)	9.4 (8 - 10.5)	1.0 (- 0.5 - 2.8)	0.025	9.2 (8.1 - 9.5)	9.7 (8.5 - 11.2)	0.1 (0.0 - 2.4)	0.083
CHr (pg)	27.8 (21.8 - 28.9)	34.8 (27.5 - 38)	5.8(1.6 - 13.9)	0.18	27.0 (25.5 - 28.3)	31.4 (26.6 - 33.2)	2.8 (1.1 - 5.7)	0.046
Ferritin (ng/mL)	435 (253 - 663)	1029 (603 - 1427.1)	594(265.3 - 848.5)	0.025	578 (328 - 999)	517 (228 - 1404)	93 (- 249.5 - 540.4)	0.655
TSAT (%)	25 (22.6 - 65.5)	45.4 (28 - 52)	14.4(4.4 - 35.1)	0.18	33 (21.9 - 45.5)	25 (17.5 - 52)	- 3.1 (- 8.9 - 8.5)	0.655

Abbreviation: CFB: changes from baseline.
[a]Significant $P < 0.05$.
[b]All tests performed using Mann Whitney U test.

Footnotes

Ethical Approval: The study was approved by the Ethics and Research Committee, University Kebangsaan Malaysia Medical Centre (Code Project FF - 2014-347).

Conflicts of Interest: The authors declare that they had no conflicts of interest.

References

1. Kidney Disease Outcomes Quality I. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis*. 2004;**43**(5 Suppl 1):S1–290. [PubMed: 15114537].
2. Macdougall IC. Poor response to erythropoietin: practical guidelines on investigation and management. *Nephrol Dial Transplant*. 1995;**10**(5):607–14. [PubMed: 7566570].
3. Nissenson AR. Achieving target hematocrit in dialysis patients: new concepts in iron management. *Am J Kidney Dis*. 1997;**30**(6):907–11. [PubMed: 9398140].
4. National Kidney Foundation anemia working group . IV. NKF-K/DOQI Clinical Practice Guidelines for Anemia of Chronic Kidney Disease: update 2000. *Am J Kidney Dis*. 2001;**37**(1 Suppl 1):S182–238. [PubMed: 11229970].
5. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH. Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. *Nephrol Dial Transplant*. 2004;**19**(1):141–9. [PubMed: 14671049].
6. Fishbane S, Galgano C, Langley RJ, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. *Kidney Int*. 1997;**52**(1):217–22. [PubMed: 9211366].
7. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood*. 2002;**99**(4):1489–91. [PubMed: 11830506].
8. Brugnara C, Laufer MR, Friedman AJ, Bridges K, Platt O. Reticulocyte hemoglobin content (CHr): early indicator of iron deficiency and response to therapy. *Blood*. 1994;**83**(10):3100–1. [PubMed: 8180408].
9. Karagulle M, Gunduz E, Sahin Mutlu F, Olga Akay M. Clinical significance of reticulocyte hemoglobin content in the diagnosis of iron deficiency anemia. *Turk J Haematol*. 2013;**30**(2):153–6. doi: 10.4274/Tjh.2012.0107. [PubMed: 24385778].
10. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. *Clin Lab Haematol*. 2006;**28**(5):303–8. doi: 10.1111/j.1365-2257.2006.00812.x. [PubMed: 16999719].

11. Jeremiah PN, Cheak BB, Ahmad GB, Kun LS. *Management of anemia in patients on dialysis*. 2013. 16 p.
12. Macdougall IC. Monitoring of iron status and iron supplementation in patients treated with erythropoietin. *Curr Opin Nephrol Hypertens*. 1994;**3**(6):620–5. [PubMed: 7881986].
13. Rocha LA, Barreto DV, Barreto FC, Dias CB, Moyses R, Silva MR, et al. Serum ferritin level remains a reliable marker of bone marrow iron stores evaluated by histomorphometry in hemodialysis patients. *Clin J Am Soc Nephrol*. 2009;**4**(1):105–9. doi: 10.2215/CJN.01630408. [PubMed: 18842949].
14. Saito M, Tsuchiya K, Ando M, Akiba T, Nihei H. [Measuring the content of reticulocyte hemoglobin (CHr) in predialysis chronic renal failure (CRF) patients]. *Nihon Jinzo Gakkai Shi*. 2003;**45**(5):430–8. [PubMed: 14509218].
15. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol*. 2006;**1 Suppl 1**:S4–8. doi: 10.2215/CJN.01490506. [PubMed: 17699374].
16. Adhikary L, Acharya S. Efficacy of IV iron compared to oral iron for increment of haemoglobin level in anemic chronic kidney disease patients on erythropoietin therapy. *JNMA J Nepal Med Assoc*. 2011;**51**(183):133–6. [PubMed: 22922860].
17. Charytan C, Levin N, Al-Saloum M, Hafeez T, Gagnon S, Van Wyck DB. Efficacy and safety of iron sucrose for iron deficiency in patients with dialysis-associated anemia: North American clinical trial. *Am J Kidney Dis*. 2001;**37**(2):300–7. doi: 10.1053/ajkd.2001.21293. [PubMed: 11157370].
18. Sunder-Plassmann G, Horl WH. Importance of iron supply for erythropoietin therapy. *Nephrol Dial Transplant*. 1995;**10**(11):2070–6. [PubMed: 8643170].