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The Correlation between Reticulocyte Hemoglobin Equivalent (RET-He), Iron Status, and Erythrocyte Indices in Chronic Kidney Disease Patients at Prof. Dr. R.D. Kandou Manado Hospital

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ABSTRACT

Background: Chronic Kidney Disease (CKD) is a public health concern with anemia being a major complication. RET-He is a cost-effective parameter to assess iron status in CKD, but further research is necessary to assess its correlation with existing parameters. This study attempted to investigate the correlation between RET-He, iron status, and erythrocyte indices among CKD patients.

Materials and Methods: A cross-sectional study involving 110 CKD patients who underwent routine hematology and iron profile tests (serum iron/SI, total iron binding capacity/TIBC, transferrin saturation/TSAT, and ferritin). RET-He was then measured using a Sysmex XN-1000 hematology analyzer. Statistical tests were used to define the correlation between RET-He), iron status, and erythrocyte indices.

Results: There was a significant positive correlation between RET-He and SI ($r = 0.349$; $p = 0.000$), TSAT ($r = 0.393$; $p = 0.000$), and ferritin ($r = 0.279$; $p = 0.003$). Among CKD patients with excess iron levels, there was a moderate correlation between RET-He and TSAT ($r = 0.404$; $p = 0.000$).

Conclusion: The study found a significant correlation between RET-He levels and iron status markers in CKD patients. RET-He is recommended as an additional parameter to assess iron status and as an additional method to estimate TSAT and ferritin levels, especially in settings where chemistry analyzers are unavailable. Further research is required to establish RET-He cut-off values for identifying excessive iron levels in CKD patients.

Keywords: Reticulocyte hemoglobin equivalent; Iron status; Erythrocyte indices, CKD

INTRODUCTION

With an increasing incidence of kidney failure, a poor prognosis, and significant costs, chronic kidney disease (CKD) is a worldwide public health concern. According to the 2018 Indonesian Baseline Health Research, CKD prevalence at age >15 years is 0.38%[1,2], and renal disease is responsible for the second-highest spending in the national insurance system after heart diseases[3].

Anaemia is a main issue with chronic kidney disease (CKD). The most significant factor that contributes to anaemia in CKD is erythropoietin deficiency, but other factors that can also play a role include iron shortage, blood loss, hemolytic conditions, uremic substances, and inflammatory states[4,5]. Erythropoietin is a glycoprotein hormone that controls the process of erythropoiesis: the production of red blood cells (RBCs). The kidneys,

especially the peritubular fibroblasts of the renal cortex, are responsible for producing this hormone. The kidneys make around 80-90% of erythropoietin, with the liver producing the remaining amounts. This hormone stimulates the differentiation and proliferation of erythroid progenitor cells and the synthesis of haemoglobin to become mature RBCs in the bone marrow[6,7]. In CKD, the process is disturbed, leading to a decrease in RBCs and anaemia[4,5]. Anaemia decreases the patient's quality of life and leads to major health problems. Therefore, the management of anaemia is critical. Data shows that about 25% of patients with CKD require recurrent blood transfusion[8].

The aim of anaemia management in CKD is to improve haemoglobin (Hb) and hematocrit (Hct) levels, specifically Hb >10 g/dL and Hct >30%. This Hb level can be achieved through conservative treatment or with erythropoietin (EPO) therapy. EPO therapy aims to reduce the need for transfusions. EPO therapy failures in CKD often occur in patients with iron deficiency comorbidity. Therefore, iron status examinations are necessary for the management of CKD[8,9].

The recommended method of determining iron status is bone marrow iron staining, but it is an invasive and risky procedure. Instead, indirect methods of determining iron status are preferred, such as serum iron (SI), total iron binding capacity (TIBC), transferrin saturation (TSAT), and serum ferritin. However, indirect methods are frequently unavailable due to their high cost, require specialized equipment, and are affected by inflammatory factors that are present in CKD patients[10-12].

Currently, an automatic haematology analyzer is equipped with a parameter describing how much iron is available for the erythropoiesis process in the bone marrow, known as reticulocyte haemoglobin equivalent (RET-He), which measures the haemoglobin content in reticulocytes[13-15]. The RET-He examination does not require an additional analyzer, so it is relatively cheaper and more efficient compared to iron status examinations, especially for hospitals that do not have a chemistry analyzer to check the iron status[16-17].

