Original Article

SIGNIFICANCE OF MEASURING RETICULOCYTE HEMOGLOBIN (RET-HE) IN CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Objective: To determine the significance of measuring reticulocyte hemoglobin (Ret-He) in chronic kidney disease patients.

Material and Methods: It was a cross sectional study conducted at Chughtai Healthcare from March 2021 to March 2022. Approval was obtained from the ethical and research committee of the institute. 102 patients, both males and females, between the ages of 10-75 years, diagnosed cases of CKD were included in the study. Informed consent was taken from all the patients. Blood specimens were collected in EDTA vials and serum separating vials and tested for serum iron, serum ferritin, total iron binding capacity (TIBC), transferrin saturation and complete blood count'(CBC). Ret-He was obtained by flowcytometry method using Sysmex XN 1000. Patients with acute infections/ inflammation, liver diseases, pregnant females, any coexisting bleeding disorder and malignancy were excluded from the study.

Results: There is a significant positive correlation between Iron and Ret-He, a positive correlation between Ret-He and Transferrin saturation and no correlation between Ret-He and ferritin along with TIBC.

Conclusion: Ret-He' is a reliable and economical test that can be used for detecting iron deficiency, especially in patients with chronic inflammatory conditions such as CKD.

Key Words: Chronic kidney disease (CKD), Reticulated hemoglobin (Ret-He), Iron deficiency anemia

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INTRODUCTION

CKD is a common health problem that is widespread throughout the world affecting approximately 8.16% of the world population [1]. In the recent past years, CKD has been considered as one of the major cause of mortality. According to a study by Jager *et al* [2]. the estimated number of individuals affected by various stages of CKD (1-5) was 843.6 million. In developing countries like Pakistan, the high cost of its treatment put a huge financial burden on healthcare system so it is of utmost importance to identify and manage the complications of CKD in time.

Most common presenting feature of CKD is anemia. A number of factors are involved in its etiology like decreased erythropoietin levels, nutritional deficiency including folic acid and vitamin B12, disturbance in iron hemostasis, chronic infections and blood loss [3]. Anemia develops during the early stages of the disease and worsens with decreasing kidney function. It is important to find out the cause of anemia for the timely and adequate management of the patient.

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Various tests are available for assessing the cause of anemia. Most commonly used biochemical marker is serum ferritin which is an acute phase reactant and its levels are influenced by infection/ inflammation, thus making it unreliable. Bone marrow biopsy, though a gold standard test, is usually not a preferred method for assessing the iron stores because it is an invasive procedure. The other biochemical tests include serum iron and transferrin saturation levels which are also affected in case of any inflammation [4]. Soluble transferrin receptor (sTfR) has been shown to have an inverse correlation with the body's iron stores but this, despite being unaffected by inflammation, is less commonly used due to its unavailability in many laboratories [5]. Red blood cell indices usually decrease in advanced stages of iron deficiency so these may be normal in early iron deficiency.

The reticulocytes are present in the peripheral blood for 24 to 48 hours before being converted into mature erythrocytes. During early stages of iron deficiency decrease iron levels would lead to lesser hemoglobin content in the reticulocytes of bone marrow and it can be easily picked up by reticulocyte hemoglobin content [6]. Ret-He is an advanced parameter in the latest automated hematology analyzer that is based on flowcytometry. It can give an idea about the available iron for

erythropoiesis in bone marrow, thus identifying earlier stages of iron deficiency [4]. It is more sensitive, easier to perform, relatively cheaper than the routine iron profile and unaffected by infection and inflammation [7].

We aim to compare the conventional method of detecting iron status through biochemical markers which is expensive, less reliable and troublesome for the patient, with the single novel hematological parameter which will be cost effective and truly represent the iron stores of bone marrow.

