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RESEARCH ARTICLE

Difference in the Levels of Ferritin, Hemoglobin, and Erythrocyte Count of Stage 5 Chronic Kidney Disease Patients after One, Three and Six Months of Hemodialysis

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ABSTRACT
Introduction: *77,892 chronic kidney disease patients in Indonesia undergo hemodialysis in 2017. However, the effects of period of undergoing hemodialysis on hemoglobin level, erythrocyte count, and ferritin level are still unclear.* **Objective:** *This research aims at examining the effects of period of undergoing hemodialysis on the levels of ferritin, hemoglobin, and erythrocyte count of stage 5 of chronic kidney disease patients.*
Methods: *In the analytical observational research with a cross sectional design, 30 men meet the inclusion criteria and are randomly divided into 3 groups: one month group (1M-G), three months group (3M-G), and six months group (6M-G). Each of the research objects has undergone hemodialysis for one, three and six months. The levels of ferritin, hemoglobin, and erythrocyte count are analyzed with an immunoflourescence method.*
Results: *The Post Hoc LSD analysis states that the ferritin levels of 3M-G (718±63.61) and 6M-G (947±66.22) are significantly higher than that of 1M-G (383.77), p< 0.01. The ferritin level of 6M-G is significantly higher than that of 3M-G, p<0.01. The hemoglobin levels and erythrocyte counts between 1M-G (9.45±0.84) & (3.26±0.55), 3M-G (9.10±1.22) & (3.21±0.50) and 6M-G (8.35±1.21) & (2.92±0.40) are insignificant, p>0.05.*
Conclusion: *After undergoing hemodialysis for three and six months, the ferritin levels improve significantly compared to that of one month of hemodialysis, and there is no significant difference in hemoglobin levels and erythrocyte counts.*

Keywords: *Hemodialysis, Ferritin, Erythrocyte, Hemoglobin*

ABSTRAK
Pendahuluan: penderita penyakit ginjal kronis di Indonesia pada th 2017 yang menjalani hemodialisis sebanyak 77892. Namun masih belum jelas efek lama menjalani hemodialisis terhadap kadar hemoglobin, jumlah eritrosit, dan kadar ferritin. **Tujuan**: penelitian ini bertujuan untuk mengetahui pengaruh lama hemodialisis terhadap kadar ferritin, hemoglobin, dan jumlah eritrosit pada penderita penyakit ginjal kronis stadium 5.
Metode: Dalam penelitian observasional analitik dengan rancangan *cross sectional,* sebanyak 30 pria yang telah memenuhi kriteria inklusi, dibagi menjadi 3 kelompok: kelompok satu bulan (1M-G), kelompok tiga bulan (3M-G), dan kelompok enam bulan (6M-G) secara random. Subjec penelitian masing-masing telah menjalani hemodialisis selama satu, tiga, dan enam bulan. Kadar ferritin, hemoglobin, dan jumlah eritrosis dianalisis dengan metode *immunoflourenscence*.
Hasil: Analisis *Post Hoc* LSD menyebutkan bahwa kadar ferritin pada 3M-G (718±63.61) dan 6M-G (947±66.22) lebih tinggi secara bermakna dibanding 1M-G (383,77), p< 0,01. Kadar ferritin pada 6M-G lebih tinggi bermakna dibanding 3M-G, p<0,01. Sedangkan kadar hemoglobin dan jumlah eritrosit antara 1M-G (9.45±0.84) & (3.26±0.55), 3M-G (9.10±1.22) & (3.21±0.50), dan 6M-G (8.35 ± 1.21) & (2.92±0.40) tidak bermakna, p>0,05.
Kesimpulan: Kadar ferritin setelah menjalani hemodialisis selama tiga dan enam bulan meningkat secara bermakna dibanding hemodialisis selama satu bulan. Sedangkan kadar hemoglobin dan jumlah eritrosit tidak terdapat perbedaan bermakna.

