

Research Article

Reticulocyte Haemoglobin Content (CHr) is a Reliable Marker of Iron Deficiency in Pre-dialytic Chronic Kidney Disease (CKD) Patients

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Abstract

Chronic kidney disease (CKD) is a non-communicable epidemic disease. The CKD patients suffer from various types of anemia including iron deficiency anemia. Measurement of reticulocyte hemoglobin content (CHr) has been proposed as a measure of available iron stores. In this study CHr and serum iron profile were compared with stainable iron in the bone marrow to evaluate the CHr as a marker of iron deficiency in pre-dialytic CKD patients. This cross sectional study was conducted at the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from January 2018 to December 2018. A total of seventy (70) pre-dialytic CKD patients were selected. All participants had undergone bone marrow study for detection of iron deficiency anemia and it was observed that, 60% CKD patients had iron deficiency by bone marrow iron stain. Serum iron profile [serum iron, total iron binding capacity (TIBC), serum ferritin, transferrin saturation (TSAT)] and reticulocyte haemoglobin content (CHr) of each patient were determined accordingly. Data analysis reveals that CHr was significantly low in iron deficient group ($p < 0.001$). The sensitivity and specificity of CHr at a cut-off 28 pg/cell was 81% and 75.0% respectively ($p < 0.001$); sensitivity and specificity of serum ferritin at a cut-off 100 ng/ml was 40.6% and 67.9% respectively ($p = 0.051$); while sensitivity and specificity of TSAT at a cut-off 20% was 54.2% and 57.1% respectively ($p = 0.465$). The study proves that reticulocyte haemoglobin content (CHr) is a reliable marker to assess iron status for pre-dialytic CKD patients.

Keywords: Anemia; Chronic Kidney Disease (CKD); Iron Deficiency; Reticulocyte Hemoglobin Content (CHr)

1. Introduction

Anemia is a common complication of chronic kidney disease (CKD) with a prevalence of twice (15.4%) as in the general population and it increases with stages of CKD, from 8.4% at stage 1 to 53.4% at stage 5 [1]. Improvement of hemoglobin level in CKD patients leads to improvement of their daily performance status, reduction in the disease progression and ultimately to decrease morbidity and mortality [2]. Along with many other causes, iron deficiency either absolute or functional, is an important cause of anemia in chronic kidney disease (CKD) patients [3]. Iron deficiency anemia (IDA) can be treated either by oral iron or parenteral iron therapy or in some cases by blood transfusion. However, increased tissue iron can aggravate disease progression by precipitation of infection and generation of hyper-reactive free radical mediated tissue injury [4, 5]. Therefore, it is important to determine the exact body iron status in CKD patients for their proper management.

In a normal healthy individual iron is stored in the liver, muscle and bone marrow. Bone marrow iron is contained mainly in the macrophages that release it to the developing erythron for the synthesis of hemoglobin [5]. Examination of bone marrow iron stores is traditionally considered as the gold standard for the diagnosis of iron deficiency anemia because most of the anemia show normal or increased iron storage in the bone marrow except iron deficiency anemia where it is depleted [6, 7]. But the procedure is cumbersome and not routinely available in most of the laboratories especially in the countries like Bangladesh. Therefore, the easily available tests are used for the diagnosis. A low level of serum iron, serum ferritin, transferrin saturation (TSAT) status and high concentration

of serum transferrin indicates iron deficiency [8]. Serum ferritin level and TSAT status are usually considered as the corner stone for the diagnosis of iron deficiency [9]. The World Health Organization (WHO) recommends that a serum ferritin level of <15 µg/l in adult and <12 µg/l in children of less than 5 years is diagnostic of iron deficiency [10]. However, the ferritin is an acute phase reactant protein and its level increases in the inflammatory condition and any chronic disease like CKD [11, 12]. Therefore, the threshold for diagnosis of IDA in CKD is higher. A serum ferritin <100 µg/L or TSAT <20% is considered diagnostic for IDA in CKD and if the serum ferritin is 100–300 µg/L, TSAT <20% is required to confirm iron deficiency, although these two parameters fail to detect functional iron deficiency in all cases of CKD patients [9]. Chronic kidney disease with occult infection may cause increased serum ferritin level irrespective of the actual iron storage [9]. Transferrin saturation (TSAT) may also be misleading since nutritional status and loss in urine can affect transferrin concentration in CKD [13]. The ideal method for detecting initial iron status should be to directly measure the iron content of erythrocytes, particularly in newly produced cells- reticulocytes.

