The role of reticulocyte hemoglobin content for the diagnosis of functional iron deficiency in hemodialyzed patients

Ali A. Alageeli, Fatmah S. Alqahtany, Farjah H. Algahtani

Keywords: reticulocytes hemoglobin hemodialysis

Abstract

The effectiveness of reticulocyte hemoglobin content (CHR) had been reported to detect early functional iron deficiency especially among Chronic kidney disease (CKD) patients. CHR is more superior to classic biochemical indices in reflecting transient iron-deficiency status, therefore improving diagnosis and treatment. This study was conducted to determine the sensitivity of CHR in the diagnosis of functional iron deficiency (FID) in hemodialyzed patients. One hundred hemodialyzed patients along with 60 healthy controls were recruited and blood specimens were collected. Venous blood was used for hematological and biochemical investigations collected via 3 ml lavender-top tubes for hematological tests including CBC, blood film, ESR and CHR, and red-top tube for biochemical tests including TIBC, SF and CRP. A statistically significant decrease was noted in CHR values between hemodialysis patients and the control group (24.8 ± 2.0 pg vs. 30.9 ± 1.3 pg, p<0.001). CHR values showed a significant correlations with RBCs, Hb- hemoglobin, Hct- hematocrit level, MCV- mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC, RDW- red cell distribution width, SI- Serum Iron, TIBC- Total iron binding capacity and TSAT- Transferrin saturation. The present study showed that CHR in comparison to the conventional hematological and biochemical markers commonly used to diagnose iron deficiency.

1. Introduction

Iron is a very important substance, which was needed by the body to transport oxygen to tissues. Majority of the body iron (2/3) is in the red blood cells (RBC), and about 1/3 is stored in the liver, spleen and bone marrow. Iron is transported into the circulation by transferrin (Knutson, 2017). Patients on hemodialysis have lower intestinal iron absorption and greater iron loss (Rostoker et al., 2016). Levels of ingested iron vary from very small amounts up to 200 mg as that was seen in end-stage renal disease (ESRD) patients given supplements (Rostoker et al., 2016). In ESRD though, there is little iron absorption, as 1 to 2% of this stored iron (Zumbrunnen-Bullough and Babitt, 2014). Some studies showed that despite therapy with recombinant human erythropoietin (rHuEPO) in hemodialysis (HD) patients, there would still be iron deficiency anemia in as much as 49.1% of HD patients (Kuo, 2018; Chavers, 2004). Thus, there is an utmost need for compensation of iron in HD patients.

In Chronic kidney disease (CKD) patients on HD, annual iron loss can amount to 2 to 4 g or even more. Correction of anemia in hemodialysis patients with Recombinant human erythropoietin (rHuEPO) can be infuriating, if iron is insufficient (Alves, 2015). Thus, it is wise to establish the diagnosis of iron deficiency in CKD patients on HD. However, there is no ideal test for monitoring iron storage. Currently, iron status is evaluated by serum ferritin levels (SF) and transferring saturation (TSAT). Although serum ferritin is still a reliable indicator of iron confession in most cells (Nakanishi, 2010), its value is limited because ferritin is an acute-phase reactant (Kell and Pretorius, 2014) and the extracellular ferritins are raised in incendiary or malicious disease (Wang, 2010).

Other biomarkers of body iron stores were assessed including transferrin saturation (TSAT), percentage of hypochromic erythrocytes (HYPO) and hemoglobin content of reticulocytes (HCr) (Buttarello, 2016; Cai, 2017). In one study, TSAT showed most suitability to determine iron stores for optimal IV iron therapy (Buttarello, 2016). However, more studies showed that both TSAT and ferritin as poor indicators of body iron load particularly in hemodialysis patients (Diebold and Kistler, 2019; Ali Rafi, 2007; Wish, 2018).
The usefulness of CHr has been reported to identify initial functional iron shortage particularly amongst CKD patients who are on EPO therapy (Kuo, 2018; G.T., Renal anemia: a nephrologist’s view. HIPPOKRATIA, 2011; Hayat et al., 2008; Reddy et al., 2013). Increasing evidence showed that CHr is more superior than classic biochemical indices in reflecting transient iron-deficiency status, thus improving diagnosis and treatment (G.T., Renal anemia: a nephrologist’s view. HIPPOKRATIA, 2011; Hayat et al., 2008; Reddy et al., 2013). This study was conducted to determine the sensitivity of CHr in the diagnosis of functional iron deficiency (FID) in hemodialyzed patients.

