

CORRELATION BETWEEN HEMOGLOBIN RETICULOCYTES AND FERRITIN IN CHRONIC KIDNEY DISEASE PATIENTS UNDERGOING HEMODIALYSIS AT PKU BANTUL

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Abstract: As many as 7 – 12% of the world's population has chronic kidney disease (CKD). CKD patients can experience various complications, one of which is anemia. Anemia can cause a variety of cardiovascular complications in CKD patients. Enforcement of the diagnosis of anemia and its type is carried out by laboratory examination, among others by examination of reticulocytes-hemoglobin (ret-he) and iron status such as serum ferritin. Examination of these parameters can help in planning the right treatment for CKD patients who have anemia. The purpose of this study was to determine the relationship of Ret-he with ferritin levels in CKD patients undergoing hemodialysis at PKU Bantul Hospital. This study used cross-sectional method. The data source used was secondary data obtained from medical records and the Indonesian Renal Registry (IRR). Sampling research used consecutive sampling method. The analysis used univariate analysis which was frequency distribution table, and bivariate analysis. Univariate analysis obtained that average levels of ret-he from 50 subjects was 28.87 ± 3.75 pg and median serum ferritin levels were 118,1 (9,76 – 1615) mL. Bivariate analysis with pearson correlation test found a significant correlation between serum ret-he and ferritin levels ($r = 0.498$, $p = 0.000$). There was a significant correlation between he-ret levels and serum ferritin levels

Keywords: chronic kidney disease, anemia, reticulocytes-hemoglobin, serum ferritin.

INTRODUCTION

Chronic kidney disease (CKD) is still a public health problem, especially the people of Indonesia with a high incidence rate, as well as high morbidity and mortality rates. Chronic kidney disease is a syndrome defined as a persistent change of kidney structure, function, or even both that lasts more than 3 months and impacts the health of the individual. Chronic kidney disease also includes a spectrum of various pathophysiological processes associated with renal abnormalities and a progressive decrease in the glomerular filtration rate (GFR), including $GFR < 60 \text{ mL/min/1.73 m}^2$, albuminuria, imaging-proven renal structural abnormalities, abnormalities renal tubules, and a history of kidney transplantation.^{1,2}

The causes of CKD vary from country to country. Data in the United States shows that the most frequent causes of CKD are type 2 diabetes mellitus (30%-50%), hypertension (27.2%), and primary glomerulonephritis (8.2%), while the most common causes of CKD in Indonesia according to the Ministry of Health are diabetes mellitus, hypertension, chronic glomerulonephritis, obesity, and the rest are unknown.^{3,4}

According to the 2013 Riskesdas, the prevalence of CKD is directly proportional to the increasing number of elderly people and the incidence of diabetes mellitus and hypertension.³ This is evident from the report of the Indonesian Renal Registry (IRR) program by the Indonesian Nephrology Association (Pernefri) on data related to dialysis, kidney transplantation, as well as epidemiological data on kidney disease and hypertension throughout Indonesia. According to the report, in 2017, the most CKD patients were aged 55-64 years with a percentage of 30.45%, then followed by CKD patients aged 45-54 years with a percentage of 29.57%, while the rest were aged less from 45 years by 23.31%, and CKD patients aged over 65 years by 16.14%.⁵

In addition to these data, IRR also presents data on the number of patients who stopped doing hemodialysis in 2017. As many as 30% of CKD patients stopped hemodialysis for more than 3 months and the cause could not be found, while 70% of CKD patients died. These data indicate that the mortality rate of CKD patients is very high. Various clinical conditions are the cause of the high mortality rate of CKD patients, one of which is anemia.

