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Iron Profile and Status of Anemia with the Associated Factors in Chronic Kidney Disease Patients

Rupesh Kumar Shreewastav,[1] Bishal Raj Joshi,[1] Rahul Yadav,[2] Anup Katwal,[2] Shailendra Shrestha[2]

[1]Department of Biochemistry, Nobel Medical College Teaching Hospital, Biratnagar, Nepal, [2]Department of Nephrology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal.

ABSTRACT

Background: One of the main complications of chronic kidney disease is anemia. Disorders of iron homeostasis seen in such patients make the management of anemia more challenging and risky. To obtain the desired result, erythropoietin and iron dose must be carefully regulated. The aim of the study is to find out the prevalence of anemia and level iron indices in patients of chronic kidney disease at a tertiary care hospital.

Methods: A cross-sectional descriptive study was conducted on chronic kidney disease patients at a tertiary care center. Demographic data like age, sex, height and weight were collected with the help of using Performa. The hematological and biochemical study variables were assayed by blood sample of the patients in the clinical laboratory services. Data were analyzed by statistical package for the social sciences Version 20.0

Results: Out of 171 patients with chronic kidney disease, 162 (94.7%) were anemic, with the highest percentage 54 (31.5%) having hypertension. The median value with inter-quartile range of hemoglobin, serum iron, serum ferritin, TIBC and transferring saturation were 8.9 gm/dl (6.7-10.0), 115.0 (µg/dl) (60.0-140.0), 599.0 ng/ml (142.6-973.0), 279.0 µg/dl (250.0-342.0) and 41.0 % (22.0-53.0) respectively. Serum creatinine and eGFR were significantly correlated with hemoglobin and iron indices.

Conclusions: The current study showed that anemia was more prevalent in patients of chronic kidney disease in our setting as compared to similar studies. Hypertension was the most common disease among them. The median value of parameters of iron profile except ferritin among them was within the reference limit.

Keywords: Anemia; chronic kidney disease; iron

INTRODUCTION

Chronic kidney disease (CKD) frequently results in anemia, which places a heavy strain on patients and the healthcare system. [1, 2]

Intravenous iron (IV) is recommended for CKD patients on dialysis, whereas oral iron is prescribed to those who are not on dialysis. [3] Even in post-erythropoietin (EPO) era, there is growing concern that IV iron delivery in dialysis patients may result in iron overload and tissue iron deposition. [4] As a result, it is essential to check iron indices in CKD patients undergoing iron therapy to prevent iron overload and its harmful side effects. Serum iron indices are primary indicators of iron status, which are used to guide anemia treatment in patients with chronic kidney disease. [5]

The purpose of the present study is to find the prevalence of anemia, level of iron indices and other associated factors in CKD patients attending a tertiary care hospital in our setting.

METHODS

A descriptive cross-sectional study was conducted prospectively at a tertiary care Hospital, Nobel Medical College Teaching Hospital (NMCTH) on the patients diagnosed with chronic kidney disease (CKD) from 15th May 2022 to 15th October 2022 after getting the ethical approval of the same from institutional review committee, NMCTH (Ref no-621/2022). The patients with CKD, who presented themselves or had been referred from other center at outpatient door or admitted to the nephrology ward NMCTH, were enrolled in this study. The patients, who were reluctant for the study, were excluded. Convenience sampling was done for the present study. The calculated sample size was

Correspondence: Dr Rupesh Kumar Shreewastav, Department of Biochemistry, Nobel Medical College Teaching Hospital, Biratnagar, Nepal. Email: rupeshaiims4@gmail.com, Phone: +9779841864446.

98 by using the formula $n=Z^2pq/d^2$ [where, n= minimum required sample size, Z= 1.96 at 95% Confidence Interval (CI), p= prevalence taken as 47.85% [6], q= 1-p, d= margin of error, 10%], however 171 of total sample in the study period was considered for the study. The study was conducted after getting the consent from the patients or attendant.

A predesigned Performa was used to collect demographic data like age, sex, height, weight and other relevant data pertaining to cause, management and mode of treatment of CKD patients. Body mass index (BMI) was calculated by the formula, BMI= Weight in Kg/ (Height in m).[2] In the clinical laboratory services (CLS), NMCTH, biochemical and hematological investigations were carried out from the blood sample of the patients. Iron profile was estimated in the blood samples of the CKD patients. The method for the estimation of serum iron in blood samples of patients was bi-reagent system by end point reaction. Iron is dissociated of transferrin by the action of an acid pH buffer. Ascorbic acid presented in reagent reduces ferric ions to ferrous ions that afterwards form a brilliant magenta complex with ferrozine. Its absorbance is measured at 540 and 580 nm and is proportional to the iron concentration in the sample. The reagent was used of Labtest Ferrozine Company.

