

## Evaluation of iron profile status in different stages of chronic kidney diseases (CKD)

### Abstract

**Background:** Iron deficiency is common in Nepal due to low socioeconomic reason in pre-dialysis chronic kidney disease (CKD) patients than in the hemodialysis population. In Nepal, little is known about iron indices in patients with CKD. **Aims:** Aim: The aim of this study was to evaluate the iron status among anemic pre-dialysis patients with different stages of CKD. **Patients and Methods:** Using a cross-sectional study design, we evaluated serum iron, Ferritin, transferrin saturation (TSAT) and haemoglobin concentration among 70 pre-dialysis CKD patients with anemia attending our outpatient nephrology clinic. CKD was defined as a glomerular filtration rate less than 60 ml/min/1.73 m<sup>2</sup> for 3 months or more, while anemia was defined as a hemoglobin concentration (Hb) less than 11 g/dl. **Results:** The mean age of the study participants was 52.5 ± 13.2 years and 40 (57.1%) of the patients were males. The most common causes of CKD were diabetic nephropathy (45%) and hypertension (34%). The mean serum iron and mean TSAT were 95 ± 34.9 µg/dl and 28.9% ± 13.8%, respectively. **Conclusions:** Anemia due to iron deficiency is a hallmark of different stages of CKD patients, mostly of moderate in severity with serum creatinine levels >3.0 mg/dl. Hemoglobin and other iron profile aggravates with progressive loss of kidney functions.

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~~Anemia is very common and earliest manifestations in different stages of CKD patients and majority of them were in stage 4 and 5.~~

**Key words:** Chronic kidney disease (CKD), Iron profile, Anemia

### Introduction

Anemia is an important and prevalent complication of chronic kidney disease (CKD) and causes increased morbidity and mortality [1]. In anemia, decrease of hemoglobin (Hb) levels is also established and in up to 68% of CKD patients with having a hemoglobin concentration (Hb) less than 11 g/dl at the time of starting dialysis [2,3]. Recently, it has been appreciated that anemia begins to develop early in the course of CKD, and the prevalence of anemia in stage 3–5 CKD was 12.0% [4]. The causes of anemia in CKD depend on number of factors such as with the decreased production of erythropoietin by the failing kidneys as one of the important causes and is the main determinant of anemia in stage 4–5 of CKD, apart from that chronic blood loss, reduced red blood cells (RBC) survival, hyperparathyroidism, bone marrow depression, folate, iron and vitamin B12 deficiencies also contribute to the development of anemia in CKD [5]. Anemia due to iron deficiency is very common in patients with chronic kidney disease. One of the important reasons for iron deficiency may be due to poor dietary intake or sometimes occult bleeding, or an imbalance between the actual iron supply and iron requirements of the

erythroid marrow. Other contributing factors for anemia in CKD include the presence of uremic inhibitors such as inflammatory cytokines, parathyroid hormone [6].

Iron an important constituent of hemoglobin (Hgb) when deficiency of iron occurs it leads to impaired Hgb production [7,8]. Homeostasis of normal iron is maintained by compensating daily loss by absorption of dietary iron through duodenum [9]. Number of evidence suggest that diabetes and hypertension to be the major causes of kidney disease worldwide [10,11].

Therefore, the aim of this study was to determine iron profile levels among anemic pre-dialysis CKD patients attending the out-patient nephrology clinic at the Birat Medical College Teaching Hospital Tankisinuwari, Morang, Nepal.

### **Patients and Methods**

A total of 120 consecutively presenting pre-dialysis CKD patients attending renal outpatient were screened and 70 (seventy) adults above 17 years age, of either sex, diagnosed with CKD (pre-dialysis) were randomly selected as cases and 50 (fifty) healthy persons as controls. Patients providing informed consent and patients with documented chronic kidney disease were included in the study. The study was carried out between October 2021 and May 2022. All participating patients gave written informed consent. About 10 ml of blood was obtained from each patient for the determination of hemoglobin concentration, RBC indices, reticulocyte count as well as white blood cell count. Serum creatinine, serum ferritin, serum total iron, and unsaturated iron binding capacity were also determined by fully automated analyzer. CKD was defined as the presence of markers of kidney damage and/or eGFR of  $<60 \text{ mL/min/1.73 m}^2$  for at least three months [12].

### **Statistical Analysis**

Comparison between means was carried out using the student's t-test while comparison between percentages was carried out using chi square test. One-way Analysis of variance (ANOVA) and Kruskal-Wallis tests were used to compare participants' parameters across the stages of CKD. The level of statistical significance was set at a P value less than 0.05.