The RET-He examination is not yet a standard procedure at Prof. Dr. R.D. Kandou Manado Hospital. Although this hospital has a haematology analyzer with the Ret-He parameter, no research has been done on this parameter. Therefore, further study is required to determine the relationship between RET-He and the iron status tests that are regularly performed.

MATERIALS AND METHODS

Subjects

This was an analytical observational study with cross-sectional study design. The samples were taken from CKD patients in the nephrology clinic who came to the Integrated Clinical Laboratory of Prof. Dr. R.D. Kandou Manado Hospital for haematology and iron status examinations (SI, TIBC, TSAT, and ferritin). For those who met the inclusion criteria, an additional RET-He examination was conducted. The time for collecting samples is throughout July 2020. The sample size was calculated using the consecutive sampling method[19].

$$n = \left[\frac{(Z\alpha + Z\beta)}{0.5\ In\ \left(\frac{1+r}{1-r}\right)}\right]^2 + 3$$

$$n = \left[\frac{(1.96 + 1.64)}{0.5\ In\ \left(\frac{1+0.499}{1-0.499}\right)}\right]^2 + 3$$

Notes:

n = minimum number of samples required

Zα = Type I Error (Zα) = set at 5% with the two-way hypothesis. Zα is set at 1.96.

Zβ = Type II error (Zβ) = set at 10% with the two-way hypothesis. Zβ is set at 1.64.

r = 0.499 [17]

Thus, the minimum number of samples required was 100 samples.

Patients had to meet the inclusion criteria for this study, which included those who had iron status examinations, specifically SI, TIBC, TSAT, and ferritin, as well as routine haematology examinations. Patients also must be older than 18 years old. On the other hand, the exclusion criteria indicated that patients referred or consulted from other clinics or units within the nephrology clinic would not be eligible to participate. Patients who had undergone kidney transplantation were additionally excluded from the study. These criteria were created to

guarantee that the study's findings were reliable and relevant to the intended demographic. All participants who met the criteria for inclusion signed informed consent forms before beginning the study. The ethical clearance was issued by the Health Research Ethics Committee of Prof. Dr. R.D. Kandou Manado Hospital with approval number 065/EC/KEPK-KANDOU/VII/2020.

Methods

Haematological and RET-He examinations were conducted using the Sysmex XN-1000 automatic haematology analyzer (Sysmex Corporation, Kobe, Japan). Meanwhile, iron status (SI, TIBC, TSAT, and ferritin) examinations were conducted using the Roche Cobas 8000 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Internal and external quality control were done for both instruments twice a year for each parameter.

Statistical analysis

The univariate analysis was utilized to determine the distribution of each variable according to the characteristics of the sample. If normal data distribution was acquired ($p > 0.05$), the results were reported using the mean ± standard deviation (SD). On the contrary, the results were reported using the median and interquartile range (IQR) when data distribution was not normal.

Then, a bivariate analysis was applied to find the relationship between RET-He and SI, TIBC, TSAT, and ferritin. Moreover, the relationships between RET-He and the RBC, haemoglobin, and hematocrit counts were also determined. The relationship was considered significant if $p < 0.05$. The degree of correlation showed a very weak correlation if r = 0.00 – 0.199, a weak correlation if r = 0.20 - 0.399, a moderate correlation if r = 0.40 - 0.599, a strong correlation if r = 0.60 – 0.799, and a very strong correlation if r = 0.80 – 1.00, as shown by Table 1[20]. Data were analyzed using SPSS version 25.0 for Windows.

Table 1: Interpretation of correlation test

r*	Interpretation
0.00 – 0.199	Very weak correlation
0.20 - 0.399	Weak correlation
0.40 - 0.599	Moderate correlation
0.60 – 0.799	Strong correlation
0.80 – 1.00	Very strong correlation

*r = correlation coefficient

RESULT

Characteristic of Subjects

There were 110 subjects in total, consisted of 53 males (48.2%) and 57 females (51.8%). The age range and mean were 20 – 79 years and 55.2 years, respectively. The distribution of sex and age were shown in Table 2.