2. Materials and Methods

One hundred adults (>18 years old), comprised of 51 males and 49 females were recruited from the hemodialysis unit of Prince Sultan ibn Abdulaziz King Fahad Hospital in Jizan, Saudi Arabia. Eligibility criteria included the age criteria more than 18 years old, and those undergone hemodialysis treatment for more than 3 months, the EPO dosing for at least three months with no changes in EPO dosing for at least 4 weeks. All patients received EPO 2,000-6,000 units three times a week.

A control group was selected from blood donors from the same institution comprising of 60 healthy individuals (43 males and 17 females). All were with normal complete blood count (CBC) and biochemical iron parameters. Informed and written consents were taken from all participants, both hemodialysis and control group.

Blood specimens were collected from all patients. Venous blood was used for all hematological and biochemical investigations and collected via 3 ml lavender-top tubes for hematological tests including CBC, blood film, ESR and CHr, and red-top tube for biochemical tests including TIBC, SF and CRP.

2.1. Reticulocyte hemoglobin content measurement

CHr was measured on the ADVIA 2120 hematology analyzer (Siemens Diagnostic Solutions, Tarrytown, New York). Measurement was carried out by fluorescence flow cytometry as the CHr which stains cells according to their RNA content (Fishbane et al., 1997).

ESR was measured by Westergren using Sedplast ESR system (Polymedco, Cortlandt Manor, NY). Serum iron, serum iron binding capacity were measured automatically using Siemens Flex reagent cartridge DF49A on Dade Behring Dimension RXL clinical chemistry autoanalyser. Serum ferritin was measured using Modular Analytic E170 analyzer. CRP was measured by NycoCard CRP single test from Axis-shield.

2.2. Statistical Analysis

Data analysis was performed using Statistical Package for Social Sciences version 18.1 (SPSS Inc, Chicago, IL, USA)(Corp., 2010). All normal distributions of continuous variables were reported as mean ± standard deviation. Correlation coefficient was used to analyze the degrees of association between two variables (Pearson correlation coefficient with p value and 95% confidence interval for r). Regression analysis were performed to describe the relationship between two variables and to predict one variable from another. P values <0.05 were considered statistically significant for all analyses.

3. Results

There were 100 hemodialysis patients, 51 (51%) males and 49 (49%) females. Mean age of the patients was 46.9 ± 9.9 years. Mean CHr was 24.8 ± 2.0 pg. Mean TSAT was 17.4 ± 8.1%. Mean TIBC was 45.7 ± 7.7 umol/L. Mean hemoglobin and biochemical parameters in these patients are shown in Table 1. Most of the patients had transferring saturation (TSAT) <20% and serum ferritin (SF) of >100 ug/L. Mean CRP was 11.1 ± 4.0 mg/L. Mean EPO dose was 3393.3 ± 1328.2. (Table 1)

In contrast to the control group, hemodialysis patients had significantly lower (Hb), (RBC), (Hct), (MCV), (MCH), (MCHC), (SI), and (TSAT)(Karagülle, 2013). A statistically significant decrease was noted in CHr values between hemodialysis patients and the control group (24.8 ± 2.0 pg vs. 30.9 ± 1.3 pg, p<0.001). (Table 1)

The correlations between CHr values and the mean results of hematological and biochemical parameters of iron status in hemodialysis patients were calculated and shown in Table 2. CHr values showed a significant negative correlation. There were significant positive correlations seen between CHr and SI (r=0.79, p<0.001), TIBC (r=0.225, p=0.025) and TSAT (r=0.64, p<0.001). (Table 2, Figures 1 and 2)

