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Hematology

UTILITY OF RETICULOCYTE HEMOGLOBIN EQUIVALENT (RET-HE) IN ASSESSMENT OF RESPONSE TO IRON THERAPY IN CHRONIC KIDNEY DISEASE PATIENTS: A ONE YEAR HOSPITAL BASED STUDY.

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ABSTRACT **Introduction:** Reticulocyte hemoglobin equivalent (RET-He) is considered as a useful alternative parameter of iron deficiency anemia in chronic kidney disease patients due to its good diagnostic performance, availability of blood analyzer, and its lower cost. This study aims to find the response to iron therapy by using a real time marker (RET-He) which can be easily obtained, cheaper and will be convenient for both pathologists and clinicians. **Methods:** 100 chronic kidney disease (CKD) patients with iron-deficient states at Silchar Medical College and Hospital, Silchar were studied from June 2019 to May 2020. RET-He was compared with conventional iron parameters for identification of iron deficiency before and after starting of iron therapy. **Results:** Receiver operating characteristic (ROC) curve for RET-He revealed the value of area under the curve was 1 ($p < 0.0001$). Using cut-off level 27.6 pg, RET-He showed 100% sensitivity and 99% specificity in diagnosing iron deficient states of CKD patients. Serum ferritin ($r=0.6553$, $p<0.001$) and transferrin saturation/TSAT ($r=0.6876$, $p<0.001$) showed correlation with RET-He. Significant improvement in hemoglobin and RET-He were found after intervention ($p <0.0001$and $p <0.0001$, respectively). **Conclusion:** RET-He increased promptly after starting of iron supplementation and erythropoietin stimulating agent therapy, therefore, could be used as an early predictor of response to iron supplementation and thus, allows avoidance of problems like iron over-dosage which can damage many potential organs and also reduces the health-related direct and indirect costs.

KEYWORDS : Ret -He, Chronic kidney disease, Iron deficiency, iron therapy

INTRODUCTION

Chronic kidney disease (CKD) affects millions of people worldwide, with high incidence and prevalence and increasing costs.[1] Analysis of National Health and Nutrition Examination Survey (NHANES) data from the year 1988-1994 (NHANES III), 1999-2004 and 2007-2010 have yielded prevalence of CKD in US populations as 11.0%, 16.8% and 14.0% respectively.[2, 3, 4] However, the burden of chronic kidney disease (CKD) in India cannot be assessed accurately. Historically, the CKD research literature has struggled to create consensus on definitions of CKD [5,6] that has made the reporting of CKD research problematic. This situation appeared to be resolved in the year 2002 when the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) created guidelines providing a clear definition and classification system for CKD.[7] These guidelines define CKD as the presence of kidney damage or glomerular filtration rate (GFR) of <60 mL/min/1.73m^2 for ≥ 3 months.

Anemia in patients with CKD is due to many factors. Erythropoiesis and iron homeostasis are impaired as a result of a complex chain of events, including the relative deficiency of erythropoietin, chronic inflammation, blood loss, decreased iron absorption and utilization, exogenous iron and erythropoietin acquisition via biologically unregulated mechanisms (blood transfusion and medicinal EPO and iron administration).[1]

While treating anemia of CKD patients, one is very curious to know the response to treatment at the earliest. This confirms that treatment is on correct line, and this also avoids inadvertent hazards of over treatment.[8]

For assessment of response to iron therapy, clinicians have been using reticulocyte count as a marker for many ages. Other parameters like hemoglobin percentage (Hb %), packed cell volume (PCV), serum iron, serum ferritin, transferrin saturation (TSAT) are also being used. But Reticulocyte count rises only after 7-10 days[9] of therapy while other mentioned markers like serum ferritin, TSAT show a response as late as 3 months.[10]

However, our aimed marker reticulocyte hemoglobin equivalent (RET-He) offers a real-time information on iron supply to erythropoiesis[15] has been postulated to be the earliest marker of response to iron therapy.

Conventional parameters such as serum ferritin, serum iron, TSAT are not free of flaws and have been found to show many false-positive reactions particularly in states of physiological alterations like pregnancy, chronic kidney disease etc.[11]

Hence, we aim to do this study to find the response to iron therapy by using a real time marker which can be easily obtained, cheaper and will be convenient for both pathologists and clinicians.

MATERIALS AND METHODS

It was a prospective observational study and the samples were taken from 100 chronic kidney disease (CKD) patients with iron-deficient states who had either attended the OPD of the Department of Medicine of a tertiary care hospital of South Assam or were admitted in that department during a period of June 2019 – May 2020.

The required blood samples were collected from the patients before starting of therapy i.e. oral/intravenous iron and erythropoietin stimulating agent therapy for evaluating complete blood counts with reticulocyte parameters, and conventional markers for iron (Serum ferritin, serum iron, TIBC, and TSAT).

