

Comparative Evaluation of Automated and Manual Reticulocyte Methods in Monitoring Erythropoiesis and Anemia in CKD Hemodialysis Patients

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Abstract

Anemia is a common complication in patients with chronic kidney disease (CKD), particularly those undergoing hemodialysis, and is associated with impaired erythropoiesis and iron deficiency. Accurate monitoring of erythropoietic activity is essential for effective management; however, conventional parameters have limitations. To assess the clinical utility of automated reticulocyte parameters, particularly reticulocyte hemoglobin equivalent (RET-He), in comparison with manual reticulocyte methods for monitoring erythropoietic activity and anemia in CKD patients undergoing hemodialysis. This observational, cross-sectional analytical study included 400 CKD patients on hemodialysis. Hematological parameters, including manual reticulocyte count, RET-He, hemoglobin (Hb), hematocrit (HCT), and RBC count, were analyzed. Statistical methods included descriptive analysis, paired t-test, Pearson's correlation, and multiple linear regression. A high prevalence of anemia was observed, predominantly of moderate severity. RET-He and manual reticulocyte count showed weak and non-significant correlations with hemoglobin levels. Paired t-test revealed a significant difference between pre- and post-erythropoietin levels, indicating limited improvement in erythropoietic response. Regression analysis demonstrated low predictive value of reticulocyte parameters, with RBC being the only significant predictor of hemoglobin. Automated reticulocyte parameters, including RET-He, provide limited independent value in assessing anemia in CKD patients. A combined approach using both conventional and advanced hematological markers is recommended for improved monitoring and management.

Keywords: Chronic kidney disease; RET-He; Reticulocyte count; Anemia; Hemodialysis

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1. Introduction

Chronic Kidney Disease (CKD) is a significant health issue around the world, and it is often linked to anemia, which is an issue that highly leads to morbidity and poor quality of life in people with the condition. Anemia development in CKD is multifactorial due to the primary causes of its occurrence that are, first, the reduced production of erythropoietin by the malfunctioning kidneys, second, the impact of iron deficiency, and third, the impact of chronic inflammation on erythropoiesis (Akinola et al., 2018). These aspects are also more complicated in patients undergoing hemodialysis, whereby there is compromised production of red blood cells and functional iron deficiency, making it more difficult to manage the patient (Alagiyawanna et al., 2020). ESAs are widely-used to treat anemia in CKD patients but the application is highly dependent on sufficient iron and erythropoietic activity monitoring (Bohlius et al., 2019). The conventional laboratory values of monitoring anemia are hemoglobin (Hb), hematocrit (HCT), and manual reticulocyte counts. Although these markers are useful, they are not able to capture early alterations in erythropoiesis and iron status (Abdelrahman et al., 2024). The manual method of reticulocyte counting, especially, is deemed less credible

because it is labor-intensive, has inter-observer error, and is not as sensitive to subtle changes in bone marrow activity (Gorte et al., 2020). In recent years the automated hematological parameters, which provide more accurate and timely information about erythropoietic activity, have been of growing interest. Among them, reticulocyte hemoglobin equivalent (RET-He) has proven to be a promising biomarker in assessing the iron status and erythropoietic activity. RET-He measures the hemoglobin levels of reticulocytes and gives real-time feedback on how iron is incorporated during erythropoiesis (Capone et al., 2017). Research has shown that RET-He could be used as an early warning of iron deficiency before the drastic alterations in hemoglobin levels have taken place (Dal Bó et al., 2023). Moreover, the automated parameters of reticulocytes have demonstrated their role in distinguishing between different forms of anemia and enhancing the diagnostic accuracy (Chakma et al., 2023). RET-He has been linked to a superior evaluation of iron status and enhanced erythropoietin therapy advice in CKD patients under hemodialysis (Karunaratne et al., 2022). Also, new reticulocyte indices have been identified as having a greater impact on improving clinical decision-making and anemia

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management plans (Czyzewska et al., 2018). Notwithstanding these developments, there are still inconsistencies in the routine adoption of such automated parameters into clinical practice, in part because of a lack of comparative evidence with conventional techniques.

