The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Iron indices in patients with functional anemia in Chronic Kidney Disease

G. Chinnapu Reddy[1], Ramakrishna Devaki[1], Pragna Rao[2]
[1]Department of Biochemistry, Kamineni Institute of Medical Sciences, Sreepuram, Narketpally, Nalgonda-508 254
[2]Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka-576104

Corresponding Author:

Ramakrishna Devaki
Associate Professor
Department of Biochemistry
Kamineni Institute of Medical Sciences
Sreepuram, Narkatpally
Nalgonda – 508 254
Andhra Pradhesh, INDIA
Tel: 091-9989561671 - Fax: 091-4024022277
e-mail: ramakrishna.devaki@gmail.com

Key words: Iron overload, Functional Iron Deficiency, Anemia, Transferrin, Ferritin, Hemodialysis.

Abstract

Background: Despite high ferritin level, HDCKD patients may have functional iron deficiency even after intravenous iron (iv) therapy. The aim of this study was to test the hypothesis that lowered serum transferrin level may contribute to functional anemia and erythropoietin hypo responsiveness by the failure to transport accumulated tissue iron to the relevant target tissue.
Materials and methods: The study subjects were divided into four groups. Group-A: HDCKD Patients receiving iv iron (n=290). Group-B: Patients not initiated on to hemodialysis (NDCKD), and received oral iron (n=38). Group-C: HDCKD patients with erythropoietin hypo responsiveness (n=9). Group-D: Healthy controls (n=36). The group-A, patients were sub-divided into five groups (A-1 to A-5) based on their serum ferritin levels.
Results: Serum ferritin and tissue iron levels in group-A and C patients were significantly greater than the group-D($p<0.0001$) and group-B patients($p<0.001$). Transferrin level of group-A and C showed lowered values and consequently a higher %TSAT when compared to group-D. The percent of patients with iron overload was 2.6%, 31%, and 44% in group-B, group-A and group-C respectively. Serum transferrin level significantly correlated with TIBC in group-A and B patients ($p<0.0001$;$p<0.05$ respectively). Transferrin level significantly correlated with TIBC in all subgroups of HDCKD($p<0.05$) with the exception in subgroup-A2 and with hemoglobin in subgroups A3 ($p<0.05$) and A5($p<0.01$) respectively.
Conclusions: The lowered transferrin level prevents the proper transport of the iron to the hematopoietic sites, which may be a reason for the low hemoglobin synthesis and also for the development of erythropoietin hypo responsiveness in some of the dialysis patients.

Introduction

The treatment of anemia in Chronic Kidney Disease patients on hemodialysis (HDCKD) as per the NKF-DOQI guidelines is by giving intravenous(iv) iron , where as in non hemodialysis CKD patients(NDCKD), oral iron is generally administered (1). The iv iron administration in HDCKD patients may lead to iron overload with deposition of iron in tissues even in the post erythropoietin (EPO) era has been accompanied by growing concern about iron overload (2). Hence, it becomes important to regularly monitor iron indices in HDCKD patients receiving iron therapy to ensure that iron overload with its toxic manifestations do not occur.

The main markers of iron status are serum ferritin, TSAT and TIBC. By determining both the serum ferritin concentration and the transferrin saturation, are used to direct anemia therapy in chronic kidney disease (CKD) and are associated with clinical outcomes in patients on dialysis (3). Serum Ferritin adequately reflects iron stores in bone marrow of HD patients and also functions as an acute phase reactant (4). Despite the ferritin values ranging from approximately 80 - 480ng/mL, the iron stores were absent on bone marrow aspiration in ESRD patients starting dialysis and may have functional iron deficiency (FID) (5). Indian patients with CKD have evidence of iron overload similar to those in developed countries (6). Despite the accumulation of tissue iron and elevated serum ferritin which occurs in HDCKD patients on iv iron therapy, the hemoglobin levels continue to be low, which point to the prevalence of FID anemia seen in CKD patients. We have examined the accumulation of tissue iron and elevated serum ferritin in HDCKD and NDCKD patients receiving iv and oral iron therapy respectively. The serum iron levels were correlated with tissue iron and ferritin levels to gauge the occurrences of functional and absolute iron deficiency. The aim of this study was to test the hypothesis that lowered serum transferrin levels may also significantly contribute to functional anemia and erythropoietin hypo response by the failure to transport accumulated tissue iron to the relevant target tissue. The cause of such a reduced synthesis of iron transporting protein may be traced to the concomitant presence of the malnutrition and inflammation syndrome seen in hemodialysis patients. Recognizing and treating functional iron deficiency and transferrin deficiency is therefore an important concept in the management of anemia in both HDCKD and NDCKD patients.

