International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-11-13

Role of reticulocyte hemoglobin content in the evaluation of iron deficiency state in hemodialysis patients

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Abstract

Background: Anemia is a major factor that limits the quality of life in dialysis patients and may affect their morbidity & mortality. Insufficient production of erythropoietin from the failed kidneys is the major cause of anemia in this population. Absolute and functional iron deficiency (demand/supply imbalance), and iron deficiency due to inflammation (reticulo-endothelial blockade) are the most important causes of iron insufficiency. Recently, the reticulocyte hemoglobin content (CHr) has been shown to be a sensitive and specific indicator for functional iron deficiency. The present study is aimed to assess the relationship between CHr with conventional parameters of iron status, particularly, the transferrin saturation (TSAT) and ferritin, as well as with C-reactive protein (CRP), in order to verify the clinical usefulness of CHr as predictor of iron deficiency state in patients undergoing chronic hemodialysis. **Methods**: Across-sectional study was conducted on 90 randomly selected chronic hemodialysis patients receiving treatment with erythropoiesis stimulating agents (ESAs). CHr, CRP, TSAT and serum ferritin, were determined along with routine hematological parameters. These parameters and their correlations with CHr for patients without inflammation (low CRP,<5 mg/dL) were compared with those for patients with inflammation (high CRP, > 5 mg/dL), to study the influence of inflammation on the iron status of the patients. **Results**: Mean hemoglobin level was 8.94±2.02 g/dL and mean CHr was 30.21±2.77 pg. There was a weak positive correlation between CHr and TSAT(r = 0.281, *P*= 0.007), CHr and Ferritin and (r = 0.229, *P*= 0.030), respectively. However, there was a strong positive correlation with mature red blood cell hemoglobin indices: MCH (rs (90) = 0.851, *P* = 0.000)and MCHC (rs (90) = 0.632, *P* = 0.000), respectively. On the other hand, there was a weak negative correlation between CHr and CRP, rs (90) = -0.369, *P* = 0.000.Median CHr level was statistically significantly lower in patients with inflammation/infection than in patients without inflammation/infection, U = 626, z = -2.45, *P* = 0.014. **Conclusions**: Thirty one patients (34.4%) comprising of the study population were identified to have IDA. The logistic regression model was not statistically significant ($\chi^2(4) = 3.965$, *P* = 0.138). Hence, the present result simply that CHr cannot be used as a predictor of iron deficiency state in hemodialysis patients.

Keywords: Anemia, Reticulocyte hemoglobin content, C-reactive protein, Erythropoiesis stimulating agents

Introduction

Anemia is a major factor that limits the quality of life in dialysis patients and may affect their morbidity & mortality [Johansen et. Al. 2010].The incidence of anemia in patient with chronic kidney disease (CKD) increases as the glomerular filtration rate (GFR) declines. Population studies such as National Health and Nutrition Examination Survey (NHANES) by the National Institutes of Health and the Prevalence of Anemia in early Renal Insufficiency (PAERI) study suggest that the incidence of anemia is less than 10% CKD stage 1and 2, 20-40% in CKD stage 3, 50-60% in CKD stage 4, more than 70% in CKD stage 5 [Drueke et. al. 2006][Fishbane et. al. 2010].Insufficient production of erythropoietin from the failed kidneys is the major cause of anemia in this population [Astor et. al. 2002]. Other factors include deficiencies of iron, folic acid & vitamin B12. Blood loss, leading to loss of 5-7 mg of iron during each

dialysis treatment and short half-life of red blood cells may also complicate the management of anemia [Rockey 2005] [Kruse et. al. 2008]. It has been demonstrated that RBCs survival is decreased from 120 days in normal individuals to 60-90 days in patients with CKD. This may be as a result of RBC trauma from micro-vascular disease as well as decreased resistance to oxidative stress. The reduction of EPO in patients with CKD also contributes to neocytolysis; a physiologic process that leads to hemolysis of the youngest RBCs in the circulation [Astor et. al. 2002]. Another type of anemia in CKD patients is pure cell aplasia that is caused by the production of anti-erythropoietin antibodies induced by administration of exogenous erythropoiesis stimulating agents (ESAs) [MacdougalI et. al. 2009].