Kata kunci : Hemodialisis, Ferritin, Eritrosit, Hemoglobin

INTRODUCTION

Chronic kidney disease (CKD) is a condition where kidney does not function well either for three months or more (Weckmann et al., 2018). This disease occurs after a number of conditions which damages the mass of kidney nephron, particularly glomerulus, that reduces glomerular filtration rate (GFR) with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m2 (Padhi et al., 2015; Vadakedath and Kandi, 2017), as marked with persistent albuminuria (Kidney Disease

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Improving Global Outcomes (KDIGO), 2018; Tonelli et al., 2013). Hemodialysis (HD) is one therapy for chronic kidney disease patients aiming at removing various waste products of metabolism and excess water from blood in prevention of toxicity and prolonging life (Prodjosudjadi, 2009; Vadakedath and Kandi, 2017). Hemodialysis initiated in chronic kidney disease with GFR > 10ml/min/1.73m2 (Cabrera et al., 2017). In HD process, patients' blood flows through semipermeable membrane with special hemodialyzer liquid to clear up various substances which are toxic to the body. However, various data state that repeated HDs which take long period frequently cause severe anemia to patients (Babitt and Lin, 2012; Kidney Disease Improving Global Outcomes (KDIGO), 2018). HD induced anemia is Fe deficiency anemia which is naturally related to the level of iron stored in the body. One form of iron store in the body is ferritin, a cell protein with an important role of storing intra-cell Fe (Wang et al., 2010). Considering Fe deficiency anemia is a syndrome which is related to Fe level, an evaluation needs to be made whether anemia incident in HD, besides its correlation with hemoglobin level and erythrocyte count, is also correlated with ferritin level pursuant to the period of dialysis.

Chronic kidney disease is a non-infectious disease which makes a health problem throughout the world. The prevalence of CKD in the work ranges from 11%-13% (Hill et al., 2016). Indonesia is a country with a high number of CKD patients. The existing data show that there are a relatively high number of 77,892 CKD patients who actively undergo hemodialysis (HD) predicted to increase by 10% annually (Indonesian Renal Registry, 2017). The causes of chronic kidney disease which come to end stage renal disease and require hemodialysis are diabetic kidney disease, hypertension, glomerulonephritis, kidney obstruction and infection, polycystic kidney disease, etc. (Prodjosudjadi, 2009).

Anemia which occurs in CKD patients are normocytic, normochromic and hypoproliferative anemia marked with a hemoglobin level decline (<12 g/dL for women and <13.5 g/dL for men and an erythrocyte count decline marked with a hematocrit level decline (Babitt and Lin, 2012; Hsu et al., 2002). Evidence shows that there is positive correlation between the period a CKD patient undergoes HD and declines of hemoglobin level and erythrocyte count (Hsu et al., 2001). This, besides in regard to erythropoietin level decline, is also caused by erythrocyte lifespan decline, Fe loss caused by chronic bleeding resulting from dysfunction of platelet, phlebotomy, and blood trapped in dialysis instrument (Bennett et al., 2009). Physiologically, erythrocyte count decline which is directly followed by oxygen pressure decline will trigger erythropoietin production by kidney and Fe release from ferritin (Babitt and Lin, 2012). Ferritin is iron reserve stored in the body and may be reused when the need for erythropoiesis increases. Therefore, systemic Fe homeostasis will be well controlled without inducing various cellular damages because of oxidative stress (Loréal et al., 2014). However, on the other hand, evidence shows that ferritin level of CKD patients who undergo hemodialysis frequently goes up since it cannot be released from its binding protein. Consequently, anemia still occurs with various impacts like quickly getting tired, left ventricular hypertrophy, hospitalization, CKD self-progressivity, and life quality decline (Babitt and Lin, 2012; Loréal et al., 2014).

The purpose of this research is to evaluate the difference in the levels of ferritin, hemoglobin, and erythrocyte count after one, three and six months of undergoing hemodialysis.