Materials and methods

Patients: The study groups comprised of CKD patients attending the Nephrology department of Kamineni hospital during the period June 2008 to September 2010. The ethical committee of Kamineni Hospital approved the study. In this study the hemodialysis (HD), non dialysis (ND) cohort of CKD patients taken into study and compared themselves and also compared with healthy controls. The inclusion criteria are the stable CKD patients on MHD at least 3 months period and the CKD stage iv and v NDCKD patients who were on oral iron therapy for a period of minimum 3 months duration. The exclusion criteria are patients with known malignancies, bleeding disorders, infection or inflammation of other cause and transplant cases. Some of the HDCKD patients remained persistently anemic with no increase in Hb% with EPO dosage of more than 500 IU/Kg/wk and were labeled as hypo responsive to erythropoietin. Indices of iron metabolism were studied in this EPO hypo responsive subgroup to detect biochemical evidence of iron overload. Antibodies for EPO were also estimated in this group. The study subjects were divided into four groups.

Group-A: HDCKD patients receiving iv iron therapy (n=290).

The group-A, hemodialysis patients were further sub-divided into five groups (A-1 to A-5) based on their serum ferritin levels.

Sub groups	N	Serum Ferritin levels
Sub group A-1	(n =94)	20 - 300 ng/mL
Sub group A-2	(n =65)	300 - 500 ng/mL
Sub group A-3	(n =58)	500 - 1000 ng/mL
Sub group A-4	(n =44)	1000 - 2000 ng/mL
Sub group A-5	(n =29)	> 2000 ng/mL

Group-B: NDCKD stage-4 & 5 patients who were not initiated on to hemodialysis and on oral iron supplementation (n=38).
Group-C: Suspected EPO resistant HDCKD patients tested for EPO antibodies (n=9). **Group-D:** Healthy controls (n=36).

Methods

A prospective study from June 2008 to September 2010 was carried out with the patients attending the Nephrology Department at Kamineni Hospital. All HDCKD patients received iv iron supplementation and erythropoietin as per the Nephrology unit's protocol. The study parameters included serum iron, unsaturated iron binding capacity (UIBC), serum ferritin, transferrin, percent saturation of transferrin, blood urea and serum creatinine. The blood samples collected after 2 weeks of iv iron supplementation in HDCKD patients and at random for NDCKD and controls subjects. All the tests were assessed on the same day by automated chemistry analyzer, Cobas Integra 400+ (Roche). TIBC was calculated as being equal to serum iron + UIBC and tissue iron levels were calculated by using the mathematical
equation (7), (8). EPO antibodies were analyzed by ELISA. Hemoglobin was estimated by using BC-3000-plus Mindray auto hematology analyzer.

Statistical Methods

All relevant statistics were performed using the Statistical package for social sciences (SPSS Ver-11.0, SPSS Inc, Chicago) software. The mean and standard error (SE) of all parameters were expressed. Analysis of variance (ANOVA) was used for comparison of mean between the groups. The relationship between the parameters obtained by Pearson's correlation matrix (r) and a value of P< 0.05 was considered (at 95% CI) to be statistically significant(9).

Results

The mean age of subjects was 50±12 years. Among these, 51% had type-2 diabetes and 61% had hypertension as a co- morbid disease. The demographics of the study patients were shown in Table-1. Serum ferritin levels in group-A (HDCKD patients receiving iv iron therapy) and group-C (HDCKD tested for EPO antibodies) patients were significantly greater than in group-D (healthy controls) and group-B (NDCKD) patients (p<0.0001, p<0.001 respectively). Similarly, tissue iron was also significantly elevated in group-A and C patients when compared to group-D and B patients (p<0.0001 for both). Transferrin levels and its saturation (% TSAT) in group-B NDCKD patients who received oral iron were not significantly different from group-D healthy subjects. However, there was a significant difference in the mean levels of transferrin between the group-A, group C HDCKD patients and the group-B NDCKD patients (p<0.0001). Transferrin levels in HDCKD patients of both groups-A and C showed lowered values and consequently a higher %TSAT when compared to group-D controls. Hence transferrin levels were lower in patients on hemodialysis who also had a higher incidence of iron overload. Hemoglobin levels were low in all groups of CKD patients when compared to the healthy controls (p<0.0001). As per NKF-K/DOQI guidelines, which define iron overload as serum ferritin >800ng/mL, 2.6% of NDCKD (Group-B) patients and 31% of HDCKD (group-A) patients had iron overload in this study. Among the EPO resistant cases (Group-C), 44% had iron overload. Absolute iron deficiency was identified by two parameters: i)

