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ORIGINAL ARTICLE

A Study of Reticulocyte Haemoglobin in Patients with Renal Disease on Hemodialysis

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ABSTRACT

Background: In patients with chronic kidney disease, normochromic normocytic anemia mainly develops due to decreased renal synthesis of erythropoietin. Patients with chronic renal failure show abnormal hematological parameters, lower indices and the degree of changes depends on the severity of chronic renal failure. **Methods:** New generation cell counters provide reticulocyte count, more accurately by fluorescent method. This also provides reticulocyte parameters such as absolute reticulocyte count (RET), reticulocyte hemoglobin content (RET-He), and reticulocyte parameters like, immature reticulocyte fraction (IFR), low fluorescence reticulocytes (LFR), medium fluorescence reticulocyte (MFR), and high fluorescence reticulocyte (HFR). These parameters provide insight into functional anemia and response to therapy. **Results:** Most cases in this study had normal reticulocyte hemoglobin, in spite of anemia suggesting a functioning marrow in end stage renal disease. Four cases had a low reticulocyte count at 0.3% to 0.5%, out of which only two cases had low RET-He too. Low RET-He levels thus suggests an early parameter to know a declining marrow response likely due to iron deficiency. Hence these cases can be evaluated for iron studies to confirm the associated functional iron deficiency. **Conclusion:** Our study emphasizes on the value of evaluation of RET-He levels even in cases of mild anemia in renal failure patients thus aiding in diagnosis of iron deficiency and therapy.

Keywords: RET-He; Chronic renal disease; Hemodialysis

INTRODUCTION

Anemia is a frequent complication of chronic kidney disease. Anemia severity generally relates to the degree of renal impairment. In patients with chronic kidney disease, normochromic normocytic anemia mainly develops due to decreased renal synthesis of erythropoietin. Patients with chronic renal failure show abnormal hematological parameters, lower indices and the degree of changes depends on the severity of chronic renal failure. Reticulocytes are immature RBCs, presence of which indicates bone marrow response. When in increased demand, reticulocyte count increases, such as in hemorrhage and hemolysis. New generation cell counters provide reticulocyte count, more accurately by flouroscent method. This also provides reticulocyte parameters such as absolute reticulocyte count (RET), reticulocyte hemoglobin content (RET-He), and reticulocyte parameters like, immature reticulocyte fraction (IFR), low fluorescence reticulocytes (LFR), medium fluorescence reticulocyte (MFR), and high fluorescence reticulocyte (HFR). These parameters provide insight into functional anemia and response to therapy. Study of reticulocyte hemoglobin in renal disease on hemodialysis reflects on bone marrow response in end stage renal disease patients.

OBJECTIVES OF THE STUDY

- To determine the reticulocyte haemoglobin level in patients with anemia on hemodialysis.
- To evaluate the reticulocyte count in patients with anemia on hemodialysis.

MATERIALS AND METHODS

Total of 47 patients with anemia on hemodiaysis were selected for this study. Blood sample was collected, and hematological parameters were done including hemoglobin value, reticulocyte parameters – reticulocyte count, IRF,

LFR, MFR, HFR and reticulocyte haemoglobin value. All blood samples of patients with end stage renal disease having anemia on hemodialysis are included. Patients on hemodialysis for other causes are excluded. The venous blood sample is obtained from the hemodialysis patients for the study. EDTA vacutainer is used for reticulocyte haemoglobin count, haemoglobin and reticulocyte parameters were measured using the Sysmex haematology analyser. The Sysmex XN 1000 automated hematology analyzer uses combined impedance and radiofrequency conductance detection, semiconductor diode laser light 90° side scatter and 0° frontal scatter detection and polymethine fluorescence nucleic acid staining 90° side-fluorescence detection. It measures many clinical variables and graphs scatter plots and histograms. Reticulocytes are newly produced, relatively immature red blood cells (RBCs). A reticulocyte helps to determine the number and or percentage of reticulocytes in the blood and is a reflection of recent bone marrow function activity. Reticulocytes are visually, slightly larger than mature RBCs. Mature RBCs have no nucleus, but reticulocytes still have some RNA.

- **Method**

Supravital stain for ribosomal RNA and examined microscopy or fluorescent dye taken up RNA and cells counted by flow cytometry.

- **Normal values are**

Reticulocyte count – 0.5 – 2.5%
IRF – 0.11 – 0.38%
LFR – 81.0 – 96.4%
MFR – 1.1 – 15.2%
HFR – 0.03 – 3.95%
Reticulocyte hemoglobin – 30 – 38 pg

RESULTS

In the present study a total of 47 individuals were considered. The mean age (in years) of participants in is 57.7 years to 8.9 years. Majority of the study participants 20 (42.6%) are in the age group of 50 to 59 years. Majority of the study participants are males (68%).

In the present study, out of 47 cases, 2 cases showed RET-He below 30, with Hb level at 10.1 and 11gm/dl. Whereas all other 45 cases with anemia ranging from 9-11gm/dl had RET-He levels of more than 30pg. Thus, 2 out of 47 cases of anemia, (4.26% of total cases) were detected to have lower RET-He levels than the lower limit (Normal range 30-38 pg). This finding correlated well with IFR, LFR, MFR and HFR levels as well as these values. A positive correlation of RET-He and IFR, MFR, HFR levels were noted by using Karl Pearson's correlation. Out of the 47 cases, 36 of them showed moderate anemia, 11 of them showed mild anemia (11- 12gm/dl). Out of 47 cases, 43 of them shows normal reticulocyte count, four cases had lower reticulocyte count than 0.5% with normal RET-He in 2 of these cases. Out of 47 cases, 45 of them showed normal reticulocyte hemoglobin. Reticulocyte parameters like IFR, MFR, HFR shows positive correlation with reticulocyte hemoglobin of the study participants. While LFR shows negative correlation was observed between the variables ($p>0.05$). Correlation of Hb level and RET-He of the study participants showed 0.057 - 0.704 out of 47 cases studied.