Recently, a novel assay ‘reticulocyte hemoglobin content (CHr)’, a measure of hemoglobin in newly formed reticulocytes, becomes available [14, 15]. This newer marker (CHr) has currently been introduced to guide iron therapy [9]. European best practice guidelines 2005 and Kidney Disease Outcomes Quality Initiative (KDOQI) 2006 guidelines have recommended this newer parameter (CHr) as marker of iron deficiency status [16, 17]. CHr has been reported to identify initial functional iron shortage particularly among CKD patients and has been proposed as a measure of available iron stores that could be superior to

the conventional tests in various studies [14-16]. It has the ability to provide a snapshot of the iron available for erythropoiesis, which can be used for early detection of iron deficiency anemia [15]. It is also cheaper than measurement of conventional iron markers such as- TSAT and ferritin. Therefore, CHr can serve as an alternative to conventional markers (TSAT and ferritin) in evaluating iron status in resource poor countries. This study was aimed to evaluate the CHr as a marker of iron deficiency in pre-dialytic CKD patients among Bangladeshi population.

2. Methodology

2.1 Study design

This cross sectional study was conducted in Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from January 2018 to December 2018. This study was approved by the ethical review committee BSMMU, Dhaka, Bangladesh.

2.2 Participants

The study was carried out among seventy (70) pre-dialytic CKD patients who were admitted at Department of Nephrology, Bangabandhu Sheik Mujib Medical University (BSMMU) hospital in Dhaka, Bangladesh during the study period. Participants were selected by purposive sampling technique following selection criteria. Adult (age ≥ 18 years) pre-dialytic CKD patients of both sexes with chronic anemia were included in this study. CKD patients with active bleeding, bleeding disorder, taking erythropoetin (EPO) or recent blood transfusion (within 120 days) were excluded from the study. Chronic kidney disease (CKD) was defined as a serum creatinine level of >1.3 mg/dl or a creatinine clearance of <60 ml/minute, present for more than 3 months [18].

2.3. Definition of chronic kidney disease (CKD)

According to KDIGO* chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for more than three (3) months [18].

[*KDIGO = Kidney Disease Improving Global Outcomes].

Criteria for chronic kidney disease (CKD)

[Either of the following present for more than 3 months]

a) Markers of kidney damage (one or more) [18]

- Albuminuria [Urine albumin excretion rate (AER)] ≥ 30 mg/24 hours; Urine albumin to creatinine ratio (ACR) ≥ 30 mg/gm.
- Urine sediment abnormalities
- Electrolyte and other abnormalities due to tubular disorders
- Abnormalities detected by histology
- Structural abnormalities detected by imaging
- History of kidney transplantation

b) Decreased glomerular filtration rate (GFR) [18]

Glomerular filtration rate (GFR) < 60 ml/minute/1.73 m² (GFR categories G3a–G5).

2.4. Procedure

After taking informed written consent from the study participants, their bone marrow was aspirated from the posterior iliac crest under local anaesthesia with all aseptic precaution. Bone marrow materials were examined for presence or absence of stainable iron deposits. According to the bone marrow aspiration report study patients were divided into two groups; iron deficient group and iron

present group. At the same time, serum iron profile [that includes- serum iron, total iron binding capacity (TIBC), serum ferritin and transferrin saturation (TSAT)] and reticulocyte haemoglobin content (CHr) of each patient were determined accordingly. Serum iron, TIBC, serum ferritin levels were measured by automated analyzer. Transferrin saturation (TSAT) was calculated from the 'serum iron level' and 'TIBC' in each case. Reticulocyte haemoglobin content (CHr) was determined by Sysmex XT-4000 automated hematology analyzer. All data were collected in a data collection sheet by taking history, examining the patients clinically and from the laboratory findings.