The collected blood sample was analyzed by using SYSMEX XN-550 hematology auto analyzers having 6 part differentials and VITROS 5600 biochemistry automated analyzer.

Once the treatment was started, samples were again collected on day 3, 7 and 30. p-value less than 0.05 was considered statistically significant and data analysis were done using GraphPad Prism 8.4.2 (679) version.

Due permission from Institutional ethics committee was taken for the study.

Inclusion Criteria:

All CKD patients above 18 years of age having iron-deficient states.

Exclusion Criteria:

1) CKD patients with vitamin B12 and folic acid deficiency.
2) CKD patients associated with hemoglobinopathies.
3) Patients having chronic inflammatory states like rheumatoid arthritis, SLE, and malignancy.

RESULTS AND OBSERVATIONS

In this study, the age of the patients with CKD ranged from 24 years to

80 years. The maximum number of study subjects in our study were in the 41-50 years of age group i.e. 31% (31 out of 100 cases) which was followed by 31-40 years having 24% of cases, while 71-80 years of age group had only 4% of cases.

In this study, male (72%) cases outnumbered female cases (28%). Diabetic nephropathy was found to be the most common cause of CKD (35%) which was followed by hypertensive nephropathy (30%), chronic glomerulonephritis (25%) and obstructive uropathy (10%) respectively. The maximum number of cases were found in the CKD stage IV which was 42%). This was followed by CKD stage V (34%), stage IIIb (15%), stage IIIa (6%), and stage II (3%) respectively.

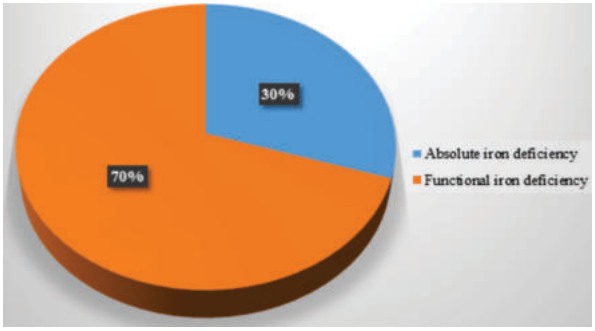

Figure 1: Pie Diagram Showing The Description Of Study Subjects According To Iron Status

From above figure 1, we observed that 30 out of 100 cases had absolute iron deficiency state as measured by serum ferritin level <100µg/L and/ or TSAT <20% and the remaining 70 cases had functional iron deficiency states as measured by serum ferritin >100µg/L and/or TSAT >20%.

Table 1: Calculation Of Mean MCH/ Mean RET-He Ratio In Both Groups

Group	Mean MCH/ Mean RET-He ratio	Value
Absolute iron deficiency group	25.4/25.1	1.012
Functional iron deficiency group	28.8/29.1	0.989

We can observe from the table 1 that the absolute iron deficiency group had a mean MCH/ mean RET-He ratio >1 which indicates iron-deficient states and needs for iron supplementation. On the other hand, the functional iron deficiency group had a mean MCH/ mean RET-He ratio <1.0 which is an indication of the physiologic unavailability of iron by the erythrone.

Table 2: Comparison Of Mean RET-He And Mean Hb Before And After Starting Of Iron Therapy In Absolute Iron Deficiency Group (Unpaired T-test)

Day	Mean RET-He (pg)	Mean Hb (gm/dL)	p-value
0	25.1	7.0	<0.0001
3	25.8	7.3	
7	26.5	7.8	
30	27.3	8.4	

A statistically significant improvement occurred in mean RET-He and mean Hb level in absolute iron deficiency group after the starting of iron therapy (p <0.0001).

Table 3: Comparison Of Mean RET-He And Mean Hb Before And After Starting Of Iron And ESA Therapy In Functional Iron Deficiency Group (Using Unpaired T-test)

Day	Mean RET-He (pg)	Mean Hb (gm/dL)	p-value
0	29.1	8.2	<0.0001
3	29.5	8.5	
7	29.7	9.0	
30	30.0	9.4	

A statistically significant change occurred in both mean RET-He and mean Hb level after starting of iron and ESA therapy for the functional iron deficiency group (p <0.0001).

Table 4: Comparison Of Mean RET-He Improvement In Both Groups Before And After Starting Of Therapy (Unpaired T-test)

Day	Mean RET-He (pg) in absolute iron deficiency group	Mean RET-He (pg) in functional iron deficiency group	p-value
0	25.1	29.1	0.0005
3	25.8	29.5	
7	26.5	29.7	
30	27.3	30.0	

The mean RET-He level increased in both groups after starting of therapy which was more in absolute iron deficiency group. At the end of day 3, day 7, and day 30 the changes in mean RET-He in the absolute iron deficiency group were 0.7 pg, 0.7 pg, and 0.8 pg respectively. Similarly, for the functional iron deficiency group, the changes in mean RET-He were 0.4 pg, 0.2 pg, and 0.3 pg at the end of day 3, day 7, and day 30 respectively.

