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Role of reticulocyte hemoglobin equivalent in early detection of iron deficiency anemia in regular hemodialysis patients

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Abstract
Background: The reticulocyte hemoglobin equivalent (RET-HE) level is a valuable measure that enables the early detection of anaemia in patients prior to its manifestation. The purpose of this work was to evaluate the role of RET-HE in early detection of iron deficiency anemia (IDA) in hemodialysis individuals to determine iron supplementation needs.
Methods: This work was performed on 200 regular hemodialysis individuals for three months or more, on recombinant human erythropoietin and older than 18 up to 70 years. Reticulocyte hemoglobin percent was measured.
Results: The mean RET-HE equivalent was 33.18±3.86. A substantial variation was existed among before and after hemodialysis as regard creatinine and urea. A statistically substantial variation was existed among RET-HE equivalent regarding validity.
Conclusion: The RET-HE measure has been regarded as a valuable alternative in assessing IDA among regular recipients of hemodialysis. RET-HE shown a rapid rise subsequent to the administration of iron supplementation. Consequently, it may serve as an early indicator for evaluating the effectiveness of intravenous iron supplements.

Keywords: Reticulocyte hemoglobin equivalent, iron deficiency anemia, regular hemodialysis, the reticulocyte hemoglobin content, chronic kidney disease

Introduction
Anaemia is a prevalent complication that often arises in individuals with chronic kidney disease (CKD), with a higher incidence seen among individuals undergoing dialysis treatment. The treatment of anaemia in individuals with CKD involves the use of recombinant human erythropoietin and IV iron supplementation [1].
Iron deficiency anaemia (IDA) is a prevalent dietary insufficiency on a worldwide scale and is the most prevalent aetiology of anaemia. The levels of haemoglobin (Hb) may stay within the normal range for a certain amount of time following the depletion of iron stores. During this time, a deficiency of iron might be detected without the presence of anaemia. However, the levels of plasma ferritin and plasma transferrin saturation are lowered during this period. After the exhaustion of iron reserves, a decline in haemoglobin levels is seen [2].
In instances of iron shortage, the production of haemoglobin is first diminished in reticulocytes. The measurement of RET-HE level serves as a valuable metric for the early detection of anaemia in patients. Additionally, this parameter may be discovered well in advance of the rise in haemoglobin levels and traditional reticulocyte count, making it valuable for both therapy and treatment monitoring purposes. The variable RET-HE is used as the first assessment to assess the functional ID and demonstrate the extent to which the patient is experiencing positive outcomes from the therapy [3, 4].
Research has shown that the measurement of reticulocyte haemoglobin content (CHr) may serve as an early marker for iron-restricted erythropoiesis. The European recommendations on anaemia therapy in CKD have examined the usefulness of Hb concentration more than 29 pg/cell as a reticulocyte measure for assessing a patient's iron requirements [5].
The RET-HE method, which has been recently developed to measure the forward scatter of fluorescence-labeled reticulocytes, appears to be a very sensitive indication for detecting IDA [3].

In women, anaemia was characterized as an Hb level less than 12.0 g/L, whereas in males, anaemia was characterized as a Hb level less than 13.0 g/L. A serum ferritin level below 26.96 pmol/L was considered to be indicative of iron deficiency in people with anaemia. The mean value of RET-HE is 30.8 pg, with a lower threshold of 28 pg [6].

The purpose of this work was to evaluate the role of RET-HE in early detection of IDA in hemodialysis individuals to determine iron supplementation needs.

Patients and Methods

This work was performed on 200 regular hemodialysis recipients for three months or more, on recombinant human erythropoietin and older than 18 up to 70 years.

The work was performed following permission from the Ethics Committee Tanta University Hospitals, and Faisal Al Saud Hospital, Dessouk city, Kafr El Sheikh Government, Ministry of Health, Egypt. An informed verbal consent was gathered from all participants.

Exclusion criteria were pregnant female, malignant diseases, liver diseases, patients with infections characterized by a high fever three days before samples and patients received blood transfusion, oral or intravenous iron supplementation within the last two months.

All participants had been exposed to clinical evaluation [duration of hemodialysis, duration of dialysis, dialysis doses, the efficiency of hemodialysis, diabetes mellitus (DM), hypertension and drug use (Calcimimetic, vitamin D, phosphate binders and proton pump inhibitors)], clinical examination [blood pressure and anthropometric measures], usual laboratory tests [Full blood picture (CBC), C- reactive protein (CRP), serum urea, urea reduction ratio, Ca, Pi, Na, K, albumin-creatinine ratio, human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), iron study and the RET-HE]

Reticulocyte hemoglobin percent measurement

All of the patients had blood samples taken. Venous blood was obtained using 5-milliliter lavender-top tubes for haematological investigations which includes CBC, blood film, ESR, and CHr, and a red-top tube for biochemical assays like SF, TIBC, and CRP. Venous blood was utilised for all haematological and biochemical studies.