Serum ferritin was estimated in blood samples of the patients in Siemens XP fully automated machine by the Dai Ferritin CLIA test, which utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact Ferritin molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the Ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of Ferritin in the sample. By reference to a series of Ferritin standards assayed in the same way, the concentration of Ferritin in the unknown sample is quantified.

Total iron binding capacity is measured by colorimetric end point method. The method measures the TIBC by first saturating the transferrin with escess of Fe^{+3}. The remaining iron is adsorbed with magnesium carbonate and once the binding process is complete the quelator is removed by centrifugation, and an assay for iron content is performed in the supernatant. From this measurement the TIBC value is obtained. When the serum iron determination is performed concurrently with the TIBC and the result substracted from the TIBC value, the difference yields the unsaturated iron-binding capacity (UIBC), or seric transferrin not bound to iron. The reagent was used from Cromatest Linear Chemicals Company. Transferring saturation (TS) was calculated by using formula, TS%= Serum iron/TIBC ×100

CLS tested the patient's sera for all the remaining biochemical parameters (Urea, Creatinine, Calcium, Phosphorus and Uric acid) using the reagents provided by the manufacturer in a fully automated analyzer (Labsystems Diagnostics, Nano Lab 200). Estimated glomerular filtration rate (eGFR) of the individual patients was calculated by using Cockroft-Gault formula.

$$CCr = \{((140\text{-age}) \times \text{weight})/ (72 \times SCr)\} \times 0.85 \text{ (if female)}$$

CCr is creatinine clearance in ml/min, age in years, weight in kg, and SCr is serum creatinine in mg/dl. Another way to describe creatinine clearance (CCr) is as an estimated glomerular filtration rate (eGFR).

The whole blood sample of these CKD patients was evaluated for hematological parameters (Hb, RBC, MCV, MCH, MCHC) using the Accurex (CBC Fully Analyzer 3 parts) analyzer. HbA1c was also calculated by whole blood sample of patients in fully automated analyzer working on the principle of high performance liquid chromatography (HPLC). The patients were considered anemic on the basis of finding of their Hb concentration (Male <12g/dl, Female <11g/dl) in their blood.

The data were entered in MS Excel 2010 and analyzed by Statistical Package for the Social Sciences (SPSS) for Windows Version 20.0. Mean and SD were used to compute descriptive statistics for numerical variables, and percentages were used for categorical variables. Median value with inter-quartile range (IQR) was calculated for the non-parametric study variables. Mann Whitney U test was applied to compare the significance between the median values of two independent groups. The correlation between the non-parametric study variables was calculated by Spearman correlation coefficients and the significance of the correlation was tested by two-tailed test. The data was considered significant if $p < 0.05$ at 95% confidence interval (CI).

RESULTS

The total number of patients, with chronic kidney disease, enrolled in the study was 171. The mean age of the patients was 53.6 year with the minimum age of 16 and maximum of 93. Out of 171 patients, 109 (63.7%) were male and 62 (36.3%) were female. The total number

of patients suffering with anemia was 162 (94.7%). The mean±SD value of body mass index (BMI) of the study patients was 25.1±5.4 kg/m^2. The maximum cause of CKD among study participants was hypertension, which was observed in 54 (31.5%) patients. Among the CKD patients, the maximum 61 (35.6%) were having the disease more than 2 year. The total number of patients, who received dialysis, was 40 (23.4%) and 152 (88.8%) patients were supplemented with iron either orally or intravenously. Out of these 171 patients, 56 (32.7%) received blood transfusion and 59 (34.5%) were supplemented with erythropoietin (EPO) as shown in Table 1.

Table 1. Baseline characteristics of study participants (n=171).