4. Discussion

In this study, hemodialysis patients had significantly lower HB, RBC, Hct. MCV, MCH, MCHC, SI, TSAT and, higher levels of RDW, TIBC and SF compared to the control group. There was significantly lower level of CHr in hemodialyzed patients compared to the control group. The mean CHr in our study group was 24.8 ± 2.0 pg, which is in congruence to the values reported by Fishbane et al. (1997) (27.5 ± 2.8 pg) done in 164 stable hemodialyzed patients but, is lower than the values reported by Fukui et al. (2002) (mean 33.9 ± 3.6 pg).

### Table 1

| Variables | Control Mean ± SD | CRF Mean ± SD | T test p values |
|-----------|------------------|---------------|----------------|
| WBC, x10³/ul | 6.3 ± 1.9 | 5.7 ± 1.8 | <0.001 |
| RBC, x10³/ul | 4.7 ± 0.5 | 3.7 ± 0.4 | <0.001 |
| Hb in g/dl | 14.1 ± 1.4 | 9.6 ± 0.8 | <0.001 |
| Hct in % | 41.6 ± 4.1 | 29.0 ± 2.6 | <0.001 |
| MCV in fL | 87.1 ± 2.9 | 80.4 ± 5.9 | <0.001 |
| MCH in pg | 29.7 ± 1.0 | 26.6 ± 2.3 | <0.001 |
| MCHC in g/dl | 34.3 ± 1.5 | 32.0 ± 1.5 | <0.001 |
| RDW in % | 13.2 ± 0.5 | 15.1 ± 1.7 | <0.001 |
| PLT, x10³/ul | 26.2 ± 4.8 | 21.3 ± 9.1 | <0.001 |
| CHr in pg | 30.9 ± 1.3 | 24.8 ± 2.0 | <0.001 |
| SI in umol/L | 20.9 ± 2.9 | 10.1 ± 5.1 | <0.001 |
| TIBC in umol/L | 42.8 ± 4.5 | 45.7 ± 7.7 | 0.004 |
| TSAT in % | 46.4 ± 6.0 | 17.4 ± 8.1 | <0.001 |
| SF in ug/L | 9.7 ± 1.2 | 52.6 ± 31.6 | <0.001 |

### Table 2

| Variables | CHr Correlation | Df= n-2 | p values |
|-----------|----------------|--------|----------|
| RBCs | 0.468 | 98 | <0.001 |
| HB | 0.557 | 98 | <0.001 |
| Hct | 0.316 | 98 | <0.001 |
| MCV | 0.548 | 98 | <0.001 |
| MCH | 0.548 | 98 | <0.001 |
| MCHC | 0.300 | 98 | 0.003 |
| RDW | -0.56 | 98 | <0.001 |
| SI | 0.789 | 98 | <0.001 |
| TIBC | 0.225 | 98 | 0.025 |
| TSAT | 0.636 | 98 | <0.001 |
| SF | 0.228 | 98 | 0.023 |
Figure 1. Correlations between CHr values and haematological parameters in CRF patients.
of 31.0 pg), Kim et al. (2008) (29.9 ± 1.9 pg), Miyata (2003) (33.1 ± 1.5 pg), Buttarello (2010) (mean of 30.6 pg) and Urrechaga (2009) (31.6 ± 3.5 pg).

This study also showed the inverse correlation between CHr and Total RBC count. This is in agreement with Mittman's report Neal Mittman et al., 1997, who suggested that the erythropoietic stimulus of routinely administered rHuEPO may result in functional iron deficiency. The positive correlations that existed between CHr and Hb, Hct, SI, TSAT and SF, making CHr as a potential marker for monitoring renal anemia especially in dialyzed patients Neal Mittman et al., 1997.

