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Correlation Between Reticulocyte Hemoglobin Equivalent (Ret-He) With Erythrocyte Indices in Predialytic Chronic Kidney Disease Patients in Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar

Christina Noviasiata Djawa[1,*], Sianny Herawati[2], Ni Kadek Mulyantari[2]

Christina Noviasiata Djawa[1,*], Sianny Herawati[2], Ni Kadek Mulyantari[2]

[1]Clinical Pathology Residency Education Program, Faculty of Medicine Udayana University, Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar, INDONESIA.

[2]Department of Clinical Pathology, Faculty of Medicine, Udayana University/ Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar, INDONESIA.

Correspondence

Christina Noviasiata Djawa

Clinical Pathology Residency Education Program, Faculty of Medicine Udayana University, Prof. Dr. I.G.N.G. Ngoerah General Hospital Denpasar, INDONESIA.

E-mail: christina.noviasiata@gmail.com

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ABSTRACT

Background: Early detection and monitoring of anemia are important in CKD patients, which significantly increases the effectiveness of clinical treatment, reduce treatment cost and improve quality of life patients. Differential diagnosis of anemia based on conventional erythrocyte indices is limited, therefore it requires to be combined with other indices to determine iron deficiency. Reticulocyte hemoglobin equivalent (RET-He) is an alternative indicator of iron deficiency that is not affected by inflammation, and has been proposed as an additional marker for iron deficiency screening. **Objective:** To determine the correlation between RET-He and erythrocyte indices (MCV, MCH, MCHC) in predialytic CKD patients. **Method:** Cross-sectional retrospective study of predialytic CKD patient data in Prof. Ngoerah General Hospital, that were tested for RET-He and erythrocyte indices in January – March 2022. Data was collected from laboratory information system (LIS) and medical record. RET-He was measured with lightscatter flow cytometry method and erythrocyte indices was calculated. **Result:** Sixty and three (63) study subjects were consisted of 37 men (58,7%) and 26 women (41,3%), with average of age was 60,68±10,52 years. The mean value of RET-He, MCV, MCH and MCHC was 30,55±2,86 pg, 89,08±7,78 fL, 28,76±2,72 pg, and 30,55±2,86 g/dL. Result of data analysis showed positive correlation between RET-He and MCV (r = 0,659), MCH (r = 0,734), and also between RET-He and MCHC (r = 0,383), with p < 0,05. **Conclusion:** There was a significant positive correlation between RET-He with erythrocyte indices in predialytic CKD patients.
Key words: RET-He, Erythrocyte indices, Predialytic CKD.

INTRODUCTION

Chronic kidney disease (CKD) is a global health problem. Based on Basic Health Research (RISKESDAS) data, the prevalence of CKD in Indonesia aged ≥ 15 years in 2018 was 0.38%, and the prevalence of CKD in Bali province was 0.44%, where 37.04% of patients underwent hemodialysis.[1]

Predialysis CKD patients often experience anemia, and one of the causes is iron deficiency. Anemia is a common complication in CKD patients and occurs when the glomerular filtration rate (LFG) is < 60 ml/min/1.73 m2. Various studies have reported a high incidence of anemia in patients with CKD, particularly iron deficiency anemia (ADB).[2] Early detection and monitoring of anemia is necessary in CKD patients. Anemia in dialysis CKD patients has been widely studied, but the identification and management of anemia in predialysis CKD patients has been reported to be suboptimal, requiring further research.[3]

The erythrocyte index is a widely used guide for anemia classification. Differential diagnosis of anemia based on the conventional erythrocyte index is limited, so it needs to be combined with other indices for iron deficiency determination.[4] Reticulocyte hemoglobin equivalent (RET-He) examination is used as an alternative indicator of iron deficiency that is not affected by acute or chronic inflammation.[5] RET-He has been proposed as an additional marker for iron deficiency screening. RET-He examination describes the real-time availability of iron in the process of erythropoiesis in the bone marrow and its incorporation into hemoglobin in the last 3-4 days.[6]

Research on the relationship between RET-He with erythrocyte index means corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in predialysis CKD patients is still limited. Therefore, this study aims to determine the relationship between RET-He and erythrocyte index, which is one of the parameters of anemia in predialysis CKD patients at Prof. Ngoerah General Hospital, so that it can help clinicians to make the diagnosis and subsequent patient management appropriately and optimally.

