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ORIGINAL PAPERS

Differences Ret-He, Reticulocyte, and Hemoglobin Levels Pre and Post Administration of Erythropoietin in Patients With Chronic Kidney Disease Undergoing Hemodialysis

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Abstract

Background: Chronic Kidney Disease (CKD) patients undergoing hemodialysis usually undergo anemia condition. Calculation of hemoglobin and other hematological parameters such as Ret-he (Reticulocyte Hemoglobin) and reticulocytes along with Glomerulus Filtration Rate (GFR) calculations determine the stage of CKD. Erythropoietin (EPO) administration is one of the important therapies for CKD patients. Initiation of EPO therapy, according to Kidney Disease Improving Global Outcome (KDIGO). **Method:** This study was an observational cross-sectional analytical study. This research was carried out from October 2023 to November 2023 at the Hemodialysis Installation of Dr. RSUD. R Sosodoro Djatikoesoemo Bojonegoro. Normality of the data using the Shapiro-Wilk test and differences in Ret-he levels, reticulocyte counts, and hemoglobin levels pre and post-administration of erythropoietin using paired t-test was used if the data were normally distributed and the Wilcoxon test if the data were not normally distributed. **Results:** This study results showed that there was an increase in ret-he levels pre and post-administration of erythropoietin with pre mean value of 30.50 ± 4.043 and post mean value was 32.20 ± 2.936. Reticulocyte levels also increase pre and post-administration of erythropoietin with a mean value of 1.63 ± 0.869 for pre-administration and 2.04 ± 0.915 for post-administration. Hemoglobin levels before giving erythropoietin with a value of 8.21 ± 0.855 and after giving erythropoietin the value was slightly increased to 8.29 ± 0.853. Ret-he and reticulocytes were significant differences between pre and post-EPO administration with p=0.000 and hemoglobin showed that there are no significant difference values pre and post-EPO with p=0.373. **Conclusion**: There is a significant difference (p-value 0.000) between Ret-He and Reticulocyte levels pre and post-erythropoietin treatment and there is no significant difference (p-value 0.373) Hemoglobin levels pre and post-erythropoietin treatment.

Keywords: Anemia, Chronic Kidney Disease, Hemoglobin, Ret-He, Reticulocyte, Hemodialysis.

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INTRODUCTION

Chronic Kidney Disease (CKD) or End Stage Renal Disease (ESDR) is a health problem in the world and is a progressive and irreversible kidney function disorder. Based on the 2018 Report of the Indonesia Renal Registry, over the last three years, CKD has experienced a significant increase and there were 25,446 new patients undergoing hemodialysis and 52,835 active patients in 2016. In 2017 there were 30,831 new patients undergoing hemodialysis and 77,892 active patients. Meanwhile, in 2018 there were 66,433 new patients undergoing hemodialysis and 132,142 active patients[1-3].

CKD requires therapy to maintain life, one of which is hemodialysis. Hemodialysis function was carried out to remove metabolic waste or certain toxins from the human bloodstream. CKD undergoing hemodialysis can be found to have anemia complications which are caused by blood loss during the dialysis process and there are also many cases of increased iron consumption with the administration of erythropoietin[3-5].

Management of anemia is carried out by examining Ret-he levels which can describe the availability of iron in the process of erythropoiesis in the bone marrow in the last few days, examining the number of reticulocytes which is an indicator of bone marrow activity, the number of reticulocytes in the peripheral blood depicts erythropoiesis almost accurately and calculating the hemoglobin number parameters along with Glomerulus Filtration Rate (GFR) calculation determines the stage of CKD which is achieved by Hb levels> 10 g/dL and Ht> 30%, either with conservative management or with erythropoietin[6,7].

Giving Erythropoietin (EPO) is an important therapy for CKD patients. Initiation of EPO therapy, according to the Kidney Disease Improving Global Outcome (KDIGO) in 2012, is carried out when the Hb concentration is <10.0 g/dL and is not used for maintenance therapy when the Hb concentration is above 11.5 g/dL in adult patients[8].

Research by Rosdewi *et al.*, 2022 at Stella Maris Hospital in Makassar found that the average Hemoglobin (Hb) value of patients before hemodialysis was 7.37 gr/dl and after hemodialysis average Hb value was 8.38 gr/dL. A cohort study conducted in 2014, showed that Hb level before therapy was 9.34 g/dL and the mean Hb level 1 month after receiving EPO was 10.35 g/dl[9]. In line with this research, the previous study showed that Erythropoiesis Stimulating Agent (ESA) therapy was able to maintain Hb levels ≥ 10 g/dL[10].