2.5 Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS) software version 23. Quantitative data were expressed as mean and standard deviation. Categorical data were expressed as frequency and percentage. Association between categorical variables was seen by chi-square test. Comparison of continuous variables was done by independent sample 't' test. A receiver operator characteristic curve (ROC curve) was used to evaluate the accuracy of the parameters. For all statistical tests, p-values less than 0.05 was considered as significant.

3. Results

A total of seventy (70) pre-dialytic chronic kidney disease (CKD) patients due to various causes of CKD with chronic anemia were studied. Of them 48.6% had diabetic nephropathy (DN), 41.4% chronic glomerulonephritis (CGN), 7.1% hypertensive nephropathy (HTN) and 2.9% obstructive nephropathy (Figure 1).

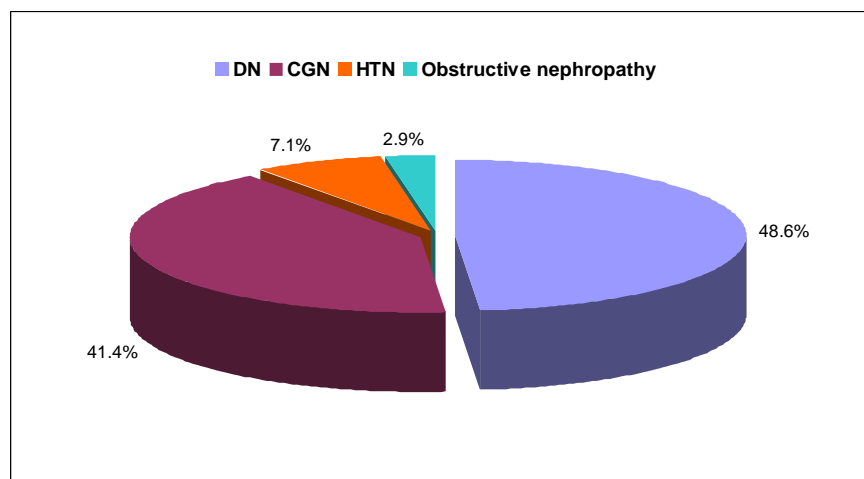


Figure 1: Pie diagram displaying the causes of CKD among the study population (N=70).

Among total 70 pre-dialytic CKD patients 46 (65.7%) were male and 24 (34.3%) were female with a male to female ratio was 1.9:1. The mean age of the study patients was 43.6 ± 11.13 years (ranged 24 - 65 years), their mean

haemoglobin level and mean serum creatinine level were 9.73 ± 0.93 gm/dl (ranged 7 - 11.3 gm/dl) and 3.55 ± 0.92 mg/dl (ranged 2.20 - 5.90 mg/dl) respectively (Table 1).

Characteristics	Mean \pm SD (Range)	Frequency (%)
Age (years)	43.6 ± 11.13 (24 - 65) years	
Gender		
Male		46(65.7%)
Female		24(34.3%)
Ratio	1.9:1	
Haemoglobin (gm/dl)	9.73 ± 0.93 (7.0 - 11.3 gm/dl)	
Serum creatinine (mg/dl)	3.55 ± 0.92 (2.20 - 5.90 mg/dl)	

Table 1: Basic data of the study patients (N=70).

According to the bone marrow aspiration report; out of 70 study patients, 42(60%) patients were iron deficient group and 28(40%) patients were iron present group. The mean(\pm SD) haemoglobin (Hb) level, reticulocyte haemoglobin content (CHr), serum ferritin level, and transferin saturation (TSAT) status in iron present group and iron deficient group were 10.55 \pm 0.32 mg/dl, 29.32 \pm 1.25 pg/cell, 279.1 \pm 171.1 ng/ml, 25.3 \pm 10.4 (%) and

9.18 \pm 0.79 mg/dl, 26.57 \pm 1.82 pg/cell, 192.8 \pm 136.2 ng/ml, 23.5 \pm 11.4 (%) respectively. The different parameters among each subgroup were compared. It was observed that, parameters like haemoglobin (Hb), reticulocyte haemoglobin content (CHr), serum ferritin were significantly different among the groups ($p < 0.05$), while difference in transferin saturation (TSAT) was not significant between the groups ($p = 0.517$) (Table 2).

