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Original Research Article

Evaluation of iron status in patients with chronic kidney disease

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Abstract

Background: Anemia is an early and common complication of non dialysis chronic kidney disease (CKD) and is considered a hallmark of chronicity of renal disease. It increases in prevalence and severity as renal function decreases, becoming much more common as the progression of disease thereby further increasing the morbidity in these patients. Therefore, earlier detection and correction of anemia may be helpful in preventing the progression of the diseases and its adverse outcomes.
Methods: The present study was designed to evaluate the iron status in pre dialysis CKD patients. Fifty diagnosed CKD subjects were randomly selected who were attending the department of Medicine and Nephrology of A J Institute of Medical Sciences hospital. Fifty age and sex matched healthy persons served as controls. Haemoglobin, serum iron, Total iron binding capacity (TIBC), transferrin saturation (TSAT), serum ferritin and serum creatinine were estimated by using commercially available kits. Statistical data were analyzed by using SPSS16.
Results: All the CKD subjects were anaemic with haemoglobin concentration below 11g/dl and 68% of them showed moderate degree of anemia. Serum iron, serum ferritin, TSAT and haemoglobin were significantly lower in CKD patients as compared to the control group (p=<0.0001) whereas TIBC was significantly higher in the CKD patients when compared to the control group (p=0.0496). Among the CKD patients, 14% had serum ferritin < 100 ng/mL and TSAT < 20% which indicated absolute iron deficiency. The most frequent causes of CKD were diabetes mellitus (64%) and hypertension (20%).
Conclusions: It is evident from the present study that iron deficiency in patients with CKD maybe multifactorial. Anemia is one of the earliest manifestations in CKD patients. Our findings suggest that various parameters of iron status if used in tandem are useful as markers for determining the severity of iron deficiency anemia in CKD patients.
Keywords: Anemia, Chronic kidney disease, Iron deficiency.

1. Introduction

The burden of Chronic Kidney Disease (CKD) in India has been recognized as a silent epidemic and a major cause of mortality and morbidity affecting large populations worldwide, the approximate prevalence of CKD being 800 per million population [1]. The alarming rate at which the CKD patient population continues to grow can be mainly attributed to the epidemiology and the associated risk factors in CKD. According to national kidney foundation/ kidney disease outcomes quality initiative (NKF/DOQI) guidelines, CKD has been defined as either a level of glomerular filtration rate (GFR)<60ml/min per 1.73m^2, which is accompanied in most cases by signs and symptoms of uraemia, or a need for initiation of renal replacement therapy [2]

The hallmark of chronicity of renal disease is characterized by anemia seen as an early and common complication which is predominantly due to decreased kidney production of erythropoietin by the diseased kidney resulting in reduced production of red cells from the erythroid marrow [3].

Iron deficiency is the major contributing factor for anemia in about 23 – 38% of CKD patients. It is one of the major risk factors for the progression of chronic kidney disease to ESRD. Iron deficiency may be absolute, resulting from occult blood loss, frequent blood sampling, low grade gastrointestinal bleeding, and decreased oral iron absorption because of dietary restrictions, loss of taste for iron-rich foods, and low or high levels of hepcidin [4]. Iron deficiency leads to a reduction in formation of red cell

haemoglobin, causing hypochromic microcytic anemia. A number of other factors may have contributory role like nutritional insufficiency or increased blood loss leading to iron, B12 and folate deficiency [5,6,7], diminished red blood cells survival, hyperparathyroidism, bone marrow fibrosis [8], mild chronic inflammation, and uremic milieu [9].

Functional iron deficiency is more common and is strongly associated with upregulation of inflammatory cytokines such as IL-6 and impaired tissue responsiveness to erythropoietin, which can inhibit iron transport from tissue stores to erythroblasts [10].

Assessment of iron stores is routinely done in CKD patients through measurement of various indicators of iron status like haemoglobin, serum iron, percentage transferrin saturation (TSAT), total iron binding capacity (TIBC) and serum ferritin. Haemoglobin is a better quantitative measure for monitoring and managing anemia in patients with CKD [2]. Patients with chronic renal disease should have enough iron to achieve and maintain target haemoglobin concentration.

Iron deficiency is accompanied by reductions in serum iron and transferrin saturation (TSAT), increase in total serum iron-binding capacity (TIBC) in conjunction with normal/increased serum ferritin [11]

The TSAT represents the percentage of the transferrin iron-binding capacity actually occupied by iron in the serum [12]. A reduction of TSAT below 16% is a reliable index of an under supply of iron to the developing red cells [13]. It is a good indicator to measure body's functional capacity to readily use stored iron for erythropoiesis, which accounts for less than 0.1% of iron in the body.