Table 1
Demographics and biochemical characteristics (Mean±SE) of the study groups

Parameter	CKD Patients (n=337)			Healthy Controls (N=36)
	HDCKD (N-290) Stage: v	HDCKD- suspected EPO hypo response (N=9); Stage: v	NDCKD (N=38) Stage: iv & v	
Age	51±15	50±17	52±12	48±14
Male	164	5	22	19
Female	124	4	16	17
Anemia (%)	95	93	94	4
Diabetes (%)	47	51	56	NA
Hypertension (%)	61	58	58	NA
No of subjects with rHuEPO supplementation	276	9	Nil	Nil
Weekly dose (mean IU) of rHuEPO	8000 U	8000 U	NA	NA
*Weekly dose (mean mg) of iron	250 mg	250 mg	50-100 mg	NA
Time of HD (months)	25±9	23±4	NA	NA
Iron(µg/dL)	84±5.7	139±70	66±7	68±6
TIBC(µg/dL)	221±7	220±55	234±12	240±10
% TSAT	39±2	50±9	30±3	35±3
Ferritin(ng/mL)	684±37	669±126	207±28	130±14
Tissue iron(mg)	250±11	301±37	60±27	4±22
Transferrin(mg/dL)	184±5	31±5	220±8	255±6
Hemoglobin(g/dL)	8.65±0.2	7.63± 0.4	8.75±.02	14.8±0.2
Urea(mg/dL)	117±3	44±17	114±9	30±4
Creatinine (mg/dL)	8.4±2.7	9.1±3.1	5.3±1.6	0.9±0.2

serum iron <35µg/dL ii) ferritin <100ng/mL. Functional iron deficiency was measured either as iron <35µg/dL or serum ferritin <100ng/mL (10). Among the group-A HDCKD patients, 18% had serum iron less than 35µg/dL and 11% of them had ferritin <100ng/mL. However, only 1.5% of these patients had absolute iron deficiency.

As per the NKF/Kidney Early Evaluation Programmed guidelines, a hemoglobin level below 11g/dL is defined as anemia. Ninety four (94%) percent of all the CKD patients in this study were anemic having hemoglobin level less than 11g/dL. The prevalence of iron deficiency by serum iron levels less than 35µg/dL revealed only 19% of the total CKD patients had iron deficiency. Hence the cause of anemia could be factors other than iron deficiency alone.

Tissue iron deposits were significantly increased in all CKD patients when compared to the controls (p<0.0001). In group-C suspected EPO resistant HDCKD patients, serum was examined for the presence of antibodies to recombinant erythropoietin. However, none of the studied patients had any antibodies to erythropoietin. Overall, the picture that emerges in this population is one of sufficient serum and tissue iron and paradoxically low hemoglobin levels combined with very high serum ferritin levels.

Correlation analysis in study groups

Serum transferrin levels significantly positively correlated with TIBC in group-A HDCKD and group-B NDCKD patients (r=0.409, p<0.0001; r=0.394, p<0.05 respectively) (Table-2). There was no significant association between these two parameters in group-

Table 2
Correlation analysis between parameters of iron status in the HDCKD (on iv iron), NDCKD (on oral iron), EPO-hypo responsive HDCKD patients

