Reticulocyte hemoglobin equivalent (RET-He) as a predictor of response to intravenous iron in hemodialysis patients: A hospital based analytical study

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Abstract: Background: Among chronic kidney disease CKD patients, iron deficiency anemia is a common. The administration of iron is important during the treatment with erythropoiesis-stimulating agents (ESA). Reticulocyte hemoglobin content (RET-He) is a diagnostic marker for IDA. Measuring the RET-He can predict the iron status in respond intravenous (IV) iron supplementation in CKD patients. The current study was detect the cut-off value of RET-He as the target of iron supplementation in patients with CKD. **Methods:** This hospital based observational and analytical study included 50 CKD patients on hemodialysis (CKD-HD) in the maintenance phase of erythropoietin therapy and not receive any iron supplementation. Blood count, RET-He, and iron were studies for all patients. For each patients, we analyzed two samples: a baseline sample and another sample after 4 weeks of IV iron administration. The patients were classified into two groups regarding the optimal correction of anemia (OCA) after IV iron thereby; (1) patients how achieved the OCA (Hb ≥13.5 g/ in dl males and ≥12 g/dl for females); (2) patents who did not achieved the OCA compared to the baseline. Operating characteristic analysis (ROC) was used to determine the cut of value for predicting the response to iron administration achieving the OCA. **Results:** Out of 50 included CKD-HD patients, 33 patients achieved the OCA and only 17 patents not achieved the OCA. There was a statistical significant increase in HER-He after 5 weeks of IV iron supplementation comparing to the baseline values. ROC curve analysis a RET-He cutoff level of 26.9 pg, iron deficiency could diagnosed by a sensitivity of 70.6%, and a specificity of 51.5%.

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1. Introduction

Chronic kidney disease (CKD) is a worldwide public health problem with a significant cost[2]. It is manifest in various ways depending upon the underlying cause and the severity of disease. Later, loss of appetite or heart diseases. Lack of red blood cells in bone marrow lead to anemia [3]–[5]. Iron deficiency anemia (IDA) is a frequent complication in chronically hemodialyzed CKD patients (CKD-HD)[6]. Iron deficiency is the most common nutritional deficiency worldwide affecting about 1.48 billion people[7]. The prevalence of anemia with estimated glomerular filtration rate (eGFR) of 60 mL/min/1.73 m2 is 9%. While, at eGF. R of 30 mL/min/1.73 m2, it increased to 33%. Patients with CKD required to receive erythropoiesis-stimulating agents (ESAs) together with iron supplementation. ESAs were first used in to treat anemia in patients with chronic renal failure who have a hemoglobin (Hb)<10 g/dL, providing the transferrin saturation (TSAT) is more than 25% and ferritin >200 ng/mL [8], [9]. The 2012 KDIGO and National Institute Health Care Excellence (NICE) suggest evaluate iron status at least every three months during ESA treatment and more frequently when increasing the ESA dose or monitoring the response to intravenous (IV) iron when iron stores may be depleted [10], [11]. Therefore, All CKD patients associated with anemia, especially those on dialysis and receive ESA treatment will require serum iron monitoring due to the confirmed concerns regarding the adverse effects associated with supplemental iron (as liver toxicity as well as hypotension, coagulopathy, and GIT iteration) or elevated doses of ESAs.

Iron-stained bone marrow aspiration was the gold standard to iron stores and diagnose IDA. It was replaced nowadays by less invasive parameters as serum ferritin (SF) and serum transferrin receptor (sTfR). Nevertheless, the sensitivity and specificity of SF and sTfR, are far from satisfactory in CKD patients because CKD is known to be large in the underlying kind of inflammation[12]. Therefore, these tests are replaced by alternative parameters that are to assess iron status in CKD-HD patients as reticulocyte hemoglobin content (CHr) using ADVIA analyzer or reticulocyte hemoglobin equivalent (RET-He) using Sysmex analyzer [13]–[16]. Compared with erythrocytes, the shorter lifespan (one or two days) of

reticulocytes makes RET-He a better biomarker to reflect iron status in the short term [17], [18]. A decreased value of RET reflects on reduced of cellular hemoglobin content and identified iron deficiency [19]. It was proved that the imbalance iron requirements, the reduction of cellular hemoglobin content in newly produced reticulocytes[14], [20].