### **Definition of terms**

TSAT was calculated using the formula  $(\text{TSAT}) = \text{Serum Iron}/\text{TIBC} \times 100$  [13]

Where  $\text{TIBC} (\mu\text{g/dL}) = \text{iron level} + \text{UIBC}$  and serum iron.

CKD was defined as the presence of markers of kidney damage and/or eGFR of  $90 \text{ mL/min/1.73 m}^2$  and/or persistent proteinuria;

Stage 2, eGFR of  $60\text{--}89 \text{ mL/min/1.73 m}^2$  and/or persistent proteinuria;

Stage 3, eGFR of  $30\text{--}59 \text{ mL/min/1.73 m}^2$ ;

Stage 4, eGFR of  $15\text{--}29 \text{ mL/min/1.73 m}^2$ ; and

Stage 5, eGFR  $<15 \text{ mL/min/1.73 m}^2$  [14]

## Results

70 diagnosed cases of pre-dialyzed chronic kidney disease patients were included in the study and 50 healthy persons as controls. Majority (48.5%) of the participants belonged to 41 to 60 year age group and mostly belongs to male category (Figure 1).

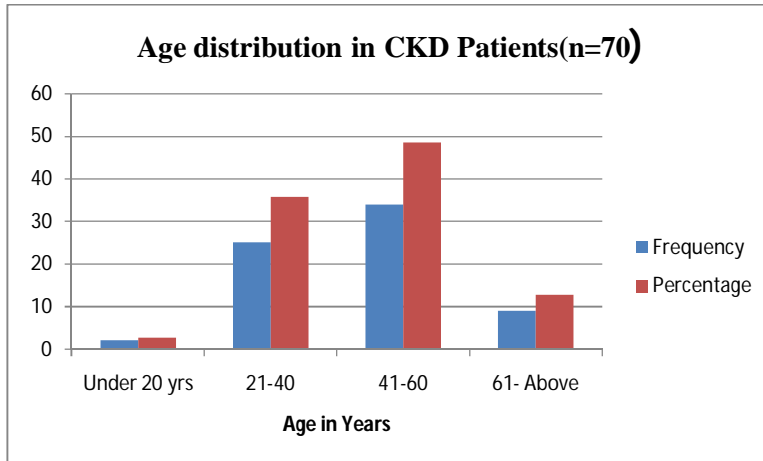


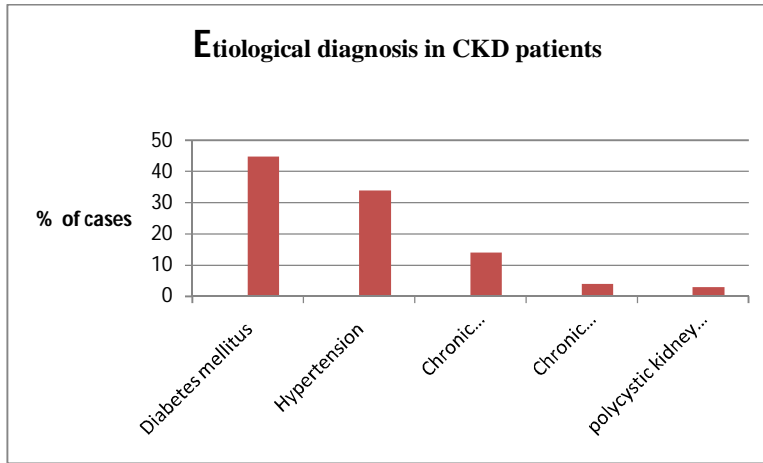
Figure 1 Age distribution in CKD patients (n=70)

The mean age of CKD patients and controls in males were  $54.8 \pm 12.9$  and  $56.4 \pm 13.8$  respectively, while there were  $50.8 \pm 11.6$  and  $52.9 \pm 12.7$  mean age of CKD and controls in female patients respectively. The difference was not statistically significant ( $P = 0.44$  in male and  $0.34$  in case of female). Out of 70 CKD patients ten (14.3%) were in Stage 1, 9 (12.8%) in Stage 2, 22 (31.4%) in Stage 3, 17 (24.2%) in Stage 4, and the remaining 12 (17.1%) were in Stage 5 (Table 1).

**Table 1 Characteristics of the study population**

Gender	CKD patients (Mean±SD)	Control (Mean±SD)	p-value
Male	$54.8 \pm 12.9$	$56.4 \pm 13.8$	0.44
Female	$50.8 \pm 11.6$	$52.9 \pm 12.7$	0.34
Stages of CKD	n (%)		
1	10(14.3)		
2	9(12.8)		
3	22(31.4)		
4	17(24.2)		
5	12(17.1)		

The etiology of CKD in the study were diabetes mellitus (45%), hypertension (34%), chronic glomerulonephritis (14%), chronic pyelonephritis (4%) and polycystic kidney disease (3%) (Figure 2).