The major clinical manifestations in such patients are fatigue (with exercise and at rest), decreased cognitive function, loss of libido and decreased sense of well-being. These symptoms tend to occur when the Hb is less than 10 g/dL, and they are even more severe at lower Hb levels [Besarab et. al. 1998] [Coyne 2008] [Kliger et. al. 2012]. More insidious are the cardiac complications of anemia, which may occur when the patient otherwise asymptomatic and contribute to the adverse cardiovascular morbidity and mortality outcomes observed among patients with CKD. Anemia may lead to exacerbation of angina because of myocardial oxygen delivery, also lead to increased heart rate (HR) and lead to left ventricular hypertrophy (LVH) [Besarab et. al.1998]. Correction of the anemia yields numerous benefits: a higher tolerance for physical activity, an improvement of cognitive and cardiovascular functions, a better quality of life, reduced hospitalization, and lower mortality [Revickiet. al. 1995][Panichiet.al 2011].Anemia is corrected with the administration of ESAs. The therapeutic goal is to reach a hemoglobin concentration between 11.0 and 12.0 g/dL [KDOQI Clinical Practice Guidelines 2007] [Locatelli et.al. 2004][Lewis et. al. 2011].Side effects such as hypertension & pure red cell aplasia should be monitored and controlled [MacdougalI et. al. 2009].

In hemodialysis patients on treatment with ESA, iron-deficient erythropoiesis is frequently observed [Fishbane et. al. 2001] with increased need to maintain iron level for maximum response of ESA [Lankhorst et. al. 2010]. The iron deficiency can be absolute (due to malnutrition, gastrointestinal bleeding, chronic blood retention in dialysis circuit and frequent blood sampling) or functional (limitation of bone marrow erythropoietic activity by inability to mobilize sufficient iron from blood store sites, although the body's total iron stores may be normal) [Elliott et. al. 2009].Most hemodialysis patients receive IV iron to help maintain sufficient iron stores as oral supplementation generally is ineffective due to patient non-compliance and gastrointestinal adverse effects [Rozen-Zviet. al. 2008][Gotloib et. al. 2006].

Early detection of iron insufficiency at the level of erythropoietic cell is necessary to optimize management of uremic anemia with recombinant human erythropoietin (rHuEPO) [Eschbach 2005]. Absolute and functional iron deficiency (demand/supply imbalance) and iron deficiency due to inflammation (reticulo-endothelial blockade) are the most important causes of iron insufficiency [Kalantar-Zadeh et. al.2003][Rambod et. al. 2008]. Under the circumstances, transferrin saturation (TSAT) & serum ferritin measurements have been noted to be insensitive & inaccurate measures to detect functional iron insufficiency [Kim et. al. 2008].

The Dialysis patients' Response to IV Iron with Elevated Ferritin (DRIVE) study examined the efficacy of IV iron administration in hemodialysis patients who had Hb less than 11 g/dL on adequate ESA therapy, TSAT less than 25% and serum ferritin of 500-1200 ng/mL. The study showed that administration of eight 125 mg-doses of iron gluconate resulted in more efficient erythropoiesis, a more rapid rise in Hb levels, a decrease in ESA requirements, and adverse events similar to those in a control group that received no IV iron. These findings suggest that there's a spectrum of responsiveness to IV iron that extends to patients with serum ferritin levels as high as 1200 ng/mL [Coyne et. al. 2007].In 2007, the NKF-K/DOQI anemia work group published an updated recommendation that the Hb target for ESA-treated patients should be 11-12 g/dL and a guideline that target should not exceed 13 g/dL [K/DOQI clinical practice guidelines 2007].The NKF-K/DOQI anemia work group suggested that ESA therapy should be used to avoid having the Hb concentration fall below 9.0 g/dL by starting ESA therapy when the hemoglobin is between 9.0-10.0 g/dL for adult CKD 5D patients [KDOQI Clinical Practice Guidelines 2012].

Recurrent or chronic inflammatory processes are common in individuals with CKD, due to many underlying factors, including uremic toxins, elevated levels of circulating pro-inflammatory cytokines, oxidative stress, carbonyl stress, protein-energy wasting (PEW), enhanced incidence of infections (especially, dialysis-access related) and others [Hoen et. al. 1995][Zimmermann et. al. 1999]. The presence

with an inflammatory state may also be closely related to accelerated atherogenesis, Protein Energy Malnutrition (PEM) and anemia [Kalantar-Zadeh et. al.2003][Kalantar-Zadeh et. al. 2005]. A number of positive acute phase reactants, such as, CRP and serum ferritin have been studied as inflammatory markers in CKD patients. Consequently, inflammation may affect the sensitivity of serum ferritin as a marker of iron status [Fleming et. al. 2001].

