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ANALYSIS OF RET-HE IN CHRONIC KIDNEY DISEASE PATIENTS AT DR.WAHIDIN SUDIROHUSODO HOSPITAL, MAKASSAR

Febrina Rovani, Asvin Nurulita, Mansyur Arif

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr.Wahidin Sudirohusodo Hospital, Makassar, Indonesia. E-mail: febrinarovani@gmail.com

ABSTRACT

Anemia, the common feature of Chronic Kidney Disease (CKD), is a multifactorial process due to disordered erythropoiesis and iron homeostasis. Determining the cause of anemia is important for adequate management. A bone marrow biopsy using Prussian Blue as the gold standard for diagnosis is invasive and more complicated to perform. Reticulocytes-Hemoglobin (Ret-He) a new parameter that indicates the hemoglobin content in reticulocytes is faster, easier, and less expensive. This study aimed to analyze the Ret-He in determining the iron status in patients with CKD. A cross-sectional study was held in the Clinical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital Makassar during April-August 2016. Forty-five (45) samples were tested for iron serum (Fe), Total Iron Binding Capacity (TIBC), and Complete Blood Count (CBC) ordered by the physician. Reticulocytes-Hemoglobin was tested using the whole blood. Subjects were around the age of 19-71 years, no significant difference was found between numbers of males and females (46.6% and 53.3%). Hemoglobin median was 8 (5.0-15) g/dL, Fe 50 (6-177) U/mL, TIBC 183 (73-379), Transferrin Saturation (Tsat) 25 (5-95)%. Spearman correlation test method showed significant correlations between Ret-He and iron serum r=0.533, p <0.001, Ret-He and TIBC r=0.321 p=0.031 Ret-He and transferrin saturation r=0.416 p=0.019. The Mann-Whitney method showed no significant difference of Ret-He in both groups (Tsat <20% and >20%). There were significant correlations between Ret-He and iron, Ret-He and TIBC, Ret-He and transferrin saturation. A further study using larger samples is suggested to consider factors affecting the result of Ret-He.

Key words: Reticulocyte hemoglobin equivalent, Fe, transferrin saturation, chronic kidney disease, iron deficiency

INTRODUCTION

Chronic Kidney Disease (CKD) is one of the health problems with increasing incidence and prevalence worldwide. The high cost of CKD treatment is a burden for healthcare systems, especially in developing countries. Anemia is a common manifestation found in CKD. This condition can develop in the early phase of the disease and lead to a decreased quality of life.[1,2] Various factors such as erythropoiesis cause anemia in CKD and iron homeostasis disturbance through a series of the complex mechanisms, such as erythropoietin deficiency, chronic inflammation, blood loss, decreased iron absorption, iron administration, and exogenous erythropoietin. Other causes of erythropoiesis disruption are an inadequate response to erythropoietin therapy, erythrophagocytosis, decreased proliferative activity of erythroid precursors in bone marrow, the red blood cell lifespan and reduced availability of iron.[1-3]

The gold standard in assessing iron deficiency is bone marrow staining using Prussian Blue but this examination is invasive, therefore hematology and biochemical parameters are commonly used. Hematology parameters can only detect advanced stages of iron deficiency, while biochemical parameters such as serum iron, transferrin, ferritin are affected by inflammation.[4,5] The development of flow cytometry in the latest automated hematology analyzers can estimate the hemoglobin content of reticulocytes (Reticulocytes Hemoglobin Equivalent/ Ret-He). Ret-He can give information on how much iron is available for the erythropoiesis in the bone marrow and can detect iron deficiency in earlier stages.[4,5] Reticulocytes have a more rapid turnover in circulation compared to mature erythrocytes, suggesting that Ret-He is more sensitive in assessing erythropoietic activity.[5-7] Ret-He test is easier to perform and relatively cheaper than other iron profile tests.[1,5,6]