We hypothesized that measuring the RET-He can predict the iron status in respond IV iron supplementation in CKD-HD patients. In addition, we aimed to determine the cut-off value of RET-He as the target of iron supplementation in patients with CKD-HD.

2. Materials and Methods

1.1. Study Subject:

This hospital based observational and analytic study was conducted at a tertiary care center in hemodialysis unite at Zagazig university hospitals during January 2017 to March 2018. The protocol and consent forms were reviewed and approved by the institutional review board of participating institution (IRB#:3440/5-3-2017).

Eligible patients were age ≥ 18 years old with CKD and on regular central dialysis (two or more times per week), folic acid (twice per peek), vitamin B12 (three times per week) supplementation, and receiving a maintenance dose of erythropoietin (50 units/kg three times per week for both intravenous and subcutaneous administration). Exclusion criteria were: (1) pregnant women; (2) patients who had a blood transfusion, oral, or intravenous iron supplement within a four weeks; (3) patients who suffered from hemoglobinopathy, thalassemia, macrocytosis, leukemia, myeloma, or myelodysplastic syndrome. All included CKD-HD patients received 100 mg IV iron sucrose given at each consecutive hemodialysis treatment for a total of 10 doses (a total of 1000 mg in five weeks) regardless of the Hb and regardless of whether patients were treated with an ESA. Two samples were analyzed for each patient; the first sample was taken before iron administration and considered the baseline sample, the second sample was taken after complete IV iron administration. The patients were classified into two groups regarding the optimal correction of anemia (OCA) after IV iron thereby; (1) patients how achieved the OCA (Hb ≥13.5 g/dl in males and ≥12 g/dl in females); (2) patients who did not achieved the OCA (Hb<13.5 g/dl in males and <12 g/dl in females) regardless the baseline Hb level.

1.2. Analytical Methods

Venous blood samples were collected in EDTA anticoagulant tubes. A centrifugal speed freezing centrifuge was used to centrifuge blood samples. Sysmex XN-2000 hematology analyzer was used to detect complete blood count, including Hb, RET-He, hematocrit (HCT), mean cellular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin content (CH), iron indexes of serum iron, SF, sTfR, transferrin saturation (TSAT) and total iron-binding capacity (TIBC).

1.3. Statistical analysis

Statistical analyses were performed using the statistical software program, SPSS, for Windows version 25.0 (SPSS; Chicago, IL, USA). Results were given as mean ± standard deviation (±SD) and parametric independent t test was performed to detect statistical differences between the two group of patients (Achieved and not achieved the OCA) when the variables showed normal distribution by Kolmogorov-Smirnov test. Mann-Whitney test was used for skewed data of the two independent groups while, Wilcoxon signed ranks test was used to assess the difference in the hematological parameters between the paired data. The correlation between RET-He and other hematological parameters was evaluated by Pearson's correlation test for parametric data or Spearman's rank correlation test for non-parametric data. We also identified the optimal cutoff value of RET-He and other hematological parameters for predicting the response to iron administration using the receiver operating characteristic analysis (ROC). The goal was to determine the diagnostic performance of the Ret He parameter against the existing diagnostic tests for iron-deficiency diagnosis (serum iron < 40ug/dl, TSAT <20%, ferritin <100 ng/ml and hemoglobin <11 g/dl. A value of $p < 0.05$ was considered statistically significant with 95% confidence interval.

3. Results

We included 50 CKD-HD patients aged 57 (±11.7) with average dialysis duration of 3.7 (1-8) years. Of them, 33 patients achieved the OCA and only 17 patents not achieved the OCA. The demographic characteristics and biochemical findings of the two groups at the baseline are shown in Table 1. Hb, RBC, and serum ferritin were statistically higher in the group who achieved the OCA, $P < 0.05$. Interestingly, serum iron and RET-He did not differ significantly between the two groups at the baseline. Table 2 showed the changes in parameters reflecting Hb content in the two groups after receiving 100 mg IV iron sucrose given at each consecutive hemodialysis treatment for a total of 10 doses.