Diurnal variation in serum iron causes wide fluctuations in TSAT levels. Nutritional status and chronic diseases have a large effect on the transferrin levels in the body [3].

The diagnostic value of serum ferritin as an estimate of body iron stores is a powerful determinant of erythropoietic mechanism. Ferritin levels may be altered by factors such as inflammatory cytokines including interleukin-1 and tumor necrosis factor-α [14].

There have been very limited studies evaluating the haematological profile in pre dialysis CKD patients hence the purpose of our analysis was to broaden the scope to include our findings for fuller exploration of iron indices in CKD.

2. Materials and methods

A hospital based case control study was conducted for a period of one year in A J Institute of Medical Sciences, Mangalore, India. The study population consisted of fifty diagnosed patients of CKD between the age of 15 – 75 years who were attending the department of Medicine and Nephrology of the hospital. Fifty healthy persons served as controls.

2.1 Inclusion criteria

Fifty cases were selected by assessment of renal function with the creatinine clearance estimated using Cockcroft-Gault equation.

2.2 Exclusion criteria

Patients on dialysis, children, those having inflammatory conditions, malignancy were excluded. Patients with burns, trauma, known haematological disorders and liver dysfunction or on drugs, haematinics, recombinant human erythropoietin (rHuEPO) and blood transfusion in the last three months that were likely to cause haematological disturbances were also excluded. Informed written consent was obtained from all the patients and study subjects after explaining the objectives of the study, risks and benefits involved. Confidentiality regarding the personal details of the study population was maintained. The study was approved by the institutional ethical committee.

2.3 Sample collection

Under aseptic precaution, 5 ml of blood was collected in plain vacutainers from each subject from a large peripheral vein (mostly antecubital vein) with a sterilized syringe. The serum was separated after centrifugation at 3000rpm for 10min and parameters were analyzed the same day.

2.4 Laboratory analysis

The collected samples were assayed for haemoglobin concentration, serum creatinine, serum iron, Total iron binding capacity, transferrin saturation and serum ferritin.

2.4.1 Estimation of Haemoglobin by Cyanmethaemoglobin method [15]

This estimation was done using commercially available kit on CAFA 200 autoanalyser at a wavelength of 540 nm. This is based on the reaction where haemoglobin (oxyhaemoglobin, methaemoglobin, carboxyhaemoglobin) is converted to cyanmethaemoglobin. The intensity of the color change produced is proportional to the haemoglobin concentration and is compared to known cyanmethaemoglobin standard.

2.4.2 Estimation of serum Creatinine by Modified Jaffe's Kinetic method [16]

This estimation was done using commercially available kit on CAFA 200 autoanalyser at a wavelength of 492 nm. Creatinine reacts with picric acid to produce a coloured compound, creatinine picrate. The change in absorbance is proportional to the creatinine concentration in the serum sample.

2.4.3 Estimation of serum Iron by the Ferrozine method [17]

This estimation was done using commercially available kit on Lablife Chem Master semiautoanalyser at a

wavelength of 560 nm. The spectrophotometric measurement of serum iron is accomplished by releasing the protein bound iron which is in the ferric form from transferrin. The released ferric iron is then reduced to ferrous iron by hydroxylamine. This ferrous iron reacts with ferrozine to produce a violet coloured complex, the absorbance of which is proportional to serum iron concentration.

2.4.4 Estimation of serum Total Iron Binding Capacity (TIBC) by precipitation with Magnesium Carbonate Method [18]

This estimation was done using commercially available kit on Lablife Chem Master semiautoanalyser at a wavelength of 560 nm. This is based on the principle that the total iron binding capacity is the sum of the serum iron concentration and the amount of additional iron required to saturate the unbound iron binding sites of transferrin. In this procedure serum is treated with excess of ferrous iron to saturate the iron binding sites on transferrin at alkaline pH. The added excess ferrous iron is adsorbed and precipitated & the iron content in the supernatant is measured to give the TIBC.

2.4.5 Estimation of Transferrin Saturation (TSAT) [19]

This was calculated as the ratio of serum iron and TIBC [(Serum iron ÷ TIBC) x 100] and expressed as a percentage.