Assessment of iron status

An assessment of iron status in hemodialysis patients is obtained by the determination of red blood cell indices, blood film, white blood cells and platelets, reticulocyte count, reticulocyte hemoglobin content (CHr), serum iron, total iron binding capacity (TIBC), transferrin saturation (TSAT), serum ferritin, C-reactive protein, percentage of hypochromic red cells and soluble transferrin receptors [16][Wish et. al. 2006][Thomas et. al. 2002].

The serum ferritin and TSAT, both reflecting total body iron stores, are commonly used to indicate iron status in hemodialysis patients. The NKF-K/DOQI guidelines recommended maintaining serum ferritin at or less than 100 ng/ml, or TSAT at or less than 20% to manage iron deficiency [26].However, some papers have reported these cut-off values as insufficient to treat functional iron deficiency in which sufficient iron is not released from the body iron stores to meet an increased demand for iron in the red blood cell production accelerated by EPO administration, despite a normal or even elevated serum ferritin level.

Recently, the reticulocyte hemoglobin content (CHr) has been shown to be a sensitive and specific indicator for functional iron deficiency. Reticulocytes are the youngest erythrocytes released from the bone marrow into the blood and they circulate for 1-2 days before becoming mature erythrocytes. CHr reflects the amount of iron available for hemoglobin production in the bone marrow and has, therefore, been proposed as a marker of iron status [Mittman et. al. 1997]. Fishbaneet al. have demonstrated that CHr has greater sensitivity and specificity for diagnosing iron deficiency than traditional iron measurements and reported that patients with CHr values less than 26 pg are iron deficient and these patients' reticulocyte counts are likely to increase in response to an IV bolus of iron dextran. At a level of26 pg,CHr has a sensitivity of 100% and a specificity of 80% in diagnosing iron deficiency. In contrast TSAT values of less than 20%, and ferritin values of less than 100 ng/ml has sensitivities of 57.1% and71.4%, respectively [Fishbane et. al. 1997] [Schmidt et. al.2005].

A reduction in the percentage of hypochromic red cells (%HYPO) (mean lifetime of 120 days) denotes a longer-term deficiency in iron supply while reduced CHr (mean lifetime of 48 h) is an indicator of current iron deficiency, providing an accurate measurement of bioavailable iron over the previous 3-4 days. In hemodialysis patients, CHr<29 pg has been demonstrated to be a more accurate measure of functional iron deficiency than the combined use of ferritin and TSAT. Furthermore, CHr measurement may serve topredict the response to intravenous (IV) iron therapy within 2-4 days after onset [Thomas et. al. 2002].Specifically, CHr is expected to provide a snap shot of functional iron status because of the reticulocyte's short lifespan, and, more recent papers have reported a correlation between CHr and other indices of iron status in iron deficiency anemia (IDA) of healthy and chronic hemodialysis patients [Kim et. al. 2008][Mittman et.. al. 1997][Tessitore et. al. 2001].

The present study is aimed to assess the relationship between CHr with conventional parameters of iron status, particularly, the transferrin saturation (TSAT) and ferritin, as well as with C-reactive protein (CRP), in order to verify the clinical usefulness of CHr as predictor of iron deficiency state in patients undergoing chronic hemodialysis.

Methods

The present cross-sectional study was conducted on 90 randomly selected chronic hemodialysis patients receiving their treatments at the El-Hamoul Hemodialysis Unit, El-Hamoul Hospital (Ministry of Health Central Hospital) - Kafr El Sheikh, Egypt. There was no exclusion criteria applied during the study. Informed consent was obtained from all participants before enrollment in to the study.

Patients were managed according to the recommendations of the NKF-K/DOQI (National Kidney Foundation, Kidney Disease Outcomes Quality Initiative) anemia guidelines [KDOQI Clinical Practice Guidelines 2012]. All of the patients were treated with a variety of erythropoietin doses given three times a week, at the time of hemodialysis treatment. In addition, the majority of patients were treated with a maintenance dose of intravenous iron (100–200 mg of iron gluconate), weekly or every other week, in order to maintain iron stores at the

recommended levels. Iron supplements were withheld for three weeks before sampling to obtain a reasonable wash-out.