MCV, MCHC, MCH, Hb were higher in the group who achieved the OCA but, the results were statistically insignificant, $P > 0.05$. Interestingly, RET-He was significantly higher in patients who achieved the OCA (MD= -2, 95%CI (-3.1 to -1.26), P<0.001) compared to the non-achieved group.

Using Wilcoxon test revealed a statistical significant increase in HER-He and other hematological parameter after 5 weeks of IV iron supplementation (1000 mg in total) comparing to the baseline values as shown in Table 3 and Figure 1.

There was a strong positive correlation between RET-He and serum iron and Hb in CKD-HD patients as seen in Figure 2 (r=0.82; p<0.001 and r=91; p<0.001, respectively). While, a week but significant correlation was observed between the RET-He and SF (r=0.45, p=0,001), TSAT (r=0.36. p=0.009) and TIBC (r= -0.54, p=0.001).

Table 4 showed the diagnostic performance of hematological parameters including RET-He, HCH, MCHC, MCV, and RDW to detect the achievement of optimal correction of Anemia in CKD-HD patients after IV iron supplementation. ROC curve analysis can use a RET-He cutoff level of 26.9 pg, Deficiency of iron could be diagnosed with a sensitivity of 70.6%, and a specificity of 51.5%. The area under the curve was 0.6.

Table 1: Characteristics of enrolled patients at the baseline.

		Achieved the OCA (N=33)		**Not Achieved the OCA (N=17)**		**Mean Difference 95% CI**	**P value**
		Mean ± SD	Range	Mean ± SD	Range		
		Demographic Data					
Age (Year)		54.9 ± 12.2	30-70	58 ± 11.4	22.75	3.1 (-4.1 to 10.27)	0.38
Dialysis duration (Year)		3 ± 1.8	1-8	4.1 ± 1.7	1-7	1.1 (0.04 to 2.16)	0.025
Gender	Female N (%)	15 (88.2%)	-	10 (30.3%)	-	-	< 0.001
	Male N (%)	2 (11.8%)	-	23 (69.7%)	-		
		CBC Data					
RET-He (pg)		27 ± 0.9	25-28.5	26.6 ± 1.3	24-29.5	-0.4 (-1.03 to 0.23)	0.262
HB (g/dl)		9.9 ± 0.6	8.6-11	9.4 ± 0.9	8.1-11	0.05 (-0.93 to -0.07)	0.034
RBCs		3.5 ± 0.2	3-3.8	3.4 ± 0.2	3-4.1	-0.1 (-0.22 to 0.02)	0.033
MCV (fl)		70.9 ± 6.3	61.8-78	69.2 ± 4.4	61.8-77	-1.7 (-5.14 to 1.74)	0.182
MCHC (g/dl)		39.5 ± 1.1	37.8-41.2	39.6 ± 1.1	37.6-41	0.1 (-0.56 to 0.76)	0.984
MCH (pg)		27.9 ± 2.6	24.4-32.1	27.4 ± 1.8	4.4-31.7	-0.5 (-1.92 to 0.92)	0.478
		Iron Indices					
Serum iron (ug/dl)		42.5 ± 8.7	27-56	38.9 ± 10.4	25.9-59	-3.6 (-9.18 to 1.98)	0.179
TIBC (ug/dl)		267.9 ± 11	255-300	282 ± 35.3	220-357	14.1 (0.73 to 27.4)	0.098
Serum Ferritin (ng/dl)		283± 84.5	108-425	227.5 ± 81.5	86-425	24.9 (-105 to -5.3)	0.027*
TSAT (%)		20.1 ± 2.6	16-25	19.3 ± 3	15-26	-0.8 (-2.44 to 0.84)	0.347

OCA: Optimal Correction of Anemia; SD: Stander Deviation; CI: Confidence Interval; RET-He: Reticulocyte Hemoglobin Equivalent; HB: Hemoglobin; RBCs: Red Blood Cells; MCV: Mean corpuscular volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin; TSAT: Transferrin Saturation. All variables were compared using Mann Whitney test.
* Independent T test; ** Chi square test

