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Comparative evaluation of iron status by reticulocyte haemoglobin content (CHr) in chronic kidney disease patients on haemodialysis and erythropoietin

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***Abstract*---**Due to the inflammatory state associated with uraemia, diagnosing iron deficiency using currently available assays is difficult in individuals with chronic kidney disease (CKD). The goal of this study was to see how useful reticulocyte haemoglobin (CHr) is as a diagnostic tool for iron deficiency and a predictor of intravenous iron therapy in a group of CKD patients in Sri Lanka who were on haemodialysis. A hundred (100) individuals with CKD on regular haemodialysis and erythropoietin participated in this descriptive cross-sectional study. Serum ferritin, transferrin saturation, and reticulocyte haemoglobin were used to divide patients into groups (CHr). A single dose of intravenous (IV) iron 500 mg was given to all individuals with CHr<29 pg.To examine the response, the CHr was measured 72 hours after the IV iron treatment. Mean haemoglobin

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was 9.27 g/dL, mean serum ferritin was 243.5 ng/mL, mean transferrin saturation was 18.6%, and mean CHr was 29.2 pg in the population. Thirty-three of the 100 patients (33%) received IV iron therapy, which resulted in a significant increase in CHr 72 hours following treatment ($p<0.001$). CHr has a sensitivity of 56 percent, specificity of 73 percent, and positive predictive value of 84 percent as a diagnostic test for iron insufficiency in CKD patients on haemodialysis. CHr (reticulocyte haemoglobin) can be utilised as an early predictor of IV iron therapy response. However, further research is needed before CHr can be considered as a diagnostic tool.

***Keywords*---**chronic kidney disease (CKD), irondeficiency, functional iron, deficiency reticulocyte, haemoglobin (CHr).

Introduction

Diagnosis of iron deficiency by a single laboratory investigation is quite difficult due to the lack of sensitivity and specificity of the commonly used investigations. Iron deficiency is described as the decrease in iron stores which occurs before overt iron deficiency anaemia develops or which is present without progression [1]. Functional iron deficiency (FID) is described as a condition with inadequate mobilisation of iron from the stores even though the demand is increased. This is seen with treatment with erythropoietin as well [2]. Chronic kidney disease (CKD) patients have impaired kidney function, hence impaired erythropoietin production by the renal tubules. Treatment with Erythropoietin may cause functional iron deficiency in long term haemodialysis [2]. The most important factors which cause resistance to administered erythropoietin are the “absolute” and “functional” iron deficiency [3]. The main goal of iron therapy in haemodialysis patients is to support erythropoietin therapy to achieve a consistent haematocrit value range between 33 and 36% [4]. Diagnosing iron deficiency with currently available tests is rendered difficult in patients with chronic kidney disease due to the presence of an inflammatory state associated [5] with uraemia Iron deficiency in CKD patients is indicated by a transferrin saturation value of 20% or a serum ferritin concentration of 100 ng/mL in non haemodialyzed patients and 200 ng/mL in haemodialyzed patients [2].

In preliminary research, reticulocyte haemoglobin content (CHr) was found to be highly accurate in identifying early iron shortage. A CHr value of less than 29 pg in patients receiving recombinant human erythropoietin implies iron limited erythropoiesis due to absolute or FID [2]. CHr-based iron therapy decreases the quantity of IV iron required while maintaining identical haematocrit levels and erythropoietin dosages [4]. The CHr tends to rise 48–96 hours after starting IV iron therapy, and so serves as an early predictor of responsiveness to IV iron therapy [6].

Materials and Procedures

This descriptive cross-sectional study involved 100 patients with CKD who were receiving regular haemodialysis and erythropoietin. The participants in the study were chosen based on inclusion and exclusion criteria.