DISCUSSION

The National Kidney Foundation in India states that, kidney diseases rank – 3rd amongst life threatening diseases. The prevalence of CKD is rising rapidly throughout the world, including India about 200,000 persons go into terminal kidney failure every year[1].

Patients with CRF classically develop a normochromic normocytic anemia[2]. The assessment of iron states for HD patients has been hindered by the inaccuracy of commonly used diagnostic tests. A novel assay, the reticulocyte hemoglobin content has recently been found to sensitively detect functional iron deficiency among non-numeric patients treated with recombinant erythropoietin (rHuEPO)[3].

In our study, we included total 47 hemodialysis participants, where majority of them are males. That is total number of male participants are 32 and that of female is 15.

In a study done by M Suesh et al, that patients with CRF shows abnormal hematological parameters, it has been proposed that in chronic renal failure, impaired production of erythropoiesis is main reason for the decrease in RBC count, Hb concentration. They have lower haematological indices, and the degree of changes depends on the severity of CRF[4].

In the present study, out of 47 cases, 2 cases showed RET-He below 30, with Hb level at 10.1 and 11gm/dl. Whereas all other 45 cases with anemia ranging from 9-11gm/dl had Ret-He levels of more than 30pg. Thus, 2 out of 47 cases of anemia, (4.26% of total cases) were detected to have iron deficiency anemia as the Ret-He levels were lower than the lower limit (Normal range 30-38 pg). Out of the 47 cases, 36 of them showed moderate anemia, and 11 of them showed mild anemia (11-12 gm/dl). Out of 47 cases, 43 of them shows normal reticulocyte count and 45 of them shows normal reticulocyte hemoglobin.

In the study conducted by Fishbane S et al, the study was to describe the mean and distribution of RET-He values and iron status in patients on hemodialysis. RET-He levels has been identified as a convenient indicator of iron deficient erythropoiesis[5]. In the present study, out of 47 cases of patients on hemodialysis, only 2 cases had lower RET-He levels. Study population included here are patients with renal failure. All patients on hemodialysis, although had mild to moderate anemia, their RET-He levels were normal in

majority of the cases (45 out of 47 cases). Therefore, RET-He levels reflected on the declining marrow response likely due to iron deficiency in these two cases. RET-He levels can also be used to evaluate the treatment response and thus the erythropoetic activity of the bone marrow, as seen in the study done by Fishbane et al. They studied the RET-He level after treatment with iron[3].

In study done by Brigg C et al, the delay in testing will increase the water content of reticulocytes, affecting the reticulocyte volume and hemoglobin concentration, but not the hemoglobin content. RET-He has attributes that may make it an ideal test of iron status for hemodialysis patients[2].

In study done by Touchie D et al, observed the relationship between the results of standard serum iron studies, Hb levels, and the RET-He in patients remaining preoperative intravenous iron (IV) therapy. They concluded that RET-He appears, to be an early marker of iron sufficiency. Based on these findings prospective studies are used to further investigate pre Ret-He as a predictor for the rate of Hb increment post IV iron therapy[6].

According to the study conducted by Scherer P et al, there was an increase of immature reticulocyte fraction (IFR, LFR, MFR and HFR) in patients with CKD[7]. In the present study also all cases had increased IRF but a normal LFR, MFR, and HFR values. These values were lower in two cases with low RET-He levels. Also, positive correlation of RET-He and LFR, MFR, HFR levels were noted by using Karl Pearson's correlation. Reticulocyte parameters like MFR, HFR shows positive correlation with reticulocyte hemoglobin of the study participants. While LFR shows negative correlation was observed between the variables ($p>0.05$). Correlation of Hb level and RET-He of the study participants 0.057 - 0.704 out of 47cases studied.

In study of Joao et al and Choi et al, that iron deficiency anemia is associated with an increase to approximately twice of IFR and reticulocyte fraction with average levels of fluorescence, and an increase of four times of reticulocyte with high fluorescence levels. The fluorescence intensity is directly related to RNA intracellular level, and therefore, with the degree of reticulocyte maturation, suggesting that iron deficiency anemia is associated with an increase in the proportion of IFR, related to increased erythropoietic activity of bone marrow[8,9]. Present study showed increased IRF in all 47 cases.

CONCLUSION

This study is limited by the evaluation of RET-He levels without correlating with treatment response or iron studies. However, in this study all cases had normal reticulocyte parameters in spite of anemia suggesting a functioning marrow in end stage renal disease. Four cases had a low reticulocyte count at 0.3% to 0.5%, out of which only two cases had low RET-He too. Low RET-He levels thus suggests an early parameter to know a declining marrow response likely due to iron deficiency. Hence these cases can be evaluated for iron studies to confirm the associated functional iron deficiency. Our study emphasizes on the value of evaluation of RET-He levels even in cases of mild anemia in renal failure patients thus aiding in diagnosis of iron deficiency and therapy.

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Conflicts of Interest

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