Blood samples for complete blood count (CBC) and CHr were collected in K3-EDTA anticoagulated tubes and analyzed using ADVIA 2120Iautomated hematology analyzer (Siemens, IL USA). All the patients were evaluated for hemoglobin content (Hb%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), CHr, serum ferritin, total iron binding capacity (TIBC), transferrin saturation (TSAT, calculated by serum Iron x100 / TIBC), serum transferrin, Urea Reduction Ration (URR) (calculated as (Urea pre-urea post) x 100 / Urea pre), and C-reactive protein (CRP).Serum iron and total iron-binding capacity (TIBC) were measured usingaLISA 500 plus automated chemical analyzer-COBAS 6000. Serum ferritin was measured with automated analyzer. Transferrin was measured with a BN II automated chemical analyzer (Siemens, IL USA).

Statistical analysis

Statistical analysis was conducted using SPSS version 20.0 for Windows (IBM, USA). Study subjects were divided into two groups according to CRP level. Group A consisting of patients with no infection or inflammation as indicated by low CRP (<5 mg/dL), and Group B, included patients with high CRP (>5 mg/dL). Categorical variables were summarized as counts or percentages and were compared by the Chi-square test. Continuous variables were expressed as mean±SD or median with ranges, and were compared by Student's t-test or the Mann-Whitney test as indicated. Spearman's correlation was performed to detect associations between CHr and other variables. A logistic regression was performed to ascertain the role of CHr as a predictor of iron deficiency anemia (IDA). IDA defined as Hb<11g/dL, Ferritin <200 ng/mL, and TSAT <20%.A two-sided probability value <0.05 was considered statistically significant.

Results

The study included 90 chronic hemodialysis patients, 55 (61.1%) males and 35 (38.9%) females with median age of 55 years (16-75 y). The demographic and mean clinical characteristics of the study population are shown in Table 1. The correlation coefficients of CHr with other hematological variables are listed in Table 2, along with the corresponding *P* values. There was a weak positive correlation of CHr with TSAT and Ferritin, *rs* (90) = 0.281, *P* = 0.007 and *rs* (90) = 0.229, *P* = 0.030, respectively. However, there was a strong positive correlation with mature red blood cell hemoglobin indices, MCH and MCHC, *rs* (90) = 0.851, *P* = 0.000 and *rs* (90) = 0.632, *P* = 0.000, respectively. On the other hand, there was a weak negative correlation between CHr and CRP, *rs* (90) = -0.369, *P* = 0.000. Scatter plots showing the correlations between CHr and other variables are displayed in Figure 1 (A-F).

Table 1: Demographic and clinical characteristics of the study population

Characteristic	Value
Age (years)	55(16-75)
Gender	
Male, n (%)	55 (61.1%)
Female, n (%)	35 (38.9%)
Hb (g/dL)	8.94± 2.02 [a]
Hct (%)	28.94 ± 6.39 [a]
MCV (fL)	84.74±6.1 [a]
MCH (pg)	26.19±2.26 [a]
MCHC (g/dL)	30.86± 0.946 [a]
CHr (pg)	30.21±2.77 [a]
Iron(µg/dL)	64.50 (19 - 194)
TIBC(µg/dL)	230 (150 - 530)
Ferritin (ng/mL)	352.0(0- 2314.0)
TSAT (%)	28.77 (6.77-84.30)
sTfR (mg/dL)	139 (82-427)
CRP (mg/dL)	9.10 (0.6-106.20)
URR (%)	61 (44-71)

Data are shown as median (range). [a] Data are expressed as mean ± SD

Table 2: Spearman correlation coefficients of CHr with hematological variables

Parameter	Correlation test (rs)	P value
Age (years)	-0.192	0.069
Hb (g/dL)	0.270	0.010*
Hct (%)	0.188	0.076
MCV (fL)	0.739	0.000*
MCH (pg)	0.851	0.000*
MCHC (g/dL)	0.632	0.000*
Iron(µg/dL)	0.355	0.001*
TIBC(µg/dL)	-0.017	0.872
Ferritin (ng/mL)	0.229	0.030*
TSAT (%)	0.281	0.007*
sTfR (mg/dL)	-0.132	0.214
CRP (mg/dL)	-0.369	0.000*
URR (%)	-0.173	0.103