The literature review indicates that there is significant gap in the literature that directly compares the manual reticulocyte counts with automated indices including RET-He, especially in CKD patients receiving hemodialysis. Even though the newer complete blood count parameters have proven to be more diagnostic, their use in clinical practice is still under development (Khodajji, 2019). Further, although the pathophysiology of iron deficiency anemia is well-defined, there is no agreement on the most sensitive and reliable biomarkers to detect and monitor iron deficiency in CKD populations (Kolarš et al., 2025). The inconsistency in the erythropoietin response also makes anemia treatment more tricky, which is why precise monitoring instruments are necessary to implement personalized treatment plans (Kraiem et al., 2018). The clinical guidelines emphasize the significance of managing anemia, but they usually use conventional parameters that might fail to identify the initial alterations in erythropoiesis (Liem et al., 2019). The development of biomarkers also suggests the possibility of the increased use of sophisticated diagnostic methods, but their implementation in the everyday hematological screening is underutilized (Loria et al., 2024). As a result, an increased demand to assess the clinical usefulness and applicability of automated reticulocyte parameters compared with conventional manual techniques is emerging.

Considering the shortcomings of the traditional methods and the growing need to obtain precise and fast diagnostic solutions, automated reticulocyte parameters, especially RET-He, may be a viable alternative to assessing erythropoietic activity in hemodialysis patients with CKD (Merlin et al., 2021). Better diagnostic approaches can lead to better treatment outcomes, maximize erythropoietin therapy, and minimize the complications of anemia (Portolés et al., 2021). Moreover, tailored solutions in the treatment of anemia presuppose dynamic and trustworthy indicators that would guarantee the use of proper therapeutic interventions (Rogers et al., 2018). Against this backdrop, the given research is designed to evaluate the clinical utility of the automated reticulocyte parameters (e.g. reticulocyte hemoglobin content (RET-He) compared to the manual reticulocyte techniques in tracking erythropoietic activity and anemia in respondent with chronic kidney disease under hemodialysis.

2. Methodology

2.1 Study Design

The current research was carried out as an analytical study with cross-sectional design aimed to compare and contrast automated and manual reticulocyte parameters in measuring erythropoietic activity and anemia in patients with CKD under hemodialysis. The design

allows assessing relationships among hematological variables at a particular time without intervention and thus give clues on the applicability of such variables in clinical practice in the usual management of patients.

2.2 Study Population and Sample Size

A total of 400 patients diagnosed with CKD but experiencing regular hemodialysis were involved in the study. The selected population was because of the high incidence of anemia and altered erythropoiesis in CKD, which is suitable in assessing the clinical usefulness of reticulocyte parameters. The size of the sample was deemed sufficient to give a statistically reliable sample and to be able to compare the manual and automated hematological approaches in a meaningful manner.

2.3 Criteria of Inclusion and Exclusion

The inclusion criteria of the study were that the patients must be confirmed to have chronic kidney disease, be undergoing regular hemodialysis, and have complete hematological data, including manual reticulocyte count (Retic), RET-He, Hb, and HCT. To ensure accuracy of data, patients whose hematological records were not complete were excluded. Also, those participants with acute infections, malignancies and other conditions were excluded which are known to independently alter erythropoiesis or hematological parameters so that the results observed could be mainly due to CKD-related anemia.

2.4 Data Collection

The data were collected with a broad range of demographics, clinical and hematological variables that were important in measuring the erythropoiesis and anemia. Demographic factors included age and gender, and clinical factors included hemodialysis time and comorbid conditions (diabetes mellitus and hypertension). Manual reticulocyte count (Retic), reticulocyte hemoglobin equivalent (RET-He), Hb, HCT, and RBC count were hematological parameters measured. Moreover, variables associated with treatments like pre-treatment and post-treatment erythropoietin (EPO) levels were also considered to assess erythropoietic response and treatment results.

2.5 Statistical Analysis

The data were entered into Microsoft Excel. Continuous variables were given in terms of mean and SD and categorical variables in terms of frequencies and percentages. The pre- and post-erythropoietin (EPO) levels and differences between the manual and the automated reticulocyte method were compared using a paired t-test. The correlation analysis by Pearson was used to evaluate the relationships between the RET-He, manual reticulocyte count, hemoglobin (Hb) and other hematological parameters. To determine predictors of hemoglobin (RET-He, Retic, RBC, and hematocrit), multiple linear regression was conducted. The p-value of less than 0.05 was regarded as a statistically substantial.

3. Results

3.1 Demographic and Clinical Characteristics

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The study involved 400 patients who were on hemodialysis and had chronic kidney disease. The study population contained 252 males (63%) and 148 females (37%). On weight change, 191 patients had weight loss, 82 gain and 127 were stable. There was also a large number of comorbidities with 309 patients (77.20%) having diabetes mellitus and 236 having hypertension. In 26 patients there was a history of heart attack and 10

had cancer (Table 1). The patient had urinary tract infection in 62 patients (15.50%), swollen legs in 4 (1%) and jaundice or hepatitis in 11 (2.80%). Autoimmune disease was not common and 23 (5.80) of the patients were diagnosed with lupus nephritis which was a sign that most of the patients had CKD-related anemia and with a significant comorbidity burden.

