ISSN 0216-3438 (Print). ISSN 2621-1122 (Online)
Jurnal Profesi Medika : Jurnal Kedokteran dan Kesehatan

ANALYSIS OF RETICULOCYTE HEMOGLOBIN EQUIVALENT LEVELS IN PATIENTS WITH CHRONIC KIDNEY DISEASE STAGE IV AND V IN DR. M. DJAMIL CENTRAL PUBLIC HOSPITAL FROM JULY-SEPTEMBER 2020

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ABSTRACT

Reticulocyte hemoglobin equivalent (RET-He) represents hemoglobin content in reticulocytes. *Reticulocyte hemoglobin equivalent* test can assess the iron status of chronic kidney disease (CKD). Iron deficiency happens in 40% CKD and can lead to anemia manifestation. The RET-He level gives a real-time assessment of iron availability for hemoglobin production, and the level will be getting lower when iron storage for erythropoiesis decreasing. Reticulocyte hemoglobin equivalent is more stable than ferritin and transferrin saturation in assessing iron status. This study aimed to determine RET-He level in patients with CKD stage IV and V. This study was a cross-sectional descriptive study. Subjects were 96 CKD stage IV and V patients that met inclusion and exclusion criteria. Subjects conducted blood tests at Central Laboratory Installation Dr. M. Djamil Central Public Hospital Padang from July to September 2020. Examination of RET-He level was analyzed by Sysmex XN-1000 flowcytometry fluorescence method. Data was presented in the frequency distribution table. The RET-He level below cut-off (<29,2 pg) indicated the need for iron supplementation therapy for CKD stage IV and V patients. Samples with RET-He level below cut-off were 48 (50%), and 48 (50%) were above the cut-off.

Keywords: RET-He; Iron Status; Anemia; CKD

INTRODUCTION

Chronic kidney disease (CKD) is characterized by progressive and permanent loss of kidney functions. A decrease in glomerulus filtration rate (GFR) ≤60 ml/min/1.73 m^2 for three months or more in adults indicates loss of normal kidney functions for half or more. Chronic kidney disease is divided into five stages based on GFR, with stage I-III being a mild-moderate stage while stage IV-V are severe stages and require hemodialysis.[1,3]

Chronic kidney disease is a global public health problem with increasing prevalence and incidence of kidney failure, poor prognosis, and high cost. The prevalence of CKD increases with the increasing number of older people with an incidence of diabetes mellitus and hypertension.[4] Prevalence of CKD stage I-V globally was 13.4%.[5] Prevalence in males was higher than female by the ratio of two to three.[6]

Anemia is a common complication in CKD.[7,10] Anemia prevalence in CKD is inversely correlated with renal function, and most CKD stage V have anemia.[11] Prevalence CKD stage IV and V in children exceeded 87%.[10] Anemia in CKD is associated with decreased quality of life and increased risk of cardiovascular disease, cognitive impairment, and even death.[9] The main cause of anemia in CKD is a relative deficiency in erythropoietin production. Other possible contributing factors include iron deficiency, blood loss, inflammation, hemolysis, and nutritional deficiencies.[12,13] Optimizing iron status is a prerequisite for effective treatment of anemia.[10]

Iron deficiency occurs in 40% of CKD patients and 40-77% of CKD patients undergoing hemodialysis (CKD-HD).[9] Patients with CKD are prone to both absolute iron deficiency and so-called functional iron

deficiency. Absolute iron deficiency occurs due to combination of inadequate iron intake, impaired iron absorption in the duodenum, and excessive iron loss through intracorporeal hemodialysis circuits or gastrointestinal bleeding. Meanwhile, functional iron deficiency is caused by extracellular iron homeostasis.[10,11] Iron deficiency reduces erythropoietin (EPO) therapy response, and therefore, CKD-HD patients who have iron-deficiency anemia should be given iron supplements simultaneously with EPO therapy.[9]

Assessment of iron status in adult and pediatric CKD patients generally uses a combination of serum transferrin and ferritin saturation. These tests have limitations on biological and analytical variabilities and parameter instability during inflammation and malnutrition. Alternative tests have been widely studied in recent years to replace these two parameters, one of which is by assessing the level of reticulocyte hemoglobin content (CHr) or reticulocyte hemoglobin equivalent (RET-He).[10]

About 2 million out of 50 million CKD patients worldwide require hemodialysis as renal replacement therapy.[2,14] Hemodialysis begins at stage CKD stage V or if there are some other indications. [8,15] Parameter of CHr or RET-He is more stable in assessing iron status of CKD patients who are undergoing hemodialysis.[7]

National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) in 2006 and the Renal Association in 2017 had recommended CHr and RET-He to assess the iron status of CKD patients.[7,16] The study of 106 CKD-HD patients conducted by Wirawan *et al*. showed a very strong positive correlation and good concordance between RET-He and CHr. This result showed that clinicians could assess iron supplementation targets if patients were examined in different laboratories using different hematology analyzers. The cut-off value of RET-He as the target of iron supplementation in CKD-HD patients in the Wirawan's study was 29.2 pg, with CHr as the gold standard.[9]

Iron availability for erythropoiesis can be calculated by measuring iron contained in reticulocytes using CHr or RET-He. Measurement of CHr can be performed using ADVIA analyzer while RET-He using a Sysmex analyzer.[9] The biological and analytical variabilities of CHr and RET-He are better than conventional iron status tests.[10,17]