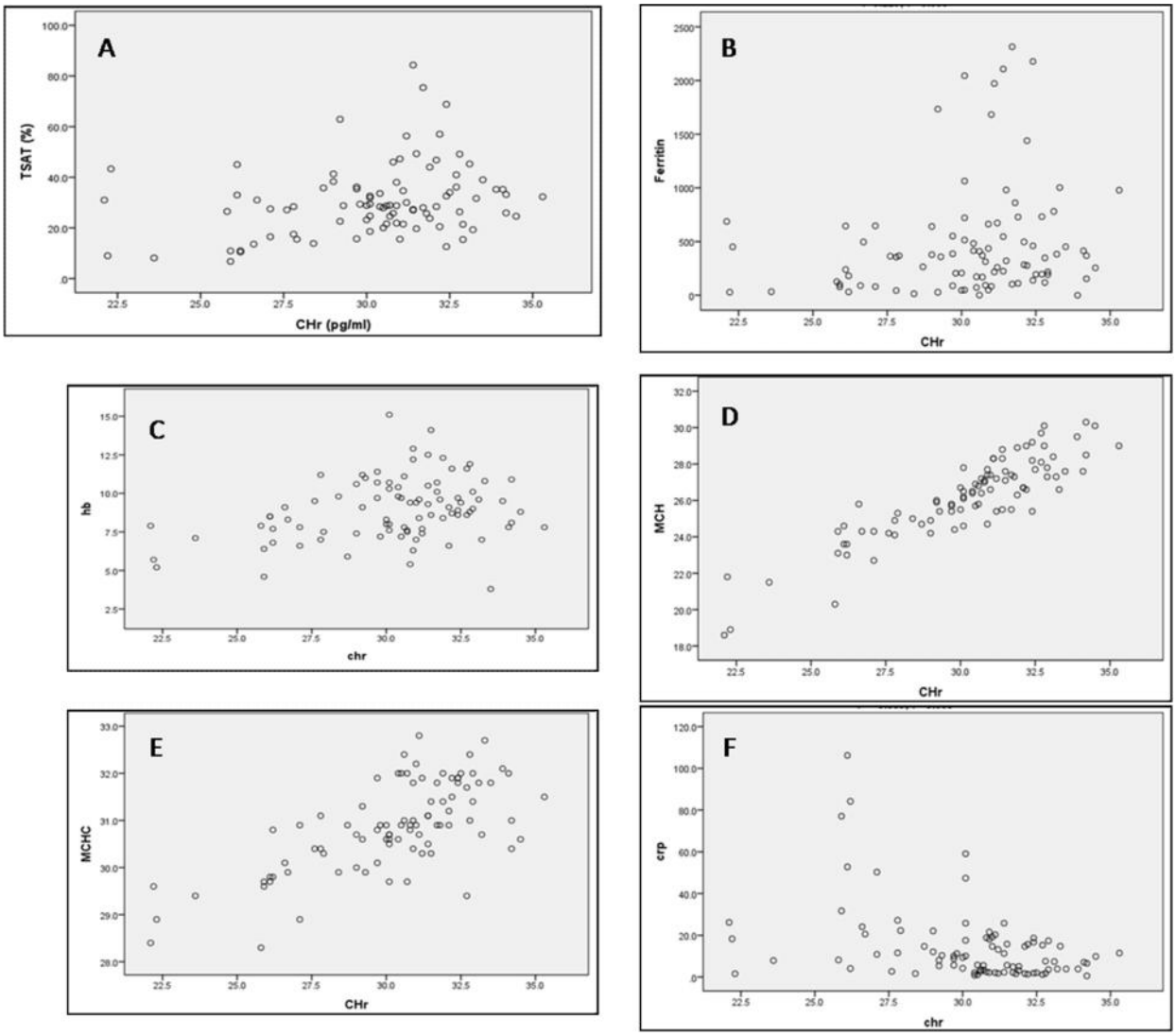

Figure 1Scatter plots showing the correlations between CHr and other hematological variables: (A) TSAT, (B) Ferritin, (C) Hemoglobin, (D) MCH, (E) MCHC, and (F) CRP.