Characteristics	Number	Percentage
Age in year (Mean±SD)		**53.6±16.5**
Male	109	63.7
Female	62	36.3
Anemic	162	94.7
Male (<12g/dl)	100	57.7
Female(<11g/dl)	62	36.3
BMI in Kg/meter^2 (Mean±SD)		**25.1±5.4**
<18.5	9	5.2
18.5-24.9	93	54.3
25-30	37	21.7
>30	32	18.8
Causes of CKD		
Diabetes Mellitus	43	25.1
Hypertension	54	31.5
CGN	45	26.4
Obstructive Uropathy	4	2.3
ADPKD	6	3.6
More than one chronic disease	19	11.1
Duration of CKD		
<6 month	59	34.6
6-11 month	24	14.1
1-2 year	27	15.7
>2 years	61	35.6
Dialysis		
Yes	40	23.4
No	131	76.6
Iron Supplementation (oral/IV)		
Yes	152	88.8
No	19	11.2
Blood transfusion		
Yes	56	32.7
No	115	67.3
EPO Supplementation		
Yes	59	34.5
No	112	65.5

The median value with inter quartile range of biochemical study parameters among all the participants were calculated. The median (IQR) value of Serum iron, serum ferritin, TIBC and transferring saturation were 115.0 µg/dl (60.0-140.0), 599.0 ng/ml (142.6-973.0), 279.0 µg/dl (250.0-342.0) and 41.0% (22.0-53.0) respectively. The median (IQR) value of other biochemical parameters is also mentioned in Table 2. The median value with inter quartile range of hematological parameters among all the participants were also tabulated. The median (IQR) value of Hb and RBC were 8.9 gm/dl (6.7-10.0) and 2.9 million cells/µl (2.2-3.4) respectively. The median (IQR) value of MCV, MCH and MCHC are shown in Table 3.

Table 2. Median value with inter quartile range of Iron profile and other study parameters among all participants (n=107).

Parameters	Median (IQR)
Serum Iron (µg/dl)	115.0 (60.0-140.0)
Serum Ferritin (ng/ml)	599.0 (142.6-973.0)
TIBC (µg/dl)	279.0 (250.0-342.0)
Transferrin saturation (%)	41.0 (22.0-53.0)
HbA1c (%)	6.3 (5.9-7.4)
Urea (mg/dl)	122.0 (85.0-179.0)
Creatinine (mg/dl)	6.7 (3.7-10.10)
eGFR (ml/min)	10.0 (6.0-18.0)
Calcium (mg/dl)	8.2 (7.8-8.75)
Phosphorus (mg/dl)	5.5 (4.8-5.9)
Uric acid (mg/dl)	5.85 (4.5-7.3)

Table 3. Median value with inter quartile range of Hematological parameters among all participants (n=107).

Parameters	Median (IQR)
Hb (gm/dl)	8.9 (6.7-10.0)
RBC (million cells/µl)	2.9 (2.2-3.4)
MCV (fl)	97.4 (95.7-99.6)
MCH (pg)	29.3 (27.4-30.8)
MCHC (%)	30.1 (28.6-31.8)

Table 4. Comparison of Median (IQR) value of Hemoglobin and Iron profile between dialysis and non-dialysis patients.

Parameters	Dialysis patients	Non-dialysis patients	P value
Hemoglobin	9.2 (7.5-10.3)	7.0 (5.3-8.4)	< 0.001
Serum Iron	130.0 (101.0-180.0)	99.5 (50.0-139.0)	0.015
Serum ferritin	973.0 (609.0-1160.0)	407.0 (85.2-797.0)	<0.001
TIBC	257.0 (250.0-290.0)	289.5 (251.0-370.0)	0.20
Transferrin saturation	51.0 (36.0-67.0)	32.5 (21.0-51.10)	0.001

(Mann Whitney U test)

The study was extended by observing the comparison of study parameters between dialysis and non dialysis patient. The median value of hemoglobin (7 g/dl) among the dialysis patients was significantly ($p < 0.001$) lower than non-dialysis patients (9.2 g/dl). Similarly, the median value of serum iron (99.5) and transferrin saturation (32.5) was significantly lower (p=0.015, p=0.001 respectively) in dialysis patients than non-dialysis. The median value of TIBC (257.0) among dialysis patients was non- significantly (p=0.2) lower than non-dialysis patients (289.5). Conversely, the median value of serum ferritin (973.0) among dialysis patients was significantly (p<0.001) higher than non-dialysis patients (407.0) as shown in Table 4.