Table 2: Comparison of changes in RBCs Indices as regard achievement of optimal correction of Anemia

	Achieved the OCA (N=33)		**Not Achieved the OCA (N=17)**		**Mean Difference 95% CI**	**P value**
	Mean ± SD	Range	Mean ± SD	Range		
RET-He (pg)	7.9 ± 1.2	5.7-9.8	5.9 ± 1.3	3.3-9.8	-2 (-3.1 to -1.26)	<0.001
RBCs	1 ± 0.5	0.4-2	0.7 ± 0.4	-0.3-2	-0.3 (-0.58 to -0.02)	0.143
MCV (fl)	3.3 ± 7.7	-6.8-18.2	4.4 ± 6.4	-9-17.7	1.1 (-3.28 to 5.48)	0.461
MCHC (g/dl)	-3.7 ± 1.2	-6--1.8	-3.9 ± 2.4	-7.7-1.7	-0.2 (-1.22 to 0.82)	0.315
MCH (pg)	0.1 ± 3.2	-5.6-4.5	1.5 ± 3.5	-5.3-9.7	1.4 (-0.58 to 3.38)	0.335
HB (g/dl)	2.5 ± 0.7	1.5-3.7	2.3 ± 0.5	1.3-3.4	-0.2 (-0.58 to 0.18)	0.446

OCA: Optimal Correction of Anemia; SD: Stander Deviation; CI: Confidence Interval; RET-He: Reticulocyte Hemoglobin Equivalent; HB: Hemoglobin; RBCs: Red Blood Cells; MCV: Mean corpuscular volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin.
All variables were compared using Mann Whitney test

Table 3: The difference in parameters reflecting the response of IV iron supplementation after 5 weeks (1000 mg in total)

	Baseline parameter before IV iron administration		**After a total of1000 mg IV iron administration**		**P value**
	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)	
	CBC Data				
RET-He (pg)	26.7 ± 1.2	27 (24-29.5)	33.3 ± 1.8	33.1 (30-36)	<0.001
HB (g/dl)	9.6 ± 0.8	9.9 (8.1-11)	11.9 ± 0.7	12 (11-13.6)	<0.001
RBCs	3.4 ± 0.2	3.5 (3.1-4.1)	4.3 ± 0.5	4.2 (3.5-5.5)	<0.001
MCV (fl)	27.6 ± 2.1	28 (24.4-32.1)	28.6 ± 2.3	28.9 (23.1-37)	0.076
MCHC (g/dl)	39.6 ± 1.1	39.4 (37.6-41.2)	35.7 ± 1.9	36 (31.4-41)	<0.001
MCH (pg)	69.8 ± 5.1	69.6 (61.8-78)	73.8 ± 4	73.9 (65.4-85.2)	0.001
	Iron Indices				
Serum iron (ug/dl)	40.1 ± 9.9	38 (25.9-59)	74.4 ± 7.3	73 (60-91)	<0.001
TIBC (ug/dl)	277.2 ± 30	270 (220-357)	276 ± 66.1	266 (219-587)	0.06
Serum Ferritin (ng/dl)	246.6 ± 86	252.5 (86-425)	266.3 ± 11.4	268.5 (238-280)	0.092
TSAT (%)	19.6 ± 2.9	19 (15-26)	34.4 ± 3.9	34 (29-44)	<0.001

OCA: Optimal Correction of Anemia; SD: Stander Deviation; CI: Confidence Interval; RET-He: Reticulocyte Hemoglobin Equivalent; HB: Hemoglobin; RBCs: Red Blood Cells; MCV: Mean corpuscular volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin; TSAT: Transferrin Saturation.

All variables were compared using Mann Whitney test.

Table 4: The diagnostic performance of different hematological parameters to detect the achievement of optimal correction of Anemia in CKD-HD patients after IV iron supplementation.