2.4.6 Estimation of Serum ferritin by quantitative turbidimetric latex assay [20]**:**

This estimation was done using commercially available kit on Lablife Chem Master semiautoanalyser at a wavelength of 650 nm. The latex particles coated with anti human ferritin are agglutinated when they react with samples containing ferritin. The latex particle agglutination which is detected as an absorbance change is proportional to the concentration of the ferritin present in the sample and can be measured by turbidimetry using a spectrophotometer.

Estimated creatinine clearance or GFR was calculated by using the Cockcroft-Gault equation [21]:

$$\text{eCrCl (ml/min)} = \frac{(140\text{-age}) \times \text{body weight (kg)} (\times 0.85 \text{ for women})}{72 \times \text{serum creatinine (mg/dL)}}$$

2.5 Statistical analysis

All data analyses were carried out using the SPSS, version 16. Results are presented as mean ± standard deviation value. Data was analyzed by Student t-test and Karl Pearsons correlation. P value <0.05 was considered as statistically significant.

3. Results

Fifty diagnosed cases of pre-dialyzed chronic kidney disease patients and fifty control subjects had analyzable data. Out of fifty CKD patients (twenty five patients in stage 3 and twenty five patients in stage 4 CKD), 68% were male and 32% were female. Majority of the patients belonged to >61 year age group (42%) Table 1.

Table 1: Age and sex distribution in CKD patients (n=50)

Category Age group (years)	Frequency	Percentage
<20	1	2%
21 – 40	12	24%
41 – 60	16	32%
>61	21	42%
Sex		
Males	34	68%
Females	16	32%

The characteristics of the study population are shown in Table 2. There was no significant age difference (P=0.318) between the mean ages of the patients (52.34±13.66 years) and the control subjects (54.74±9.98 years). The mean BMI was significantly lower (p=<0.0001) in CKD group (24.23±3.21 kg/m^2) when compared to the control subjects (30.98±4.43 kg/m^2). The mean systolic blood pressure (SBP) was significantly higher (p=<0.0001) in CKD group (144.6±19.29 mm Hg) when compared to the control subjects (126.08±7.58 mm Hg). The mean diastolic blood pressure (DBP) was significantly higher (p=<0.0001) in CKD group (90.2±11.15 mm Hg) when compared to the control subjects (81.92±6.97 mm Hg). The mean serum creatinine levels were higher in CKD group (4.15±1.26 mg/dL) when compared to control subjects (1.04±0.19) and was statistically highly significant.

Table 2: Characteristics of study population

Characteristics	Cases (n=50)	Controls (n=50)	P Value
Mean age (years) (Mean ± SD)	52.34±13.66	54.74±9.98	0.318
BMI (Kg/m^2) (Mean ± SD)	30.98±4.43	24.23±3.21	<0.0001**
Mean SBP (mmHg) (Mean ± SD)	144.6±19.29	126.08±7.58	<0.0001**
Mean DBP (mmHg) (Mean ± SD)	90.2±11.15	81.92±6.97	<0.0001**
Serum Creatinine (mg/dL) (Mean ± SD)	4.15±1.26	1.04±0.19	<0.0001**
eGFR (ml/min) (Mean ± SD)	28.67 ± 9.83	114.02±7.43	<0.0001**

** p < 0.001; Highly significant, *p<0.05; Significant

The most frequent causes of CKD were diabetes mellitus (64%), hypertension (20%), chronic glomerulonephritis (12%) and others (4%) (Table 3, Figure 1).

Table 3: Etiology of chronic kidney disease in the study

Etiology of CKD	Frequency	Percentage
Diabetes mellitus	32	64%
Hypertension	10	20%
Chronic glomerulonephritis	6	12%
Others	2	4%

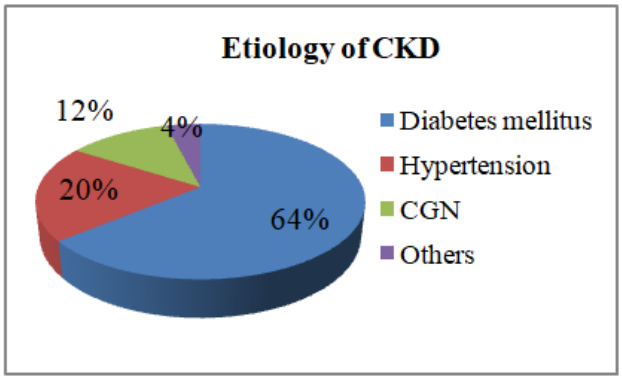

Figure 1: Etiology of CKD patients (n=50)