Effect of inflammation

The clinical characteristics of the two subgroups, Group A (without inflammation, CRP < 5 mg/dL) and Group B (with inflammation, CRP > 5 mg/dL) are compared in Table 3. Mann-Whitney U-test was performed to determine whether statistically significant differences in CHr levels exist between patients with and without inflammation/infection (as indicated by elevated CRP levels). Distribution of the CHr for the two groups was similar, as assessed by visual inspection. Median CHr level was significantly lower in Group B patients with inflammation/infection than in Group A patients without inflammation/infection, U = 626, z = -2.45, P = .014.Spearman correlations of CHr with hematological parameters for groups with and without inflammation are presented in Table 4. In the presence of inflammation, CHr was found to be better correlated to MCHC in addition to MCV and MCH, and fairly correlated to Hb, serum Iron and ferritin, compared to Group A.

Table 3: Comparison of clinical characteristics for groups with and without inflammation

Variable	Patients without inflammation (CRP <5)	Patients with inflammation (CRP >=5)	P value
Age (years)	55 (18-73)	55 (16-75)	0.875
Hba (g/dL)	9.01±2.21 [a]	8.90±1.94 [a]	0.555
Hct (%)	29.50 (12.00-40.20)	28.99 (15.60-49.40)	0.842
MCV (fL)	86.10 (65.30-93.60)	84.27 (65.40-96.60)	0.144
MCH (pg)	26.79±2.14 [a]	25.87±2.28 [a]	0.068
MCHC (g/dL)	31.23±0.82 [a]	30.67±0.95 [a]	0.006*
Iron(µg/dL)	73 (31-194)	62 (19-151)	0.164
TIBC(µg/dL)	230 (150-380)	240 (150-530)	0.523
Ferritin (ng/mL)	364 (0-2314)	348 (27.8-2045)	0.842
TSAT (%)	28.80 (11-84.30)	28.4 (6.77-62.90)	0.176
sTfR (mg/dL)	126 (86-301)	146 (82-345)	0.014*

Table 4: Spearman correlations of CHr with hematological parameters for groups with and without inflammation

Variable	Patients without inflammation (CRP <5)		Patients with inflammation (CRP >=5)	
	Correlation	**P value**	**Correlation**	**P value**
Age (years)	0.243	0.188	-0.388	0.002*
Hb (g/dL)	0.113	0.546	0.354	0.006*
Hct (%)	0.086	0.646	0.243	0.064
MCV (fL)	0.711	0.000*	0.747	0.000*
MCH (pg)	0.784	0.000*	0.864	0.000*
MCHC (g/dL)	0.374	0.038*	0.666	0.000*
Iron(µg/dL)	0.329	0.070	0.364	0.005*
TIBC(µg/dL)	-0.086	0.644	0.075	0.571
Ferritin (ng/mL)	0.176	0.343	0.276	0.034*
TSAT (%)	0.329	0.070	0.239	0.069
sTfR (mg/dL)	-0.176	0.342	0.007	0.961
URR (%)	-0.301	0.100	-0.119	0.371

Figure 2 depicts the range of CHr levels for groups A and B. A logistic regression was performed to ascertain the role of CHr as an indicator for the likelihood that participants have iron deficiency anemia (IDA), while adjusting for CRP. IDA is defined as Hb<11 g/dL, Ferritin <200ng/mL, and TSAT <20%. Thirty one patients (34.4%) of the study population were identified to have IDA. However, the logistic regression model was not statistically significant, ¡ ^{2}(4) = 3.965, P = 0.138. Hence, our data suggests that CHr cannot be used as a predictor of iron deficiency state in dialysis patients.