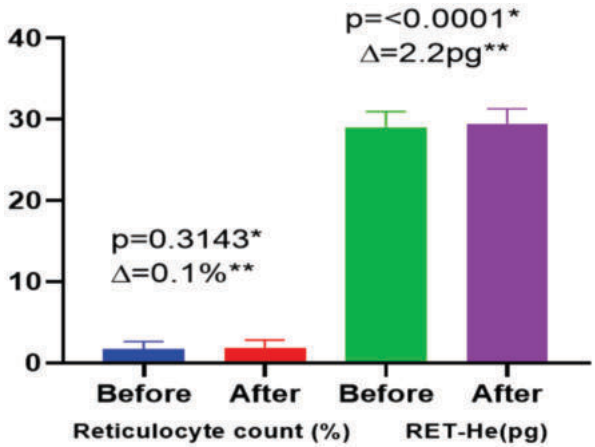

Figure 2: Comparison of improvement in mean reticulocyte count and mean RET-He in functional iron deficiency group before and after starting of iron and ESA therapy (day 0 vs day 3) (*paired t-test, **mean improvement)

A statistically significant improvement of mean RET-He was observed at day 3 in the functional iron deficiency group after initiation of iron and ESA therapy (p <0.0001). However, the mean reticulocyte count did not increase significantly at day 3 after starting of therapy (p=0.3143).

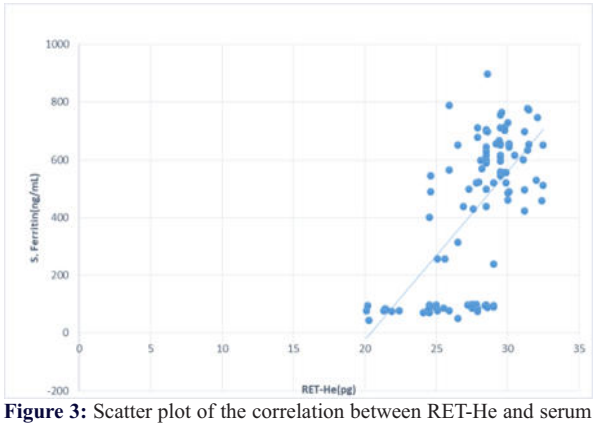

Figure 3: Scatter plot of the correlation between RET-He and serum ferritin in all 100 cases using Spearmen's correlation analysis, (r=0.6553, p<0.001)

Scatter plot of RET-He against serum ferritin shows a moderate positive correlation between these two parameters (r=0.6553, p<0.001).

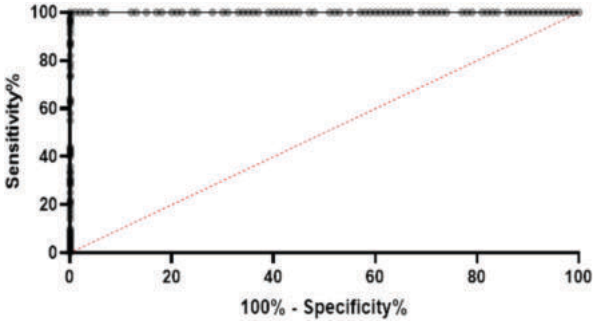

Figure 4: Receiver operator characteristic (ROC) curve for RET-He in

predicting iron deficiency anemia of all 100 cases

Area under curve is 1, p <0.0001. Best cut-off value is 27.6pg with sensitivity 100% and specificity 99%.

DISCUSSION

In the present study, the maximum number of CKD patients (31%) were in the age group of 41-50 years. The mean age was 49.6 years in our study. Similar findings were also found in an Indian study conducted by Rajapurkar MM et al.[12]

We found that the maximum cases were male which is similar to other studies like Rajapurkar MM et al., Modi GK et al., and Chaudhuri ST et al.[12,13,14]

Diabetic nephropathy was the leading cause of CKD in our studies which is consistent with other studies done in the past. In one study conducted by Iyawe IO et al., they found that hypertensive nephropathy was the most common etiology followed by diabetic nephropathy.[15]

The present study reveals the maximum number of cases were in the CKD stage IV (42%) followed by stage V (34%), stage III (21%, stage IIIa=6% and stage IIIb=15%), and stage II (3%) respectively. These findings are similar to the study done by Iyawe IO et al. and Afshar R et al.[15,16]

We observed that among CKD patients, maximum cases were in a functional iron-deficient state. In the absolute iron deficiency group, maximum patients were female contributing to other factors of iron deficiency. Our findings are similar to other previous studies like Dalimunthe NN et al.[10] Data from the Dialysis Outcomes and Practice Pattern Study (DOPPS) 2003 and National Health and Nutritional Examination Survey also reported that iron deficiency was prevelant in CKD patient who were on hemodialysis.[17] Thus, we can say that anemia remains a major source of morbidity in our cohort.

