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CORRELATION BETWEEN THE PERCENTAGE OF HYPOCHROMIC ERYTHROCYTES AND FERRITINE LEVELS IN CHRONIC KIDNEY DISEASE PATIENTS UNDERGOING HEMODIALYSIS IN PKU BANTUL HOSPITAL

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Abstrak:

The incidence of CKD in the world is more than 500 million patients and up to 1.5 million patients need to undergo renal replacement therapy in the form of hemodialysis. Chronic kidney disease is inseparable from several complications, one of which is anemia. Confirmation of the diagnosis of anemia in CKD patients can be confirmed through various examinations such as the classic examination of serum ferritine levels, but ferritine is known to be involved in the inflammatory process that occurs in CKD. The aims of this study were to determine the relationship between the percentage of hypochromic erythrocytes and ferritine levels, to determine the average percentage of hypochromic erythrocytes and to determine the average ferritin level in CKD patients undergoing hemodiliasis at PKU Bantul Hospital. This study was conducted using a cross-sectional method involving 50 CKD patients at PKU Bantul Hospital. Blood samples were taken to check ferritine levels and hypochromic erythrocyte percentage. Data analysis was carried out with two types, univariate analysis and bivariate analysis which were carried out with the Spearman correlation test. Based on the results of univariate analysis, the median percentage of hypochromic erythrocytes of the research subjects was 0.95%. The median ferritine in research subjects was 105.5 ng/mL. based on the results of bivariate analysis, there was no significant relationship between the percentage of hypochromic erythrocytes and ferritine levels (p=0.130, r=-0.217). There was no significant relationship between the percentage of hypochromic erythrocytes and ferritine levels.

Keywords: anemia; ferritine serum, chronic kidney disease, reticulocytes hemoglobin

Introduction

Chronic kidney disease (CKD) according to Kidney Disease: Improving Global Outcome is a degenerative function disorder that causes fluid and electrolyte balance disorders that have an impact on all normal body systems. Abnormalities in this function can be interpreted as a decrease in the performance of the renals themselves related to blood processing in the body. This disorder is characterized by the finding of a decrease in the glomerular filtration rate (GFR) to <60 mL/min/1.73m^2. Abnormalities in the structure are characterized by the discovery of one or more signs of renal damage, namely albuminuria, abnormalities of urine sediment and the discovery of signs of damage on histological examination and imaging.[1]

Chronic kidney disease is a serious concern in various countries in the world, it can affect all ages and the incidence of the disease continues to show an increase every year. The World Health Organization (WHO) noted that there was an increase in the incidence of chronic kidney disease by 41.4% in the years between 1995-2025. The incidence of CKD in the world is recorded at more than 500 million patients and those who must undergo renal replacement therapy in the form of hemodialysis are up to 1.5 million patients.[2] The Indonesian Nephrology Association (Pernefri) estimates that there are 70,000 CKD patients in Indonesia, and it is estimated that this number will continue to increase every year by around 10%.[3] The prevalence of CKD over the age of 15 in Indonesia is estimated at 0.2% in 2013 and increased in 2018 to 0.38%. The prevalence in males (0.3%) is higher than females (0.2%).[4]

Chronic kidney disease is inseparable from several complications, one of which is anemia. The main cause of anemia in CKD is inadequate erythropoietin, as well as iron deficiency due to inadequate absorption and mobility. Therefore, anemia therapy in CKD must focus on a balance between two things, namely stimulating erythropoiesis and maintaining iron levels, therefore it is very important to know the condition of iron in the body accurately.[5]

Classic laboratory markers of iron status such as serum iron, transferrin and ferritine have drawbacks when used in CKD patients. Chronic kidney disease is a proinflammatory state and involves biologic variability in iron status. Ferritine is well known to be involved in this inflammation. New markers for iron status have been introduced that may be useful when serum ferritine and transferrin saturation are insufficient. These tests include reticulocyte hemoglobin content and hypochromic erythrocyte percentage (%Hypo). A direct consequence of the imbalance between erythropoiesis and actual supply is a reduction in the hemoglobin level of red blood cells, the first of which causes hypochromic reticulocytes and mature hypochromic erythrocytes. The reticulocyte hemoglobin (CHr) content and %Hypo reflects current iron availability for the erythropoietic system and are reliable markers of functional iron deficiency.[6]