For CKD patients who undergo hemodialysis and develop anemia, EPO therapy is given which aims to increase the erythropoiesis process which will stimulate the formation of new erythrocyte cells, so researchers are interested in analyzing and proving the effect of giving EPO on increasing Ret-he levels, reticulocyte counts and hemoglobin levels.

METHOD

This research was conducted using a cross-sectional method. The aim for this study was to determine the differences in ret-he, reticulocyte and hemoglobin levels before and after administration of erythropoietin in Chronic Kidney Disease patients undergoing hemodialysis. This research was carried out in October-November 2023 at the Hemodialysis Installation at Dr. R Sosodoro Djatikoesoemo Bojonegoro. The data for this research is primary data, namely blood samples before and on the 4th day after administration of erythropoietin.

The research population was CKD patients undergoing hemodialysis at RSUD Dr. R Sosodoro Djatikoesoemo Bojonegoro. Research samples were patients with chronic kidney disorders who underwent hemodialysis at RSUD Dr. R. Sosodoro Djatikoesoemo Bojonegoro, who met the inclusion and exclusion criteria categories, were taken by consecutive sampling with a total sample of 30 people.

TOOLS AND MATERIALS

1. The tools used in this research were Sysmex
2. The ingredients in this research are EPO alpha 3000 IU, Respondent Characteristics Questionnaire, Whole Blood.

RESEARCH PROCEDURE

Examination of Ret-He, Reticulocyte and Hemoglobin levels

First, a blood sample of ± 5 ml is taken from the patient, to be checked for Ret-he levels, reticulocyte count, and hemoglobin using a Sysmex Xn-550 device before hemodialysis is carried out. After the hemodialysis procedure, EPO alfa 3000 IU is given which is on the national formulary, then on the 4th day the patient's blood is taken again for examination so that they can determine the Ret-he level, reticulocyte count and hemoglobin level after giving EPO. Researchers then analyzed data on Ret-he levels, reticulocyte counts, and hemoglobin levels before and after administering EPO according to the results of the observations.

Data Analysis

Data is processed using SPSS software. Data is presented in the form of descriptive statistics, in the form of tables or graphs. Data distribution used the Shapiro-Wilk test, then to determine the differences in ret-he, reticulocyte and hemoglobin levels before and after administration of erythropoietin in CKD patients the Wilcoxon test was used.

RESULTS

Characteristics of Research Subjects

The total respondents in this study consisted of 30 male and female samples, who met the inclusion and exclusion criteria. The hematology examinations are presented in Table 1. Table 1 shows that the number of patients undergoing hemodialysis was dominated by male research subjects, 20 people (66.7%) with the most research subjects aged 51-57 years, 7 people (23.3%) with age The youngest is 22 years old and the oldest is 72 years old. There is a profile of the patient's erythrocyte index, hematocrit, MCV, MCH before hemodialysis.

Descriptive analysis for differences in ret-he levels pre and post administration of erythropoietin showed that there are increasing average value for Re-Het, Hemoglobin, and reticulocyte.

Table 1. Characteristic Respondent

Characteristic	**N (%)**	**Median (Min-Max)**	**Mean±SD**
Gender			
Male	20 (66,7%)		
Female	10 (33,3%)		
Age		52 (22-72)	50,4 ± 1,905
22-29 years	3 (10,0%)		
30-36 years	1 (3,3%)		
37-43 years	5 (16,7%)		
44-50 years	4 (13,3%)		
51-57 years	7(23,3%)		
58-64 years	5 (16,7%)		
65-71 years	4 (13,3%)		
72-78 years	1(3,3%)		
Eritrocyte Pre Hemodialysis (/µl)		3,00 (2-4)	2,87 ± 0,441
Hematocryte Pre Hemodialysis (%)		25,50 (21-30)	25,81 ± 2,609
MCV Pre Hemodialysis (Fl)		90,35 (75-100)	89,06 ± 6,796
MCH Pre Hemodialysis (Pg)		29,00 (24-33)	28,90 ± 2,369

Table 2. Difference Test Variable Pre and Post EPO Treatment

Variable	Pre	Post	p
Ret-he (Pg)	30,50 ± 4,043	32,20 ± 2,936	0.000*
Reticulocyte (%)	1,63 ± 0,869	2,04 ± 0,915	0.000*
Hemoglobin (g/dl)	8,21 ± 0,855	8,29 ± 0,853	0.373

**Significant*

DISCUSSION

Based on the characteristics of respondents in this study, CKD patient in this study was dominated by male respondents (66.7%) compared to female respondents (33.3%). This data shows that the incidence of chronic kidney disease is more common in men because men predominantly often experience systemic disease and kidney problems due to men's lifestyle with smoking habits which can cause the kidneys to work harder[11].

