Usefulness of Reticulocyte Hemoglobin Equivalent in Management of Regular Hemodialysis Patients with Iron Deficiency Anemia

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Introduction. Reticulocyte hemoglobin equivalent (RET-He) is a new parameter for evaluating iron status. This study aims to assess diagnostic value and investigate RET-He as early predictor of response to intravenous iron supplementation.

Methods. Seventy-two regular hemodialysis patients at Adam Malik Hospital were studied from April to May 2011. RET-He was compared with conventional iron parameters for identification of iron deficiency. Fifteen patients with iron deficiency anemia were selected to receive 100 mg iron sucrose intravenous during every dialysis session (2x/weeks) for 4 weeks.

Results. Receiver operating characteristic (ROC) curve for RET-He revealed the value of area under the curve was 0.818 ($p < 0.0001$). Using cutoff level 31.65 pg, RET-He showed 81.5% sensitivity and 61.6% specificity. Serum ferritin ($r = 0.499$, $p < 0.0001$) and transferrin saturation/TSAT ($r = 0.592$, $p < 0.0001$) were correlated to RET-He. Significant improvement in hemoglobin, hematocrit and RET-He were found after intervention ($p = 0.023$, $p = 0.049$ and $p = 0.019$, respectively).

Conclusion. RET-He is a useful marker of iron deficiency and early predictor of response to intravenous iron supplementation in regular hemodialysis patients.

Key words: anemia, iron deficiency, renal dialysis, reticulocyte, ferric compounds.

INTRODUCTION

Anemia is a common complication in patients with chronic kidney disease (CKD), especially those undergoing regular hemodialysis. The major cause of anemia in this specific population is an inadequate quantity of endogenous erythropoietin, but iron deficiency is also frequently found [1]. Iron deficiency in regular hemodialysis patients can be absolute (e.g., malnutrition, gastrointestinal bleeding, chronic blood retention in the dialysis circuit and frequent blood collection) or functional (e.g., limitation of bone marrow erythropoietic activity to mobilize sufficient iron from body storage sites; in this situation the body's total iron stores may be normal) [2].

Treatment of iron deficiency is an important component of care for patients on hemodialysis with numerous benefits such as a higher tolerance for physical activity, an improvement of cognitive and cardiovascular function, a better quality of life, reduced hospitalization, and lower mortality. It has been well established that intravenous iron supplementation is essential for the great majority of regular hemodialysis patients with iron deficiency anemia [2-3]. It is essential to select which patient need iron supplementation because parenteral iron administration has potential immediate risk (e.g., toxic effect and anaphylactic reaction) and long-term risk (e.g., decreased polymorphonuclear leu-

kocyte function, hepatic dysfunction, cardiovascular and infectious morbidities) [4].

Several international guidelines like the European Best Practice Guidelines and United States Kidney Disease Outcome Quality Initiative suggest using conventional widely available biochemical markers (serum ferritin and serum transferrin saturation/TSAT) for recognizing iron deficiency [2]. However, their validity for diagnosis of iron deficiency in CKD patients is still debatable. These conventional parameters are indirect markers; serum ferritin is an acute phase protein and thus can be increased in chronic inflammatory disease such as uremia [2-3]. TSAT has a wide diurnal variation and is also affected by nutritional status [5]. Therefore, additional parameters that can directly estimate bone marrow iron availability have been investigated, such as percentage of hypochromic erythrocytes (%HYPO), reticulocyte hemoglobin content (CHr), erythrocyte zinc protoporphyrin (Er-ZPP) and reticulocyte hemoglobin equivalent (RET-He) [2, 3].

RET-He estimates the amount of hemoglobin in the reticulocyte, with reasonably good reflection of how much iron was available for erythropoiesis in bone marrow [6-7]. Since reticulocyte has a more rapid turnover in circulation than mature erythrocyte, it is hypothesized that reticulocyte may be more sensitive in detecting erythropoietic activity. RET-He can be measured with an existing

common blood analyzer installed with upgraded software [8]. RET-He is advantageous in that it has no interference with inflammatory conditions, has early response to iron therapy and lower cost compared to conventional parameters. Previous study from Brugnara *et al.* (2006), Miwa *et al.* (2010) and Buttarello *et al.* (2010) showed that RET-He can be used as alternative parameters for iron deficiency anemia in regular hemodialysis patients [2, 6, 8]. Therefore, this study aims to assess diagnostic value of RET-He in identification of iron deficiency in regular hemodialysis patients and to investigate RET-He as early predictor of response to intravenous iron supplementation.