Parameter		HDCKD (Group A)		NDCKD (Group B)		EPO-Hypo responsive HDCKD (Group C)	
		r	P	r	P	r	P
Ferritin	% TSAT	0.282	<0.0001	0.366	<0.05	0.314	NS
	Tissue iron	0.879	<0.0001	0.896	<0.0001	0.982	<0.0001
	Urea	0.039	NS	0.462	<0.004	0.388	NS
	Creatinine	-0.01	NS	0.309	NS	-0.101	NS
Tissue iron	Ferritin	0.879	<0.0001	0.896	<0.0001	0.982	<0.0001
	% TSAT	0.314	<0.0001	0.379	<0.05	0.318	NS
	Urea	0.145	<0.05	0.444	<0.01	0.450	NS
	Creatinine	0.098	NS	0.311	NS	-0.010	NS
S.Iron	% TSAT	0.601	<0.0001	0.815	<0.0001	0.972	<0.0001
	Ferritin	0.039	NS	0.462	<0.01	0.388	NS
Urea	% TSAT	0.097	NS	0.429	<0.01	0.166	NS
	Tissue iron	0.145	<0.05	0.444	<0.01	0.450	NS
	Creatinine	0.690	<0.0001	0.812	<0.0001	0.691	<0.058
Hb	Ferritin	-0.14	<0.05	-0.141	NS	-0.004	NS
	%TSAT	-0.08	NS	0.013	NS	0.237	NS
	Tissue iron	-0.17	<0.05	-0.172	NS	-0.181	NS
	Transferrin	0.158	NS	0.276	NS	-0.297	NS
	Creatinine	-0.06	NS	-0.179	NS	-0.697	<0.055
TIBC	Ferritin	-0.22	<0.0001	-0.345	<0.05	0.271	NS
	Tissue iron	-0.19	<0.001	-0.328	<0.05	0.283	NS
	Transferrin	0.409	<0.0001	0.394	<0.05	0.280	NS
	%TSAT	-0.05	NS	-0.273	NS	0.780	<0.05
	Urea	0.023	NS	-0.242	NS	0.099	NS

P<0.05 considered as significant,; NS= Not significant,; Entered p values are against the controls. %TSAT= Percentage Transferrin Saturation,; TIBC= Total Iron Binding Capacilty,; Hb= Hemoglobin.

C EPO resistant and group-D control subjects. It was observed that hemoglobin and transferrin did not correlate each other in all study groups. Serum ferritin significantly positively correlated with tissue iron in controls, HDCKD and NDCKD patients.
In group-A HDCKD patients, hemoglobin did not correlate with serum ferritin, % TSAT, tissue iron. In group-B NDCKD patients who were on oral iron supplementation; hemoglobin correlated with serum ferritin, tissue iron levels. Therefore, while in NDCKD patients, serum ferritin is an index of iron availability, in HDCKD patients; ferritin may not an appropriate marker of iron status. TIBC correlated negatively with ferritin and tissue iron in HDCKD and NDCKD (group A&B) patients and controls ($p<0.001$ for all groups). TIBC also correlated with % TSAT in NDCKD and controls subjects but not in HDCKD patients (Table-2).
The iv iron receiving group-A HDCKD patients were distributed into 5 different sub-groups based on their serum ferritin levels. When serum ferritin was less than 500ng/mL (group A-1 and A-2), ferritin levels correlated significantly with %TSAT ($r = 0.288$, 0.453 and $p <0.05$, $p<0.0001$) respectively. Tissue iron deposits correlated with % TSAT when ferritin was less than 500ng/mL (groupA-1and A-2), but as serum ferritin increased further, there was no correlation between ferritin and transferrin or % TSAT, indicating that factors other than iron were responsible for elevation of serum ferritin levels beyond 500ng/mL. The serum iron significantly positively correlated ($p<0.0001$) with the %TSAT in all the subgroups (A-1 to A-5), with hemoglobin in subgroup A-3 and with transferrin in subgroups A-3 ($r=0.441$,$p<0.05$) and A-5 ($r=0.620$,$p<0.01$) respectively (Table-3). Transferrin levels significantly correlated with TIBC in all subgroups [A1($r=0.321$, $p<0.05$); A3($r=0.362$,$p<0.05$); A4($r=0.537$,$p<0.05$) and A5($r=0.512$,$p<0.05$) respectively], with the exception in subgroup A2, who had ferritin levels 300-500ng/mL.
Total iron binding capacity significantly correlated with the serum iron in subgroups A-2, A-3, A-4 and A-5, except in subgroup A-1. Tissue iron in all sub-groups (A-1 to A-5) significantly correlated with serum ferritin ($p<0.0001$ for all groups). Blood urea in subgroup A-1 correlated well with the serum ferritin ($p<0.01$) and also with tissue iron ($p<0.05$) and transferrin saturation ($p<0.05$).