METHODS

The study was conducted at the Clinical Pathology Laboratory of Prof. Ngoerah Denpasar Hospital from June 2022 - December 2022. This study was an observational analytic study with a retrospective cross-sectional study approach. The target population of the study was all patients with a diagnosis of predialysis CKD, with the affordable population being all CKD patients undergoing treatment at Prof. Ngoerah Hospital in the period January 2022 - March 2022. The number of research samples was 63 samples. Samples were taken using the consecutive method. The research data were obtained from secondary data sourced from the

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Laboratory Information System (LIS) and patient medical records at Prof. Ngoerah Hospital.

The diagnosis of CKD was based on the Kidney Disease Improving Global Outcomes (KDIGO) 2013 criteria. The inclusion criteria in this study were CKD patients who had not undergone regular dialysis, were ≥ 18 years old, and were examined for RET-He and complete blood on the same day. Exclusion criteria were liver disease, infectious and inflammatory diseases, malignancy, pregnancy, and PRC transfusion < 3 months.

RET-He examination was performed on a Sysmex XN-3000 device with the light scatter flow cytometry method. Erythrocyte index examination (MCV, MCH, MCHC) based on automatic calculation in Sysmex XN-3000 device. Using venous blood samples (whole blood) with ethylenediaminetetraacetic acid dipotassium (K2-EDTA) anticoagulant tubes.

Data were analyzed with SPSS version 26. Data normality test using Kolmonogorov-Smirnov test. The correlation test between parameters was carried out with the Pearson correlation test, with a p-value <0.05 considered significant.

RESULTS

This study involved 63 research subjects who met the inclusion and exclusion criteria. Data on the characteristics of the research subjects are shown in Table 1. The study subjects consisted of 37 men (58.7%) and 26 women (41.3%) with an average age of 60.68 ± 10.52 years. The frequency of predialysis CKD patients based on stage is stage 3 CKD (30.2%), stage 4 CKD (44.4%), and stage 5 CKD (25.4%). Of the 63 study subjects, the median hemoglobin (Hb) level was 10.8 g/dL (5.5 - 12.9 g/dL). The mean RET-He value was 30.55±2.86 pg. The mean MCV value was 89.08±7.78 fL. The mean MCH value was 28.76±2.72. The mean value of MCHC was 32.36±2.86 g/dL.

Data normality test using the Kolmonogorov-Smirnov test gave the results of normally distributed data for RET-He, MCV, MCH, and MCHC parameters. From the results of data analysis using the Pearson correlation test, a significant strong positive correlation was obtained between RET-He and MCV (r = 0.659; p 0.000), RET-He and MCH (r = 0.734; p 0.000). There was also a significant weak positive correlation between RET-He and MCHC (r = 0.383; p 0.002). The scatter plot of the relationship between RET-He and the erythrocyte index can be seen in Figure 1.

DISCUSSION

The study subjects were 63 predialysis CKD patients at Prof. Ngoerah Hospital who met the inclusion and exclusion criteria. The study participants had an average age of 60.68 ± 10.52 years. The prevalence of CKD increases with decreasing eGFR and increasing age.[7] The risk of CKD increases in patients aged >60 years by 2.2 times compared to the population aged <60 years. This is because increasing age causes a gradual decrease in the number of nephrons, resulting in functional and structural changes in the kidney, such as decreased tubular function and decreased speed of glomerular excretion.[8]