The purpose of this study was to examine the relationship between the percentage of hypochromic erythrocytes and ferritine levels in CKD patients undergoing hemodialysis. The subjects of this study were chronic kidney disease patients with anemia who undergoing hemodialysis at the Hemodialysis Installation at PKU Bantul Hospital, Yogyakarta.

Research Method

This research is an analytic observational research with cross-sectional data collection method. The data used are secondary data from CKD patients who routinely undergo

hemodialysis therapy. The direct variable observed was the percentage of hypochromic erythrocytes (% Hypo). The iron status data in the form of ferritine were obtained secondary from the medical records of CKD patients who routinely undergo hemodialysis.

The study was conducted at the Hemodialysis Unit of PKU Bantul Hospital in November 2020 – December 2020. This study uses a target population of CKD patients in hospitals from 2019 to 2020. Meanwhile, the affordable population in this study were patients diagnosed with chronic kidney disease and were undergoing hemodialysis at PKU Bantul Hospital from January 2019 to January 2020. Data were obtained secondary from the Indonesian Renal Registry (IRR). The inclusion criteria used are 1) Chronic kidney disease patients undergoing hemodialysis and suffering from anemia at PKU Bantul Hospital for at least 3 months, 2) Undergo hemodialysis 2 times a week, and 3) The 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 is the consecutive sampling technique, and the sample size formula used in this study is the sample size formula for numerical-numeric correlative analytics.[7] The formula used to determine the sample size in this study is to use a correlation analysis sample size determination.[8]

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

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

$$n = 35$$

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 studies (r) = 0.513[9]

Therefore, 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 were univariate and bivariate analysis. Univariate analysis was used to describe the description of a variable, while bivariate analysis was 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 ferritine levels), while the use of bivariate analysis to explain the relationship between reticulocytes hemoglobin with serum ferritine. To examine the relationship between reticulocyte hemoglobin and serum ferritin in bivariate analysis, correlative statistical tests were used. The normality test used was the Shapiro-Wilk test, because the required sample size was 50 samples. The correlation test used was the Pearson correlation test. The correlation test was said to be meaningful if the p value is < 0.05, and it was said to be meaningless if the p value was > 0.05.[10]

Results

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

Table 1. The characteristics of research subjects (N=50)

Characteristics	Frequency	Percentage
Gender		
Male	29	58
Female	21	42
Age		
<45 y.o	3	6
45-59 y.o	28	56
60-74 y.o	18	36
75-90 y.o	1	2
Occupation		
Entrepreneur	5	10
Private Employee	11	22
Farmer	14	28
Other	20	40
HD Duration		
≤36 months	32	64
>36 months	18	36
Comorbid		
Hypertension	35	70
Diabetes melitus	17	34
Urinary Tract Stones	6	12
Others	2	4

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

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

Tabel 2. Iron profile of the research subjects

Variable	Median (min-max)
Hemoglobin (g/dL)	
Male (30)	8,40 (6,50-11,50)
Female (20)	7,80 (5,70-10,20)
Total (50)	7,85 (5,70-11,50)
Feritine (ng/mL)	
Male (30)	74,39 (9,76-732,9)
Female (20)	105,7 (30,8-562,8)
Total (50)	105,5 (9,76-732,9)
Percentage of Hypochromic Erythrocytes (%)	0,70 (0,10-6,10)
Male (30)	0,15 (0,10-21,70)
Female (20)	0,95 (0,10-21,70)
Total (50)	

Table 2 describes the description of the laboratory variables of the research subjects, namely hemoglobin, ferritine, and the percentage of hypochromic erythrocytes. In the laboratory variable description, male hemoglobin has a median of 8.40 g/dL with the highest value being 11.5 g/dL and the lowest being 6.5 g/dL. While in the laboratory variable description, female hemoglobin has a median of 7.80 g/dL with the highest value of 10.2 g/dL and the lowest value of 5.7 g/dL. The standard deviation (SD) of hemoglobin levels has a value smaller than the average value, which means that the distribution of the data is small and there is no large enough variation gap in the variable data. From the hemoglobin level data in Table 2, it can be concluded that all research subjects were anemic.