Table 3
Correlation between the indices of iron status in sub-groups of HDCKD patients.

Parameter	Subgroup	A-1		A-2		A-3		A-4		A-5	
		r	p	r	p	r	p	R	P	r	p
Ferritin	%TSAT	0.288	<0.01	0.453	<0.0001	0.055	NS	0.017	NS	NS	NS
	Tissue iron	0.942	<0.0001	0.996	<0.0001	0.794	<0.0001	0.997	<0.0001	NS	NS
	Urea	0.268	<0.01	0.039	NS	0.011	NS	-0.03	NS	NS	NS
	Creatinine	0.242	<0.05	0.117	NS	0.012	NS	0.023	NS	NS	NS
Tissue iron	Ferritin	0.942	<0.0001	0.996	<0.0001	0.794	<0.0001	0.997	<0.0001	NS	NS
	% TSAT	0.263	<0.01	0.449	<0.0001	-0.12	NS	0.005	NS	NS	NS
	Urea	0.254	<0.05	0.055	NS	0.087	NS	-0.02	NS	NS	NS
S.Iron	% TSAT	0.89	<0.0001	0.694	<0.0001	0.696	<0.0001	0.59	<0.0001	0.82	<0.0001
	S. Iron	0.007	NS	0.935	<0.0001	0.276	<0.05	0.775	<0.0001	0.521	<0.01
TIBC	Transferrin	0.321	<0.05	0.180	NS	0.362	<0.05	0.537	<0.05	0.512	<0.05
	% TSAT	0.364	<0.0001	0.449	<0.0001	-0.33	<0.01	0.033	NS	-0.01	NS
	Urea	-0.16	NS	-0.10	NS	0.419	<0.001	0.197	NS	-0.40	NS
	Creatinine	-0.02	NS	-0.11	NS	0.454	<0.0001	0.150	NS	-0.31	NS
Hb	Iron	-0.08	NS	-0.08	NS	0.525	<0.001	-0.02	NS	0.120	NS
	Transferrin	-0.19	NS	0.134	NS	0.441	<0.05	0.345	NS	0.620	<0.01
	Creatinine	-0.23	NS	-0.30	<0.05	0.274	NS	0.389	<0.05	0.205	NS

P<0.05 considered as significant,; NS= Not significant,; Entered p values are against the controls. %TSAT= Percentage Transferrin Saturation,; TIBC= Total Iron Binding Capacilty,; Hb= Hemoglobin.

Discussion

Chronic Kidney Disease (CKD) is one of the worldwide public health issues and anemia is the most common complication of advanced CKD (11). The prevalence of CKD in India in different communities is about 0.16% and other renal disorders is about 0.7% (12). The recent population based study assessed the incidence at 150-200 cases per million population per year in India (13). The anemia in CKD is mainly caused by insufficient production of erythropoietin by diseased kidneys. Iron deficiency, chronic inflammation, hyperparathyroidism, and blood loss may also contribute to anemia in these patients. Recombinant human erythropoietin (rHuEPO) has been used in the treatment of the anemia of CKD since 1986 (14). The imbalance in the iron availability leads to iron deficiency in most of the CKD patients, especially in stage-5 NDCKD subjects (15). Iron is an essential component for hemoglobin formation, must be assessed and adequate iron stores should be available before erythropoietin therapy is initiated. Iron supplementation is essential for an adequate response to erythropoietin in patients with CKD because the demands for iron by the erythroid marrow frequently exceed the amount of iron that is immediately available for erythropoiesis (as measured by percent transferrin saturation) as well as iron stores (as measured by serum ferritin).