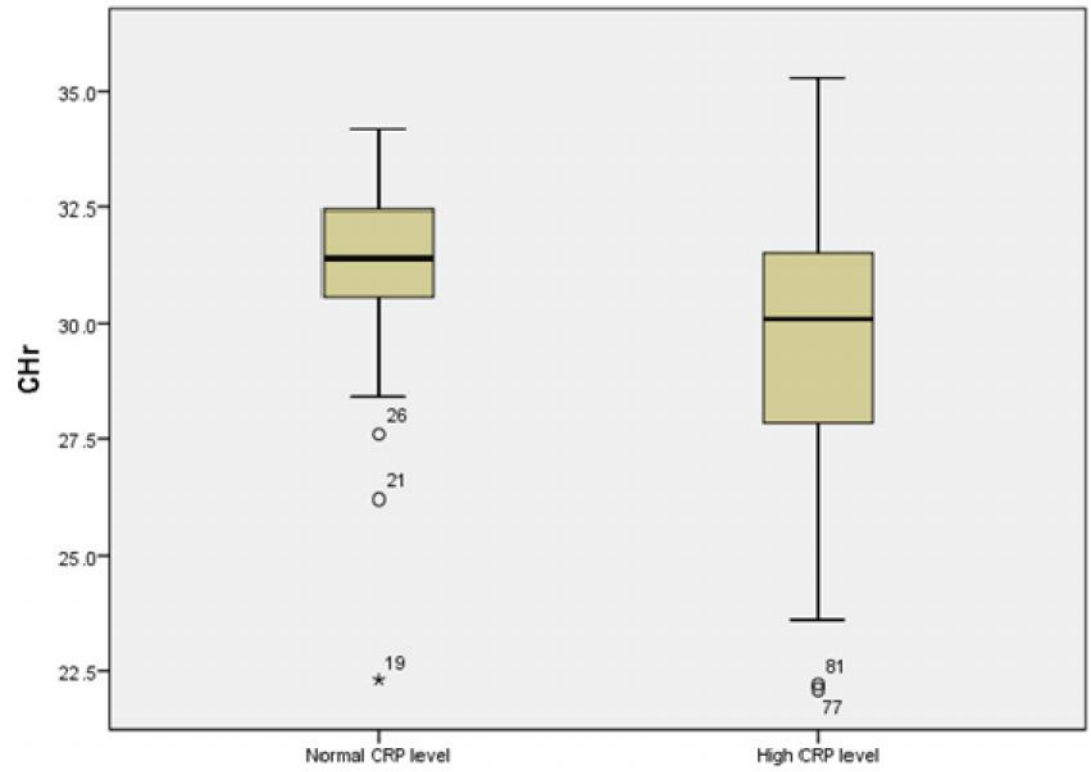

Figure 2 CHr levels in patients: (A) without inflammation, and (B) with inflammation.

Discussion

Functional iron deficiency has been defined as an impaired iron supply from body iron stores despite a normal or elevated serum ferritin, due to accelerated need for iron for erythropoiesis stimulated with administered rhEPO. Recognition of functional iron deficiency is important because patients with this condition will have a sub-optimal response to ruEPO therapy requiring a higher dose to achieve equivalent Hb levels [Thomas et. al. 2002].

The most common tests used to assess iron status are TSAT and ferritin. Several national and international guidelines suggest using such widely available biochemical markers for recognizing iron deficiency, but these tests may sometimes be difficult to interpret in dialysis patients. Both ferritin and TSAT may be affected by factors that are unrelated to iron status such as inflammation. TSAT% is strongly influenced by the daily fluctuation of serum iron levels, and the serum ferritin value is an acute phase protein and, therefore, is increased in chronic inflammatory diseases such as uremia [Rambod et. al. 2008]. The guidelines for treating anemia in patients undergoing hemodialysis and receiving ESAs and IV iron agree on the lower values of TSAT (<20%) at which therapy has to be started, but disagree on the upper value of ferritin which should not be exceeded to avoid the risk of acute and chronic toxicity [KDOQI Clinical Practice Guidelines 2012].

CHris expected to be a sensitive marker of functional iron deficiency because it directly reflects the iron-deficient erythropoiesis at the erythrocyte level, regardless of total body iron stores. CHr is also affected by inflammation, but not to the extent seen with TSAT and ferritin. Furthermore, these tests provide indirect information regarding the amount of iron available in the bone marrow for erythropoiesis. In the present study, an attempt has been made to assess the effect of inflammation on CHr and conventional iron indices as ferritin and TSAT. However, there were statistically significant differences in CHr levels between patients with and without inflammation/infection as indicated by CRP levels. Median values of CHr and other iron indices were CHr31.4pg, TSAT 28.4%, and Ferritin 348 ng/mL in patients with inflammation, compared to CHr 30.1 pg, TSAT 28.8%, and Ferritin 364 ng/mL in patients without inflammation. Therefore, CHr values are also affected by inflammation, like the other iron indices TSAT and ferritin. These results agree with that of Kaneko et al., who have concluded that although CHr reflects the iron status more sensitively, TSAT is a better clinical marker for iron supplementation therapy. Kaneko et al. have demonstrated that TSAT is superior to CHr for the

treatment of iron deficiency and reducing the amount of rhEPO required in Japanese chronic hemodialysis patients with IDA, and concluded that CHr is a sensitive marker that rapidly reflects the change of iron status, but may be insufficient as a target of iron supplementation therapy [Kaneko et. al. 2003].