A study conducted by Dalimunthe and Lubis showed that Ret-He was a useful parameter for assessing iron deficiency and was able to predict the response of intravenous iron therapy in patients undergoing regular hemodialysis.[6] They reported

that with a cut-off value of 31.65 pg, Ret-He showed the sensitivity of 81.5% and specificity of 61.1%. Brugnara *et al*. said that Ret-He could diagnose iron deficiency at a cut-off value of 27 pg with a sensitivity and specificity of 93.3% and 83.2%, respectively.[5,7]

A study on Ret-He and iron status has never been done in Makassar, so researchers were interested to analyze Ret-He in assessing the iron deficiency in CKD patients compared to iron profile parameters that were routinely used in the Dr.Wahidin Sudirohusodo Hospital, Makassar. It is expected that the results of this study can be a reference for clinicians in choosing an effective and efficient test to assess iron status in CKD patients.

METHODS

This study was a cross-sectional study conducted in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Hospital Makassar from April to August 2016. Samples were blood specimens sent to the clinical pathology laboratory for serum Fe and TIBC testing and used whole blood samples. Serum Fe and TIBC tests were performed using the ABX Pentra C400 (Horiba Ltd, Kyoto, Japan). The Transferrin Saturation (Tsat) was obtained by manual calculation of serum Fe/TIBC x 100%. Ret-He was obtained using whole blood samples analyzed by flowcytometry method using Sysmex XN-1000 hematology analyzer (Sysmex America.Inc, Lincolnshire, Illinois) after patients signed a informed consent.

The data were analyzed using SPSS version 22 to assess the distribution of serum Fe, TIBC, transferrin saturation, and Ret-He.

The data were also analyzed for significant correlations between serum Fe and Ret-He, transferrin saturation, and Ret-He using Spearman correlation test as the data found were not normally distributed and for assessing the differences in Ret-He between the group of transferrin saturation ≥ 20% and group of transferrin saturation < 20%, the statistical test used was the Mann-Whitney analysis test.

RESULTS AND DISCUSSION

This study was conducted on 45 subjects with CKD, and the number of subjects did not differ significantly between males and females. Age distribution was between 19-71 years with a median of 52 years.

Table 1. Sample characteristics (n = 45)

Variable	N (%)	Median (min-max)
Age		52 (19-71)
<50	18 (40%)	
≥50	27 (60%)	
Sex		
Male	21 (46.6%)	
Female	24 (53.3%)	
Hemoglobin (g/dL)		8.0 (5.0 - 15.0)
Ret-He (pg)		31.2 (21.4 - 36.6)
Fe (µg/dL)		50 (6 - 177)
TIBC (µg/dL)		183 (73 - 379)
Tsat (%)		25 (5-95)

Most patients had anemia, with a hemoglobin range of 5.0-15.0 g/dL. The range of serum Fe level was 6-177 (µg/dL), TIBC (µg/dL) was 73-379, transferrin saturation was 0.05-0.95%, and Ret-He was 21.4-36.6 pg (Table 1).

Chronic kidney disease can occur in young adults to older adults, but the disease is most commonly found in older adults (≥ 50 years).[8] The prevalence of anemia is increased in CKD patients, especially those undergoing hemodialysis.[9,10] Anemia is defined as hemoglobin levels of less than 12 g/dL in females or less than 14 g dL in males.[9,11] A study conducted by Vali *et al*. in Manado reported that the hemoglobin range in CKD patients was 5.7-16.3 g/dL.[12] These results were consistent with this study which found that the hemoglobin in CKD patients ranged from 5.0 - 15.0 g/dL.

This study showed a range of Fe, TIBC, transferrin saturation and Ret-He levels ranging from low to normal, but none of them had high-normal levels. These results were consistent with a study conducted by Babbit *et al*. which stated that anemia in patients with CKD was a multifactorial process in which the most common cause was erythropoietin deficiency but not least due to Fe deficiency.[7,9,10]

The Spearman correlation test showed a correlation value of 0.533 with p <0.001 (Fe and Ret-He), which meant a significant positive correlation between Ret-He with Fe. This test showed a value of r=0.533 which indicated a moderate strength correlation. This result showed that serum Fe level was directly proportional to the level of Ret-He.