Table 1. Demographic and clinical characteristics (N = 400)

Variable	Category	n	%
Gender	Male	252	63.00
	Female	148	37.00
Weight change	Loss	191	47.80
	Gain	82	20.50
	No change	127	31.80
Diabetes mellitus	Yes	309	77.20
	No	91	22.80
Hypertension	Yes	236	59.00
	No	164	41.00
Heart attack	Yes	26	6.50
	No	374	93.50
Cancer	Yes	10	2.50
	No	390	97.50
UTI	Yes	62	15.50
	No	338	84.50
Swollen legs	Yes	4	1.00
	No	396	99.00
Jaundice/hepatitis	Yes	11	2.80
	No	389	97.20
Autoimmune disease	Lupus nephritis	23	5.80
	No	377	94.20

Figure 1 visually depicts the demographic population and comorbidity of the study population.

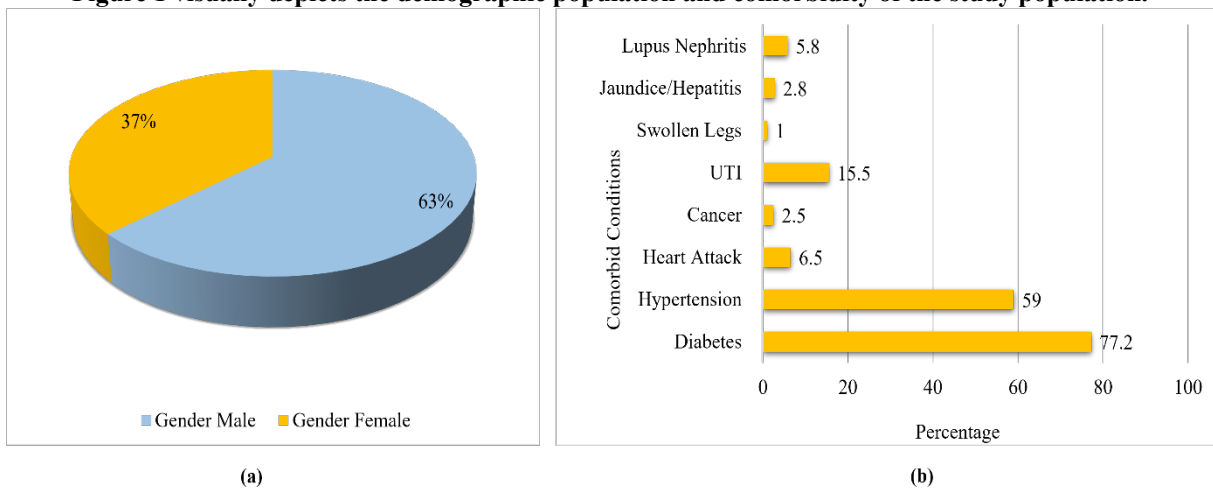


Figure 1. Study Population Characteristics: (a) Gender Distribution; (b) Prevalence of Comorbid Conditions

Figure 1 shows that the proportion of male patients was greater than that of females and indicates that most patients had multiple comorbid conditions in the study population. The most frequently observed conditions were diabetes and hypertension, where other clinical conditions were relatively less prevalent among the patients, evidencing a significant comorbidity burden among patients.

3.2 Distribution of Anemia Severity Based on Hemoglobin Levels

The distribution of severity of anemia showed that most patients were moderately anemic with 252 patients (63%) distributed under the moderate anemia (Table 2). None had serious anemia; 129 patients had mild anemia (32.20%). Only a small percentage of patients, 12 (3%), had the normal range of hemoglobin level. In general, 97 percent of the study population had some level of

anemia, which revealed that anemia is a significant health problem in CKD patients undergoing hemodialysis.

Table 2. Severity distribution of anemia among study participants (N = 400)

Category	Hemoglobin range	n	%
Severe anemia	<8 g/dL	7	1.80
Moderate anemia	8.00–10.90 g/dL	252	63.00
Mild anemia	11.00–12.90 g/dL	129	32.20
No anemia	≥13.00 g/dL	12	3.00

Figure 2 shows the distribution of the severity of anemia among CKD patients under hemodialysis treatment.