METHODS

This analytical observational research employs cross sectional design with 30 medical records of the research subjects, who are stage 5 chronic kidney disease patients who meet the inclusion criteria. The inclusion criteria include male, above 17 years old, routinely undergoing hemodialysis (2-3 times a week), not taking herbal drugs, not taking vitamin C and Iron, and no transfusion in the last 2 months. The subjects are randomly divided into three groups, each consisting of 10 subjects. The one month group (1M-G) consists of patients who had undergone hemodialysis for one month; the three months group (3M-G) consists of patients who had undergone hemodialysis for three months; and the six months group (6M-G) consists of patients who had undergone hemodialysis for six months. The levels of ferritin, hemoglobin, and erythrocyte count are measured in the end of research upon approval of the Ethic Commission, Faculty of Medicine, UNISSULA Semarang.

Ferritin, Hemoglobin, and Erythrocyte Count Examination

Patients' blood samples are left for 30 minutes and spun with a centrifuge for 5 minutes at 4000 rpm. The serum is immediately moved into a sample cup of 500 micron in size and put in container. The samples are then taken to the laboratory of clinic of the Academy of Health Analyst, Pekalongan for examination of ferritin level with immunoflourescence method. The erythrocyte count and hemoglobin level data are taken from patients' data worked on in the laboratory of

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Table 1. Research Subjects' Baseline Data of Each Group.

Criteria	Groups			P value (Anova)
	1M-G	3M-G	6M-G	
Sex	Male	Male	Male	
Age (mean)	32.40 (± 5.11)	31.20 (± 4.73)	32.95 (± 4,58)	0.708
Frequency of Dialysis (mean)	9.60 (± 2.06)	32.80 (± 7.00)	67.20 (± 10.11)	0.000
Transfusion in the last two months	No	No	No	

Table 2. Mean Ferritin Level, Erythrocyte Count and Hemoglobin Level of Chronic Kidney Disease Patients after Undergoing Hemodialysis

Variables	Period of Hemodialysis			p (Levene)
	1M-G n=10, χ(±SD)	3M-G n=10, χ(±SD)	6M-G n=10, χ(±SD)	
Ferritin (ng/mL)	383.77 (± 65.87)	718.31 (± 63.61)	947.98 (± 66.22)	0.944
Hemoglobin (g/dl)	9.45 (± 0.84)	9.10 (± 1.22)	8.35 (± 1.21)	0.234
Erythrocyte (x 10^6 cell/mm^3)	3.26 (± 0.55)	3.21 (± 0.50)	2.92 (± 0.40)	0.691

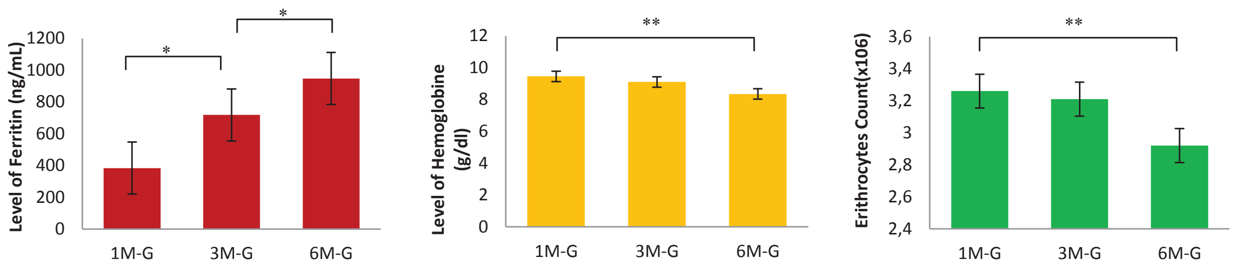

Figure 1. One Way Anova and Post Hoc Statistical Analysis for Level of Hemoglobine, Erithrocyte Count, and Ferritin Level. **One Way Anova analysis, p>0.05; *Post Hoc Analysis, p<0.01

Kraton Hospital, Pekalongan Regency automatically performed with hematology analyzer instrument Xi 1000.

Statistical Analysis

The statistical Saphiro Wilk and Levene tests are conducted on the data for data distribution and homogeneity. Considering the data obtained are normal and homogenous, the One Way Anova is employed for the statistical test, followed with Post Hoc Test. The statistical analysis result will be considered significant is the p value <0.05.