In the laboratory variable description, male ferritine has a median of 74.39 ng/mL with the highest value of 732.9 ng/mL and the lowest of 9.76 ng/mL. Meanwhile, in the laboratory variable description, female ferritine has a median of 105.7 ng/mL with the highest value of 562.8 ng/mL and the lowest of 30.8 ng/mL. The median ferritine level of the research subjects had a lower value than their respective standard deviations, which means that the distribution of data is large, and the gap of data variation is quite large as well. The median serum ferritine level of all study subjects was 105.5 ng/mL with the highest value being 732.9 ng/mL and the lowest being 9.76 ng/mL. by only looking at the ferritin levels of the research subjects, the condition of iron cannot be ascertained to be sufficient or excessive, because serum ferritine is an acute phase protein that is influenced by the inflammatory process.[11]

In the description of the laboratory variables, the percentage of male

hypochromic erythrocytes has a median of 0.7% with the highest value of 6.1% and the lowest of 0.1%. While in the description of the laboratory variables, the percentage of female hypochromic erythrocytes has a median of 0.15% with the highest value of 21.7% and the lowest of 0.1%. By using a cutoff > 6% to indicate iron deficiency, the percentage of hypochromic erythrocytes of male and female subjects showed a normal percentage. The two hypochromic erythrocyte percentage levels in the research subjects have values that are greater than their respective standard deviations, which means that the data distribution is large, and the data variation gap is quite large as well.

Based on the bivariate analysis that has been carried out, the relationship between the percentage of hypochromic erythrocytes and serum ferritin levels can be explained in Table 3.

Table 3. Relation of ret-he with serum ferritine levels

		Feritine
percentage of hypochromic erythrocytes	r	= -0,217
	P value	= 0,130
	n	= 0,50

The bivariate analysis step begins with a normality test, then a correlation test is performed. Normality test was carried out using the Shapiro-Wilk test, the distribution of ferritine levels and percentage of hypochromic erythrocytes was abnormal with a significance value of 0.000. Since both data were not normal, the data transformation was carried out and the data distribution of ferritin levels and hypochromic erythrocyte percentage were 0.457 and 0.705, respectively. Because the two data have normal data distribution after the transformation, the Pearson correlation test is applied. After the correlation test, it was found that the value of p = 0.130 and r = -0.218, which means that there is no significant relationship between the percentage of hypochromic erythrocytes and ferritine levels.

Based on the results of univariate analysis, the median value of the percentage of hypochromic erythrocytes of the research subjects was 0.95%. Examination of the percentage of hypochromic erythrocytes is used to provide an overview of iron in the body. The median percentage of hypochromic erythrocytes of the research subjects was in the normal category (cut-off >6%) which means that the picture of iron levels in the body is normal and the anemia that occurs is not caused by iron deficiency, giving rise to a picture of normochromic normocytic anemia typical of chronic disease.[12]

Discussion

The results of the research conducted are in line with the research conducted by Rehu, Ahonen, and Punnonen in 2011. A study involving 129 patients with anemia of chronic disease due to several underlying diseases such as CKD had an average percentage of hypochromic erythrocytes of 1%, this shows that the average patient with anemia of chronic disease such as CKD generally does not experience iron deficiency anemia; therefore, it can be concluded that anemia caused by occurs in the average study subject is not anemia caused by iron deficiency.[13] Another study conducted by Shastry and Belukar in 2019 on 300 CKD patients found that 94% of the study subjects had normochromic normocytic anemia, followed by hypochromic microcytic anemia at 3.4% and macrocytic anemia at 2.6%. The results of this study showed that most of the research subjects who were CKD patients experienced anemia typical of chronic diseases that were

not caused by iron deficiency.[14] This result is also supported by the research conducted by Sundhir et al. in 2018, which explains that the most common type of anemia experienced by CKD patients is anemia characteristic of chronic disease, which is 76% of patients. The anemia is characterized by normochromic normocytic erythrocyte morphology.[15]