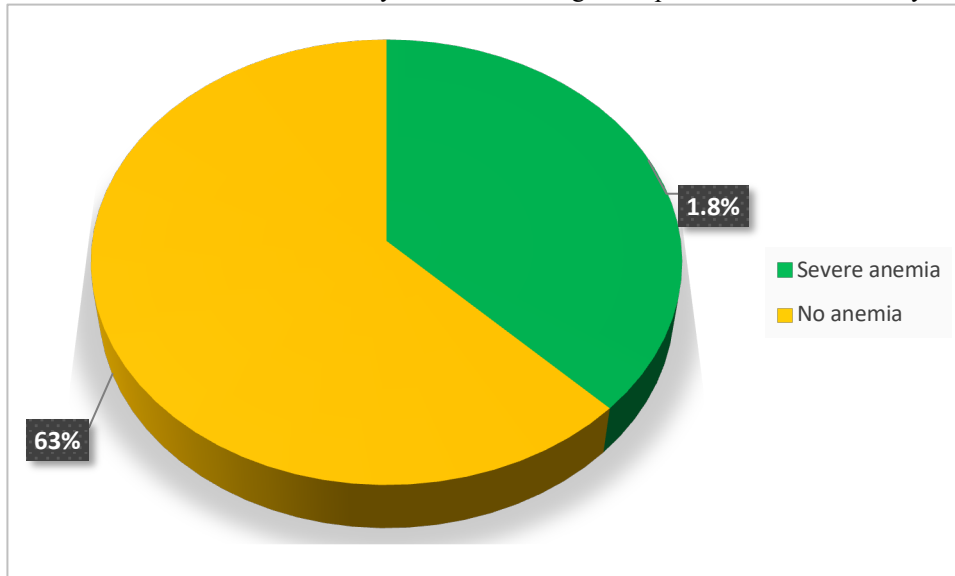


Figure 2. Distribution of Anemia Severity

The figure above indicates that the majority of patients are in the moderate anemia range, next in line was mild anemia, and severe anemia and normal hemoglobin were relatively small. This underscores the prevalence of anemia among the study population.

3.3 Descriptive Statistics of Hematological and Erythropoietic Parameters

The mean age of the research group were 54.10 ± 14.23 years, and the average dialysis time was 11.52 ± 11.07 years, which is a sign of a group of chronically treated

patients (Table 3). The mean pre-EPO and post-EPO values were 1.53 ± 1.19 and 1.42 ± 0.83 , respectively. The average number of reticulocyte was 1.49 ± 0.94 and the average reticulocyte hemoglobin equivalent was 24.91 ± 2.56 . The hemoglobin level was 10.38 ± 1.47 g/dL, which confirmed the prevalence of anemia, and the mean RBC and hematocrit were 3.87 ± 0.55 and $35.15 \pm 9.78\%$, respectively, indicating the disruption of erythropoietic activity in hemodialysis patients with CKD.

Table 3. Descriptive statistics of hematological and erythropoietic parameters (N = 400)

Parameter	Mean \pm SD
Age (years)	54.10 ± 14.23
Duration of dialysis (years)	11.52 ± 11.07
Pre-EPO	1.53 ± 1.19
Post-EPO	1.42 ± 0.83
Manual reticulocyte count	1.49 ± 0.94
RET-He	24.91 ± 2.56
Hemoglobin (g/dL)	10.38 ± 1.47
RBC	3.87 ± 0.55
Hematocrit (%)	35.15 ± 9.78

The average values of the most significant hematological and erythropoietic parameters are given in Figure 3.