**Figure 2 Etiological diagnosis in CKD patients (n=70)**

The mean serum creatinine was significantly higher in the CKD group compared to the control ( $6.9 \pm 2.8$  vs  $1.2 \pm 0.2$ ;  $P < 0.0001$ ) while e GFR was significantly lower in compared to control ( $37.82 \pm 27.96$  vs  $112.23 \pm 39.84$ ;  $P < 0.001$ ). The mean of serum iron ( $95 \pm 34.9$ ), TIBC ( $331 \pm 68.1$  vs  $310 \pm 43.1$ ;  $P < 0.005$ ) and transferrin saturation (TSAT) ( $28.9 \pm 13.8$ ) in CKD patients. Serum iron was significantly lower in CKD group as compared to the control group, whereas TIBC were significantly higher in CKD patients than in the control group **Table 2**. The ferritin level was significantly higher in the study group ( $220.12$  ng/mL) than in the control group ( $150.6$  ng/mL).

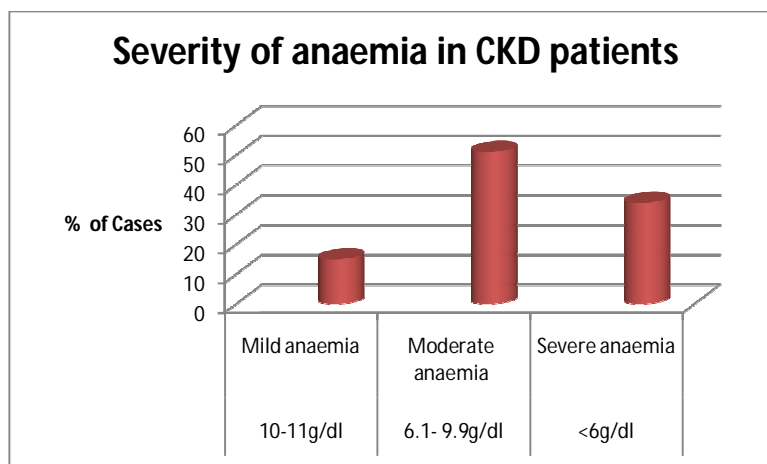
**Table 2: Biochemical parameters among control and CKD patients**

Sr.No	Parameter	Cases n=(70)	Control n=(50)	P- value
1.	Serum iron ( $\mu\text{g}/\text{dl}$ ) Mean $\pm$ SD	$95 \pm 34.9$	$109.1 \pm 25.9$	<b>0.017</b>
2.	TIBC ( $\mu\text{g}/\text{dl}$ ) Mean $\pm$ SD	$331 \pm 68.1$	$310 \pm 43.1$	<b>0.05*</b>
3.	Transferrin saturation (%) Mean $\pm$ SD	$28.9 \pm 13.8$	$34.2 \pm 5.2$	<b>0.01</b>
4.	Serum Ferritin (ng/ml)	$220.12 \pm 112.78$	$150.6 \pm 64.57$	<b>&lt;0.0001*</b>
5.	Serum Creatinine (mg/dl) Mean $\pm$ SD	$6.9 \pm 2.8$	$1.2 \pm 0.2$	<b>&lt;0.0001*</b>
6.	e GFR ( $\text{mL}/\text{min}/1.73 \text{ m}^2$ )	$37.82 \pm 27.96$	$112.23 \pm 39.84$	<b>&lt;0.0001*</b>

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\*p value is significant.

All the cases were anemic with hemoglobin concentration (Hb) below 11g/dl and the mean hemoglobin concentration was (8.68±24.9) g/dl compared to control (12.89±8.64). The mean Hb level in males was 8.1±2.4 g/dl and in females was 6.9±1.6 g/dl. The proportions with mild, moderate and severe anemia among CKD subjects were 15%, 51% and 34% respectively (Figure 3).



**Figure 3: Severity of anemia in CKD patients (n=70)**

### Discussion:

Iron deficiency is very common in CKD patients. Anemias caused by iron deficiency are widespread in most parts of the world, mostly prevalent in the central and West Africa and South Asia including Nepal [15]. Total serum iron (95±34.9) and the serum ferritin (220.12±112.78) in the present study were in accordance with the study by Deori et al 2016 [16], and by Singh et al 1999 [17] respectively (Table 3). Ferritin is one of the acute phase reactants and is frequently increased in patients with different stages of CKD, irrespective of their iron stores [18, 19]. Increased ferritin levels in CKD are due to systemic inflammation because ferritin synthesis is in response to inflammatory cytokines [20].