The ADVIA 2120 haematology analyzer (Siemens Diagnostic Solutions, Tarrytown, New York) was used to quantify CHr. Fluorescence flow cytometry (BD Biosciences, San Jose, CA) has been employed for measuring as it is the CHr and labels cells according on their RNA content [7]. To determine the presence of ID, RET-HE was contrasted to traditional iron measures (serum ferritin and/or TSAT). Individuals with IDA (haemoglobin ≤10 g/dl or hematocrit ≤30%) with ferritin in their serum. Westergren used the Sedplast ESR system (Polymedco, Cortlandt Manor, NY) to measure the ESR. Utilizing the Siemens Flex reagent cartridge DF49A on the Dade Behring Dimension RXL clinical chemistry autoanalyser, serum iron and serum iron binding capacity had been automatically determined. The Modular Analytic E170 analyzer has been utilized to detect serum ferritin levels. The NycoCard CRP single test from Axis-shield was used to quantify CRP.

Statistical analysis

The computer was given data, and IBM SPSS software package version 20.0 (IBM Corp, Armonk, NY) was used for analysis. Numbers and percentages were used to describe the qualitative data. The normality of the distribution is confirmed using the Kolmogorov-Smirnov test. The terms range (Minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) have been utilized to characterize quantitative data. Plotting sensitivity (TP) on the Y axis against 1-specificity (FP) on the X axis at various cut off levels produced the ROC curve. The diagnostic performance of the test is shown by the area under the ROC curve. A score of more than 50% indicates a satisfactory performance, while a score of about 100% indicates the best result for the exam. The performance of two tests may also be compared according to the ROC curve. The findings' significance was assessed at the 5% level.

Results

That there were (56.0%) of the instances in the stud had been males and (44.0%) had been females. The mean age was 52.50±9.07 SD. There were 20.0% were smokers. The mean duration of the disease was 4.13±1.65 SD. According to etiology of CKD, 30.5% had diabetic nephropathy, 36.5% had systemic hypertension and 8.5% had analgesic nephropathy. The mean erythropoietin (EPO) dose/week was 7220.0±2327.8 SD. Table 1

Table 1: Distribution of the studied cases according to baseline characters EPO dose/week/IV

		(n =200)
Age (Years)		52.50±9.07
Sex	Male	112(56.0%)
	Female	88(44.0%)
Smoking history	Non-smokers	160(80.0%)
	Smokers	40(20.0%)
Duration of disease (Year)		4.13±1.65
Etiology of CKD	Diabetic nephropathy	61(30.5%)
	Systemic hypertension	73(36.5%)
	Lupus nephritis	3(1.5%)
	Polycystic kidney	13(6.5%)
	Reno vascular disease	1(0.5%)
	Analgesic nephropathy	17(8.5%)
	Chronic GN	8(4.0%)
	Chronic interstitial nephritis	4(2.0%)
	Gout	5(2.5%)
	Obstructive uropathy	15(7.5%)
Drug history	EPO dose/week (IU)	7220.0±2327.8
	4000	57 (28.5%)
	8000	125(62.5%)
	12000	18(9.0%)

Data are presented as mean ± SD or frequency (%). EPO: Erythropoietin. CKD: chronic kidney disease.

The mean RET-HE equivalent was 33.18±3.86, serum ferritin was 1133.5±691.3, serum iron was 84.65±33.12, transferring saturation was 47.53±26.92, total iron-binding capacity (TIBC) was 12.76±2.37, Hb was 9.05±1.44, mean corpuscular volume (MCV) was 74.60±7.36, mean cell hemoglobin (MCH) was 26.69±1.74, mean corpuscular hemoglobin concentration (MCHC) was 31.39±1.67, reticulocyte count was 0.52±0.17, platelets (PLTS) was 144.6±27.45, white blood cells (WBCs) was 6.10±1.96, KT/V was 1.2±0.35, CRP was 38.82±13.99, albumin/creatinine ratio was 1347.4±790.6, serum intact parathyroid hormone
(iPTH) was 164.0 – 2984.0, serum phosphorus was

4.69±1.03, serum Ca was 8.53±0.77 SD, serum Na was 133.05±7.6 and serum K was 4.70±0.97 SD. Table 2

Table 2: Distribution of the studied cases according to laboratory investigations.