Of the 63 study participants, 58.7% were male and 41.3% were female. The results of this study are in line with the research of Salman et al. (2016), which obtained the results of male gender (64.1%) than females in the predialysis CKD population.[9] Various factors such as hypertension, hyperglycemia, lifestyle, kidney structure, and hormonal differences can cause men to experience more predialysis CKD.[10]

In this study, the median value of hemoglobin parameters was 10.80 g/dL (5.5 - 12.9 g/dL). This is in line with research by Salman *et al.* (2016), who found that the mean hemoglobin level in predialysis CKD patients was 10.90 ± 2.40 g/dL.[9] According to KDIGO, the diagnosis of anemia in adult and child CKD patients over 15 years of age if the hemoglobin

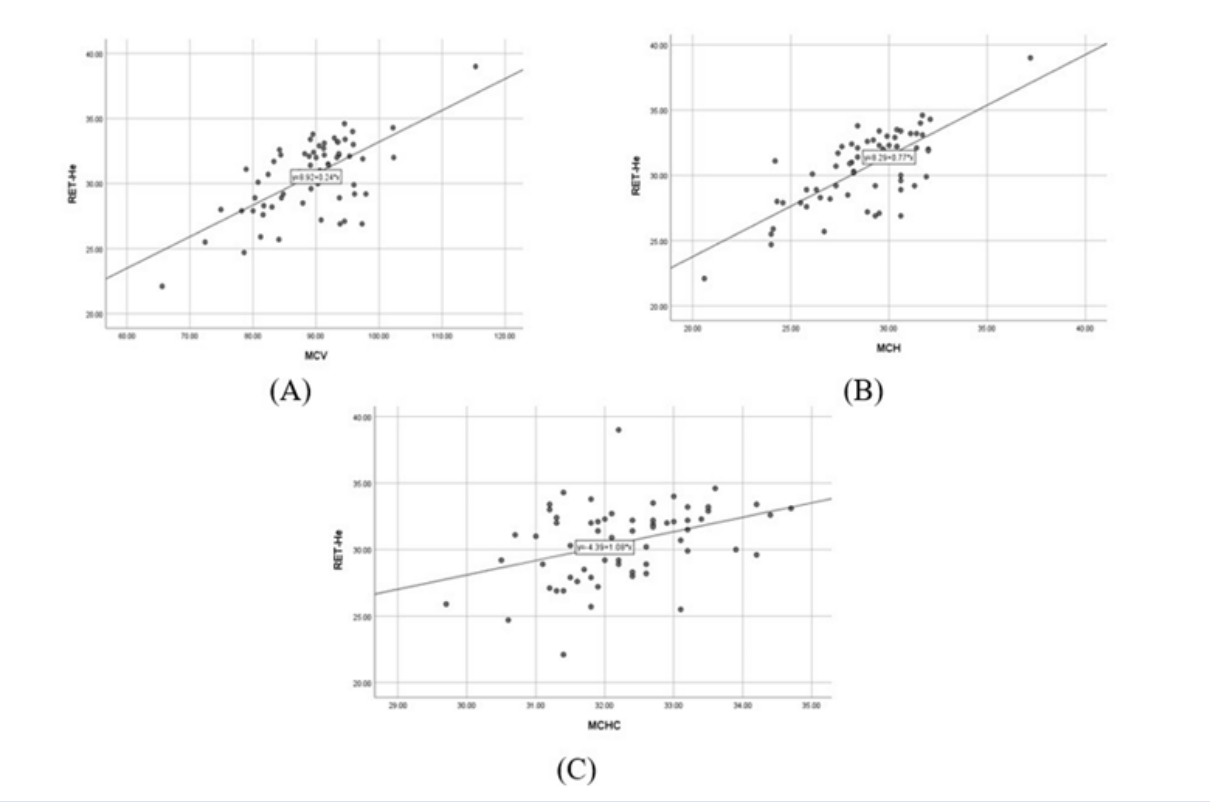

Figure 1: (A) Scatter plot of the relationship between RET-He and MCV, (B) Scatter plot of the relationship between RET-He and MCH, (C) Scatter plot of the relationship between RET-He and MCHC.