Criteria for acceptance

- Over the age of eighteen (18) years
- Use of erythropoeitin for three months or longer without any dose changes in the previous month
- CKD patients on haemodialysis for three
- months or longer

Criteria for exclusion

- Patients who are not receiving optimal dialysis, i.e. two weekly dialysis sessions
- Have you had any blood transfusions in the recent three months?
- Within the recent month, IV (intravenous) iron therapy
- Any haematological condition other than iron deficiency anaemia that has been previously recognised
- Patients with any inflammatory or infectious disorders, cancers, or haemoglobinopathies that are clinically apparent.
- Any patient who has experienced drug reaction when receiving IV iron therapy.

Before starting the normal haemodialysis session for the week, the participants' blood samples were obtained for a full blood count, C reactive protein, serum ferritin, transferrin saturation, and reticulocyte haemoglobin concentration.
Blood samples for complete blood count and reticulocyte haemoglobin (CHr) were drawn into EDTA tubes by venepuncture and analysed in the manual mode on the Mindray BC 6800 analyser within 6 hours after collection. Prior to the analysis, the analyser was calibrated to international standards and quality control samples were done daily. With proper quality control, serum ferritin was measured using the chemilunest method in the Immulite biochemistry analyser, and transferrin saturation was measured using the Feren Method in the Olympus AU 680 biochemistry analyser.

Results

Table 1
Classification ofanaemia depending on the Hb value in both sexes according to WHO recommendations

	Male			Female		Total	
Hb(g/dL)	n	%	Hb(g/dL)	n	%	n	%
8severe	11	18.33	8severe	7	17.5	18	18

8–10.9moderate	39	65	8–10.9moderate	26	65	65	65
11–12.9mild	10	16.67	11–11.9mild	5	12.5	15	15
>13normal	0	0	>12normal	2	5	2	2

Table 2
Distribution of serum ferritin in the cohort of CKD patients

Serumferritinng/mL	Number	%
<30	7	7
31–100	29	29
101–200	28	28
201–500	24	24
501–600	3	3
601–700	3	3
701–800	2	2
>800	4	4

For the study, a total of one hundred (100) patients with end-stage renal failure on regular haemodialysis were recruited, with sixty (60) males and forty (40) females. The ratio of males to females was 1.5:1. All of the patients were over the age of eighteen (18), and the majority of them (n = 54, or 54 percent) were over the age of fifty (50), with a male predominance (38/54). Patients between the ages of 51 and 60 (51–60) accounted for the greatest number of patients (n = 27, 27%). Haemoglobin (Hb) levels in the population ranged from 4.3 to 12.7 g/dL, with a mean of 9.27 g/dL. The World Health Organization's recommendations were used to classify the degree of anaemia (WHO).The majority of the patients (n = 64, 64%) had blood ferritin levels below 200 ng/mL. The remaining 36 individuals had serum ferritin levels of 200 ng/mL. (Table 2). Out of the 100 patients in the study, 58 (58%) had transferrin saturation of 20% or higher, while 42 (42%) had values of 20% or higher. In 74 (74 percent) of the patients, both levels were reduced below the conventional cutoff limits. The average blood ferritin level in the study cohort was 243.6 ng/mL.

The CHr was the most important metric in this investigation, and a cutoff of 29 pg was utilised in the classification, as indicated above. According to the CHr, 53 percent (n = 53) of patients in the study population were iron sufficient, while 47 percent (n = 47) were iron deficient. Based on serum ferritin and CHr levels, the study group was further separated into four (4) primary groups (Table 3). When the sample was further tested according to serum ferritin, the CRP value was taken into account, and twenty (20) patients were eliminated from the original hundred (100) patients due to a high CRP value ([10 mg/L]).