Ret-He describes the quality of the newly produced reticulocytes. Continuous production of reticulocytes without adequate iron supply will result in hypochromic microcytic erythrocytes.

Table 2. Correlation of Ret-He and serum Fe, TIBC, and Tsat

	Ret-He
Fe (U/mL)	r : 0.533 p : <0.001 n : 45
TIBC (U/mL)	r : 0321 p : 0.031 n : 45
Tsat (%)	r : 0.416 p : 0.019 n : 45

Reticulocytes will become mature erythrocytes within 1-2 days after release from the bone marrow, so hemoglobin content of reticulocytes reflects iron availability for erythropoiesis in recent days.[2,4] Dalimunthe *et al*. in 2011 reported that Ret-He increased rapidly after intravenous iron therapy so that it could be used as an early marker of iron therapy response.[6,7]

Table 2 also showed the results of Spearman correlation test with a correlation value of 0.321 and p=0.031 (TIBC and Ret-He). This result indicated a significant positive correlation between TIBC and Ret-He levels. Based on this test, r=0.321 meant a weak correlation. In patients with iron deficiency, TIBC levels tend to increase, but may also be normal and even decrease. Patients with decreased TIBC levels commonly have inflammation, low albumin or both.[13]

A significant correlation between Transferrin Saturation (Tsat) and Ret-He was also shown in Table 2 with a correlation value of p=0.019. Transferrin saturation and Ret-He were positively correlated with a moderate strength (r=0.416).

This result was consistent with the results of Miwa *et al*., the study indicating a strong correlation between Ret-He, and transferrin saturation (r=0.543).[14] Transferrin saturation has several acute phases of reactivity. Transferrin may be elevated in inflammation or infection, and it will decrease transferrin saturation if the circulated iron is constant. Low transferrin may be due to reduced transferrin synthesis in malnutrition or chronic disease, thus increasing transferrin saturation if the circulated iron is constant.[15]

The difference of Ret-He in two sample groups based on Tsat; the group with transferrin saturation <20% and ≥20% was also analyzed. The results of the Mann-Whitney test in Table 3 showed no significant difference in Ret-He between the two groups (p=0.056). It might be due to the small number of samples so that the iron status was not highly variable.

Several studies have suggested that response to iron supplementation can be assessed at 2 to 4 weeks after intravenous iron supplementation while using conventional parameters such as ferritin and transferrin saturation according to NKF-KDOQI guidelines (2006), monitoring is performed every three months.[6,7,16] Brugnara *et al*. reported that Ret-He can be used to monitor the initial response of iron therapy so that iron overload can be prevented.[7]

Table 3. Comparison of Ret-He in Tsat <20% and 20% group

	n	Ret-He Median (min-max)	p*
Tsat <20%	16	30 (25.7-36.1)	0.056
Tsat ≥20%	29	32.1 (21.4-36.6)	

Canals *et al*., conducted a study using Ret-He for the identification of iron deficiency anemia in 504 samples. Their study reported that Ret-He levels were only slightly decreased in anemia of chronic disease when compared with controls, whereas patients with iron deficiency anemia had a significant decrease.[17]

The limitations of this study were the small number of samples and incomplete data on the patient's therapy. The study was conducted with a cross-sectional design, in which Ret-He test for each patient was only performed once, whereas Ret-He is more useful in monitoring the response of iron therapy.

CONCLUSION AND SUGGESTION

Ret-He had a significant correlation with Fe and transferrin saturation. The correlation strength of Ret-He, and Fe as well as Ret-He and transferrin saturation, were moderate while Ret-He and TIBC had a weak correlation. These results suggested that Ret-He test may be used to assess the iron status of patients with CKD, but should be performed along with other iron parameters to provide a more rapid and precise diagnosis.

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