On the other hand, observation of a weak negative correlation between CHr and CRP, *rs* (90) = -0.369, *P* = 0.000, and a weak positive correlation between CHr and TSAT (r = 0.281, *P* = 0.007), and CHr and Ferritin (r = 0.229, *P*= 0.030) respectively, agree, to a lesser extent, with those ofHakeng et al., whoalso demonstrated a strong and inverse relationship between CHr and CRP, significant positive relationship between CHr and TSAT, but no relationship with serum ferritin [Hakeng et. al. 2004].

There are several aspects of the present study which show a marked deviation or disagreement from other similar studies. Mittman et al. have demonstrated a comparable, if not as dramatic results using CHr<28 pg as a reference point, but with a lower sensitivity and specificity than Fishbane has reported [Fishbane et. al. 1997]. In these studies, most of the patients who had desirable values for ferritin and TSAT demonstrated substantial increase in CHr following iron dextran infusion. This would indicate that these patients were, in fact, functionally iron-deficient despite having normal values for traditional iron measures [Mittman et. al. 1997].

Tsuchiya et al. have demonstrated relationship of CHr with each outcome measure and that CHr was the significant multivariate predictor of iron deficiency. They also concluded that CHr measured simultaneously with Hct, is a sensitive and specific marker of iron status in dialysis patients [Tsuchiya et. al. 2003]. However, logistic regression of the present data demonstrates that CHr cannot be used as a predictor of iron deficiency anemia in these patients.

Kim JM et al. demonstrated the usefulness ofCHr for the assessment of iron deficiency and response to treatment using acut-off value of 32 pg[Kim et. al. 2008].Thomas et al. demonstrated that CHr value <29 pg predicts functional iron deficiency in patients receiving ESA therapy. A reticulocyte haemoglobin equivalent (Ret-He) value <25 pg is suggestive of classical iron deficiency and also predicts functional iron deficiency in those receiving ESA therapy. A Ret-He value <30·6 pg appears to have the best predictive value for likelihood of response to intravenous iron therapy in chronic kidney disease (CKD) patients on haemodialysis[Thomas et. al. 2013].

The present study differs from previous ones in the evaluation of higher sensitivity of CHr compared to TSAT in the assessment of iron status, and reveals no superiority of CHr. This could be due to the clinical status of the patients enrolled in to the study, or due to a difference in the methodology used. Also, hypochromia diagnosed by low MCHC and low CHr can be associated with diseases other than IDA, as low MCV and MCHC are not specific to iron deficiency. Although IDA is the most common microcytic anemia, less commonauses of microcytosis include thalassemic syndromes, and some disorders of heme synthesis including hereditary sideroblastic anemia and acquired disorders of heme synthesis, such as lead poisoning. On occasion, anemia of inflammation can be microcytic [Mast et. al. 2002]. Consequently, CHr cannot be used as a predictor of iron deficiency anemia in chronic dialysis patients. Some studies have suggested use of combination of parameters in assessing iron status in hemodialysis patients, to overcome the effect of inflammation on ferritin and TSAT. Vidyashankar et al. have concluded that in the presence of inflammation, the combination of CHr and hypochromic red cells add to specificity [Vidyashankar et. al. 2013].

CHr is less widely available, and appears to offer no increase in diagnostic sensitivity and specificity over serum ferritin & TSAT, and its usefulness as a marker of iron deficiency is still practically undependable. Therefore, KDIGO continues to recommend the use of serum ferritin and TSAT to define iron stores and iron availability. For all their imperfections, these metrics remain our best routinely-available tools to assess iron status and to manage iron supplementation. In the absence of superior, cost-effective and easily applicable alternatives, this approach seems reasonable [Kliger et. al. 2013]**.**

Conclusion

The present cross-sectional study demonstrates that CHr shows a significant, yet weak association with conventional iron status tests (TSAT and Ferritin) in chronic dialysis patients. However, it cannot be used as a predictor of iron deficiency anemia in those patients. Further, CHr levels are significantly affected by state of inflammation. Larger, population-based studies from multiple centers of dialysis are recommended for better assessment of effectiveness of CHr, as the present results disagree with most previous studies which conclude CHr to bea highly beneficial marker.

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