MATERIALS AND METHODS

PATIENTS

This study was conducted at hemodialysis center in Adam Malik Hospital, Medan, Indonesia. Seventy-two regular hemodialysis patients were studied from April till May 2011. The inclusions criteria were patients on hemodialysis for three months or more, older than 18 years and who agreed to participate. Patients were excluded if any of the following events occurred during the study: clinically significant bleeding or blood transfusion, received oral or parenteral iron supplementation, evident of inflammation, infectious disease or malignancy. This study was given clearance by Ethical Committee of Faculty of Medicine Sumatera Utara University.

STUDY DESIGN

RET-He was compared with conventional iron parameters (serum ferritin and/or TSAT) for identification of iron deficiency. Patients with iron deficiency anemia (hemoglobin ≤ 10 g/dL or hematocrit $\leq 30\%$ with serum ferritin < 100 ng/mL and/or TSAT $< 20\%$) were selected to receive 100 mg iron sucrose intravenous during every dialysis session (2 $\times$ /weeks) for 4 weeks. Hemoglobin, hematocrit and RET-He were measured before and following iron supplementation.

STATISTICAL ANALYSES

Receiver operating characteristic (ROC) curve was utilized to illustrate the diagnostic performance of RET-He. Cutoff value of RET-He was determined and followed by diagnostic performance analysis.

Correlations between conventional parameters and RET-He were tested with Pearson correlation analysis, in the case of no normal distribution data, Spearman's correlation analysis would be used. Changes in hemoglobin, hematocrit and RET-He before and after intervention analyzed using paired t test with p values < 0.05 were considered statistically significant.

RESULTS

ANEMIA IN PATIENTS WITH CHRONIC KIDNEY DISEASE

There were 72 regular hemodialysis patients enrolled in this study, 54 patients (75%) with anemia. Seven patients (43.7%) with anemia were defined as iron deficiency based on serum ferritin < 100 ng/mL and TSAT $< 20\%$, while serum ferritin level in 9 other patients (56.2%) were extremely high > 500 ng/mL (Figure 1). Table 1 summarizes baseline characteristic data on each parameter analysed.

DIAGNOSIS PERFORMANCE OF RET-He

ROC curve analysis in this study demonstrated the ability of RET-He in diagnosis iron deficiency with area under the curve of 0.818 ($p < 0.0001$) (Figure 2). Using cutoff level 31.65 pg, RET-He showed 81.5% sensitivity and 61.1% specificity.

CORRELATION BETWEEN RET-He AND CONVENTIONAL IRON PARAMETERS

Correlations between RET-He and serum ferritin were analyzed with Spearman's correlation due to no normal distribution data of serum ferritin. The result showed there were moderate correlations between RET-He and serum ferritin ($r = 0.499$, $p < 0.0001$). RET-He and TSAT data have normal distribution, therefore Pearson correlation analysis used to test the correlation between RET-he and TSAT. This study found a moderate correlation between a RET-He and TSAT ($r = 0.592$, $p < 0.0001$).

CHANGES IN PARAMETERS FOLLOWING IRON SUPPLEMENTATION

There were 16 patients (29.6%) defined as iron deficiency anemia in this study but 1 patient was lost of follow-up. A total of 15 subjects received

100 mg iron sucrose intravenous during every dialysis session (2×/weeks) for 4 weeks. Mean hemoglobin, hematocrit and RET-He before intervention were 8.65 ± 1.09 g/dL, $28.02 \pm 4.25\%$ and 29.98 ± 3.85 pg, respectively. After 4 weeks intervention they were 9.98 ± 1.77 g/dL, $31.76 \pm 5.31\%$ and 32.6 ± 3.24 pg, respectively. Mean improvement in

hemoglobin, hematocrit and RET-He were 1.33 g/dL, 3.7% and 2.6 pg, respectively (Figure 3). In this study, hemoglobin rises 1-2 g/dL only in 6 patients (40%), 4 patients (26.7%) showed rise in hemoglobin <1 g/dL, 3 patients (20%) with decrease hemoglobin and 2 patients (13.3%) showed no changes in hemoglobin level (Figure 4).