The haemoglobin concentration of all the cases was <11g/dL in the CKD group and the mean haemoglobin concentration was 8.91±1.43 g/dL. The mean Hb level in males was 9.24±1.23 g/dl and in females was 8.21±1.60 g/dl. The proportions with mild (10-11g/dl), moderate (6.1-9.9g/dl) and severe anemia (<6g/dl) among CKD subjects were 20%, 68% and 12% respectively. (Figure 2)

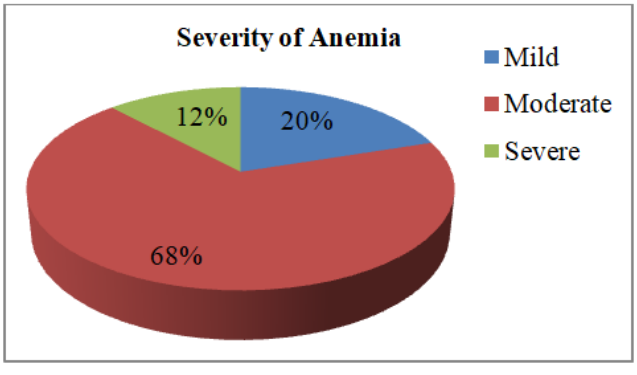

Figure 2: Severity of anemia in CKD patients (n=50).

The mean (±SD) of serum iron, serum ferritin, TIBC, transferrin saturation (TSAT) and Haemoglobin in CKD patients and control groups were shown in Table 4. Serum iron, serum ferritin, TSAT and Haemoglobin were significantly lower in CKD group as compared to the control group (p=<0.0001). TIBC was significantly higher in the CKD group when compared to the control group (p=0.0496)

Table 4: Estimated biochemical parameters compared between CKD cases and controls

Parameters	Cases (n=50)	Controls (n=50)	P value
Serum Iron (µg/dL) (Mean ± SD)	85.59±19.61	121.15±10.15	<0.0001**
Serum Ferritin (µg/L) (Mean ± SD)	108.11±40.95	305.32±49.61	<0.0001**
TIBC (µg/dL) (Mean ± SD)	348.46±45.69	332.54±33.45	0.0496*
TSAT % (Mean ± SD)	24.66±5.18	37.69±5.45	<0.0001**
Haemoglobin (g/dL) (Mean ± SD)	8.91±1.43	13.38±0.74	<0.0001**

** p < 0.001; Highly significant, *p<0.05; Significant

According to NKF/DOQI guidelines, either serum ferritin <100 ng/ml or TSAT <20% indicates iron depletion [2]. 86% of CKD patients showed transferrin saturation (TSAT) level >20%, 12% patients had <16% and 2% patients between 16-20%. Among the CKD group, 14% had serum ferritin < 100 ng/mL and TSAT < 20% which indicated absolute iron deficiency.

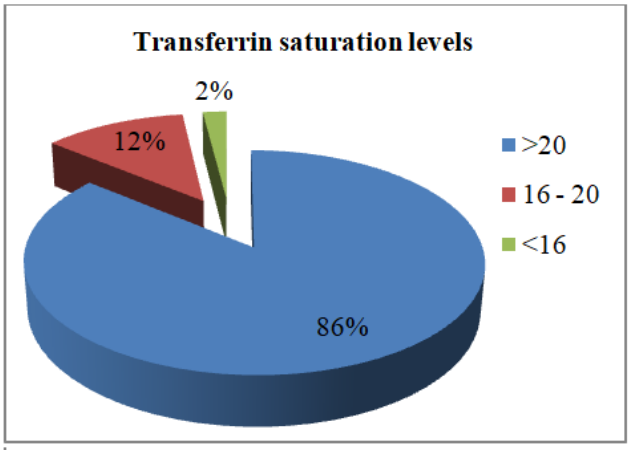

Figure 3: TSAT levels in CKD patients

4. Discussion

Anemia in kidney disease is a complex process that reflects an interaction of the erythropoietic process of bone marrow with iron availability and inflammation. Correction of anemia in CKD significantly improves the quality of life and decreases cardiovascular morbidity and mortality. The NKF-K/DOQI practice guidelines recommend maintaining ferritin >100ng/ml & transferrin saturation (TSAT) >20% to ensure adequate iron supply [2].