The general picture of anemia in CKD patients on hemodialysis that emerges from this study is one of functional anemia characterized by low hemoglobin levels in the presence of elevated serum ferritin and tissue iron levels. In order to correct the anemia, iv iron is given along with EPO and hemopoietic vitamins. The optimal supplementation of iron in HD patients efficiently improves patients response to EPO therapy, replete patients ongoing iron losses and helps to maintain hemoglobin and hematocrit ranges within target levels, as per K/DOQI guidelines published in 2006 (16) . Of the total 299 HDCKD subjects, 131 (44%) had ferritin levels more than 500ng/mL. K/DOQI guidelines of the year 2006 say that iron not to be supplemented when ferritin levels elevated more than 500ng/mL (16). However, other factors need to be considered while stopping administration of iv iron. The target hemoglobin for HDCKD patients at present is between 11-12 g/dL (17). Majority of the CKD patients (94%) in this study were anemic having hemoglobin level less than 11g/dL. Hemoglobin levels in patients receiving iv iron still remained low (<9g/dL) while tissue iron deposits and serum ferritin levels increased in this study. Though there was significant rise in the average hemoglobin levels (~12g/dL) in the same population of western studies (18), the rise in hemoglobin levels in this study was negligible. This prompt the need of hour to look into various factors resulted in low hemoglobin levels.

The percentage of patients who had absolute iron deficiency was only 1.5% indicating the majority of dialysis patients exhibits FID with high serum ferritin levels. (19) et al showed that FID associated with elevated ferritin levels in dialysis patients, and there was significant improvement in FID status with iv iron supplementation. The estimation and regular monitoring of iron indices is an important requirement in the management of anemia of CKD patients. While serum iron, TIBC and ferritin are regularly estimated in patients, the estimation of tissue ferritin and transferrin are not widely done. The average range of serum iron levels in all the CKD groups is within the normal range (66-139 µg/dL). The two fold increase of tissue iron than controls, leading to iron overload state and normal serum iron levels observed with decreased transferrin synthesis due to associated inflammation and malnutrition in CKD cases may be the reason for lack of significant correlation between TIBC and % TSAT observed in HDCKD and NDCKD patient groups. Hemoglobin is an important indicator of iron status alone, and also how well the tissue iron is being mobilized to target cells and being utilized for heme synthesis. TSAT is an indicator of circulating iron and the positive association with serum iron, serum ferritin and tissue iron ($p<0.0001$) reflects that the availability of sufficient iron in the form of iron bound with transferrin. The presence of large quantities of tissue iron in all patients of dialysis indicates that iron therapy has loaded iron into the patients, which may result in other complexities associated with MIA syndrome. Our study shows that estimation of hemoglobin or ferritin alone does not give the correct picture of the iron status which supports many of previous studies. Ferritin levels below 500ng/mL may be considered as expressing the stored iron levels. However, only about 16% of (45 out of 299) the total HDCKD patients in this study had serum ferritin levels above 500ng/mL and TSAT <35% are probably an indication of an inflammatory response. Within the sub groups of HDCKD patients, as ferritin levels increased, the hemoglobin levels decreased, indicating that ferritin was not a reliable iron status maker in HDCKD patients as also supported by atomic absorption studies (20).

Soluble transferrin receptor (sTfR) is a product of the transferrin receptor and its concentration in plasma is proportional to the total concentration of cellular TfR. A number of factors may affect the concentration of sTfR in plasma or sera: acute or chronic inflammation and the anaemia of chronic disease and malnutrition. sTfR may be a good indicator of iron deficiency anemia in iron deficient thalassemic patients and in subjects without inflammation (21), (22). However, diagnostic accuracy of sTfR as an indicator of iron deficiency anemia is not well established and it may be an appropriate marker for erythropoiesis than iron deficiency (23). Transferrin saturation and ferritin currently remain better methods for the evaluation of iron status in rHuEPO-treated chronic hemodialysis patients(24;25).

Human hepcidin is a 25–amino acid peptide. The hepatocytes are the main sources of hepcidin, while the bacteria-activated neutrophils and macrophages are other sources. The structure of the bioactive hepcidin is a simple hairpin with 8 cysteines that forms 4 disulfide bonds in a ladder-like configuration. The regulation of iron export from cytosol to plasma is controlled by hepcidin and it has no effect on the iron dynamics of reticulocytes (26). Transferrin circulates iron in a soluble, non-toxic form and delivers it to developing reticuloytes in bone marrow and play a key role in iron metabolism. Individual cells modulate their uptake of transferrin-bound iron depending on their iron requirements (27). Transferrin regulates the iron homeostasis as

component of a plasma iron sensing system that modulates hepcidin expression. Transferrin synthesis by the liver in experimentally induced acute inflammation rats is normal (28) and in HDCKD patients on one year MHD, concentration of total serum transferrin remained constant (29), though there was 2.5 fold decrease in serum transferrin levels when compared with healthy controls.