RESULTS

The population of target CKD patients with dialysis is those who visit and undergo hemodialysis at Keraton Hospital, Pekalongan Regency, in which 114 patients are selected with inclusion and exclusion criteria, resulting in 30 research subjects. The subjects of the three groups are studied for baseline data as presented in table 1.

The baseline data of the research subjects as presented in table 1 show that there is no difference in characteristics between the groups, except that the frequency of hemodialysis conforms to the period they undergo hemodialysis. Therefore, the three groups are comparable, thus further research may be conducted.

This research result shows that the lowest hemoglobin level and erythrocyte count are of the 6M-G group, followed with the 3M-G group, and the highest is of the 1M-G group. On the other hand, the highest ferritin level is of the 6M-G group, followed with the 3M-G group, and the lowest is of the 1M-G group. A statistical analysis needs to be conducted to observe whether such difference is significant. Considering that the data are normally and homogenously distributed, an Anova is conducted for the statistical test. The Anova test results show that there is no significant difference in hemoglobin level and erythrocyte count between the three groups, p > 0.05, and there is significant difference in ferritin level between the three groups, p<0.05. A Post Hoc LSD test is conducted as described below in order to observe which ferritin group has significant difference.

Ferritin Level

The results of Post Hoc LSD analysis show that the ferritin level of 6M-G group is significantly higher than that of the 1M-G and 3M-G groups, $p<0.01$. This analysis result explains that there is difference in ferritin level of chronic kidney disease patients who undergo hemodialysis for one, three and six months (figure 1).

DISCUSSION

The results of this research show that the mean hemoglobin level of stage 5 CKD patients who have undergone hemodialysis for one month is low (9.45 g/dl). The mean hemoglobin levels with hemodialysis for three months (9.10 g/dl) and six months (8.35 g/dl) also show low value, even lower than before. However, such hemoglobin level decline does not show significant difference. This explains that the longer the period of undergoing hemodialysis, the hemoglobin level will gets declining, although slowly. The results of this research are in line with the study conducted by Hsu, et al. that anemia is a complication of chronic renal insufficiency. The severer the renal insufficiency, the severer the anemia is. A research involving 15,873 samples of adults with renal insufficiency shows that anemia (Hb<12g/dl) in men occurs when creatinine clearances are below 70mL/min, and anemia (Hb<11g/dl) in women occurs when creatinine clearances are below 50 ml/min. Hb level declines by 1 g/dl when creatinine clearances are below 30 mL/min (Hsu et al., 2002). Similar results are also reported by Fadrowski, et al., stating that 340 averagely 11 years old patients have mean GFR 42ml/min per 1.73 m2 and mean hemoglobin level 12.5 g/dl. With this GFR (<43), hemoglobin declines by 0.3 g/dl for each GFR decline by 5ml/min per 1.73m2. On the other hand, for GFR above 43 ml/min per 1.73 m2, the hemoglobin level may only decline by 0.1 g/dl for each 5-ml/min per 1.73 m2 of GFR decline (Fadrowski et al., 2008). This explains that the severer the renal insufficiency, the lower the hemoglobin is.

In line with hemoglobin level, the results of this research also show that erythrocyte count in chronic kidney disease patients who have undergone hemodialysis for one month (3.26x106) is low, and gets even lower after three months (3.21x106) and six months (2.92x106). Although insignificantly different, the results of this research show that the longer the hemodialysis, the erythrocyte count tends to get lower. This explains that hemodialysis does not restore erythrocyte count because of stage 5 chronic kidney disease, or, on the contrary, even has erythrocyte count to decline. The results of this study conform to the study conducted by Hsu et al. that hematocrit value declines when creatinine clearance is below 60 mL/min in men and below 40 mL/min in women. Furthermore, Hsu's evidence also shows that GFR decline <30 mL/min/1.73 m2 in women and <20 mL/min/1.73m2 in men causes hematocrit to decline up to 33% (Hsu et al., 2001). This shows that the lower the glomerular filtration rate (GFR), the lower the erythrocyte count and hematocrit value are. The decline of hemoglobin level and erythrocyte count in chronic kidney disease is affected by some factors, such as an increase of ureum level which serves as erythropoiesis inhibitor and erythrocytes lifespan shortening (Babitt and Lin, 2012). In addition, in kidney disease patients who undergo hemodialysis, the decline of hemoglobin level and erythrocyte count is correlated with Fe homeostasis disorder.