MATERIAL AND METHODS

It was a cross sectional study conducted at Chughtai Healthcare from March 2021 to March 2022. Approval was obtained from the ethical and research committee of the institute (IRB letter number CIP / IRB / 1068). A total of 102 patients, both male and female known cases of CKD with anemia were included in the study by using non probability consecutive sampling. Sample size was calculated using OpenEpi, Version 3, open source calculator using RET-He as a reference parameter. Consent was taken from subject patients.

Blood sample from each patient in volume of 3ml was collected in EDTA vial and 3.5ml in serum separating vials. These samples were tested for serum ferritin, serum iron and total iron binding capacity by chemiluminescence microparticle immunoassay technique, using Abbott-Alinity Ci instrument. CBC of all these samples were run on Sysmex XN 1000. Ret-HE was analyzed using flowcytometry technique on Sysmex XN 1000 hematology analyzer.

Patients who were suffering from acute infections/ inflammation, liver diseases, any coexisting bleeding disorder, malignancy and pregnant females were excluded from the study. Moreover, patients who gave history of iron therapy and blood transfusion in the last 3 months were not included in the study.

Hemoglobin (Hb) less than 13g/dl in males and less than 11.5g/dl in females is defined as anemia. Reference ranges used in our laboratory were: Serum iron: 50-170 µg/dl, serum ferritin: 10-120 ng/ml for females and 20-250 ng/ml for males, serum TIBC: 250-400 µg/dl. Transferrin saturation was obtained by using formula serum iron ÷ total iron binding capacity × 100. A cutoff of 30.8 pg for Ret-He was taken as it had specificity and sensitivity of 90% for detecting iron deficiency coexisting with anemia of chronic disorder according to a study by Wardah A *et al* [8].

Statistical package for social sciences version 22.0 was used to analyze the data and to assess the correlations between reticulated hemoglobin and serum iron, serum ferritin, TIBC and transferrin saturation by spearman correlation. P value less than 0.05 was considered significant.

RESULTS

Total number of patients was 102 out of which 64 (63%) were males and 38 (37%) were females. Age range was 27-92 years with mean age of 55 years.

Mean GFR calculated was 14.59 ml/min/1.73m^2. Ranges and means for Hb, Ret-He, serum iron, total iron binding capacity (TIBC), transferrin saturation and serum ferritin are shown in Table-I.

The spearman correlation test showed a correlation value of 0.530 (Iron and Ret-He) with a p value of <0.001. Positive correlation between Iron and Ret-He is noted. Our study showed that serum Iron was directly proportional to the level of Ret-He. There was also a positive correlation between Ret-He and Transferrin saturation. No correlation was found between Ret-He and ferritin along with TIBC. Correlation between our study variables and Ret-He is given in the Table-II.

Table-I: Range, means and standard deviation (SD) of different Hb, Ret-He and iron profile in our study.

Parameter	Range	Mean ± SD
Hb (g/dl)	4.2-12.8	10.28 ± 1.86
Ret-He (pg/cell)	19-38	28.7 ± 3.65
Serum Ferritin (ng/ml)	13-1232	308 ± 288.34
Serum Iron (µg/dl)	9-131	64.9 ± 25.9
TIBC (µg/dl)	95-513	272 ± 77.8
Transferrin saturation (%)	6-60	25.68 ± 77.8

Table-II: Correlation between different variables and Ret-He.

Variables	Ret-He
Serum Iron	r = 0.530 p = **0.000** n = 102
Serum Ferritin	r = 0.001 p = 0.987 n = 102
Transferrin Saturation	r = 0.273 p = **0.000** n = 102
TIBC	r = 0.009 p = 0.145 n = 102