The hemodialysis procedure is linked to an increased risk of inflammation thus elevations of CRP levels, which somewhat increases when patients start HD. However, CRP responds only in acute phases of inflammation but it subsides quickly in concentration Gabay and Kushner, 1999, thus making CHr as a promising test to measure ID in hemodialysis patients (Fishbane et al., 1997; Hurrell, 2012; Saito, 2014; Menno, 1998; Nairz, 2016; Wessling-Resnick, 2010; Kotze1 et al., 2009; Van Wyck, 1989; Tarng, 1999; Muñoz, 2008).

The present study showed that CHr in comparison to the conventional hematological and biochemical markers commonly used to diagnose iron deficiency yielded a good and acceptable correlation. CHr can be a very good predictor of iron deficiency and enable to diagnose iron deficiency anemia more rapidly during a routine blood testing with little incremental cost. Our study confirms that CHr is not influenced by the inflammatory process that exists during hemodialysis.

Figure 2. Li near regression analysis showing the correlations between CHr values and biochemical parameters in CRF patients.
Acknowledgement

This work was supported by the College of Medicine Research Center, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

References

Knutson, M.D., 2017. Iron transport proteins: Gateways of cellular and systemic iron homeostasis. J Biol Chem 292 (31), 12735–12743.

Rostoker, G., Vaziri, N.D., Fishbane, S., 2016. Intravenous Iron Overload in Dialysis Patients at the Beginning of the 21st Century. Drugs 76 (7), 741–757.

Zumbrennen-Bullough, K., Babitt, J.L., 2014. The iron cycle in chronic kidney disease (CKD): from genetics and experimental models to CKD patients. Nephrol Dial Transplant 29 (2), 263–273.

Kuo, K.L., et al., 2018. Association of Anemia and Iron Parameters With Mortality Among Patients Undergoing Prevalent Hemodialysis in Taiwan: The AIM - HD Study. J Am Heart Assoc 7, (15) e009206.

Chauve, B.M., et al., 2004. Prevalence of anemia in erythropoietin-treated pediatric as compared to adult chronic dialysis patients. Kidney Int 65 (1), 266–273.

Alves, M.T., et al., 2015. Resistance of dialyzed patients to erythropoietin. Rev Bras Hematol Hemoter 37 (3), 190–197.

Nakanishi, T. et al., 2010. Importance of ferritin for optimizing anemia therapy in chronic kidney disease. Am J Nephrol 32 (5), 439–446.

Kell, D.B., Pretorius, E., 2014. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics, 748–773.

Wang, W. et al., 2010. Serum ferritin: Past, present and future. Biochim Biophys Acta 1800 (8), 760–769.

Buttarello, M. et al., 2016. Evaluation of the hypochromic erythrocyte and reticulocyte hemoglobin content provided by the Sysmex XE-5000 analyzer in diagnosis of iron deficiency erythropoiesis. Clin Chem Lab Med 54 (12), 1939–1945.

Cai, Jie et al., 2017. Evaluation of the Efficiency of the Reticulocyte Hemoglobin Content on Diagnosis for Iron Deficiency Anemia in Chinese Adults. Nutrients 2017, 9.

Diebold, M., Kistler, A.D., 2019. Evaluation of iron stores in hemodialysis patients on maintenance ferric Carboxymaltose dosing. BMC Nephrol 20 (1), 76.

Ali Raﬁ, A.K., 2007. Mohammed Abdelrahman, Monitoring Iron status in End-Stage Renal Disease Patients on Hemodialysis. Saudi J Kidney Dis Transplant 18 (1), 73–78.

Wish, J.B. et al., 2018. Positive Iron Balance in Chronic Kidney Disease: How Much is Too Much and How to Tell?. Am J Nephrol 47 (2), 72–83.

G., T., Renal anemia: a nephrologist’s view. HIPPOKRATIA 2011: p. 39–43.

Hayat, A., Harsa, D., Salihu, M.O., 2008. Erythropoietin stimulating agents in the management of anemia of chronic kidney disease. Patient Preference and Adherence 2, 195–200.

Reddy, G.C., Devaki, R., Rao, P., 2013. IRON INDICES IN PATIENTS WITH FUNCTIONAL ANEMIA IN CHRONIC KIDNEY DISEASE. The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine 24, 3–4.