Table 2: Characteristics of Subject by Sex and Age

Characteristics	Number (percentage)	
	n	*(%)*
Sex		
Male	53	*(48.2)*
Female	57	*(51.8)*
Total	110	*(100.0)*
Age		
20 - 35	8	*(7.3)*
36 - 55	46	*(41.8)*
56 - 65	30	*(27.2)*
66 or more	26	*(23.6)*
Total	110	*(100.0)*

Age Mean ± SD = 55.2 ± 12.2

Data distribution

The Kolmogorov–Smirnov test was conducted to determine the data distribution. It was found that the RET-He, hemoglobin, MCV, MCH, RBC and hematocrit levels data were normally distributed, thus were reported as Mean ± SD. Meanwhile, the SI, TIBC, TSAT, and ferritin data were not normally distributed, thus the median with an interquartile range (IQR) were reported. The results of the normality test were presented in Table 3.

Table 3: Normality Test's Results for Observed Parameters

Parameters	n	p	Mean ± SD	Median (IQR)
RET-He (pg)	110	0.059	32.4 ± 2.47	-
Hb (g/dL)	110	0.200	8.5 ± 1.38	-
Hct (%)	110	0.200	24.3 ± 4.13	-
RBC ($x10^6$)	110	0.200	2.8 ± 0.51	-
MCV (fl.)	110	0.200	84.8 ± 4.42	-
MCH (pg.)	110	0.200	30.0 ± 1.66	-
SI (%)	110	0.000	-	76.0 (77.1 - 95.1)
TIBC (ug/dL)	110	0.000	-	195.5 (202.6 - 229.1)
TSAT (%)	110	0.000	-	35.0 (37,5 - 45.7)
Ferritin (ng/dL)	110	0.001	-	1012.5 (987.1 - 1309.0)

Normal data distribution if $p > 0.005$

Correlation Tests

Correlation between RET-He, Haemoglobin (Hb), Erythrocyte Count (RBC), and Haematocrit (Hct)

Pearson's correlation test was done to determine the correlation between RET-He, Hb, RBC, and Hct. Insignificant negative correlations were found in the Pearson's correlation test between RET-He and Hb, RET-He and RBC, and RET-He and Hct. Table 4 indicates the results of Pearson's correlation test. The correlation scatterplots were presented in Figure 1a – 1c.

Table 4: Correlation between RET-He, Erythrocyte Indices, and Iron Status

	RET-He		
	n	p	r
Erythrocyte Indices			
Hb	110	0.448	-0.073 (very weak)
RBC	110	0.009	-0.250 (weak)
Hct	110	0.105	-0.156 (very weak)
Iron Status			
SI	110	0.000	0.349 (weak)
TIBC	110	0.699	-0.037 (very weak)
TSAT	110	0.000	0.393 (weak)
Ferritin	110	0.003	0.279 (weak)