**Table 3: Comparison of serum iron profile in CKD**

Sr.No	Parameter	Present study	Talwar et al 2002[24]	Singh et al 1999[25]	Jairam et al 2010[26]	Deori Et al 2016[16]
1.	Serum iron	95	55.1	124.93±	153.4± 31.6	98± 37.08

	(µg/dl)			123.6		
2.	TIBC (µg/dl)	331	-	-	476.39± 137.3	345.22± 75.43
3.	Serum ferritin (ng/dl)	220	30.4	259.33± 122.05	331.7± 39.56	-
4.	Transferrin saturation (%)	28.9	-	-	-	30.78± 14.45

The most important etiological factor was diabetes (45%) and hypertension (34%) which was almost similar to the study of William Mcclellan et al [21](Table 4).

**Table 4: Comparison of serum iron profile in CKD**

Sr.No	Parameter	Present study	Ashfar R et al [22]	William Mcclellan et al [21]	Seuga K et al[23]
1.	Etiological factor	Diabetes mellitus (45%), hypertension (34%), chronic glomerulonephritis (14%), and polycystic kidney disease (3%)	Diabetes 49.1%, hypertension 28.3%, glomerular disease 17.1% and polycystic kidney disease 5.6%	Diabetes 49.5%, hypertension 33.0%	-
2.	Severity of Anemia	Mild, moderate and severe anemia were 15%, 51% and 34% respectively	55% moderate and 45% mild	-	50% moderate and 50% mild
3.	Hb (g/dl)	8.68±24.9	11.11±2.26		

In our study we observed a significant decrease in hemoglobin as the CKD stage progresses, and majority of the CKD patients were anemic having hemoglobin level less than 11g/dl, the moderate degree of anemia is in 51% of CKD patients like Ashfar R et al [22,23]this statistically significant and correlated well with the different stages of CKD.

The mean hemoglobin levels among CKD cases was  $8.68 \pm 2.49$  while mean hemoglobin in stage 2 was 12.9 g/dl, in stage 3 was 10.2 g/dl while in stage 4 was 8.3 g/dl. Talwar et al, [24] and Singh et al, [25] observed lower hemoglobin similar to present study i.e. 7.1 g/dl and 6.93 g/dl among CKD cases. A high prevalence and strong association of anemia has been reported in all stages of CKD by Poudel et al [26].

The overall iron deficiency increased through stage 3 to stage 5 of CKD, and most of the patients in this of CKD receive erythropoietin and parenteral iron to prevent anemia and morbidities associated with anemia which may cause an iron overload in patients with functional iron deficiency. Apart from that inflammation which affects iron status is more prevalent in CKD patients, we could find out its presence in our study participants.

There was some limitation of our study is that sample size was small and inability to investigate the full spectrum of etiologies of anemia in observed population due to high test cost of investigations and the absence of funding for the study.

### Conclusion

Anemia due to iron deficiency is a hallmark of different stages of CKD patients, mostly of moderate in severity with serum creatinine levels  $>3.0$  mg/dl. Hemoglobin and other iron profile aggravates with progressive loss of kidney functions i.e. decrease in eGFR levels and increase in serum creatinine levels

### References

1. Moranne O, Froissart M, Rossert J, Gauci C, Boffa JJ, Haymann JP, et al. Timing of onset of CKD-related metabolic complications. *J Am Soc Nephrol*. 2009;20:164–71
2. Kovesdy CP, Trivedi BK, Kalantar-Zadeh K, Anderson JE. Association of anemia with outcomes in men with moderate and severe chronic kidney disease. *Kidney Int* 2006;69:560-4.
3. Valderrabano F, Horl WH, Macdougall IC, Rossert J, Rutkowski B, Wauters JP. PRE-dialysis survey on anaemia management. *Nephrol Dial Transplant* 2003;18:89-100.
4. Regidor D, McClellan WM, Kewalramani R, Sharma A, Bradbury BD (2011) Changes in erythropoiesis-stimulating agent (ESA) dosing and haemoglobin levels in US non-dialysis chronic kidney disease patients between 2005 and 2009. *Nephrol Dial Transplant* 26: 1583–1591.
5. Babitt JL, Lin HY. Mechanisms of anemia in CKD. *J Am Soc Nephrol* 2012;23:1631-4.
6. Mehdi U, Toto RD. Anaemia, diabetes, and chronic kidney disease. *Diabetes Care*. 2009; 32(7):1320-6.
7. Hörl WH, Jacobs C, Macdougall IC, et al. European best practice guidelines 14-16: Inadequate response to epoetin. *Nephrol Dial Transplant* 2000;15Suppl 4:43-50.
8. Ganz T Systemic iron homeostasis. *Physiol Rev*. 2013;93(4): 1721–1741. [PubMed: 24137020]
9. Cui Y, Wu Q, Zhou Y. Iron-refractory iron deficiency anemia: New molecular mechanisms. *Kidney Int* 2009;76:1137-41