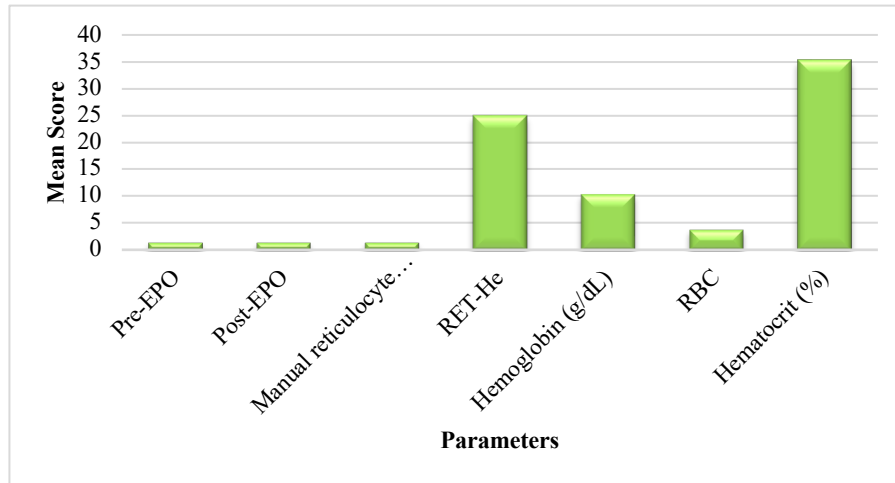


Figure 3. Mean Values of Hematological and Erythropoietic Parameters

The above figure demonstrates changes in terms of hematological parameters, whereby the mean values of RET-He and hematocrit were relatively higher, whereas the reticulocyte-related indices and the levels of erythropoietin were relatively lower.

3.4 Comparative Analysis of Erythropoietic Response Using Paired t-test

The analysis of t-test revealed a statistically substantial variance between pre-EPO and post-EPO values ($t =$

2.39, $p = 0.02$), which displayed that there was a statistically significant change in erythropoietic response after treatment. Nevertheless, the result of the comparison of the manual reticulocyte count and optimized reticulocyte count was not significant ($t = -0.79$, $p = 0.43$) which indicated that both the methodologies produced similar results in the determination of erythropoietic activity (Table 4).

Table 4. Paired comparison of erythropoietic parameters using t-test (N = 400)

Comparison	Mean 1	Mean 2	t-value	p-value
Pre-EPO vs post-EPO	1.53	1.42	2.39	0.02
Manual reticulocyte vs Optimized reticulocyte	1.49	1.51	-0.79	0.43

3.5 Correlation Analysis of Hematological Parameters

Correlation analysis showed that RET-He had a very weak and non-significant positive correlation with hemoglobin and manual reticulocyte count had a very weak and non-significant negative correlation (Table 5).

The correlation between manual reticulocyte count and RET-He was not significant as well. Conversely, there was moderate positive correlation between post-EPO and pre-EPO levels. RBC had a weak positive correlation with hemoglobin, but this correlation was statistically significant, unlike the case of hematocrit.

Table 5. Correlation analysis of hematological parameters (N = 400)

Variable pair	r	p-value
RET-He vs Hb	0.04	0.42
Retic vs Hb	-0.03	0.62
Retic vs RET-He	0.01	0.78
Pre-EPO vs post-EPO	0.64	<0.01
RBC vs Hb	0.13	0.01
HCT vs Hb	0.03	0.58

3.6 Regression Analysis of Predictors of Hemoglobin

Multi-linear regression analysis revealed that the general model was not significant ($p = 0.10$) and the explanatory power of the model was low, which means that the predictors included could not explain a significant percentage of the variability of hemoglobin levels (Table

6). RBC was also found to be the only statistically significant independent predictor of hemoglobin with no substantial predictors of hemoglobin being RET-He ($p = 0.31$), manual reticulocyte count ($p = 0.58$), and hematocrit ($p = 0.7$).

Table 6. Multiple linear regression analysis for predictors of hemoglobin (N = 400)

Predictor	B	Std. Error	Beta	t	p-value
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Constant	8.27	0.95	—	8.74	<0.01
RET-He	0.03	0.03	0.05	1.01	0.31
Reticulocyte count	-0.04	0.08	-0.03	-0.55	0.58
RBC	0.35	0.14	0.13	2.60	0.01
Hematocrit	0.00	0.01	0.02	0.34	0.73
R²	0.02				
Adjusted R²	0.01				
p-value	0.10				

4. Discussion

The study compared the clinical value of automated reticulocyte parameters, especially RET-He, with the manual reticulocyte parameters in measuring the erythropoietic activity and anemia in patients with CKD under hemodialysis. The results indicated that there was a high prevalence of anemia with most patients in the moderate category indicating that anemia continues to be a major burden in this group of people. The levels of hemoglobin were not optimal despite the erythropoietin therapy, which implies that it is not easy to reach satisfactory erythropoietic response in CKD patients. The descriptive analysis revealed that although the mean of the values of the parameters of hematocrit and RET-He were relatively high in comparison to the other parameters of hematology, manual reticulocyte counts and erythropoietin levels were relatively low. This implies that although treatment is being performed, erythropoiesis could be compromised by other underlying factors like iron deficiency, inflammation, and decreased responsiveness to erythropoiesis-stimulating agents. This observation was also confirmed by the paired t-test analysis which confirmed statistically substantial yet clinically small difference between pre- and post-EPO levels, which proved that there is limited therapeutic effects on enhancing erythropoietic activity. The correlation analysis showed that both the RET-He and manual reticulocyte count were very weak and nonsignificant with the hemoglobin levels. This implies that the two parameters do not have sufficient strength to solely represent the severity of anemia in hemodialysis patients with CKD. Nevertheless, the correlation coefficient was marginally higher with RET-He which indicates that it has a slight advantage over manual reticulocyte count in detecting changes in erythropoietin. The regression analysis further revealed that the overall predictive model was of low predictive power with only RBC being a statistically significant predictor of hemoglobin levels. These results indicate the multifactoriality of anemia in CKD and imply that using a single biomarker might not be sufficient to study anemia comprehensively.