Statistically significant correlation if $p < 0.005$

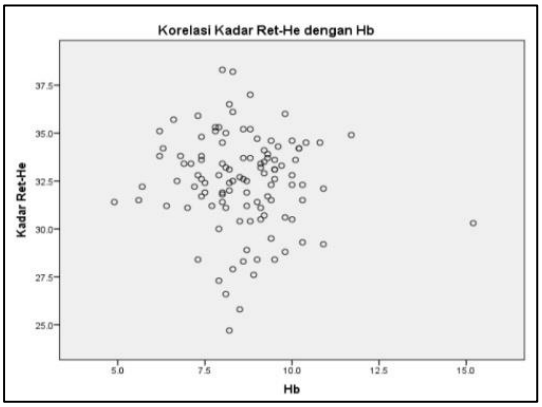

Figure 1a. Correlation between RET-He and Hb

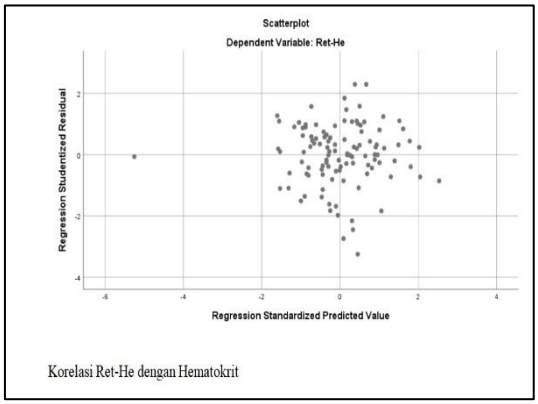

Figure 1b. Correlation between RET-He and Hct

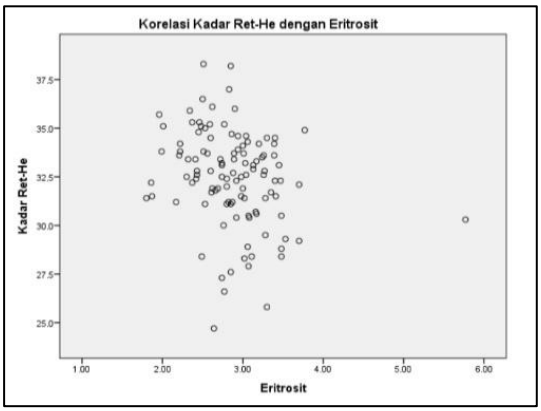

Figure 1c. Correlation between RET-He and RBC

Figure 1. Pearson's correlation scatterplots between Ret-He, Hb, Hct, and RBC

Correlation between RET-He, SI, TIBC, TSAT, and Ferritin

Spearman's ρ (rho) correlation test was done to determine the correlation between RET-He, SI, TIBC, TSAT, and Ferritin. Significant positive correlations were found between RET-He and SI, RET-He and TSAT, and RET-He and ferritin. Meanwhile, RET-He and TIBC showed an insignificant negative correlation. Table 4 presents the results of Spearman's ρ correlation test and Figure 2a – 2d illustrate the correlation scatterplots.

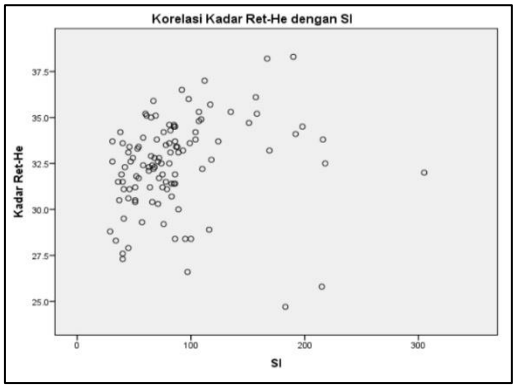

Figure 2a. Correlation between RET-He and SI

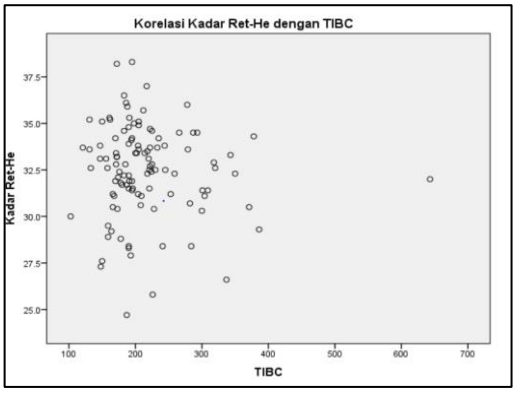

Figure 2b. Correlation between RET-He and TIBC

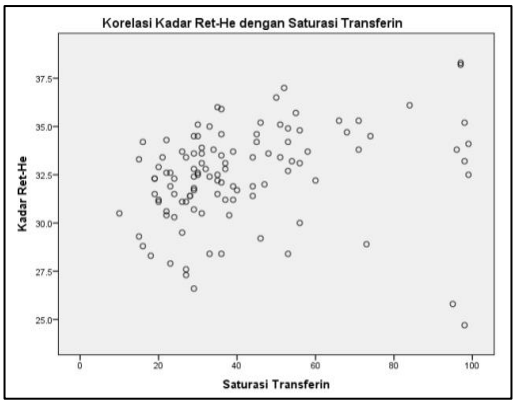

Figure 2c. Correlation between RET-He and TSAT

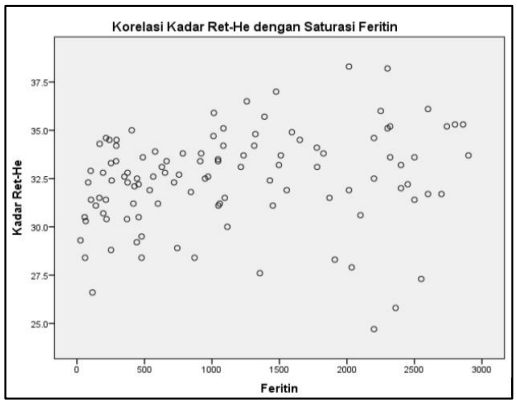

Figure 2d. Correlation between RET-He and Ferritin

Figure 2. Spearman's ρ correlation scatterplots between RET-He, SI, TIBC, TSAT, and Ferritin