Table 3
Mean CHr values before and after IV iron treatment in CKD patients on haemodialysis and erythropoietin

	Mean	Number	Standard deviation
CHr initial (pg)	25.727	33	2.836
CHrafter (pg)	31.357	33	2.785

Table 4
Increase in CHr values in CKD patients on haemodialysis and erythropoietin before and after IV iron treatment

CHr<29 pg	Group1A n=32(40%) (absolutelyirondeficient group)	Group2A n=6(7.5%) (functionallyirondeficient group)
CHr>29 pg	Group1B n=25(31.3%)	Group2B n=17(21.2%) (ironsufficientgroup)
Total	57 (71.3%)	23 (28.8%) a

IV iron therapy was administered to the groups with CHr values less than or equal to 29 pg. Three (3) patients were lost to follow up, one (1) patient died, and another patient was admitted with sepsis, therefore only thirty three (33) patients were administered IV iron and remained until the completion of the trial. Only 72 hours after IV iron treatment, there was a considerable increase in blood CHr (p value 0.001). (Table 4). After 72 hours of IV iron treatment, a large percentage of patients (n = 20/33, or 60.6%) had a CHr value that had raised by [5 pg]. The sensitivity and specificity of CHr were further assessed using Receiver Operator Curves (ROC). The test had a sensitivity and specificity of 54 percent and 73 percent for the already defined cut off value of 29 pg on CHr, but based on the ROC coordinates, a slight increase (up to 31) of the cut off value would result in better sensitivity and specificity values of 63 percent and 61 percent, respectively. However, more research in a wider population is required before these findings can be drawn.

Table 5
Increase in CHr values in CKD patients on haemodialysis and erythropoietin before and after IV iron treatment

Increase in CHr by pg	Number	%
By1–2pg	1	3

By2–3pg	3	9
By3–5pg	9	27
By5–10pg	17	52
By[10pg	3	9

Discussion

One of the first and most serious side effects of chronic renal disease is anemia [9]. Despite the fact that there are various factors that contribute to CKD anemia, iron deficiency anemia is one of the most significant inhibitors of erythropoietin therapeutic response [10]. The prevalence of CKD in India is 2.3–9.5% [11]. Published data on anaemic CKD patients are not available currently due to the lack of national registries and the less focus on the reporting of non-communicable diseases. The mean Hb within the population was recorded as 9.27 g/dL. [12].

Lacson et al. [13],Vidyashankar P et al. [5], Abdul Halim et al. [14], Naomi Niari et al. [15], Kim et al. [16], and Chiao Lin Chaang et al. [17] all found that mean haemoglobin levels differed across the globe. When compared to the other trials, ours had the lowest mean Hb value, which could be attributable to severe iron insufficiency and a lack of effective IV iron treatment. Hb levels in people with CKD, on the other hand, fluctuated often beyond or below the recommended goal levels in a short period of time, according to the studies mentioned above. Serum ferritin is the most often used test for evaluating storage iron, while transferrin saturation is the most commonly utilised marker of iron availability to support erythropoiesis, due to its widespread availability [12]. The average blood ferritin level in this study's group was 243.5 ng/mL. However, the findings ranged from 7.1 to 1562 ng/mL, indicating that confounding factors caused wide heterogeneity across individuals. Most studies [3, 5, 7, 14, 15, 17–19] found that serum ferritin was high in CKD patients on haemodialysis and that it was not a useful indication of iron status [3, 5, 7, 14, 15, 17–19].

In our study, the average transferrin saturation was 18.6%, indicating iron deficiency. All relevant studies showed slightly higher values than this, with [20 percent [7, 14, 17, 19] being the highest. Patients in our study group were divided into subgroups based on blood ferritin and CHr levels. All three indicators indicated that 32 patients were iron deficient (Group 1A). Surprisingly, all of these patients had been on oral iron therapy for at least three months. Oral iron supplements have been found in a number of studies to fail to maintain adequate iron reserves in erythropoeitin-treated haemodialysis patients. In individuals with transferrin saturation of 30 percent and serum ferritin of 500 ng/mL, KDIGO clinical practise guidelines recommend a trial of IV iron [12]. The CHr was the most relevant parameter assessed in this investigation. The average CHr value of 100 haemodialysis patients was 29.2 pg, with a minimum of 18.4 pg and a maximum of 36.5 pg in this study. Despite the fact that multiple studies have found different cut off values for CHr, we used the 29 pg cut off value to predict functional/absolute iron deficiency [2].