The study parameters (Hb and iron profile) were also compared between the patients receiving iron therapy only and the patients receiving iron with erythropoietin (EPO) and/or blood transfusion (BT). It was noted that the median value of Hb, serum iron, serum ferritin and transferrin saturation was (9.3, 125, 840 and 46 respectively) higher in patients receiving iron with EPO and/or BT than the group of patients having iron therapy only, whereas the median value of TIBC (301.0) was found be higher among group of patients receiving iron therapy only than other group of patients receiving iron with EPO and/or BT. The data is shown in Table 5.

Table 5. Comparison of Median (IQR) value of Hemoglobin and Iron profile between patients supplemented with Iron therapy and Iron with EPO and/or blood transfusion.

Parameters	Iron therapy only	Iron with EPO and/or BT	P value
Hemoglobin (gm/dl)	7.5 (6.4-8.9)	9.3 (8.4-10.3)	< 0.001
Serum Iron (µg/dl)	98.0 (54.0-146.0)	125.0 (69.0-132.0)	0.520
Serum ferritin (ng/ml)	277.0 (77.6-641.0)	840.0 (577.0-1100.0)	<0.001
TIBC (µg/dl)	301.0 (252.0-385.0)	256.0 (236.0-297.0)	0.012
Transferrin saturation (%)	31.1 (20.0-51.1)	46.0 (25.0-55.0)	0.059

We have also analyzed the correlation between different study variables among all of the study patients and it was noted that serum creatinine was negatively and significantly correlated with Hb (r =-0.591, p=<0.001) and TIBC (r = -0.31, p= 0.006), whereas it was positively and significantly correlated with serum iron (r = 0.32, p= 0.004), serum ferritin (r = 0.38, p= 0.001) and transferring saturation (r = 0.45, p= <0.001). Similarly, eGFR was negatively and significantly associated with serum iron (r = -0.29, p= 0.009), serum ferritin (r = -0.39, p= <0.001) and transferring saturation (r = -0.43, p= <0.001), whereas it was it was positively and significantly associated with Hb (r = 0.61, p=<0.001) and TIBC (r = 0.32, p= 0.004) as shown in Table 6.

Table 6. Correlation analysis between indices of iron profile and study parameters among CKD patients.

Parameter	Subgroup	Correlation r	p-value
Creatinine	Hb	-0.591	<0.001
	S Iron	0.321	0.004
	S ferritin	0.381	0.001
	TIBC	-0.31	0.006
	TS	0.456	<0.001
eGFR	Hb	0.614	<0.001
	S Iron	-0.295	0.009
	S ferritin	-0.391	<0.001
	TIBC	0.32	0.004
	TS	-0.432	<0.001
Hb	S Iron	-0.2	0.073
	S ferritin	-0.24	0.035
	TIBC	0.12	0.26
	TS	-0.28	0.13

DISCUSSION

The total number of study participants in the present study with chronic kidney disease was 171, out of that 63.7% were male and 36.3% were female with mean age of 53.6 year. Out of 171 patients, 94.7% patients were found anemic, in which 57.7% were male and 36.3%

female. A study carried out in Bangladesh reported that 58.41% of the total patients with chronic kidney disease were anemic with more number of male (64.4%) patients suffered from CKD than female (35.5%).[7] Reddy GC *et. al.* reported that the majority of CKD patients in this study (94%) were anemic, with hemoglobin levels below 11 g/dL.[8] Another study conducted in USA revealed that those with CKD had anemia twice as often as the general population (7.6% vs. 15.4%). From 8.4% at stage 1 to 53.4% at stage 5, the prevalence of anemia rose with CKD level.[9] Sofue T. *et. al.* carried out a study among CKD patients in Japan and concluded that anemia was present in 40.1% of CKD stage G4 patients and 60.3% of CKD stage G5 patients, respectively.[10] Similar research was done in Nepal at TUTH, where it was reported that 47.85% of all patients with CKD had anemia. [6] In the present study, the major proportion of the patients developed CKD due to hypertension (31.5%) followed by chronic glomerulonephritis (26.4 %). Similar results were found in a Scotland study, which found that hypertension (71.2%) and chronic heart disease (34.5%) were the two most common associated diseases among people with CKD.[11] It has been concluded in many reports that diabetes and hypertension are the leading causes of CKD. [12,13]