In this study, transferrin levels decreased significantly ($p<0.0001$) in HDCKD patients on iv iron (184±5 mg/dL) and in group-C EPO hypo responsive patients (31±5 mg/dL) when compared to controls. There was also significant difference ($p<0.0001$) in transferrin levels between the HDCKD, EPO-resistant HDCKD and NDCKD patients. The significantly lowered transferrin levels prevent the proper transport of the iron to the hematopoietic sites, which may be a reason for the low hemoglobin synthesis and also for the development of erythropoietin hypo responsiveness in some of the dialysis patients (Fig 1). The absence of EPO antibodies in serum group-C HDCKD patients, against the supplemented rHuEPO, may reflect the other factors like decreased iron availability due to significantly reduced transferrin levels may involved in resulting EPO hypo responsiveness seen in these patients. Lowered transferrin results from the presence of the Malnutrition-Inflammation–Atherosclerosis syndrome which decreases protein synthesis in the liver. This action is mediated by IL-6, a pro–inflammatory cytokine (30) (31). Hence it is important to ensure adequate mobilization of tissue iron deposits to increase hemoglobin levels rather than repeatedly administering iron to CKD patients. Recognizing functional iron deficiency and transferrin deficiency is therefore an important concept in the evaluation of anemia in CKD patients.

Conclusion

The existence of functional iron deficiency anemia, characterized by low hemoglobin levels in the presence of elevated serum ferritin and tissue iron levels observed in HDCKD patients. The average %TSAT in HDCKD patients is 44%, which indicates the adequate iron availability. The excess iron increases the tissue iron deposits and elevates the pro-inflammatory cytokines, causing decreased transferrin levels. The lack of correlation between TIBC and %TSAT in HDCKD, NDCKD groups may need further study to establish the possible causes. Serum transferrin levels decreased in all CKD patients, leading to decreased tissue iron mobilization to erythroid cells, which in turn may cause decreased hemoglobin synthesis and elevated tissue iron levels.

ACKNOWLEDGMENT

We thank Kamineni Institute of Medical Sciences for constant support and encouragement.

Conflict of interest

The authors declare that they have no competing interests.

Reference List

Reference List

1. Seiler S. Anemia of chronic renal failure: new treatment alternative. CANNT J 2000; 10(3):35-38.
2. Robbins KC. Iron overload in the erythropoietin era. Nephrol Nurs J 2000; 27(2):227-231.
3. Kovesdy CP, Estrada W, Ahmadzadeh S, Kalantar-Zadeh K. Association of markers of iron stores with outcomes in patients with nondialysis-dependent chronic kidney disease. Clin J Am Soc Nephrol 2009; 4(2):435-441.
4. Birgegard G, Hallgren R, Killander A, Stromberg A, Venge P, Wide L. Serum ferritin during infection. A longitudinal study. Scand J Haematol 1978; 21(4):333-340.
5. Fudin R, Jaichenko J, Shostak A, Bennett M, Gotloib L. Correction of uremic iron deficiency anemia in hemodialyzed patients: a prospective study. Nephron 1998; 79(3):299-305.
6. John GT, Chandy M, Thomas PP, Shastry JC, Jacob CK. Iron stores in patients on hemodialysis after renal transplantation. Natl Med J India 1993; 6(3):108-110.
7. Van Wyck DB, Stivelman JC, Ruiz J, Kirlin LF, Katz MA, Ogden DA. Iron status in patients receiving erythropoietin for dialysis-associated anemia. Kidney Int 1989; 35(2):712-716.
8. Kalantar-Zadeh K, Regidor DL, McAllister CJ, Michael B, Warnock DG. Time-dependent associations between iron and mortality in hemodialysis patients. J Am Soc Nephrol 2005; 16(10):3070-3080.
9. Altman DG. Statistics in the medical literature: 3. Stat Med 1999; 18(4):487-490.
10. Loge JP, Lange RD, Moore CV. Characterization of the anemia associated with chronic renal insufficiency. Am J Med 1958; 24(1):4-18.
11. Patel TV, Singh AK. Anemia in chronic kidney disease: new advances. Heart Fail Clin 2010; 6(3):347-357.
12. Mani MK. The management of end-stage renal disease in India. Artif Organs 1998; 22(3):182-186.
13. Agarwal SK, Srivastava RK. Chronic kidney disease in India: challenges and solutions. Nephron Clin Pract 2009; 111(3):c197-c203.
14. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med 1987; 316(2):73-78.