		(n = 200)
RET-HE equivalent (pg)		33.18±3.86
<28		19 (9.5%)
28 - 30.8		37 (18.5%)
>30.8		144 (72.0%)
Iron profile		
Serum ferritin (ng/mL)		1133.5±691.3
<26.96 anemia		14 (7.0%)
≥26.96		186 (93.0%)
Serum iron (µg/dL)		84.65±33.12
Transferrin saturation		47.53±26.92
TIBC (µg/dL)		12.76±2.37
CBC		
Anemia (<12.0 if women or <13.0 if men)		9.05±1.44
MCV		74.60±7.36
MCH		26.69±1.74
MCHC		31.39±1.67
Reticulocyte count (%)		0.52±0.17
PLTS		144.6±27.45
WBCs		6.10±1.96
KT/V		1.2±0.35
CRP		38.82±13.99
Albumin/creatinine ratio		1347.4±790.6
Serum iPTH		467.5±531.9
Serum phosphorus		4.69±1.03
Serum Ca		8.53±0.77
Serum Na		133.05±7.6
Serum K		4.70±0.97
HIV	Negative	200 (100.0%)
	Positive	0 (0.0)
HBV	Negative	200 (100.0%)
	Positive	0 (0.0)
HCV	Negative	175 (87.5%)
	Positive	25 (12.5%)

Data are presented as mean ± SD or frequency (%). CBC: complete blood count. RET-HE: reticulocyte hemoglobin equivalent. TIBC: Total iron-binding capacity. MCV: mean corpuscular volume. MCH: mean cell hemoglobin. MCHC: means corpuscular hemoglobin concentration. PLTS: platelets. WBCs: White blood cells. iPTH: intact parathyroid hormone. HIV: human immunodeficiency virus hepatitis B virus. HCV: HCV hepatitis C virus.

A highly statistically substantial variation was existed among before and after regarding creatinine and urea. Table 3.

Table 3: Renal function before and after dialysis session

Renal function	Before (n = 200)	After (n = 200)	p
Creatinine	10.67±1.64	3.79±1.0	<0.001*
Urea	166.4±23.92	50.25±10.36	<0.001*

Data are presented as mean ± SD. p: p value for comparing between before and after. *: Statistically significant at $p \leq 0.05$.

A statistically substantial variation was existed among RET-HE equivalent as regard validity. Figure 1

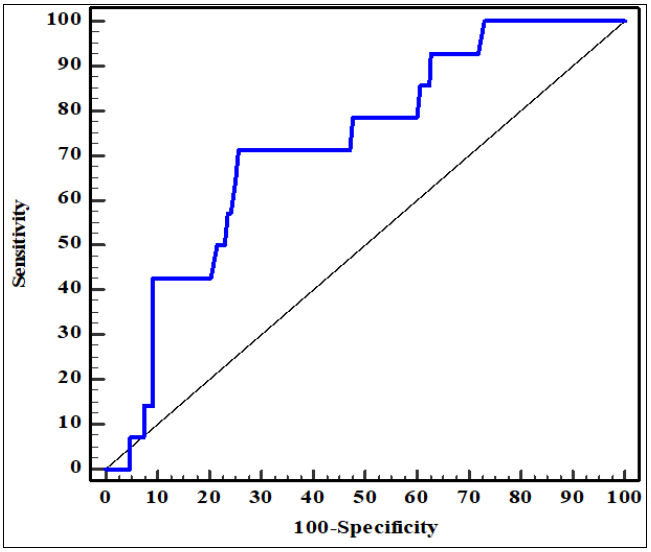

Fig 1: ROC curve for RET-HE equivalent to prognoses patients with serum ferritin <26.96 (n = 14 vs. 186)

Discussion

Erythropoietin insufficiency is the most frequent cause of anaemia in people with CKD, which is followed by deficiencies in iron. Chronic kidney inflammation damages the juxta-glomerulus, the site of erythropoietin synthesis; as a result, erythropoietin production declines, which in turn results in a reduction in the production of erythrocytes. Furthermore, hemodialysis exacerbates iron shortage. Repetitive phlebotomy, recurrent tests for blood, and clotting or loss of blood in the extracorporeal circuit are among the factors that contribute to ID in those with CKD receiving hemodialysis [8].

The result of erythropoiesis, known as RET-HE, has a brief half-life of 1-2 days preceding transforming into erythrocytes. With a solid understanding of the quantity of iron needed for erythropoiesis in the bone marrow, RET-HE can determine the amount of haemoglobin present in reticulocytes. It is hypothesised that RET-HE's short lifetime makes it more susceptible to erythropoiesis activities. Reduced erythropoiesis linked to a drop in erythropoietin as a result of long-term inflammation of the juxta glomerulus and hemodialysis procedures is indicated by low levels of RET-HE [9].