Table 1: Characteristics of the study sample.

Parameter	n (%)	Average± SD	Median (minimum-maximum)
Age (Years)		60.68±10.52	
Gender, n (%)			
Man	37 (58.7)		
Woman	26 (41.3)		
CKD stage, n (%)			
Stage 3	19 (30.2)		
Stage 4	28 (44.4)		
Stage 5	16 (25.4)		
Erythrocyte Index			
MCV (fL)		89.08±7.78	
MCH (pg)		28.76 ± 2.72	
MCHC (g/dL)		32.26 ± 1.01	
RET-He (pg)		30.55±2.86	
Hemoglobin (g/dL)			10.80 (5.50-12.90)

Table 2: Correlation test results between RET-He and erythrocyte index.

Variable	r	p value
MCV	0.659	0,000*
MCH	0.734	0,000*
MCHC	0.383	0.002*

Note: r: correlation coefficient; p: significance value, *: statistically significant

concentration is < 13 g/dL (male) and < 12 g/dL (female). Complications of anemia generally appear when the glomerular filtration rate is less than 60 ml/min/1.73 m2. The prevalence of anemia in CKD becomes higher with the loss of kidney function. The mechanism of anemia in CKD is due to reduced erythropoietin (EPO) production and iron-limited erythropoiesis. Some patients with anemia in CKD experience iron deficiency anemia (ADB) caused by absolute and functional iron deficiency.[2]

In this study, the mean value of RET-He was 30.55 ± 2.86 pg. In the research of Saputra *et al.* (2019), the average result of RET-He in hemodialysis CKD patients with anemia was 29.60 pg.[11] The results of another study by Trung *et al.* (2022) found the average RET-He was 30.2 ± 3.69 pg in CKD patients.[12] RET-He examination can provide information on the availability of iron for the process of erythropoiesis in the bone marrow in real-time and assess the quality of newly produced cells, RET-He reflects hemoglobin synthesis by newly formed erythrocyte cells in the bone marrow in real-time. Therefore, changes in iron status can be assessed early by RET-He.[6,12] The cut-off used to determine iron deficiency is 29 pg.[5] In this study, 20 (37%) of 63 patients had RET-He values < 29 pg.

The erythrocyte index examination in this study showed that the mean MCV value was 89.08 ± 7.78 fL, mean MCH 28.76 ± 2.72 pg, and mean MCHC 32.26 ± 1.01g/dL. The results of research conducted by Salman et al. (2016) in predialysis CKD patients with anemia obtained a mean MCV value of 84.9 ± 7.4 fL, mean MCH 28.3 ± 2.7 pg, and mean MCHC 33.2 ± 1.6 g/dL.[9] Based on the statistical analysis, a significant positive relationship was found between RET-He and MCV value (r = 0.659; p 0.000), MCH value (r = 0.734; p 0.000), and MCHC value (r = 0.383; p 0.002). RET-He describes the amount of hemoglobin in reticulocytes. If the amount of iron is inadequate, then an adequate amount of hemoglobin cannot be stored in reticulocytes. RET-He is therefore considered useful for assessing the status of hemoglobin synthesis and evaluating the level of iron used for hemoglobin synthesis.[13]

The erythrocyte index is used as a screening test for anemia and to identify anemia based on its morphology, but this test is affected by decreased iron levels.[14] MCV is the average volume of erythrocytes. The cell size of erythrocytes and reticulocytes is directly correlated with the hemoglobin content of the cell, so MCV indirectly reflects the hemoglobin content of the cell.[15]MCH is the average amount of hemoglobin per erythrocyte cell, and MCHC can be interpreted as the average hemoglobin concentration in one unit volume of erythrocytes. The formation of hemoglobin itself is highly dependent on the availability of iron in the body as a material to form the heme substance in hemoglobin used in oxygen distribution.[11]