	Cut-off value	**AUC (95% CI)**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
RET-He (pg)	>26.9	0.61 (0.449-0.733)	70.6 (44.0 - 89.7)	51.5 (33.5 - 69.2)	42.9 (24.5 - 62.8)	77.3 (54.6 - 92.2)
MCH (pg)	>27.3	0.561 (0.414-0.701)	70.5 (44.0 - 89.7)	48.5 (30.8 - 66.5)	41.4 (23.5 - 61.1)	76.2 (52.8 - 91.8)
MCHC (g/dl)	>39.3	0.502 (0.357-0.646)	58.8 (32.9 - 81.6)	54.6 (36.4 - 71.9)	40.0 (21.1 - 61.3)	72.0 (50.6 - 87.9)
MCV (fl)	>74.4	0.616 (0.468-0.750)	41.2 (18.4 - 67.1)	90.9 (75.7 - 98.1)	70.0 (34.8 - 93.3)	75.0 (58.8 - 87.3)

RET-He: Reticulocyte Hemoglobin Equivalent; MCV: Mean corpuscular volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin.

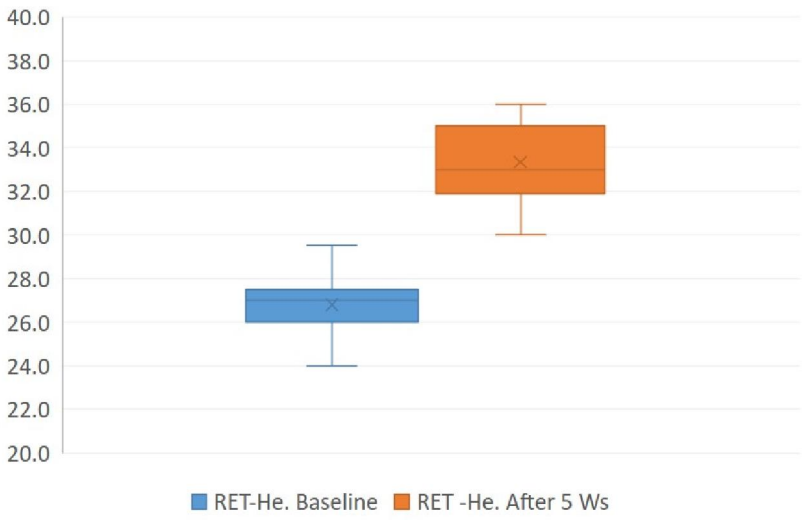

Figure 1: Difference between the baseline HER-He and after 5 weeks of IV iron supplementation

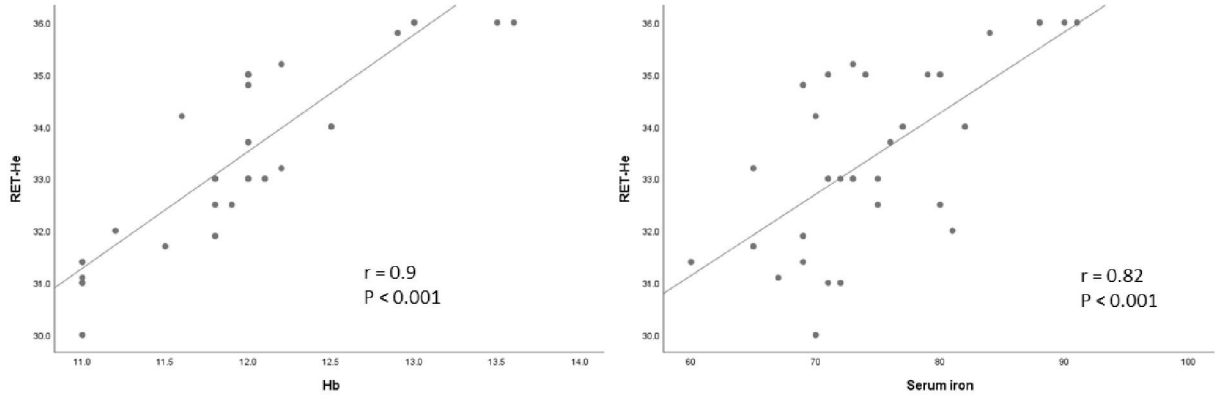

Figure 2: Correlation between RET-He and serum iron and Hb after IV iron administration