Parameters	Bone marrow aspiration report: Stainable iron		p-value
	Present (n=28) Mean \pm SD	Absent (n=42) Mean \pm SD	
Hb (gm/dl)	10.55 \pm 0.32	9.18 \pm 0.79	<0.001
CHr (pg/cell)	29.32 \pm 1.25	26.57 \pm 1.82	<0.001
Serum Ferritin (ng/ml)	279.1 \pm 171.1	192.8 \pm 136.2	0.022
TSAT (%)	25.3 \pm 10.4	23.5 \pm 11.4	0.517

Unpaired t-test was done

Table 2: Values of different parameters in iron present and iron deficient group (N=70).

Table 3 shows comparison of reticulocyte haemoglobin content (CHr), serum ferritin and transferin saturation (TSAT) with bone marrow iron status at different cut off values [19]. Data analysis revealed that, among 28 patients with stainable iron deposits in the marrow; 7 patients had CHr \leq 28 pg/cell, 21 patients had CHr $>$ 28 pg/cell and out of the 42 patients with no stainable iron in the marrow; 34 patients had CHr \leq 28 pg /cell, 8 patients had CHr $>$ 28 pg/cell. On the other hand serum ferritin level $<$ 100 (ng/ml), 100-500 (ng/ml), $>$ 500 (ng/ml) were found respectively in 10, 15 and 3 patients of detectable iron present in the

marrow group, while serum ferritin $<$ 100 (ng/ml), 100-500 (ng/ml), $>$ 500 (ng/ml) were found respectively in 20, 21 and 1 patients having no detectable iron deposits in the marrow group. In this series transferin saturation (TSAT) \leq 20% and $>$ 20% were found in 12 and 16 patients respectively among detectable iron present in the marrow group, while TSAT \leq 20% and $>$ 20% were found in 23 and 19 patients respectively among no detectable iron deposits in the marrow group. The difference between two groups was statistically significant only regarding reticulocyte haemoglobin content level ($p < 0.001$) (Table 3).

Parameters	Bone marrow aspiration report: Stainable iron		p-value
	Present (n=28) No. (%)	Absent (n=42) No. (%)	
Reticulocyte haemoglobin content (CHr)			
≤28 πγ/χελλ	7(25.0)	34(81.0)	<0.001
>28 pg/cell	21(75.0)	8(19.0)	
Serum Ferritin			
<100 (ng/ml)	10(35.7)	20(47.6)	
100-500 (ng/ml)	15(53.6)	21(50.0)	0.267
>500 (ng/ml)	3(10.7)	1(2.4)	
Transferin saturation (TSAT)			
≤20	12(42.9)	23(54.8)	0.329
> 20	16(57.1)	19(45.2)	

Chi-square test was done; Values in the parentheses denote the corresponding percentage

Table 3: Comparison of reticulocyte haemoglobin content (CHr), serum ferritin and transferin saturation (TSAT) with bone marrow iron status at different cut off values (N=70).

The ability of reticulocyte haemoglobin content (CHr) to predict bone marrow iron stores was compared with that of serum ferritin and transferin saturation (TSAT) by receiver operator characteristic (ROC) curve analysis. It was

observed that the best cut off point of CHr (blue color) was 28.0 pg/cell for iron deficiency in pre-dialytic CKD patients (Figure 2).