In this study, a strong and significant positive correlation was found between RET-He and MCH. This can be explained because MCH is a reflection of the hemoglobin content in each erythrocyte cell with picogram units, which are obtained through calculations based on the formula (HGB/RBC) x 10. RET-He is a measurement of the amount of hemoglobin in reticulocytes, which indicates cell hemoglobinization, thus reflecting the quality of new reticulocytes produced by the bone marrow 1-2 days earlier. This similarity makes MCH and RET-He have a direct relationship.[11]

Based on previous research by Lian *et al.*, there was a strong and significant positive relationship (p < 0.05) between RET-He with MCV (r = 0.747), MCH (r = 0.818), and MCHC (0.775) in 238 patients with iron deficiency anemia (age range 2-80 years).[16] Another study by Duzenli and Altinkaynak found a positive and significant correlation between RET-He and MCV (r = 0.530; p < 0.01), MCH (r = 0.619; p < 0.01), and MCHC (r = 0.719; p < 0.001) in children with iron deficiency anemia. This study also found a strong correlation between RET-He with MCV (r = 0.643; p < 0.01), MCH (r = 0.652; p < 0.01), and a weak positive correlation between RET-He with MCHC (r = 0.246; p < 0.05) in pediatric patients with iron deficiency.[17] Based on research conducted by Himawan et al. (2020), there is a strong positive relationship between RET-He levels with MCV (r = 0.831), MCH (r = 0.850), and MCHC (r = 0.690), which is significant with a p-value = 0.000 in anemic pregnant women.[14]

The results of this study are also supported by research from Auerbach *et al.*, who evaluated the correlation between RET-He and MCH and found a strong and significant correlation (r =0.777; P<.001) in 556 patients with diagnosed and treated anemia. This also shows the specificity of the RET-He examination in determining the amount of hemoglobin contained in erythrocytes and representing MCH levels.[18]

Previous research by Saputra *et al.* (2019), where the correlation value obtained between RET-He and MCH in hemodialysis CKD adult patients with anemia was 0.391 (p = 0.020), showed that there was a positive and significant correlation. However, there was no correlation between RET-He and MCV.[11] Research by Trung et al. (2022) showed a significant decrease in RET-He levels in CKD patients with iron deficiency compared to those without iron deficiency (27.99±4.18 vs 31.8±2.2; p<0.001) and also found a decrease in MCHC (316.89±16.84 vs. 329.44±24.35; p=0.001), which showed a positive correlation between RET-He and MCHC in CKD patients with iron deficiency.[12]

In this study, the significant positive relationship between RET-He and MCV, MCH, and MCHC suggests that an increase or decrease in RET-He is in line with an increase or decrease in conventional erythrocyte indices. RET-He is related to conventional parameters and is reliable in identifying anemia. This similarity makes the erythrocyte index, and RET-He have a direct relationship and are used as alternative parameters to assess iron status in CKD patients.[11] The decrease in hemoglobin concentration and erythrocyte index is due to a decrease in RET-He synthesis, and the decrease in RET-He levels is indirectly due to the absence of iron. Thus, low RET-He is associated with iron-limited erythropoiesis and/or iron deficiency anemia.[17] RET-He examination can be performed simultaneously in routine blood tests without additional blood sampling, can be calculated automatically, and provides results quickly.[15]

CONCLUSIONS

There is a significant strong positive relationship or correlation between RET-He to MCV and MCH, and there is a weak positive relationship or correlation between RET-He and MCHC in predialysis CKD patients.

ETHICAL STATEMENT

This study has received ethical approval from the Research Ethics Commission of the Faculty of Medicine, Udayana University Number: 2629/UN14.2.2.VII.14/LT/2022.

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None

CONFLICTS OF INTEREST

None

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