Anemia is the most common occurrence in patients with advanced CKD undergoing hemodialysis.⁶ Anemia can cause various cardiovascular complications such as pericarditis, pericardial pleural effusion, and heart valve disorders in CKD patients undergoing hemodialysis.⁷ Anemia is generally more common in stage 4 CKD and worsens as CKD progresses. In advanced CKD patients undergoing hemodialysis, the percentage of anemia is 90%.⁸

Anemia in CKD is caused by various problems such as reduced erythrocyte lifespan, iron and nutritional deficiency, reduced erythropoietin production, and various pro-inflammatory mediators that are generally increased in CKD that can affect erythropoiesis.⁹ These problems are closely influenced by the high and low glomerular filtration rate due to hemodialysis. Normally, 90% of erythropoietin is produced in the kidneys, precisely in the juxtaglomerulus, while the remaining 10% is produced in the liver. Under normal circumstances, the body responds to anemia by affecting the peritubular fibroblasts of the kidney by increasing the production of erythropoietin. In CKD patients, this process is disrupted. As a result, anemia occurs with low erythropoietin concentrations.¹⁰

Other studies have shown that iron deficiency can be found in anemia in CKD, where iron itself plays an important role in erythropoiesis in the bone marrow. Iron deficiency is caused by blood loss during routine hemodialysis, where iron content

can be seen by checking serum ferritin levels and transferrin saturation.⁶

According to Pernefri 2011, the examination of iron in CKD patients is to calculate transferrin saturation and serum ferritin levels. However, this standard has not provided efficient results in conditions of iron deficiency in the early stages, because it is also influenced by infectious-inflammatory conditions, so other tests are needed to get better results. Research conducted by Lorenz et al. 2015 showed that reticulocyte hemoglobin (Ret-he) can be used as a hematological parameter to evaluate iron deficiency in pregnant women according to gestational age, prevent anemia, and long-term neurocognitive deficits in premature infants.

Ret-he examination is useful for detecting iron deficiency at an early stage because reticulocytes released from the bone marrow into the bloodstream can circulate for 1-2 days before becoming mature erythrocytes, and hemoglobin levels in reticulocytes can be measured at any time.⁷ Because the Ret-he examination is not influenced by infectious and inflammatory conditions such as the examination of serum ferritin levels, the advantage of the ret-he examination is that it can be carried out in conjunction with other blood tests such as a complete blood count, so that the treatment of CKD patients with anemia becomes more efficient. This study aims to determine the relationship between Ret-he and ferritin levels in CKD patients undergoing hemodialysis at PKU Bantul Hospital.

RESEARCH METHODS

This study used an analytic observational design with cross-sectional data collection methods. Sources of data used in this study is secondary data from the results of examination of blood samples of patients with chronic kidney disease (CKD) undergoing hemodialysis. Adapun alat dan bahan yang digunakan dalam penelitian ini adalah formulir terstruktur dan data IRR. Variabel yang diteliti adalah retikulosit

hemoglobin (Ret-he) dan status besi berupa serum feritin.

Sampling of the research was obtained from medical record data of PKU Bantul Hospital Yogyakarta and Indonesian Renal Registry (IRR) data and was carried out in January 2021 - March 2021.

The target population of this study were CKD patients at PKU Bantul Hospital, Yogyakarta, while the affordable population in this study were patients diagnosed with CKD and undergoing hemodialysis at PKU Bantul Hospital, Yogyakarta. The inclusion criteria used are 1). Chronic kidney disease patients undergoing hemodialysis and suffering from anemia at PKU Bantul for at least 3 months, 2). Undergo hemodialysis 2 times a week, and 3). Aged 18 years old. Patients who were pregnant, received iron therapy in the last 3 weeks, and had a history of transfusion in the last 3 months were not included in this study.

The sampling technique used in this study was consecutive sampling technique, and the sample size formula used in this study was the sample size formula for numerical-numeric correlative analytics.^{6,11}

$$n = \left[\frac{Z\alpha + Z\beta}{0,5 \ln \left(\frac{1+r}{1-r} \right)} \right]^2 + 3$$

$$n = \left[\frac{(1,96 + 1,28)}{0,5 \ln \left(\frac{1 + 0,513}{1 - 0,513} \right)} \right]^2 + 3$$

$$n = 35$$

Information:

1. n = number of samples
2. Type 1 error Z alpha: set at 5%, with a two-way hypothesis then Z alpha: 1.96
3. Type 2 error Z beta: set at 10%, with a two-way hypothesis then Z beta: 1.28
4. Correlation coefficient of previous research (r) = 0.513⁶

So it can be concluded that the number of samples needed in the study amounted to 35 samples. To avoid errors in the study, the number of samples was rounded up to 50 people.