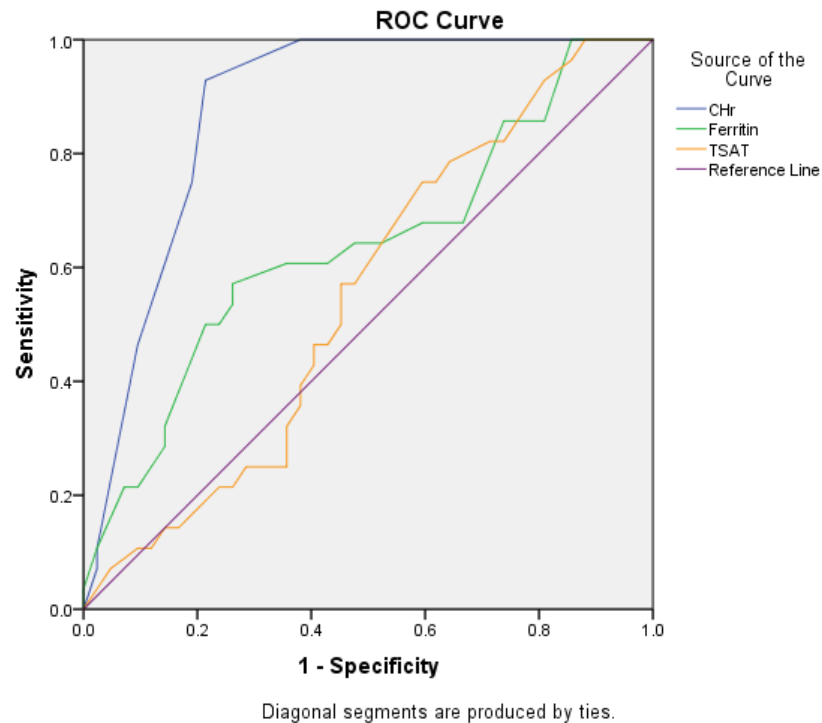


Figure 2: Receiver operator characteristic (ROC) curve showing comparison of reticulocyte haemoglobin content (CHr), serum ferritin and transferrin saturation (TSAT) for the detection of absent bone marrow iron stores.

ROC curve analysis revealed that sensitivity and specificity of reticulocyte haemoglobin content (CHr) at a cut off 28 pg/cell were 81.0% and 75.0% respectively with an area under curve (AUC) 0.879 ± 0.04 [95% confidence interval (CI); 0.785-0.954, $p < 0.001$]. Serum ferritin at a cut off 100 ng/ml had sensitivity and specificity were 40.6% and 67.9%

respectively with an AUC 0.639 ± 0.69 (95% CI; 0.503-0.775, $p = 0.051$). Transferrin saturation (TSAT) at a cut off 20% had sensitivity and specificity was 54.2% and 57.1% respectively with an AUC 0.552 ± 0.069 (95% CI; 0.417-0.687, $p = 0.465$) (Table 4).

Iron measured	AUC*	Cut off value	Sensitivity (%)	Specificity (%)	p-value	95% CI
CHr (pg/cell)	0.879 ± 0.04	28.0	81.0%	75.0%	0.001	0.785-0.954
Ferritin (ng/ml)	0.639 ± 0.69	100.0	40.6%	67.9%	0.051	0.503-0.775
TSAT (%)	0.552 ± 0.069	20.0	54.2%	57.1%	0.465	0.417-0.687

*AUC= Area under curve

Table 4: Sensitivity and specificity of iron measures for detecting iron deficiency in pre-dialytic CKD patients (N=70).

4. Discussion

Iron is an important mineral needed for many essential functions in our body including transport of oxygen from lung to tissues. The body iron is mainly stored in the red blood cells (RBC) and is transported in the circulation by transferrin [20]. Patients with chronic kidney disease (CKD) have lower intestinal iron absorption [21]. Hence the conventional iron markers are not always adequate to diagnose iron deficiency in CKD patients. Reticulocyte haemoglobin content (CHr) appears to be a better tool to predict iron deficiency anemia among pre-dialytic CKD patients [14-16]. In this background current study aimed to evaluate the effectiveness of reticulocyte haemoglobin content (CHr) as a marker of iron deficiency in pre-dialytic CKD patients among Bangladeshi population.

In this study, a total of 70 pre-dialytic CKD patients with chronic anemia were included. Regarding causes of CKD it was found that; 48.6% study patient had diabetic nephropathy (DN), 41.4% had chronic glomerulonephritis (CGN), 7.1% had hypertensive nephropathy (HTN) and 2.9% had obstructive nephropathy. Among the study population; stainable iron deposits were detected in 28 (40%) patients and the rest 42(60%) patients had no stainable iron in the marrow. Out of 28 patients who had stainable iron deposits in the marrow; 7 patients had $CHr \leq 28$ pg/cell, 21 patients had $CHr > 28$ pg/cell and out of 42 patients with no stainable iron in the marrow; 34 patients had $CHr \leq 28$ pg /cell, 8 patients had $CHr > 28$ pg/cell. In this series; serum ferritin level < 100 (ng/ml), 100-500 (ng/ml), > 500 (ng/ml) were found respectively in 10, 15 and 3 patients of detectable iron present in the marrow group, while serum ferritin < 100 (ng/ml), 100-500 (ng/ml), > 500 (ng/ml) were found respectively in 20, 21 and 1 patients