In the present study, the mean MCH/mean RET-He ratio in absolute iron deficiency group was 1.012 and that for functional iron deficiency group, it was 0.989. A study conducted by Capone D et al., where they mentioned that MCH/RET-He ratio guides iron supplementation.

A value < 1.0 is indicative of physiologic unavailability of iron for hemoglobin production and a value > 1.0 is indicative of iron deficiency and the consequent need for iron supplementation.[18]

In this study, we found that in the absolute iron deficiency group, there was a significant improvement of mean RET-He and mean Hb occurred after the starting of iron therapy similar to other studies conducted by Dalimunthe NN et al., Miwa N et al., and Chuang CL et al.[10,19,20]

Similar to the absolute iron deficiency group, we found that there was a statistically significant gradual improvement occurred in the level of mean RET-He and mean Hb (p<0.0001) in the functional iron deficiency group also. A study conducted by Mittman N et al. concluded that changes in RET-He correlated strongly and directly with concomitant changes in RBC count, hemoglobin and hematocrit, suggesting that rising RET -He was indicative of erythropoietic response. They also mentioned that those who were iron-deficient by standard measure showed a sustained response of RET-He and those who were iron sufficient by usual iron indices also responded to iron therapy with a rise in level RET-He suggesting that in fact, they were functionally iron-deficient despite "normal" conventional iron parameters.[21]

We found a significant improvement in the level of mean RET-He in both absolute iron deficiency and functional iron deficiency groups after starting of therapy. We observed that the mean improvement of RET-He occurred at the end of day 2 after initiation of therapy in both groups. The mean improvement of RET-He in the absolute iron deficiency and functional iron deficiency group was 25.1±2.8 pg vs 25.8±3.0 pg and 29.1±1.9 pg vs 29.5±1.8 pg respectively.

These findings are similar to other studies like Mittman N et al., Santen S et al., and Mehta S et al.[21,22,23] Mehta S et al. added that this improvement was as early as 2 days after initiation of treatment irrespective of the status of the iron stores of the patients.[23] We observed that mean reticulocyte count did not show significant improvement in the functional iron deficiency group after starting of iron and ESA therapy (p=0.3143) unlike that of mean RET-He improvement (p<0.0001) at the end of day 2. The mean improvement of reticulocyte count and RET-He was 0.2% (1.8±0.9% vs 2.0±1.0%) and 0.4 pg (29.1±1.9 vs 29.5±1.8 pg) respectively. These findings are similar to other previous studies like Turner J et al., Singh T et al. and Eschbach JW et al.[9,24,25]

Turner J et al. and Singh T et al. documented that the highest count of reticulocyte was observed after 7-10 days and 6-7 days of therapy.[9,24]

In our study, reticulocyte count of the functional iron deficiency group did not show significant improvement on day 3 after the starting of therapy, while our aimed marker RET-He improved significantly as early as at the end of day 2.

We observed that RET-He and S. ferritin had showed a moderate positive correlation. Our findings are similar to previous studies like Dalimunthe NN et al.[10] However, Miwa et al. and Mehta S et al observed a weak positive and strong positive correlation between the parameters respectively.[19,23]

Area under curve for RET-He was 1 in our study. We found the best cut-off value of RET-He for identifying iron deficiency anemia was 27.6 pg using ROC curve analysis with a sensitivity of 100% and specificity of 99%. This was similar to the findings of other studies performed around the globe like Dalimunthe NN et al. and Miwa et al.[10,19] A high AUC indicates a positive association of the parameter with the disease condition. This is true from the finding that RET-He had a high sensitivity, i.e. a high ability to detect true positive cases of iron deficiency anemia.

CONCLUSIONS

RET-He is considered as a useful alternative parameter of iron deficiency anemia in CKD patients due to its good diagnostic performance, availability of blood analyzer, and its lower cost. Reticulocyte count, the most commonly used conventional parameter in the assessment of response to iron therapy in CKD patients, increases significantly after 7-10 days which is not very helpful for the clinicians to make the treatment protocol. The use of the RET-He parameter in clinical practice allows avoidance of problems like iron over-dosage which can damage many potential organs and also reduces the health-related costs. Yet, potential still exists for RET-He to be employed as a follow-up parameter. Further study is warranted to investigate and validate its feasible utility in clinical settings.

Conflict Of Interest: None declared

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