Iron Status in CKD Patients

A total of 110 samples were examined; of these, 3 (2.7%) were defined as having absolute iron-deficiency anaemia, 2 (1.8%) as having functional iron-deficiency anaemia, 32 (29.1%) as having iron-sufficient anaemia, and 73 (66.4%) as having an iron-excess anaemia. Due to the limited number of samples, the groups with functional iron deficiency anemia and absolute iron deficiency anemia were excluded from the analysis.

The iron excess group was established based on a TSAT level of more than 50% and/or ferritin level of more than 500 ug/mL. Normality test was then utilized to identify the distribution of data in the iron-sufficient and iron-excess groups. Data distribution based on iron status is presented by Table 5.

Table 5: Normality Test's Results for Observed Parameters Based on Iron Status

	n	p	Mean ± SD	Median (IQR)
Iron-sufficient group				
RET-He (pg.)	32	0.473	31.8 ± 2.00	-
TSAT (%)	32	0.616	29.4 ± 7.10	-
Ferritin (ng/dL)	32	0.039	289.3 ± 137.2	-
Iron-excess group				
Ret-He (pg.)	73	0.021	32.8 ± 2.59	-
TSAT (%)	73	0.003	-	44.0 (43.1 – 54.1)
Ferritin (ng/dL)	73	0.008	1593.4 ± 700.2	-

Normal data distribution if p > 0.005

Correlation between RET-He, TSAT, and Ferritin in Iron-sufficient and Iron-excess Groups

In the iron-excess group, there was a statistically significant moderate correlation between RET-He and TSAT. On the contrary, it showed a statistically insignificant negative correlation within the iron-sufficient group. Meanwhile, there was a positive but not significant correlation between RET-He and ferritin in both groups. The equation y = -13.5 + 1.89x for linear regression was obtained. Table 6 demonstrates the results of correlation tests.

Table 6: Correlation Test between TSAT and Ferritin based on Iron Status

	RET-He		
	n	p	r
Iron-sufficient group			
TSAT	32	0.418	-0.148 (very weak)
Ferritin	32	0.658	0.081 (very weak)
Iron-excess group			
TSAT	73	0.000	0.404 (moderate)
Ferritin	73	0.322	0.118 (very weak)

Statistically significant correlation if p < 0.005

DISCUSSION

In this study, 110 samples from CKD patients were analyzed to assess their iron status, with categorization into different types of anemia based on iron levels. Critical insights into the prevalence of iron deficiency and excess within this population were provided by the findings. Due to the limited sample sizes in the absolute and functional iron-deficiency anemia groups, further analysis was excluded for these groups, leaving two primary groups: iron-sufficient and iron-excess anemia. The iron-excess group was defined by a Transferrin Saturation (TSAT) level greater than 50% and/or a ferritin level exceeding 500 µg/mL. This classification is essential for understanding the complexities of anemia in chronic kidney disease (CKD) and highlights the importance of a comprehensive assessment of iron status in this context.

The relationships between Reticulocyte Hemoglobin Equivalent (RET-He), Hemoglobin (Hb), Erythrocyte Count (RBC), and Hematocrit (Hct) were assessed using Pearson's correlation test. The results revealed insignificant negative correlations among these parameters, indicating that increases in RET-He do not correspond to significant changes in Hb, RBC, or Hct levels. This finding is particularly intriguing, given that RET-He is typically regarded as a marker for erythropoiesis and iron availability [31,32]. The absence of a significant correlation may suggest that RET-He does not directly reflect changes in these traditional hematological parameters under the studied conditions. One possible explanation for these findings lies in the complex interplay between erythropoiesis and iron metabolism. While RET-He serves as a valuable indicator of reticulocyte activity and offers insights into bone marrow function, it may not consistently correlate with peripheral blood parameters, especially in populations with varying underlying health conditions or in the presence of confounding factors such as inflammation or chronic disease[33]. Future research should aim to explore these relationships further, particularly in larger and

more diverse populations, while also investigating potential confounders that may influence these correlations.