having no detectable iron deposits in the marrow group. In this current study transferrin saturation (TSAT) $\leq 20\%$ and $> 20\%$ were found in 12 and 16 patients respectively among detectable iron present in the marrow group, on the other hand TSAT $\leq 20\%$ and $> 20\%$ were found in 23 and 19 patients respectively among no detectable iron deposits in the marrow group. Values of various parameters [haemoglobin (Hb), reticulocyte haemoglobin content (CHr), serum ferritin and transferrin saturation (TSAT)] among bone marrow iron deficient and iron present groups shows reticulocyte haemoglobin content (CHr) and serum ferritin were statistically significant between the groups ($p < 0.05$). In this context, Vidyashankar *et al.* in their study found reticulocyte haemoglobin content (CHr) and serum ferritin were statistically significant which was similar to our findings [22].

In this study comparison of reticulocyte haemoglobin content (CHr), serum ferritin, and TSAT at different cut off values with bone marrow iron status was done accordingly [19]. It was revealed that only reticulocyte haemoglobin content (CHr) was statistically significant ($p < 0.001$). This finding was consistent with a couple of previous study [23, 24].

We found that the sensitivity and specificity of CHr at a cut-off 28 pg/cell was 81.0% and 75.0% respectively. The sensitivity and specificity of CHr in this present study was almost similar to the related previous studies [23-25]. We observed sensitivity and specificity of serum ferritin at a cut-off 100 ng/ml was 40.6% and 67.9% respectively which was comparable with a previous study [26]. In this study TSAT at a cut-off 20% had diagnostic sensitivity and specificity of 54.2% and 57.1% respectively. In accordance

Chuang *et al.* in their study on CKD patients found that TSAT <19 had diagnostic sensitivity and specificity of 58.8% and 78.4% respectively [26]. The sensitivity of TSAT at a cut-off 20% of our findings had similarity but specificity was different from the study mentioned which may be due to different cut off value of TSAT or due to chronic inflammation or may be other causes.

In this current study, area under the ROC curve of CHr at a cut-off 28 pg/cell was 0.879 ± 0.04 ($p < 0.001$), while that of serum ferritin at a cut-off 100 ng/ml was 0.639 ± 0.69 ($p = 0.051$) and TSAT at a cut-off 20% was 0.552 ± 0.069 ($p = 0.465$). So the area under the ROC curve of CHr exceeded that of ferritin and TSAT, indicating that it had the best overall sensitivity and specificity for diagnosing iron deficiency in the population tested. Mast *et al.* found that the area under the ROC curve was greater for CHr than for serum ferritin and TSAT which was an agreement to our findings [25].

This study demonstrated a strong association of reticulocyte haemoglobin content (CHr) with iron deficiency in pre-dialytic CKD patients. An early detection of iron deficiency among pre-dialytic CKD patients is important for specific management to correct anemia of these patients. Conventionally iron status of CKD patients is assessed by serum ferritin and transferrin saturation (TSAT). Serum ferritin is an acute phase protein and could be raised among individuals with any infection. On the other hand transferrin saturation (TSAT) is measured by indirect method and hence is a poor indicator of body iron load. Therefore a novel biomarker is needed which is not influenced by any other physiological or inflammatory conditions. Reticulocyte haemoglobin content (CHr) could be a better

option to predict iron deficiency in pre-dialytic CKD patients.

5. Conclusion

This study concluded that the reticulocyte haemoglobin content (CHr) is an ideal test to assess iron status for pre-dialytic CKD patients. The CHr, at a cut-off value of 28 pg/cell could be an accurate predictor of iron deficiency in pre-dialytic CKD patients among Bangladeshi adults.

Limitations

It was a single centre study with a relatively small sample size.

Recommendation

A population based prospective study with large sample size should be done to establish the reticulocyte haemoglobin content (CHr) as a marker of iron deficiency in pre-dialytic CKD patients.

Conflicts of Interest

The authors declare that they had no conflicts of interest regarding the publication of this paper.

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