In the present study, the median value of serum iron, serum ferritin, TIBC, TS and hemoglobin among all the CKD was 115 µg/dl, 599 ng/ml, 279 µg/dl, 41% and 8.9 gm/dl respectively. The normal reference range for iron indices in serum are, iron=600-200 µg/dl, ferritin= 30-200 ng/ml, TIBC= 250-400 µg/dl, TS%= 20-45%.[14] A finding different from our study, which was carried out in Assam, India showed the mean value of serum iron, serum ferritin, TIBC, TS and hemoglobin among CKD patients as 33.11 µg/dl, 169.38 ng/ml, 237.24 µg/dl, 14.31% and 9.07 gm/dl.[14] Nearly similar finding with the present study was observed in an another research work conducted in Andhra Pradesh, India, which revealed the mean value of serum iron, serum ferritin, TIBC and TS among CKD patients as 84 µg/dl, 684 ng/ml, 221 µg/dl and 39 % respectively.[8] Despite the reduced hemoglobin concentration among CKD patients in the current study, the reason for having normal serum iron and TS levels may be due to study participants' oral or intravenous iron intake with EPO and/or BT. The other biochemical parameters were within normal reference range except for urea, creatinine and eGFR among CKD patients in the current study. Along with hemoglobin concentration, RBC concentration was also found to be reduced in study participants in the present study. The mean value of RBC concentration among CKD-IV patients was reported to be 3.69 cells/cmm in a study conducted by Gogoi SB *et. al.*[14] The level of hemoglobin was significantly found to be reduced in CKD patients in many of the study reports similar to current study. [9, 15-18]

In this study, the median value of Hb and iron profile were compared between CKD patients receiving dialysis and those not, and it was found that patients receiving dialysis had significantly higher levels of Hb, serum iron, ferritin and TS with non-significant reduction of TIBC. Similarly, while comparing the median value of Hb and iron profile between CKD patients receiving iron therapy only versus iron with EPO and/or blood transfusion (BT), it was noted that patients taking iron with EPO and/or BT had significantly higher levels of Hb, serum iron, ferritin and TS whereas TIBC was significantly reduced in the same. Similar, finding was observed in a study conducted in Iran, which concluded as after hemodialysis, hemoglobin levels were found to be considerably higher than those before hemodialysis (11.1 ±1.1 vs. 11.9± 1.2 g/dL, P 0.001, 7% increase).[19] The reason for getting higher level of Hb and iron profile in the patients receiving dialysis in the current study is that they are mostly supplemented with iron therapy along with EPO and or blood transfusion, which contributed to elicit the better output of the parameters. Numerous earlier investigations found that supplementing with iron therapy with EPO and/or blood transfusions significantly raised the amount of hemoglobin and the iron profile in dialyzed or non-dialyzed CKD patients.[20-25]

The present piece of work was extended by analyzing the correlation study between the parameters among all of the CKD patients and it was seen that serum creatinine was negatively and significantly associated with Hb and TIBC but with positive and significant correlation with serum iron, ferritin and TS whereas eGFR was positively associated with Hb and TIBC with negative and significant correlation with serum iron, ferritin and TS. Similarly, Hb was negatively correlated with iron, ferritin and TS but positively associated with TIBC. Similar finding was observed in one study conducted in India which revealed that serum creatinine was negatively correlated with Hb, ferritin and TIBC and Hb was negatively correlated with iron, ferritin. [8] Gogoi SB *et. al.* had reported in his study that Hb was negatively correlated with creatinine whereas it was positively correlated with eGFR. [14] In their research of patients with non-dialysis-dependent chronic kidney disease, Kovesdy CP *et. al.* found a statistically significant association between blood creatinine levels and iron indices ($p<0.05$). [26]

The limitations of the present study are the data had been included from one centre and a particular point of time. The results would have been more meaningful

if the data on CKD patients had been taken from other centers in Nepal too and the patients had been followed up for a larger period of time.

CONCLUSIONS

The prevalence of anemia was higher among chronic kidney disease patients in our center as compared to other similar studies. Anemia was more observed in male than female. The most common associated condition was hypertension with normal median value of parameters of iron profile except ferritin level among the patients. The median value of Hb and iron profile except total iron binding capacity was comparatively higher in patients receiving dialysis (Vs non-dialysis) and supplemented with iron therapy with erythropoietin and or blood transfusion (Vs iron therapy only). Serum creatinine, eGFR and Hb were significantly associated with iron indices.

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CONFLICTS OF INTEREST

We declare no conflicts of interest.

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