10. Charmaine EL, Matthew JO, Deanna MR, Janet EH. The growing volume of diabetes-related dialysis: a population based study. *Nephrology Dialysis Transplantation*. 2004;19:3098-103.
11. Haroun MK, Jaar BG, Hoffman SC, Comstock GW, Klag MJ, Coresh J. Risk factors for chronic kidney disease: a prospective study of 23, 534 men and women in Washington County, Maryland. *J Am Soc Nephrol*. 2003;14:2934-41
12. Abefe SA, Abiola AF, Olubunmi AA, Adewale A. Utility of predicted creatinine clearance using MDRD formula compared with other predictive formulas in nigerian patients. *Saudi J Kidney Dis Transpl* 2009;20:86-90.
13. Mohammed A. Evaluation of Iron Status of Chronic Kidney Disease in Ahmadu Bello University Teaching Hospital, Zaria. A Dissertation Submitted to Postgraduate School of Ahmadu Bello University; May 2015. <http://kubanni.abu.edu.ng/jspui/bitstream/123456789/8082/1/> [Last assessed on 2017 Mar 25].
14. Kidney Disease Improving Global Outcome (KDIGO). 2012 clinical practice guideline of evaluation and management of CKD. *Kidney Int Suppl* 2013;3:1-150
15. Camaschella C. Iron-deficiency anaemia. *New England J Med*. 2015; 372:1832-43.
16. Deori R, Bedanta B. Iron status in chronic kidney disease patients. *International journal of research in medicine sciences*. 2016; Aug; 8(4):3229-3234.
17. Singh NP, Aggarwal L, Singh T, Anuradha S and kohli R. Anemia, Iron studies and erythropoietin in patients of chronic renal failure. *JAPI*. 1999; 47(3):284-290
18. Kim T, Rhee CM, Streja E, Obi Y, Brunelli SM, Kovesdy CP, et al.: Longitudinal trends in serum ferritin levels and associated factors in a national incident hemodialysis cohort. *Nephrol Dial Transplant* 32: 370–377, 2017
19. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH: Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. *Nephrol Dial Transplant* 19: 141–149, 2004
20. Kwak EL, Larochelle DA, Beaumont C, Torti SV, Torti FM: Role for NF-kappa B in the regulation of ferritin H by tumor necrosis factor-alpha. *J Biol Chem* 270: 15285–15293, 1995
21. McClellan W, Aronoff SL, Bolton WK, Hood S, Lorber DL, Tang KL, et al. The prevalence of anemia in patients with chronic kidney disease. *Curr Med Res Opin*. 2004; 20(9):1501-10
22. Afshar R, Sanavi S, Salami J, Ahmadzadeh M. Hematological profile of chronic kidney disease (CKD) patients in Iran, in pre-dialysis stages and after initiation of hemodialysis. *Saudi J Kidney Dis Transplantation*. 2010;21:368-71.
23. Suega K, Bakta M, Dharmayudha TG, Lukman JS, Suwitra K. Profile of anemia in chronic renal failure patients. *Acta Med Indones*. 2005;37(4):190-4.

24. Talwar, V.K. and Gupta, H.L., 2002. Clinicohaematological profile in chronic renal failure. *The Journal of the Association of Physicians of India*, 50, pp.228-233.
25. Singh, N.P., Aggarwal, L., Singh, T., Anuradha, S. and Kohli, R., 1999. Anaemia, iron studies and erythropoietin in patients of chronic renal failure. *The Journal of the Association of Physicians of India*, 47(3), pp.284-290.
26. Jairam A R. Das,<sup>1</sup> P. K. Aggarwal, H. S. Kohli, K. L. Gupta, V. Sakhuja, and V. Jha. Iron status, inflammation and hepcidin in ESRD patients: the confounding role of intravenous iron therapy. *Indian journal of nephrology*. 2010; 20(3):125-131.
27. Poudel B ,Yadav B Jha B , Raut K B , Pandeya D R.Prevalence and association of anemia with CKD: A hospital based crosssectional study from Nepal.*Biomed Res- India* 2013 Volume 24 Issue 1;99-103

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