The outcomes of the current study are partially in line with those of previous studies that have stated the clinical significance of automated reticulocyte parameters. According to Sany et al. (2020), RET-He is applicable in diagnosing iron deficiency in patients under hemodialysis and can give early information on the availability of iron. On the same note, Sharma et al. (2020) highlighted the benefits of automated

hematological analysers in enhancing diagnostic accuracy and minimising variability related to manual techniques. Nevertheless, the current study showed weak correlations as opposed to some studies that showed significant correlations between RET-He and hemoglobin levels, indicating that the usefulness of RET-He could be different based on patient characteristics and clinical conditions. The futility of optimizing iron therapy in CKD patients to enhance treatment outcomes, as Sood et al. (2023) emphasized, is consistent with the current results that show inappropriate erythropoietic response in spite of EPO therapy. The current study findings were also supported by Tamunonengiye-ofori et al. (2022) who also reported the high prevalence of anemia among CKD patients. Moreover, clinical guidelines note the necessity of proper monitoring of anemia based on reliable biomarkers, but also recognize the shortcomings of conventional parameters and the need to develop better methods of diagnosis (Yamamoto et al., 2017). The growing contribution of sophisticated medical technologies in hematology has also been identified as an important factor in improving the accuracy of the diagnosis and management of patients (Zunic, 2018). The findings of this study have important clinical implications. The poor predictive capacity of both manual and automated reticulocyte parameters indicates that a set of biomarkers might be required to be effective in monitoring anemia in CKD patients. Even though RET-He has potential as an early predictor of iron availability, its independent role does not seem to play a role in the current study. Thus, a combination of the automated parameters with more traditional markers could offer a more holistic approach to the anemia treatment and lead to better treatment results.

Nevertheless, there are some limitations to this study. The cross-sectional design limits the possibility to develop the causal relationship among variables. There was also a possibility of variability in clinical conditions, treatment regimens, patient response to therapy, which could have affected the results obtained. There was also a lack of adequate assessment of the presence of various confounding factors including inflammation and nutritional status which could have influenced the validity of the results. To gain a clearer picture of the dynamic nature of changes in erythropoietic activity and how automated reticulocyte parameters change over time, future studies should concentrate on longitudinal studies. To confirm the clinical relevance of RET-He and other emerging biomarkers, further research involving

bigger and more heterogeneous populations should be conducted. Also, the combination of the innovative diagnostic methods with individual treatment plans could help to improve the success of anemia control in hemodialysis patients with CKD.

5. Conclusion

The current research compared the clinical usefulness of automated reticulocyte parameters, specifically reticulocyte hemoglobin equivalent (RET-He), with the traditional reticulocyte methods in the monitoring of erythropoietic activity and anemia in the patients with CKD on hemodialysis. The outcomes showed the high prevalence of anemia, and most of the patients had moderate severity even on continuing erythropoietin therapy, which underscores the current difficulty in managing anemia in this group. RET-He and manual reticulocyte count had weak and insignificant correlations with hemoglobin levels, which means that they are not very effective to use as isolated indicators of anemia severity. Though the relationship between RET-He and manual reticulocyte count was slightly higher than the other, both of the two parameters were not significant independent predictors in regression analysis. The regression model in general had low explanatory power and highlights the multifactorial nature of anemia in CKD patients. RBC was the sole significant predictor out of the parameters which were evaluated, but its influence was modest. These results indicate that although automated reticulocyte parameters have some benefits, they cannot be used in isolation to substitute the traditional ones. Rather, it might be more helpful to consider an integrated strategy that would include both conventional and newer hematological indicators to have a more detailed evaluation of erythropoietic activity. On the whole, the research highlights the importance of combined diagnostic approaches to enhance anemia management and maximize treatment in CKD patients receiving hemodialysis.

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