The analysis used in this research is univariate and bivariate analysis. Univariate analysis is used to describe the description of a variable, while bivariate analysis is used to analyze the relationship between two variables. The use of univariate analysis in this study to explain the characteristics of the research subjects (including gender, age, occupation, duration of hemodialysis, and comorbid diseases) and the hematological parameters of the research subjects (reticulocyte hemoglobin and serum ferritin levels), while the use of bivariate analysis to explain the relationship between reticulocytes hemoglobin with serum ferritin. In order to examine the relationship between reticulocyte hemoglobin and serum ferritin in bivariate analysis, correlative statistical tests were used. The normality test used is the Shapiro-Wilk test, because the required sample size is 50 samples. The correlation test used is the Pearson correlation test. The correlation test is said to be meaningful if the p value is < 0.05 , and it is said to be meaningless if the p value is > 0.05 .¹²

RESULTS AND DISCUSSION

Based on the results of univariate analysis, the characteristics of the research subjects are presented in table 1.

Table 1. Characteristics of research subjects (N = 50)

Characterics	Frequency	Percentage (%)
Gender		
Male	30	60
Female	20	40
Age		
< 45 y.o	2	4
45 – 59 y.o	30	60
60 – 74 y.o	17	34
75 – 90 y.o	1	2
Profession		
Entrepreneur	8	16
Retired	4	8
Housewife	5	10
Farmer		
Civil Servant	11	22
Others	3	6
	19	38
Hemodialysis duration		
≤ 36 months	28	56
> 36 months	22	44
Comorbid		
Hypertension	35	70
Diabetes melitus	17	34
Urinary stones	6	12

The age range of the research subjects is categorized based on the classification of old age according to WHO, namely middle age or middle age (45-59 y.o), elderly or elderly (60-74 y.o), old or old elderly (75-90 y.o), and very old or very old (> 90 y.o).

The category of duration of hemodialysis is divided into 2 categories, namely 36 months and > 36 months. The duration of hemodialysis was categorized based on previous studies looking for a relationship between the length of hemodialysis and the quality of life of patients undergoing hemodialysis.

Table 2. Iron profiles in the research

Variables	Mean $\pm$ SD	Median (Min – Max)
Hemoglobin (g/dL)		-
Male (30)	8,5 $\pm$ 1,16	-
Female (20)	7,9 $\pm$ 0,92	-
Total (50)	8,2 $\pm$ 1,10	-
Ret-he (pg)		
Male (30)	29,2 $\pm$ 3,7	-
Female (20)	28,2 $\pm$ 3,8	-
Total (50)	28,8 $\pm$ 3,7	-
Feritin serum (ng/mL)	-	
Male (30)	-	81,7 (9,76 – 1615)
Female (20)	-	167,3 (27,85 – 1047)
Total (50)	-	118,1 (9,76 – 1615)

Table 2 describes the description of the laboratory variables of the research subjects, namely Hemoglobin, Ret-he, and Serum Ferritin. The average hemoglobin level of the research subjects was 8.2 g/dL, with the highest hemoglobin level being 11 g/dL and the lowest hemoglobin level being 5.7 g/dL. This study showed that all research subjects were anemic.

The same results were also found in previous studies. In a study conducted by Hidayat et al. (2012)¹⁰ about the relationship between the incidence of anemia in CKD patients at Dr. M Djamil Hospital Padang, of 67 research subjects, 66 of them (98.5%) had anemia, where the average hemoglobin level was 7.3 g/dL with the lowest hemoglobin level was 3.4 g/dL and the highest level was 12.3 g/dL. The same thing was also found in a study conducted by Agustina and Wardani (2019),⁶ from 20 CKD patients at RSU KH Batu, all patients had anemia, where the average hemoglobin level was 7.38 g/dL the lowest hemoglobin level was 6.0 g/dL and the highest level was 9.0 g/dL.