15. Fishbane S. Iron supplementation in renal anemia. Semin Nephrol 2006; 26(4):319-324.
16. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. Am J Kidney Dis 2006; 47(5 Suppl 3):S11-145.
17. Volkova N, Arab L. Evidence-based systematic literature review of hemoglobin/hematocrit and all-cause mortality in dialysis patients. Am J Kidney Dis 2006; 47(1):24-36.
18. Kinney R. 2005 Annual Report: ESRD Clinical Performance Measures Project. Am J Kidney Dis 2006; 48(4 Suppl 2):S1-106.
19. Kopelman RC, Smith L, Peoples L, Biesecker R, Rizkala AR. Functional iron deficiency in hemodialysis patients with high ferritin. Hemodial Int 2007; 11(2):238-246.
20. Spada PL, Rossi C, Alimonti A, Bocca B, Cozza V, Ricerca BM, Bocci MG, Vulpio C, De SP. Ferritin iron content in hemodialysis patients: comparison with septic and hemochromatosis patients. Clin Biochem 2008; 41(12):997-1001.
21 Khatami S, Dehnabeh SR, Mostafavi E, Kamalzadeh N, Yaghmaei P, Saeedi P, Shariat F, Bagheriyan H, Zeinali S, Akbari MT. Evaluation and comparison of soluble transferrin receptor in thalassemia carriers and iron deficient patients. Hemoglobin 2013; 37(4):387-395.
22. Engle-Stone R, Nankap M, Ndjebayi AO, Erhardt JG, Brown KH. Plasma ferritin and soluble transferrin receptor concentrations and body iron stores identify similar risk factors for iron deficiency but result in different estimates of the national prevalence of iron deficiency and iron-deficiency anemia among women and children in Cameroon. J Nutr 2013; 143(3):369-377.
23. Infusino I, Braga F, Dolci A, Panteghini M. Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anemia. A meta-analysis. Am J Clin Pathol 2012; 138(5):642-649.
24. van der Weerd NC, Grooteman MP, Bots ML, van den Dorpel MA, den Hoedt CH, Mazairac AH, Nube MJ, Penne EL, Gaillard CA, Wetzels JF, Wiegerinck ET, Swinkels DW, Blankestijn PJ, Ter Wee PM. Hepcidin-25 in chronic hemodialysis patients is related to residual kidney function and not to treatment with erythropoiesis stimulating agents. PLoS One 2012; 7(7):e39783.
25. Chiang WC, Tsai TJ, Chen YM, Lin SL, Hsieh BS. Serum soluble transferrin receptor reflects erythropoiesis but not iron availability in erythropoietin-treated chronic hemodialysis patients. Clin Nephrol 2002; 58(5):363-369.
26. Eguchi A, Mochizuki T, Tsukada M, Kataoka K, Hamaguchi Y, Oguni S, Nitta K, Tsuchiya K. Serum hepcidin levels and reticulocyte hemoglobin concentrations as indicators of the iron status of peritoneal dialysis patients. Int J Nephrol 2012; 2012:239476.
27. Frazer DM, Anderson GJ. The regulation of iron transport. Biofactors 2013.
28. O'Shea MJ, Kershenobich D, Tavill AS. Effects of inflammation on iron and transferrin metabolism. Br J Haematol 1971; 21(3):357.
29. Formanowicz D, Formanowicz P. Transferrin changes in haemodialysed patients. Int Urol Nephrol 2012; 44(3):907-919.
30. Kalantar-Zadeh K, Regidor DL, McAllister CJ, Michael B, Warnock DG. Time-dependent associations between iron and mortality in hemodialysis patients. J Am Soc Nephrol 2005; 16(10):3070-3080.
31. Balla J, Jeney V, Varga Z, Komodi E, Nagy E, Balla G. Iron homeostasis in chronic inflammation. Acta Physiol Hung 2007; 94(1-2):95-106.