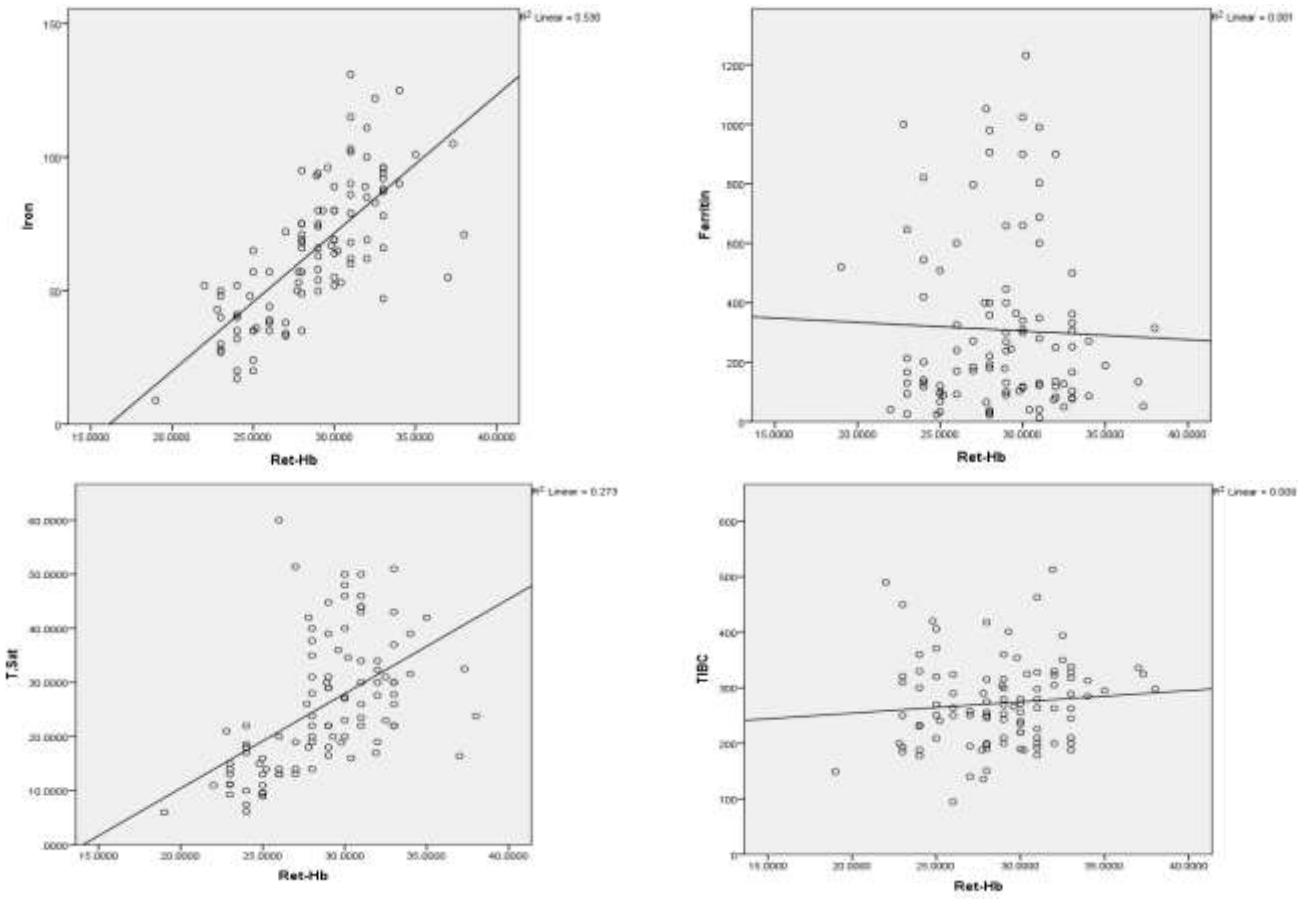

Figure-I: R squared analysis between different variables and Ret-He

DISCUSSION

Patients suffering from chronic kidney disease and anemia have more risk of morbidity and mortality [9]. According to KDOQI guidelines, CKD is described as functional / anatomical deformities or eGFR less than 60 ml/min/1.73m^2 for consecutive 3 months [10]. Study conducted by Imran S *et al*. shows overall prevalence of CKD in 25.6% of cases [11]. Prevalence of CKD in Pakistan is reported as 5.0% to 12.5% to 31.2% [12].