Fishbane et al. in the United States found a mean CHr value of 27.5 2.8 pg in 164 haemodialysis patients on erythropoietin (4). Another study conducted in the United States by Neal Mittman et al. found a mean CHr value of 28.3 pg in 364

haemodialysis CKD patients [19]. When compared to our study these 2 studies demonstrated a slightly lower mean CHr among CKD haemodialysis patients. This could be due to the larger number of patients in these studies and the ethnic variation. Another prospective randomized controlled study conducted by Abdul Halim et al. in Malaysia which involved 121 patients [14] revealed a higher mean CHr value of 32.9 pg. Fishbane et al. in the United States found a mean CHr value of 27.5 2.8 pg in 164 haemodialysis patients on erythropoietin (4). Another study conducted in the United States by Neal Mittman et al. found a mean CHr value of 28.3 pg in 364 haemodialysis CKD patients [19]. These two investigations showed a somewhat lower mean CHr among CKD haemodialysis patients as compared to ours. This could be due to the larger number of patients and ethnic diversity in these studies. Another prospective randomised controlled study conducted by Abdul Halim et al. in Malaysia which involved 121 patients [14] revealed a higher mean CHr value of 32.9 pg.

In our study 97% of patients showed an increase in CHr by 2 pg at 72 h. Fishbane et al. in the United States found a mean CHr value of 27.5 2.8 pg in 164 haemodialysis patients on erythropoietin (4). Another study conducted in the United States by Neal Mittman et al. found a mean CHr value of 28.3 pg in 364 haemodialysis CKD patients [19]. These two investigations showed a somewhat lower mean CHr among CKD haemodialysis patients as compared to ours. This could be due to the larger number of patients and ethnic diversity in these studies. Another prospective randomised controlled study conducted by Abdul Halim et al. in Malaysia which involved 121 patients [14] revealed a higher mean CHr value of 32.9 pg.

In contrast to our study, IV iron was given as 10 divided doses over a 6-month period in this study. Chiao Lin Chuang et al. also confirmed that a trial of IV iron supplementation accompanied with monitoring ofCHr within 1 month was worthwhile for clinical practice [17]. The biggest advantage of CHr was in group 2A patients (06 patients in our study) as CHr<29 pg identified the functional iron deficiency in this group. As functional iron deficiency cannot be diagnosed by conventional parameters, the value of CHr was immense and they could be treated with IV iron without any delay. We found that sensitivity and specificity of CHr of <29 pg in assessing iron deficiency in CKD patients on haemodialysis were 56% and 73% respectively. The positive predictive value of the assay was 84%. A study done by Kim et al. revealed that the best cut off value for detecting iron deficiency in haemodialysis patients was 32.4 pg with a sensitivity of 96% and specificity of 84% [16]. In our study also we found that increasing the cut off value of CHr to 31 pg will increase the sensitivity and specificity. When compared to other studies even though the sensitivity was low, the specificity and positive predictive value were higher in our study. Due to the higher positive predictive value of CHr in our study, it may be considered as a diagnostic tool to indicate iron deficiency in CKD population.

Conclusion

CHr increases promptly after IV iron supplementation, and therefore it could be used as an early predictor of response to intravenous iron therapy. Furthermore, serum ferritin is not a good indicator of iron deficiency in CKD patients on

haemodialysis. However due to the limitations of our study further evaluation is necessary to consider CHr as a diagnostic tool to detect iron deficiency in CKD and cut off value of CHr to diagnose iron deficiency in Sri Lankan population should be further evaluated. A multicentre study involving a larger number of patients would be more beneficial in the future to arrive at definitive conclusions.

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