4. Discussion

Hemoglobin the most abundant protein inside red blood cells. It contains heme prosthetic groups combined with an iron atom at its center. The life span of the RBCs is considered to be constant (120 day), unless there are structural changes in the hemoglobin or decrease in iron that lead to dysfunction and intracellular fragmentation. Anemia is extremely common among CKD patients because the damaged kidneys did not make enough of erythropoietin hormone[21]. In CKD disease, IDA can happen even before the kidneys fail, and it is very common in people on dialysis[21]. This condition needs erythropoietin administration associated with iron supplementation to maintain the normal level of Hb. The response to iron admiration is mandatory to be assessed using a highly sensitive and specific test to avoid iron toxicity or abnormal iron storage leading to organ injury. The needed iron to the erythropoiesis, due to the short lifespan (one or two days) of reticulocytes in the circulation [22]. The present study included 50 CKD-HD patients revealed that RET-He reported by the Sysmex XN analyzer was significantly increase after 4 weeks of a total 1000 mg IV iron administration compared to the baseline values as shown in Figure 1. In addition, RET-He could accurately and significantly classify CKD-HD patients with IDA into two groups; patients who achieved and not achieved the OCA after five weeks of IV iron supplementation according to weather their Hb increased by ≥13.5 g/d.l in males and ≥12 g/d.l in females. Urrechaga and colleagues included 40 CKD-HD patients to assess the effectiveness of RET-He as a predictor for IDA. Patients were classified as responders or non-responders according to whether their Hb increased to at least 10 g/L after supplementation of 100 mg iron sucrose at each dialysis session for four weeks. RET-He was

significantly higher in responder group than non-responder group. DOPPS reported an 18% increased cause mortality with a high dose of ≥ 400 mg/month and a 12% increased risk of all-cause mortality with ≥ 300 mg/month compared with 100–199 mg/month [23]. In addition, the hospitalization risk increased by 12% among patients receiving more than 300 mg/month compared with 100–199 mg/month[23].

Positive correlation between RET-He and serum iron, SF, TSAT and Hb was observed in CKD-HD patients While, a negative correlation was observed between the RET-He and TIBC. Mehta et al also observed a significant positive correlation between RET-He and SF (r= 0.7860; P < 0.0001) [24]. Despite, our study included adults patients, a same observed correlation was found in aged 1-6 years (r = 0.464, P < 0.01)[25]. Almost studies have positive correlation of RET-He with transferrin saturation [26]–[28].

The ROC curve analysis revealed that AUC for RET-He was 0.891, 95% CI 0.44 to 0.73 with sensitivity of 70.6% and lower specificity of 51% considering (serum iron < 40 lg/dl, TSAT <20%, ferritin <100 ng/ml and hemoglobin <11 g/dl) were the reference tests. The best cutoff value of RET-Hb for diagnosis of IDA was 26.9 pg. Using iron-stained bone marrow aspiration as a reference test, Mehta et al. reported an AUR for RET-He of 0.89 with a sensitivity (98.8%) and specificity (84.2%). The best cutoff value of RET-Hb for diagnosis of IDA was 22.4 pg [24]. Studding IDA in children with mean age of 2.9 years, showed a higher AUC for RET-He (0.79) than serum ferritin (0.57) and the best cut off value of RET-He was 26 pg with 83% sensitivity and 75% specificity[29]. Similar results also reported by Mateos et al. showed a 94% sensitivity and 80% specificity of RET-Hb to detect IDA in children with cut off value of 25 pg[30].

Our study has many strength points include (1) a suitable sample size, (2) using Sysmex XN-2000 hematology analyzer in our hematological analysis, (3) we used the OCA as a point of classification of patients.

Nonetheless, this study does not address the impact of cumulative doses of IV iron supplementation.

In conclusion, RET-He seems to be useful in assessing functional iron deficiency and improve anemia in patients receiving HD and could help to guide clinicians in their iron management decisions.

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