According to Rocha *et al* [13], serum ferritin remains a reliable marker for the assessment of iron stores in patients of CKD undergoing dialysis. However, they also suggested to screen patients for any inflammatory condition to rule out the possibility of falsely raised serum ferritin levels. In addition to serum ferritin, all the other available tests to assess iron deficiency have some confounding factors that limit their use in everyday practice.

Ret-He or reticulocyte hemoglobin content (CHr) is a novel marker that has been used to identify initial functional iron deficiency in CKD patients and identified as a better parameter than the conventional tests in different studies [4,5,6]. Our study evaluated the significance of this new parameter, Ret-He, in CKD patients. There is no additional sampling needed for performing this test since EDTA sample collected for CBC can be used for the purpose. It is a rapid, single, reliable and cost effective parameter that can be used for assessing iron deficiency in infection or inflammation.

As par world health organization (WHO), males having Hb less than 13g/dl and females having Hb less than 11.5 are considered anemic. 95 out of 102 patients (93.2%) in our study were anemic. This prevalence is almost similar to the prevalence (93.1%) reported in Vietnam [14].

In a study by Abdul Gafor A.H *et al* [15], CHr and Hb ($p = 0.0001$, $r = 0.3$), TSAT ($p = 0.001$, $r = 0.3$), and ferritin levels ($p = 0.001$, $r = 0.3$) showed a significant correlation. This was in accordance with our study since a significant correlation was found between Ret-He and TSAT ($p = 0.000$, $r = 0.273$) and serum iron ($p = 0.000$, $r = 0.530$) but no significant correlation was found between Ret-He and ferritin ($p = 0.987$, $r = 0.0001$) and TIBC ($p = 0.145$, $r = 0.009$). Another study by Nguyen Trung K *et al* [16] showed a strong positive correlation between serum iron and RET-He ($r = 0.513$, $P < 0.001$), TSAT and RET-He ($r = 0.589$, $P < 0.001$) while there was a moderate positive correlation between Ferritin and RET-He ($r = 0.399$, $P < 0.001$) and a weak negative correlation between TIBC and RET-He ($r = -0.182$, $P = 0.037$).

These findings are also in accordance with our findings.

In another study by CC Kariyawasan *et al* [17], a significant positive correlation was found between serum ferritin and CHr (r=0.578, p value <0.001), between serum iron and CHr (p value<0.001, r =0.209), and transferrin saturation and CHr (p value <0.001, r=0.266). There was a significant negative correlation between CHr and TIBC (p value <0.001, r = -0.224). This is in contrast to our study as we did not found any correlation among Ret-He, serum ferritin and TIBC.

CONCLUSION

We found out a significant positive correlation between Iron and Ret-He, a positive correlation between Ret-He and Transferrin saturation and no correlation between Ret-He and ferritin along with TIBC. We conclude that unlike the conventional iron studies, Ret-He is an easy, rapid, reliable, non-invasive and cost effective test that can be used for identifying iron deficiency, especially in patients with chronic infection / inflammation such as CKD. Identifying iron deficiency in these patients is of utmost importance for the timely and effective management of anemia.

LIMITATIONS

It was a single center study and sample size was small. Also we did not include non-anemic CKD patients in our study. Further studies should be done to find out the correlation between Ret-He and iron parameters in non-anemic CKD individuals.

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CONFLICT OF INTEREST: None

AUTHOR CONTRIBUTION

Isma Imtiaz: Data collection, statistical analysis and article writing.
Ayesha Younas: Literature search and article writing.
Ayisha Imran: Drafted the study design and proof reading.
Abuzar Siddique: Literature search and sample collection.
Nauman Aslam Malik and Akhtar Sohail Chughtai: Overall supervision of the study.

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