Based on the WHO criteria, it is said to be anemic in men if the hemoglobin level is <13 g/dL, and it is said to be anemia in women if the hemoglobin level is <12 g/dL.¹³ Meanwhile, according to Pernefri, CKD patients are said to be anemic if the hemoglobin level is <14 g/dL in men and anemia if the hemoglobin level is <12 g/dL in women.⁷ The most common cause of

anemia in CKD patients is erythropoietin deficiency, followed by iron deficiency. Chronic inflammation of the kidney causes damage to the juxtaglomerulus, where erythropoietin is produced, so that erythropoietin production decreases which in turn causes a decrease in erythrocyte production. In addition, iron deficiency is also exacerbated by hemodialysis. Causes of iron deficiency in CKD patients undergoing hemodialysis include repeated phlebotomy, repeated blood tests, and clotting or blood loss in the extracorporeal circuit.^{13–15}

The average levels of ret-he in 50 research subjects were 28.8 ± 3.7 pg, with the highest levels of ret-he at 38.9 pg and the lowest levels of 19.6 pg. Research conducted by Dalimunthe and Lubis (2016)¹⁶ on CKD patients undergoing hemodialysis found that the cutoff ret-he 31.65 pg showed good diagnostic results with a sensitivity of 81.5% and specificity of 61.6%. By using a cutoff of 31.65 pg, there were 36 study subjects (72%) who had ret-he levels below normal and there were 14 study subjects (28%) who had ret-he levels above normal. This finding is in line with the research conducted by Dalimunthe and Lubis (2016)¹⁶ where out of 72 CKD patients who routinely underwent hemodialysis, the average ret-he level of the study subjects was 29.98 ± 3.85 pg. Research conducted by Miwa et al. (2010)¹⁷ in 153 iron-deficient patients undergoing

hemodialysis found a slightly higher mean ret-he level of 32.4 ± 4.0 pg.

Ret-he is a product of erythropoiesis which has a short life before turning into erythrocytes, which is 1-2 days.¹⁸ Ret-he can provide an estimate of the amount of hemoglobin present in reticulocytes with a good idea of how much iron is required for erythropoiesis in the bone marrow. The short lifespan of ret-he is hypothesized to be more sensitive in detecting erythropoiesis activity. Low levels of ret-he indicate a disturbance in erythropoiesis associated with a decrease in erythropoietin due to chronic inflammation of the juxtaglomerulus and hemodialysis processes.^{13,19}

The median total serum ferritin level of research subjects was 118.1 ng/mL, with the highest serum ferritin level of 1615 ng/mL and the lowest level of 9.76 ng/mL. This finding is in line with the study conducted by Chinudomwong et al., (2020)²⁰, in which of 120 patients with iron deficiency anemia due to infection, the median serum ferritin level of the study subjects was 125 ng/mL. The same result was also found by Toki et al. (2017)²¹ in 211 deficiency anemia patients, where the median serum ferritin level was 12.6 (0.0 – 2598.5) ng/mL.

Pernefri (2017) determined that absolute iron deficiency is when serum ferritin levels are < 200 ng/mL and TSAT $< 20\%$, while functional iron deficiency is when serum ferritin levels are 200 ng/mL and TSAT $< 20\%$. Using these criteria, there were 30 study subjects (60%) who had serum ferritin levels < 200 ng/mL and there were 20 study subjects (40%) who had serum ferritin levels 200 ng/mL. Ferritin is widely recognized as an acute-phase protein and a marker of acute and chronic inflammation, and its elevated levels are not specific for a broad range of inflammatory conditions, such as CKD, rheumatoid arthritis and other autoimmune diseases, acute infections, and malignancies. The increase in ferritin levels in these conditions reflects an increase in total body iron stores, but these stores are sequestered and cannot

meet the paradoxical need for hematopoiesis, where this process will contribute to the process of anemia in inflammatory conditions. This relative iron deficiency in inflammation and malignancy is thought to develop as a protective mechanism to prevent consumption of serum iron by pathogens and tumors.²²

Based on the bivariate analysis that has been carried out, the relationship between ret-he and serum ferritin levels can be explained in table 3.