Depletion of tissue iron stores in CKD patients with serum ferritin <100 ng/mL and transferrin saturation (TSAT) <20% is characterized as absolute iron deficiency. Functional iron deficiency anemia is adequate tissue iron defined as a serum ferritin level ≥100 ng/ml and TSAT < 20% [22]. To this end our study shows comparatively low iron associated with lower ferritin, lower TSAT and haemoglobin with higher TIBC among patients with CKD when compared to control group, indicating prevalence of true iron deficiency. In the present study, 14% CKD patients had absolute iron deficiency, which is similar to the findings of Lukaszyk E *et al* [23] and Deori R *et al* [24] which were 17% and 26% respectively but contradictory to Talwar *et al* [25] where 65% had iron deficiency anemia.

Our study showed moderate degree of anemia in 68% of CKD patients and these findings were comparable to studies done by Ashfar R *et al* [26], Deori R [24] and Seuga *et al* [27] where moderate degree of anemia was seen in 55%, 48% and 50% respectively.

Present study found a significant negative correlation between haemoglobin, serum iron, TIBC and TSAT with serum creatinine however correlation of serum

ferritin with serum creatinine was insignificant. Deori R [24] observed significant correlation between serum iron and TSAT with serum creatinine of CKD patients. Ashfar R *et al* [26] found a positive correlation between creatinine clearance and haemoglobin concentration. However, in our study the correlation between creatinine clearance and haemoglobin concentration was insignificant among CKD patients.

The primary etiology of CKD was diabetes mellitus followed by hypertension which was similar to studies done by Afshar *et al* [26], Deori R [24] and Mcclellan W *et al* [28]. Irrespective of the etiology, the common consequence following long-term reduction of renal mass, involves hyperfiltration and hypertrophy of the remaining viable nephrons. Eventually as the pressure and flow increases it predisposes to maladaptive hypertrophy leading to fibrosis. Hypoxia as a consequence of peritubular capillaries loss has been frequently observed in chronic kidney disease. The specialized peritubular cells that produce erythropoietin are partially or completely depleted or injured as renal disease progresses, so that erythropoietin production is inappropriately low relative to the degree of anemia. The possible mechanism of decreased levels of haemoglobin and underlying heart failure may be due to renal hypoxia causing renal ischemia [29, 30].

Obesity increases the risk for diabetes and hypertension, the two primary causes for progression of CKD [31]. Obesity itself may be an independent risk factor for kidney disease as a result of glomerular hyperfiltration and activation of the renin-angiotensin system [32]. The findings in our study was consistent with the study done by A Shankar *et al* [33] where higher BMI levels were found to be positively associated with CKD among Asian men in a population-based sample of Malay adults from Singapore. Another study showed that weight gain was significantly associated with an increased risk for development of CKD which was similar to findings in our study [34].

Hypertension is an independent risk factor in the progression of early kidney disease to ESRD [35]. High blood pressure may be a cause or a consequence of CKD and can be associated with adverse outcomes such as worsening renal function by declining GFR and development of cardiovascular disease [36]. A recent study by Fox *et al* [37] demonstrated that predictors for new-onset kidney disease like higher SBP and hypertension (defined as SBP ≥140 mmHg, or DBP ≥90 mmHg or history of antihypertensive medication use) were significantly related to the development of kidney disease which was consistent with the findings in this present study. In a study done by Schaeffner ES *et al*. [38], a clear association was seen between elevated BP and the development CKD in healthy men but DBP showed no statistically significant association. Another study showed that when considered together, a higher SBP and lower DBP was a better predictor for the risk for future mortality in older men with CKD [39].

Arterial hypertension in patients with CKD is associated with overactivity of the sympathetic nervous system [40]. Increased efferent sympathetic nerve activity is due to activation of afferent stimuli that arise from the diseased kidneys. There is evidence that blood pressure control reduces the sympathetic activity thus improving the cardiovascular outcomes, therefore suggesting that it might be beneficial to CKD patients [41].

However further elaborate studies with a larger sample size in different levels of CKD in pre-dialysis patients along with post treatment follow-up are needed to ascertain the actual role of these parameters in understanding the relationship between the anemia and progression of CKD.

5. Conclusion

Our study shows that anemia is under-recognized common problem and increasing attention has to be paid to understand and treat pre-dialysis patients. We observed that a substantial number of patients with CKD do not have sufficient iron stores to support erythropoiesis as judged by NKF-K/DOQI targets. A proactive multidisciplinary approach to early identification of anemia in these patients, timely referral and appropriate intervention can have a positive impact on the patient outcome. Early intervention of the potential risk factors like hypertension and diabetes can also be considered to improve the quality of life.

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