In a complementary analysis, the correlation between RET-He, Serum Iron (SI), Total Iron Binding Capacity (TIBC), Transferrin Saturation (TSAT), and ferritin levels was examined using Spearman's ρ correlation test. This analysis revealed significant positive correlations between RET-He and SI, RET-He and TSAT, also RET-He and ferritin levels, suggesting that as RET-He increases, so do the levels of these iron status markers. These findings align with the hypothesis that RET-He reflects the availability of iron for erythropoiesis, as it measures the hemoglobin content in newly produced red blood cells[31,32]. Conversely, the negative correlation between RET-He and TIBC was found to be insignificant. In contrast, previous research by Davidkova et al. indicated a weak but significant positive correlation between RET-He levels and TSAT, suggesting that RET-He levels increase as TSAT levels rise[19]. Similarly, Rovani et al. reported a significant positive correlation between RET-He and TSAT[15]. This finding aligns with the understanding that TIBC can be influenced by various factors, such as nutritional status and inflammation, which may not directly correlate with reticulocyte hemoglobin content[34,35]. Several factors could contribute to the absence of a significant negative correlation between RET-He and TIBC in this study. For instance, confounding factors like inflammation or iron metabolism disorders may obscure the relationship between these two variables in this cohort. Additionally, the timing of blood sample collection relative to dietary intake or treatment interventions might have impacted the observed levels, further contributing to the insignificant correlation.

The correlation results between Reticulocyte Hemoglobin Equivalent (RET-He), TSAT, and ferritin levels in both iron-sufficient and iron-excess groups provide valuable insights into the dynamics of iron metabolism in patients with chronic kidney disease (CKD). In the iron-excess group, a statistically significant moderate correlation was observed between RET-He and TSAT, suggesting that individuals with excess iron exhibit higher TSAT levels alongside increased RET-He levels. The significant correlation between RET-He and TSAT aligns with the findings of Poventud-Fuentes et al and Rovani et al [15,36]. This relationship suggests that increased availability of excess iron may enhance erythropoiesis, reflecting a physiological adaptation to elevated iron levels[36,37]. In contrast, the correlation between RET-He and TSAT in the iron-sufficient group was found to be statistically insignificant and negative. This unexpected finding implies that, in patients with adequate iron stores, other factors may play a more crucial role in regulating erythropoiesis than TSAT levels do. Regarding ferritin correlations, both groups exhibited a positive relationship between RET-He and ferritin; however, this correlation was not statistically significant. This suggests that although there is a general trend indicating higher ferritin levels are associated with increased RET-He, the relationship in this study remains weak. In contrast, Rovani et al. reported a significant positive correlation ($r = 0.533$; $p < 0.001$) between RET-He and ferritin[15]. The lack of strength in our findings may be attributed to limitations such varying levels of inflammation, which can influence the observed correlation[38].

Limitations

This research faced several limitations related to data sampling. Firstly, the study could not investigate chronic kidney disease (CKD) patients without anemia, as no individuals were identified within this group. Additionally, patients undergoing specific therapies, particularly those receiving erythropoietin, were excluded from the study. These factors should be considered when interpreting the results or planning similar research in the future. Moreover, potential confounding variables, such as inflammation and other comorbidities affecting iron metabolism, were not addressed in this study. The relatively small sample size may also restrict the generalizability of the findings. Looking ahead, future research should focus on exploring the underlying mechanisms that contribute to the observed correlations. Larger studies could further investigate how factors like inflammatory markers interact with iron parameters to influence anemia management in CKD patients.

CONCLUSION

In conclusion, this study underscores the intricate relationship between Reticulocyte Hemoglobin Equivalent (RET-He) and iron status in chronic kidney disease (CKD) patients at Prof. Dr. R.D. Kandou Manado Hospital. While RET-He demonstrates a moderate correlation with Transferrin Saturation (TSAT) in iron-excess cases, its limited correlation with ferritin highlights the complexity of iron metabolism in CKD. Based on these findings, a RET-He examination is suggested as an alternative method to estimate TSAT and ferritin levels in CKD patients. Additionally, the RET-He parameter might be used as an additional parameter for determining iron status, especially in hospitals that are still lacking chemistry analyzers. To identify the RET-He cut-off value for identifying iron overload in CKD patients, further study is required. It is also important to consider the patient's medications. All these suggestions need to be considered to improve the quality of CKD management.

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