Physiologically, a decline of hemoglobin level, erythrocyte count, and oxygen pressure will trigger erythropoiesis (Babitt and Lin, 2012). However, erythropoiesis will not occur if there is no erythropoietin produced by kidney and ferritin as iron store (Bradley et al., 2017). Erythropoietin serves as an accelerator which drives formation of erythrocyte in bone marrow, while iron serves as the fuel. Therefore, if both compounds interact well, erythropoiesis will occur well and efficiently. This is correlated with ferritin which is the biggest iron storage in the body, particularly in liver, spleen, and bone marrow, which serves as a buffer in case of deficiency or excess of easily mobilized iron (Bradley et al., 2017).

The results of this research show that the mean ferritin level increases in chronic kidney disease patients after one, three and six months of undergoing hemodialysis. The increase of ferritin level is very significant after three and six months of hemodialysis. Some previous researches also show that ferritin level of kidney failure patients who undergo hemodialysis also increases (Babitt and Lin, 2012; Kidney Disease: Improving Global Outcomes (KDIGO) Anemia Work Group, 2012). Moreover, the study conducted by Hasuike et al. states that an increase of ferritin level > 100ng/ml with low hemoglobin level has bad prognosis (Hasuike et al., 2010). Another study conducted by Kang, et al. states that high ferritin level is an important risk factor in chronic kidney disease incident (Kang et al., 2016). This is caused by inflammation reaction induced by an increase of ferritin level. Therefore, the overall results of this research show that stage 5 chronic kidney disease patients who have undergone hemodialysis for one, three and six months have lower hemoglobin level and erythrocyte count and, on the contrary, high ferritin level. Referring to the various researches, we may state that an increase of ferritin level in stage 5 chronic kidney disease patients who undergo hemodialysis may trigger further chronic kidney disease incident that may be an endless circle (circulus viteusus) which ends with mortality.

The erythropoiesis failure in chronic kidney disease patients who have undergone hemodialysis for one month to six months as described above is correlated with functional Fe deficiency. This is mainly caused by a failure of Fe release from ferritin, as representation of total iron store in the body. The increase of ferritin in this research explains that Fe stored in ferritin is unable to optimally drive erythropoiesis, thus anemia still occurs. Referring to this condition, we may conclude that Fe stored in ferritin cannot be mobilized to drive erythropoiesis. Considering that hepcidin is peptide which may be induced by Fe, the failure to mobilize Fe from its storage is more correlated with hepcidin (Loréal et al., 2014). Hepcidin is a hormone composed of 25 amino acids rich of cysteine, produced by liver and is the main regulator of systemic Fe homeostasis (Tsagalis, 2011). As evidence, a decline of Fe serum, an increase of Fe macrophage, a decline of Fe absorption from intestine in anemia because of inflammation is the impact of an increase of expression of hepcidin induced by inflammation. On the contrary, an increase of Fe serum, a decline of Fe macrophage, and an increase of Fe absorption from intestine in hereditary hemochromatosis is the result of deviation of regulation of mutated hepcidin gene expression (Wang et al., 2010). Besides functional Fe deficiency, in chronic kidney damage, low hemoglobin level and erythrocyte count may also be caused by kidney failure to produce erythropoietin to trigger erythropoiesis in bone marrow (Babitt and Lin, 2012). One limitation of this research is that it does not examine hepcidin level, thus further research needs to be conducted.

CONCLUSION

Stage 5 chronic kidney disease patients who have undergone hemodialysis therapy for one, three and six months have their hemoglobin level and erythrocyte count declined, but have their ferritin level elevated.

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CONFLICT OF INTEREST

There is no conflict of interest which needs to be declared.

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