In this study we found that mean serum ferritin was 1133.5±691.3 SD, mean serum iron was 84.65±33.12 SD, mean transferrin saturation was 47.53±26.92 SD and mean TIBC was 12.76±2.37 SD. This result is consistent with research by Chinudomwong *et al.* [10], whereby the median blood ferritin level of 120 individuals with CKD with IDA owing to infections was measured. Moreover, Gupta *et al.* [11] reported that the study group's mean baseline blood iron level was 101.5±9.2 µg/dl, whereas the control group's baseline value was 66.65±28.52 µg/dl. There was a statistically substantial variation. The study group's serum ferritin level was significantly greater (513.82±505.07 ng/ml) compared to the control group's (88.66±132.92 ng/ml). In comparison to the control group, individuals with CKD with a greater TSAT score (32.2±15.2%) had a lower TIBC (297.2±100.1 µg/dl).

In this study we illustrated that mean Hb was 9.05±1.44 SD, mean MCV was 74.60±7.36 SD, mean MCH was 26.69±1.74 SD, mean MCHC was 31.39±1.67 SD, mean reticulocyte count was 0.52±0.17 SD, mean PLTS was 144.6±27.45 SD and mean WBCs was 6.10±1.96 SD.

Agustina and Wardani [12], from 20 CKD patients, each participant had anaemia; the greatest Hb level was 9.0 g/dL, the smallest was 6.0 g/dL, and the average was 7.38 g/dL. Furthermore, Venkatesan *et al.* [13] found that there were substantial variations in the study groups' Hb levels and anaemia severity. According to Alzahrani *et al.* [14], prior to therapy, hemodialysis patients' RBC indices [RBCs counts, haemoglobin, HCT%, and MCH] all showed a substantial decline when contrasted with the control group.

In this study we demonstrated that mean CRP was 38.82±13.99 SD with range (11.0 – 72.0).

Elmenyawi *et al.* [15] found that there is significant increase in CRP in HD individuals contrasted with healthy controls (p<0.05). Sirken *et al.* [16] the researchers discovered a positive correlation between elevated levels of CRP and the presence of relative EPO resistance among those undergoing dialysis.

The present investigation revealed that the AUC for RET-HE was determined to be 0.724 (95% CI 0.599 – 0.849), with a cutoff value of 31.4 pg. The sensitivity and specificity of this cutoff were calculated to be 71.43% and 71.51%, respectively.

In their study, Chinudomwong *et al.* [10] determined the ideal cutoff for RET-HE in the diagnosis of IDA using ROC analysis. The researchers identified this cutoff point based on the optimal balance between sensitivity and specificity. The ROC curve showed an AUC of 0.876 (95% CI 0.854-0.897, p<.001) when using a cutoff of ≤30 pg. At this cutoff, IDA was identified with a sensitivity of 74.2% and a specificity of 97.4%. In research conducted by Shalahuddin *et al.* [12], it was shown that while utilizing a cutoff value of 31.65 pg, a total of 36 study individuals (72%) exhibited RET-HE levels less than the established normal range, while 14 participants (28%) had RET-HE levels beyond the normal range. In their study, Sany *et al.* [17] conducted ROC analysis to assess the effectiveness of RET-HE value in detecting deficiencies in iron, based on the aforementioned diagnostic criteria for ID (TSAT <20% and serum iron <9 umol/L). The researchers analysed the data from those instances to establish the cutoff level of RET-HE. When the threshold value for RET-HE was established at 27.0 (pg), the sensitivity and specificity were determined to be 90.4% and 80.8% respectively.

In this study we demonstrated that the mean RET-HE equivalent was 33.18±3.86 SD with range (24.30 – 40.50). Shalahuddin *et al.* [12] found the mean RET-HE levels in a sample of 50 participants were 28.8±3.7 pg, with the maximum recorded RET-HE level being 38.9 pg and the smallest measured level being 19.6 pg. Additionally, in research conducted by Dalimunthe and Lubis [18], it was discovered that the mean ret-he equivalent level of the study participants was 29.98±3.85 pg. In their study, Sany *et al.* [17] discovered a noteworthy association between RET-HE and serum iron, transferrin, ferritin, and TSAT% levels in individuals undergoing HD (p<0.001).

Our study recommended that further multicenter research with larger sample size is required in order to endorse RET-HE as a routine diagnostic parameter for identifying IDA among those with CKD. Additionally, RET-HE has the potential to serve as a measure for monitoring initial responses to iv iron supplements in individuals undergoing dialysis.

Conclusion

RET-HE has been recognized as a viable alternative measure for assessing IDA in frequent individuals on hemodialysis. This is attributed to its favourable diagnostic performance, the widespread availability of blood analyzers, and its relatively cheaper cost. The RET-HE shown a rapid rise subsequent to the administration of iron supplements. Consequently, it may serve as an early indicator of the individual's reaction to IV iron supplements.

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Conflict of Interest: Nil

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