Table 1
Baseline characteristic data of patients

Variables	with anemia (n = 54)		without anemia (n = 18)
	with iron deficiency (n = 16)	without iron deficiency (n = 38)	
Age (year)*	49.25 ± 11.58	45.18 ± 12.97	43.11 ± 17.67
Men (number)	15	16	12
Hemoglobin (g/dL)*	8.60 ± 1.07	8.00 ± 1.35	11.36 ± 1.16
Hematocrit (%)*	27.92 ± 4.12	24.69 ± 4.49	35.02 ± 3.70
RET-He (pg)*	29.88 ± 3.74	35.07 ± 3.45	33.95 ± 2.79
Serum ferritin (ng/mL)*	484.375 ± 717.82	1362.53 ± 702.77	1397.92 ± 623.51
TSAT (%)*	15.48 ± 4.09	51.90 ± 22.20	57.46 ± 29.25

* $\bar{x} \pm SD$ (standard deviation); RET-He (Reticulocyte hemoglobin equivalent); TSAT (transferrin saturation).

Figure 1. Partition of patients. (TSAT-transferrin saturation).

Figure 2. ROC curve analysis for RET-He in diagnosis of iron deficiency anemia in regular hemodialysis patients.

Figure 3. Mean hemoglobin, hematocrit and RET-He before and after iron administration (*paired t test; **mean improvement; Hb (hemoglobin); Ht (hematocrit); RET-He (reticulocyte hemoglobin equivalent)).

Figure 4. Distribution of hemoglobin and RET-He. (a) Before, (b) after 4 weeks of iron supplementation. (Hb (hemoglobin); RET-He (reticulocyte hemoglobin equivalent)).

DISCUSSION

Anemia is present in the majority of patients with CKD, especially on hemodialysis [1]. Anemia is typically defined as a level of circulating hemoglobin of less than 12 g/dL in women or 14 g/dL in men or a hematocrit of less than 37% in women or 40% in men [1, 9]. United States Renal Data System (USRDS) 2010 reported incidence of anemia in CKD stage 1-4 was 51.8% and mean hemoglobin in end stage renal disease was 9.9 g/dL [1]. Cipto Mangunkusumo Hospital, Jakarta (2010) reported that all of their new hemodialysis patients (100%) had anemia with mean hemoglobin 7.7 g/dL [1]. Majority of patients in this study (75%) had anemia, with mean hemoglobin 8.9 g/dL.

The major cause of anemia in CKD patients is erythropoietin deficiency, but iron deficiency is also frequently found [1]. The National Health and Nutritional Examination Survey (2004) reported iron deficiency in 57.8-58.8% men and 69.9-72.8% women with CKD. Data from The Dialysis Outcomes and Practice Pattern Study (DOPPS) 2003 reported that iron deficiency was found in 31-38% of CKD patients on hemodialysis [10]. In this study, approximately 30% of anemia in CKD patients was due to iron deficiency.

In general, serum ferritin <100 ng/mL and/or TSAT <20% were defined as absolute iron deficiency and serum ferritin ≥100 ng/mL and/or TSAT <20% were considered functional iron deficiency [1]. In CKD patients, serum ferritin cutoff level for absolute

iron deficiency is markedly higher as result of chronic inflammation, infection, malnutrition or malignancy and not necessarily the result of iron overload. Previous study reported that serum ferritin level 100 ng/mL has a low sensitivity and specificity and also may even underestimate the frequency and the severity of iron deficiency in hemodialysis patients [11]. In the United States, approximately 60% of dialysis patients have serum ferritin >500 ng/mL, and 22% have >800 ng/mL [12]. In this study, there were only 7 patients (43.7%) defined as iron deficiency based on serum ferritin <100 ng/mL and TSAT <20% with mean serum ferritin >100 ng/dL that could be due to chronic inflammation in CKD patients (Table 1). Therefore, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) (2006) recommend testing of hemoglobin, serum ferritin and TSAT or hemoglobin content (CHr) together because this combination provides important insight into external iron balance and internal iron distribution [13]. CHr is the only new iron parameter recommended by NKF-KDOQI to assess iron deficiency anemia in CKD patients. Previous study by Frank *et al.* (2004) showed a good correlation between RET-He and CHr, with the advantages that RET-He can be measured using existing blood analyzer with installation of XE RET master software, while CHr can only be measured with Bayer ADVIA hematology analyser (Siemens Healthcare Diagnostic Inc) [14]. In Indonesia, RET-He were measured together with complete blood count with total cost only 20% of conventional iron parameters.