The finding of normochromic normocytic anemia in CKD patients is caused by an inflammatory process. Inflammation that occurs will cause an active immune system which will increase the production of pro-inflammatory cytokines. Proinflammatory cytokines such as IL-6 and IL-1β will significantly affect the decrease in EPO production which will disrupt the erythropoiesis process and at the same time induce apoptosis in erythroid colony forming unit (CFU-E) cells. Increased apoptosis in CFU-E cells will reduce the process of erythrocyte development and manifest in a decrease in erythrocyte production but without changes in the shape of erythrocytes.[16]

Based on the results of the univariate analysis, the median ferritine of 50 research subjects was 105.5 ng/mL. The established reference values for ferritin levels are 30-300 ng/mL in men and 10-200 ng/mL for women. It is widely agreed that a decrease in ferritine levels to <12 ng/mL is interpreted as a decrease in body iron stores. However, in CKD patients, ferritine is not considered strong enough to be the main indicator of body iron. CKD is a pro-inflammatory state, therefore ferritine levels are often found in CKD patients up to >500 ng/mL, therefore a higher cut-off value is used to establish absolute iron deficiency anemia in CKD, which is <200 ng/mL.[17] In addition to determining iron deficiency anemia in CKD patients, it is considered better not only to measure ferritine but also transferrin saturation. Absolute iron deficiency anemia can be established when there is a ferritine level <200 ng/mL and a transferrin saturation <20%. Whereas in functional iron deficiency anemia, the ferritine level is 200 ng/mL and transferrin saturation is <20%.[18]

Based on the results of the analysis carried out, it can be seen that the median ferritine of the sample patients has a value below the cut-off absolute iron deficiency anemia, which is 105.5 ng/mL. It was found that from all study subjects there were 35 (70%) study subjects who had ferritine <200 ng/mL and 15 (30%) study subjects had ferritine 200 ng/mL. Similar findings were also shown in a study conducted by Nalodo et al., in 2018 where in a study involving 258 CKD patients had an average ferritine of 103 ng/mL.[19]

Chronic kidney disease is a proinflammatory state and involves biologic variability in iron status. Ferritine is well known to be involved in this inflammation. Inflammatory reactions that occur in CKD patients will result in the activation of the immune system and an increase in the production of inflammatory cytokines. These cytokines have an effect on increasing ferritine production by macrophages, giving rise to the characteristic inflammatory anemia in the form of hyperferritinemia. Of course, this characteristic does not always occur in all CKD patients, depending on the inflammatory process that affects it. Therefore, ferritine levels present in CKD patients cannot be the sole clue to accurately determine iron levels.[20]

Based on the results of bivariate analysis of the relationship between the percentage of hypochromic erythrocytes and ferritine, there is no significant relationship between the percentage of hypochromic erythrocytes and ferritine with a value of r = -

0.217 (negative relationship) and p value = 0.129 where it can be concluded that there is no negative relationship between the two, namely the higher ferritine is followed by a low percentage of hypochromic erythrocytes and vice versa.