Reticulocyte hemoglobin equivalent (RET-He) level represents the hemoglobin content in reticulocytes, providing real-time information on iron availability for erythropoiesis and assessing the quality of newly produced cells.[18] Reticulocytes have greater volume, higher hemoglobin content, and lower hemoglobin concentration than mature red blood cells (RBC). Reticulocytes can only synthesize hemoglobin while in the bone marrow. Thus, once they enter the peripheral blood, they have the maximum hemoglobin content they can have.[19] Therefore, the changes of iron status can be detected much earlier than through the hemoglobin content of mature RBC.[18]

The RET-He test has various advantages over ferritin and transferrin saturation. This test can be performed simultaneously with routine hematologic examination and not affected by acute phase conditions such as inflammation, pregnancy, or other serious comorbidities. The RET-He test is used to monitor intravenous EPO and/or iron therapy. Increased level of RET-He indicates the success of therapy.[18]

MATERIAL AND METHODS

A cross-sectional descriptive study was conducted at the Central Laboratory Installation of Dr. M. Djamil Central Public Hospital Padang from July 2020 to September 2020.

The study population was CKD stage IV and V patients who examined routine hematological parameters. The samples were study populations that fulfilled the inclusion and exclusion criteria. Inclusion criteria were CKD stage IV and V patients. Exclusion criteria were CKD stage IV and V patients who: 1) received blood transfusions (whole blood or packed red cell), 2) consumed iron supplementation therapy, 3) under EPO therapy within two days before the examination.

Phlebotomy was performed aseptically in the vein of the cubital fossa region by trained phlebotomists. Volume 3 ml of venous blood was inserted into the K3EDTA anticoagulant tube. The sample was homogenized immediately

by slowly turning the tube eight times and avoiding foam formation. Measurement of RET-He was performed simultaneously with routine hematology examination. Level of RET-He was measured by fluorescence flowcytometry method in Sysmex XN-1000 hematology analyzer.

Data were analyzed descriptively to figure out RET-He level in patients with CKD stage IV and V. Data was presented in the frequency distribution table. In this study, the cut-off value of RET-He was using reference by Wirawan *et al.*, which was 29.2 pg. The RET-He level below cut-off (<29.2 pg) indicated the need for iron supplementation therapy in stage IV and V CKD patients.

RESULTS

Subjects' Characteristics

Study subjects were 96 patients with CKD stages IV and V with clinical characteristics shown in table 1.

Tabel 1. Subjects' Characteristics

Variable	n (%)	Median (Min-Max)	Mean (SD)
Age (year)		55 (18-80)	53.8 (13.2)
≦ 30	6 (6.3 %)		
31-50	26 (27%)		
51-70	59 (61.5%)		
≧71	5 (5.2%)		
Gender			
Male	50 (52%)		
Female	46 (48%)		
CKD Stage			
Stage IV	3 (3%)		
Stage V	93 (97%)		

Study subjects consisted of 52% male and 46% female. The mean age was 53.8 (13.2) years, with a median of 18-80 years. There were 3% CKD stage IV patients and 93% CKD stage V patients.

Hemoglobin and RET-He in CKD Stage IV & V Patients

All subjects (100%) had anemia. Study subjects with RET-He ≧29.2 pg were 48 patients (50%), and RET-He <29.2 pg was 48 patients (50%). The mean of RET-He level in this study was 28.8 (4.1), with a median of 29.1 (16-38.9) pg. Hemoglobin and RET-He levels in patients with CKD stage IV and V can be seen in table 2.

Tabel 2. Hemoglobin and RET-He in CKD Stage IV and V Patients

Variable	n (%)	Median (Min-Max)	Mean (SD)
Hemoglobin (g/dL)		8.25 (3.9-11.7)	8.2 (1.8)
Male : Hb <14	50 (100%)		
Female : Hb <12	46 (100%)		
RET-He (pg)		29.1 (16-38.9)	28.8 (4.1)
RET-He ≧29.2	48 (50%)		
RET-He < 29.2	48 (50%)		

DISCUSSION

Anemia was found in all study subjects. Anemia in CKD has multifactorial etiologies. The main cause of anemia in CKD is erythropoietin production impairment due to renal failure.[20] Effective erythropoiesis depends on adequate availability of both erythropoietin and iron. Iron deficiency is common in CKD. The reasons for iron deficiency include occult blood loss, infection, systemic inflammatory conditions, surgical procedures, venipuncture, impaired absorption secondary to elevated hepcidin concentrations, and in dialysis, retention of blood by the dialysis apparatus. It is estimated that hemodialysis patients may lose ≥2,000 mg of iron per year due to these factors.[13]

The RET-He level <29.2 pg in this study was found in 48 people (50%). Wirawan *et al.*'s study got RET-He cut-off of 29.2 pg as the target for iron supplementation in CKD-HD patients.[9] The RET-He level below this cut-off (<29.2 pg) indicated the need for iron supplementation in patients with CKD stages IV and V. The RET-He level can measure iron status so that anemia severity in CKD caused by iron deficiency can be avoided.

This study had several limitations. First, the number of CKD stage IV patients was very small, so it could not represent the RET-He level of CKD stage IV patients as a whole. We intended to examine the RET-He level of all

stages of CKD. Still, CKD patients in RSUP Dr. M. Djamil mostly were referral patients from other health facilities, in which majority of the patients were CKD stage V that had or would undergo hemodialysis.

CONCLUSION

Low RET-He levels in patients with CKD stage IV and V were found to be 50%. The iron status examination is needed at all stages of CKD. Measurement of RET-He level is an alternative test for assessing the iron status, and it can be performed simultaneously with routine hematology examination, making it more practical and cost-effective than the conventional iron status tests.

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