Table 3. The relationship between ret-he and serum ferritin levels

			serum ferritin levels
Reticulocytes- hemoglobin levels	r	=	0,498
	p value	=	0,000
	n	=	50

The bivariate analysis step begins with a normality test, then a correlation test is performed. The normality test was carried out using the Shapiro-Wilk test, the distribution of serum ferritin level data was abnormal with a significance value of 0.000, while the distribution of ret-he data was normal with a significance value of 0.836. Because the ferritin content data is not normally distributed, it is necessary to transform the data. After data transformation, the ferritin content data became normally distributed with a significance value of 0.160. Because the two variables were normally distributed, the correlation test was carried out using the Pearson correlation test. After the correlation test, it was found that the value of $p = 0.000$, which means that there is a significant relationship between ret-he and serum ferritin levels, and the value of $r = 0.498$, which means the direction of the relationship is positive with a moderate strength of correlation. From these results it can be concluded that the higher the ret-he level, the higher the serum ferritin level, and vice versa.²³

The results of this analysis are in line with research conducted by Dalimunthe and

Lubis (2016)¹⁶, in which 72 CKD patients who routinely undergo hemodialysis, found a significant relationship between ret-he and serum ferritin levels with a value of $r = 0.499$, $p < 0,0001$. The same results were also found in a study conducted by Mehta et al. (2016)²⁴, and Toki et al. (2017)²¹ in patients with iron deficiency anemia, there was a significant relationship between ret-he and serum ferritin levels with values of $r = 0.786$, $p = 0.0000$; $r = 0.654$, $p < 0.05$. These results are slightly different from the research conducted by Miwa et al. (2010),¹⁷ where the results showed that there was a weak significant relationship between ret-he and serum ferritin levels ($r = 0.279$, $p < 0.01$).

The difference between the results of the bivariate analysis and the research conducted by Miwa et al. (2010) can be influenced by several factors, one of which is the difference in the number of research subjects involved. This study involved 50 research subjects, while the research conducted by Miwa et al. (2010) involved 153 research subjects. The difference in the number of research subjects resulted in different variations in the data for each variable so that it could cause differences in research results.

The relationship of ret-he with serum ferritin levels may be explained by the following mechanism. Ret-he and serum ferritin are iron parameters that can be used to establish the diagnosis of iron deficiency. Ret-he describes the amount of hemoglobin in reticulocytes, namely premature erythrocytes resulting from erythropoiesis in the bone marrow that circulate in the circulation for 1-2 days before turning into mature erythrocytes. Serum ferritin is an iron parameter that describes the body's iron reserves in the form of Fe^{2+} , this iron reserve will later be used as material for erythropoiesis in the bone marrow. In erythropoiesis, reticulocytes remain in the bone marrow for 2-3 days before being released into the circulation. If the amount of iron is inadequate, then an adequate amount of hemoglobin cannot be stored in

the reticulocytes. Therefore, ret-he is thought to be useful for assessing the status of hemoglobin synthesis and evaluating the level of iron used for hemoglobin synthesis. In CKD, this process is disrupted because the production of erythropoietin in the kidneys is decreased due to chronic inflammation.²⁵

Chronic inflammation in CKD causes an increase in serum ferritin as an acute phase protein. The increase in ferritin levels in these conditions reflects an increase in total body iron stores, but these stores are sequestered and cannot meet the paradoxical need for hematopoiesis, where this process will contribute to the process of anemia in inflammatory conditions. Because an increase in serum ferritin can obscure the results of the examination, serum ferritin cannot be used as a specific marker to establish the diagnosis of iron deficiency.¹⁸ Ret-he is not affected by inflammatory conditions, so it is considered useful as an indicator of iron deficiency in CKD patients undergoing hemodialysis. The ret-he test can be advantageous because it can be calculated quickly, automatically, and simply on peripheral blood only without the use of additional reagents.¹⁷

CONCLUSION

The conclusion of the study conducted on patients with chronic kidney disease at PKU Bantul Hospital was that there was a significant relationship between ret-he and serum ferritin levels in CKD patients undergoing hemodialysis at PKU Bantul Hospital with a moderate correlation strength ($p = 0.000$, $r = 0.498$).

The researcher also suggested that transferrin saturation should be checked to determine the type of anemia experienced by the patient, and suggested PKU Bantul Hospital to perform a complete blood count and iron status examination of CKD patients undergoing hemodialysis on a regular basis to support the diagnosis of anemia and as a treatment consideration for the anemia.

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