Similar results have been presented in a study conducted by Bovy et al in 1999 where in this study discussed the comparison of the percentage of hypochromic erythrocytes with some classic iron predictors such as serum iron, transferrin saturation, and ferritine levels in CKD patients undergoing hemodialysis. In this study, the relationship between the percentage of hypochromic erythrocytes and ferritine levels was obtained with a p value of 0.28 with a negative direction correlation. Consequently, it can be concluded that there is no significant relationship between the percentage of hypochromic erythrocytes and ferritine levels.[21] The absence of a relationship between the percentage of hypochromic erythrocytes and ferrtine levels can be caused by an inflammatory process that occurs in CKD patients where the inflammatory process will directly affect ferritine levels. Ferritin levels are often found to be elevated in inflammatory conditions. In addition to studies in CKD patients, ferritine levels were also found to be elevated and not correlated with the percentage of hypochromic erythrocytes in other conditions that were also included in the inflammatory state as described in a study conducted by Krafft, Huch, and Breymann in 2003 where they examined several indicators of iron in pregnant women. A total of 64 postpartum pregnant women 48 hours before birth without iron deficiency were observed on conventional iron indicators, namely serum ferritine, serum iron, and transferrin saturation as well as new iron indicators: transferrin receptors, hypochromic erythrocyte percentage, and reticulocyte hemoglobin. They measured and compared various indicators of iron before and after birth which is believed to be a process that involves inflammation as evidenced by an increase in the inflammatory parameter, namely C-reactive protein after birth ($p < 0.01$). Ferritine levels were found to increase significantly before birth compared to after birth (9.7 ± 3.2 increased to 16.9 ± 10.3) with $p < 0.01$. There was no significant difference in the percentage of hypochromic erythrocytes before and after the birth process, and even tended to stay constant (4.0 ± 5.48 vs. 3.8 ± 5.31). These results indicate that there is no significant relationship between ferritine and the percentage of hypochromic erythrocytes. In addition, the results of this study increasingly show that ferritine is a variable that is not good at being a predictor of body iron. This could be because ferritin levels tend to increase in inflammatory conditions such as malignancy, chronic disease and childbirth. The conclusion that can be drawn from this study is that a new marker of iron in the form of hypochromic erythrocyte percentage is a good marker for predicting iron in the body that is not affected by the inflammatory process that occurs.[22]

These results are quite different from the findings of a study conducted by Amir et al., in 2019. The study stated that there was a strong negative correlation between ferritine and the percentage of hypochromic erythrocytes with p=<0.001 and r=-0.703. This study aims to determine the reliability of the percentage of hypochromic erythrocytes in being a marker of iron deficiency compared to the classical marker of ferritine. In this study, 160 research subjects were assigned to donate blood. Blood donation is believed to be one of the iatrogenic factors in the occurrence of iron deficiency in healthy adults. Blood donation is not a process that

causes a significant inflammatory reaction, therefore the ferritine marker can be used as an indicator of iron in the body. In the study of Amir et al., it can be concluded that there will be a significant relationship between ferritine and the percentage of hypochromic erythrocytes if there is no inflammatory process that can increase ferritine levels.[23]

Ferritine is the body's main protein of iron storage. The use of ferritine as an indicator of body iron availability is very common. Ferritine is also the most common marker for establishing iron deficiency anemia. The reason for using ferritine for diagnosis in iron deficiency anemia is because serum ferritine is the indicator that decreases most rapidly if there is a decrease in iron in the body. However, as is known, ferritine cannot be separated from the influence of inflammation that occurs in the body. Under normal conditions without inflammation, the ferritine value has a high similarity with the hemosiderin value which is the gold standard for iron deficiency. However, in inflammatory conditions, ferritine levels are often found which tend to increase so that this finding will interfere with the true interpretation.[24] Meanwhile, the percentage of hypochromic erythrocytes was a predictor of iron which was not affected by inflammation. The percentage of hypochromic erythrocytes is considered to be a reliable, inexpensive and rapid marker of being a predictor of iron in the body.[23]

Conclusions

The conclusion that can be drawn based on research that has been carried out on patients with chronic kidney disease at PKU Bantul Hospital is that there is no significant relationship between the percentage of hypochromic erythrocytes and ferritin levels (p = 0.129). The median percentage of hypochromic erythrocytes in CKD patients at PKU Bantul Hospital was 0.95%. The median ferritine level in CKD patients at PKU Bantul Hospital was 105.5 ng/mL.

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