Fishbane, S. et al., 1997. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int 52 (1), 217–222.

Corp., I., IBM SPSS Statistics for Windows, Version 19.0, 2010, IBM Corp.: Armonk, NY.

Karagüle, Mustafa et al., 2013. Clinical Signifi cance of Reticulocyte Hemoglobin Content in the Diagnosis of Iron Defi ciency Anemia. Turk J Hematol 30, 153–156.

Fukui, Y., et al., Reticulocyte hemoglobin content as a marker of iron status in patients receiving maintenance hemodialysis. Clin Exp Nephrol (2002) 6:147–153, 2002. 6: p. 147–153.

Kim, J.M., Ihm, C.H., Kim, H.J., 2008. Evaluation of reticulocyte haemoglobin content as marker of iron defi ciency and predictor of response to intravenous iron in haemodialysis patients. Int J Lab Hematol 30 (1), 46–52.

Miyata, K.M.A.H.Y., 2003. Assessment of iron defi ciency in chronic hemodialysis patients: investigation of cutoff values for reticulocyte hemoglobin content. Clin Exp Nephrol 7, 52–57.

Buttarello, M. et al., 2010. Diagnosis of iron defi ciency in patients undergoing hemodialysis. Am J Clin Pathol 133 (6), 949–954.

Urrechaga, E., Borque, L., Escanero, J.F., 2009. Potential utility of the new Sysmex XF 5000 red blood cell extended parameters in the study of disorders of iron metabolism. Clin Chem Lab Med 47 (11), 1411–1416.

Neal Mattman, M., Rajanna Sreedhara, MD, Robert Mushnick, MD, Jyoti Chattopadhyay, PhD, and P. David Zelmanovic, Mehdi Vaeghe, MD, and Morrell M. Avram, MD, FACP, Reticulocyte Hemoglobin Content Predicts Functional Iron Deficiency in Hemodialysis Patients Receiving rHuEPO. American Journal of Kidney Diseases., 1997. 30(6): p. 912-922.

GABAY, C. and I. KUSHNER, Acute Phase proteins and other systemic responses to inflammation. The New England Journal of Medicine, 1999. Volume 340 Number 6 :453: p. 449-454.

Hurrell, R.F., 2012. Influence of inflammatory disorders and infection on iron absorption and efficacy of iron-fortified foods. Nestle Nutr Inst Workshop Ser 70, 107–116.

Saito, H., METABOLISM OF IRON STORES. Nagoya J. Med. Sci. 2014. 76: p. 235 – 236.

Menno P. Kooistra;“, E.C.N., Ad van Es3 and N.M.M.-B., Albert Struyvenberg/and Joannes J. M. Marxv”, Iron absorption in erythropoietin-treated haemodialysis patients:effects of iron availability, inflammation and aluminium. Nephrol Dial Transplant 1998; 13: p. 82–88.

Nairz, M. et al., 2016. Iron defi ciency or anaemia of inflammation? : Differential diagnosis and mechanisms of anaemia of inammation. Wien Med Wochenschr 166 (13–14), 411–423.

Wessling-Resnick, M., 2010. Iron homeostasis and the inflammatory response. Annu Rev Nutr 30, 105–122.

MJ Kotze1, D.v.V., SJ van Rensburg2, R Erasmus, PATHOGENIC MECHANISMS UNDERLYING IRON DEFICIENCY AND IRON OVERLOAD:NEW INSIGHTS FOR CLINICAL APPLICATION. The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine, 2009. 20(2): p. 108-123.

Van Wyck, D.B., et al., 1989. Iron status in patients receiving erythropoietin for dialysis-associated anemia. Kidney Int 35 (2), 712–716.

Tang, D.C. et al., 1999. Erythropoietin hyporesponsiveness: From iron deficiency to iron overload. Kidney International 55, 5107–5118.

Muñoz, M. et al., 2008. Efficacy and safety of intravenous iron therapy as an alternative/adjunct to allogeneic blood transfusion. Vox Sang 94 (3), 172–183.