Erythropoietin is a kidney hormone that has functions to maintain the number of erythrocytes. According to Tandra in 2018, Erythropoietin is an erythropoiesis-stimulating agent that stimulates the blood factory in the bone marrow. The role of erythropoietin is well known from the hematological side, namely in increasing the formation of red blood cells through stimulation of the erythropoiesis process. Giving erythropoietin to CKD patients will increase the formation of red blood cells through stimulation of the erythropoiesis process[12]. Erythropoietin treatment also can increase Ret-He levels within 4 days because erythropoietin will stimulate the production of erythrocytes whose formation starts from proerythroblast, basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast cells, and ultimately will mature into reticulocytes, and ret-he itself is in reticulocyte cells that describe the quality of newly produced reticulocytes. Therefore, within 4 days after administration of erythropoietin, ret-he levels can increase.

The result from this research is in line with research by Alvesa Mt et al (2015) that described that the provision of erythropoietin therapy is related to ret-he levels.

Ret-he content is a sensitive indirect marker of iron deficiency, reflecting recent changes in erythropoiesis. Measurement of Ret-he in peripheral blood samples is useful for assessing the amount of functional iron available in the bone marrow for the production of new red blood cells during the previous 3-4 days [13].

In the erythropoiesis process, an increase in the number of reticulocytes indicates the level of bone marrow response to anemia. The mechanism of increasing the number of reticulocytes in CKD patients after administration of erythropoietin in the erythropoiesis process under the influence of recombinant erythropoietin is that the erythroid stem cells will divide and differentiate. Furthermore, maturation will occur in the bone marrow where the proerythroblast cells will become basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, and will eventually mature into reticulocytes. Reticulocytes that have just been removed from the bone marrow still contain ribosomes and RNA and continue to produce hemoglobin. After 1 – 2 days in the peripheral blood the reticulocytes will lose their ribosomes and RNA and will become mature erythrocytes[14]. Reticulocytes increased in CKD patients 4 days after being given erythropoietin. The results of this study support the research results of Suega (2012) where erythrocyte production increased 2-3 times in 10 days in healthy bone marrow thanks to the hormone erythropoietin[15].

This erythropoietin hormone is produced by interstitial cortical cells around the proximal tubule (peritubular) of the kidney. The reduced volume of this hormone in the body causes disruption in the formation of red blood cells, which directly causes a decrease in hemoglobin levels in the blood[16,17]. According to research by Elmi et al (2014) erythropoietin therapy was proven to have a significant effect in increasing hemoglobin levels after 1 month of receiving EPO therapy increased, namely 10.35 g/dL. According to KADIGO (2012) the correction phase of EPO therapy begins with 2000-4000 IU subcutaneously, repeated 2-3 times a week for 4 weeks. The expected response from the patient is an increase in Hb of 1-2 g/dL in 4 weeks or an increase in Ht of 2-4% in 2-4 weeks. If the target response is achieved, the EPO dose is maintained until the target Hb is reached (> 10 g/dL). However, if the target response is still not reached, the EPO dose is increased by 50%. If after increasing the dose the Hb rises > 2.5 g/dL or the Ht rises > 8% in 4 weeks, the EPO dose is reduced by 25%. This result is not in line with this study because the pre-test and post-test hemoglobin levels in this study were examined for a period of 4 days so there was no significant difference in hemoglobin. Apart from that, the increase in hemoglobin levels in CKD patients is influenced by several factors, including age, gender, frequency of hemodialysis, and nutritional status (BMI).

Age over 35 years is the gateway to entering a high-risk age period in terms of reproduction and the function of organs in carrying out their functions has decreased, such as a decrease in the ability to absorb iron, so that the body's ability to produce hemoglobin cannot be maximized even though it has received EPO therapy. The process of erythrocyte formation takes 6-7 days to proerythroblast cells will become basophilic normoblasts, polychromatophilic normoblasts, and orthochromatophilic normoblasts, in the end they will mature into reticulocytes which takes 3-4 days in the peripheral blood. Further research is needed to determine the development of Ret-he levels, reticulocyte counts, hemoglobin levels after being given erythropoietin therapy and sampling adjusted to the half-life or half-life of erythropoietin.

CONCLUSION

Based on the results of this research, it can be concluded that there is an increase in Ret-he levels, reticulocyte count, hemoglobin levels after administration of erythropoietin but, only Ret-he and reticulocyte that shows significant difference values between pre and post-EPO treatment.

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.
There are no conflicts of interest regarding this article."

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