Previous study by Canals *et al.* (2005), Brugnara *et al.* (2006), Grazia *et al.* (2007), Maconi *et al.* (2008), Miwa *et al.* (2010), Urrechaga *et al.* (2011) and Buttarello *et al.* (2010) found good diagnostic performance of RET-He in diagnosis iron deficiency with various cutoff level between 25-32.4 pg [2, 6, 8, 15-18]. This study also showed good diagnostic performance of RET-He with cutoff level 31.65 pg (Figure 1).

Study by Miwa *et al.* (2010) showed there was a good correlation between RET-He and TSAT ($r = 0.543$, $p < 0.01$) but a weak correlation between RET-He and serum ferritin ($r = 0.279$, $p < 0.01$) [8]. This study also showed there were lower correlations between RET-He and serum ferritin ($r = 0.499$, $p < 0.0001$) compared to RET-He and TSAT ($r = 0.592$, $p < 0.0001$). Table 1 demonstrated high serum ferritin in CKD patients with or without iron deficiency anemia that may be due to serum ferritin as acute phase protein, while the value of RET-He has no interference with inflammatory condition.

Significant improvement in hemoglobin, hematocrit and RET-He following iron supplementation were found in this study (Figure 3). Similar result was found in the study of Chuang *et al.* (2003) and Miwa *et al.* (2010) where a significant improvement in hemoglobin was found after 4 weeks of iron supplementation [3, 8]. The increase in hemoglobin is not only due to improvement of iron status but also it may reflect resolution of an inflammatory state [12]. Iron therapy has anti inflammatory effect which increases anti inflammatory cytokines interleukin (IL)-4 and decreases pro inflammatory cytokine tumor necrosis factor (TNF)- α [11]. Based on these findings, intravenous iron supplementation accompanied with monitoring of hemoglobin, hematocrit and RET-He within 4 weeks is worthwhile for clinical practice. While monitoring of iron status using serum ferritin and TSAT should only be measured after 3 months intervals [11]. Therefore, early recognition of non-responsiveness to intravenous iron supplementation, improving therapeutic efficacy as well as avoiding the inadvertent hazard related to overtreatment with iron can be achieved using RET-He.

There are several limitations of this study. No further evaluation was conducted to find the possible cause of no improvement in hemoglobin, hematocrit or RET-He in several patients after iron supplementation. Measurement of inflammatory marker such as C reactive protein (CRP) as evidence of chronic inflammatory condition in regular hemodialysis patients was not conducted.

Further multicenter research is needed in order to recommend RET-He as regular diagnostic parameter for iron deficiency anemia in CKD patients. RET-He could also be a potential parameter to monitor early responses to intravenous iron supplementation in dialysis patients.

CONCLUSIONS

RET-He is considered as a useful alternative parameter of iron deficiency anemia in regular hemodialysis patients due to good diagnostic performance, availability of blood analyzer and lower cost. RET-He increased promptly after iron supplementation, therefore it could be used as early predictor of response to intravenous iron supplementation.

Conflict of Interest: The authors declare there is no conflict of interest in this study.

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Introducere. Echivalentul hemoglobinei în reticulocit (RET-He-Reticulocyte hemoglobin equivalent) este un nou parametru pentru evaluarea rezervelor de fier. Studiul își propune analiza valorii diagnostice și rolul RET-He ca predictor precoce al răspunsului terapiei marțiale intravenoase.

Metode. În aprilie-mai 2011 au fost recrutați 72 de pacienți cu hemodializă din Spitalul Adam Malik. RET-He a fost comparată cu parametrii uzuali ai metabolismului fierului pentru a identifica deficitul de fier. 15 pacienți cu anemie feriprivă au primit 100 mg de fier intravenos cu ocazia fiecărei sesiuni de dializă (bisăptămânal timp de 4 săptămâni).

Rezultate. Curba ROC a RET-He pentru diagnosticul deficitului de fier a avut valoarea 0.818 ($p < 0.0001$). Folosind o valoare prag de 31.65 pg, RET-He a avut 81.5% sensibilitate și 61.6% specificitate pentru diagnosticul deficitului de fier. Feritina serică și saturația transferinei au fost corelate pozitiv cu RET-He ($r = 0.499$, $p < 0.0001$ respectiv $r = 0.592$, $p < 0.0001$). După terapia marțială valorile hemoglobinei, ale hematocritului și ale RET-He au fost semnificativ statistic îmbunătățite ($p = 0.023$, $p = 0.049$ și respectiv $p = 0.019$).

Concluzii. RET-He este un marker util pentru diagnosticul deficitului de fier și un predictor precoce al răspunsului terapiei marțiale la pacienții cu hemodializă.

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