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Original Article

Serum Ferritin <70 µg/L Predicts Functional Iron Deficiency in Patients with Chronic Kidney Disease

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ABSTRACT. Anemia is a common complication of chronic kidney disease (CKD) which is treated by erythropoiesis-stimulating agents. However, most of the patients do not respond adequately due to the development of functional iron deficiency (FID). The study was conducted to explore the value of inflammatory markers, high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) along with serum ferritin (SF) in the diagnosis of FID. Seventy-seven clinically diagnosed patients of CKD (Stage 3, 4, and 5) of either sex, age >18 years with hemoglobin <11 g/dL were included in the study. Complete hemogram with peripheral smear, serum iron, total iron binding capacity, transferrin saturation, SF, transferrin receptors (sTfR), hsCRP, IL-6, and erythrocyte sedimentation rate were estimated and statistically analyzed. sTfR/log ferritin (taken as gold standard) detected 31/77 patients as having iron-deficient erythropoiesis. Nineteen patients were detected as having FID. SF at a cut-off <70 µg/L showed the best sensitivity (83.87%) and specificity (73.91%) in detecting FID in these patients and identified 14/19 cases of FID. The 5 FID cases who were missed had raised hsCRP. The presence of raised hsCRP reduced the sensitivity to 79.16%. SF <70 µg/L emerged as the most sensitive and specific in the identification of iron-deficient erythropoiesis. SF >12 µg/L - SF <70 µg/L was able to identify 14/19 cases of FID. Furthermore, hsCRP further stratified the subgroup of CKD patients in which FID could be detected with higher sensitivity and specificity.

Introduction

Chronic kidney disease (CKD) is a major public health problem with anemia as the most common complication occurring early in the course of disease. It affects >50% CKD patients who are not on erythropoietin (EPO) and in 49% taking EPO.[1] Its most common cause is EPO deficiency which can be effectively treated with erythropoiesis-stimulating agents (ESAs). However, most of the patients do not respond adequately due to development of functional iron deficiency (FID).[2] As CKD is a pro-inflammatory state, it predisposes to the development of FID. It is present in >23% of

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hemodialysis (HD) patients.[3]

It becomes important to establish the presence of FID as iron deficiency not only impairs immunological status and reduces the capacity to control infections but also results in decreased response to ESAs. On the other hand, hyperferriemia or iron overload due to over treatment stimulates bacterial growth by making iron available for growth of bacteria as well as increases oxidative stress.[4]

The conventional biochemical parameters of iron used to diagnose FID include serum transferrin saturation (TSAT) and serum ferritin (SF).[5] The current diagnostic criteria for FID as recommended by the Kidney Disease Outcomes Quality Initiative (KDOQI) 2006 is TSAT <20% and SF >100 µg/L.[6] However, this criteria tends to miss the diagnosis of FID due to the variable effect of inflammation on SF and TSAT and hence, are unreliable in detection of FID. However, TSAT is a derived value and is subject to variations in serum iron (SI) and total iron binding capacity (TIBC) levels. Furthermore, its levels vary with inflammation and show diurnal variation.[6] SF correlates well with bone marrow iron and is considered as a noninvasive substitute to reflect body iron stores. In uncomplicated clinical settings, SF <12 µg/L is diagnostic of depleted iron stores.[5] However, in inflammatory conditions like CKD the use of SF to predict iron deficiency is unreliable as it is an acute phase reactant (APR) with elevated levels present despite reduced or absent iron stores. Similar to SF, high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) are APR and levels are raised in inflammatory settings like CKD.

The ratio of sTfR/log ferritin (sTfR-F) index is a new parameter and is considered the best parameter for biochemical identification of FID[7] as it covers the full spectrum of body iron status with SF reflecting iron stores and sTfR reflecting iron-deficient erythropoiesis. It has been found to be superior to sTfR in predicting iron deficiency with higher sensitivity and specificity.[8] However, sTfR assays lack standardization, are expensive with no consensus on a predictive cut-off to identify iron deficiency.[9]

The study was conducted to explore the role of the inflammatory markers along with SF in improving the efficacy of currently recommended KDOQI criteria for the diagnosis of FID.

Materials and Methods

The study was conducted in the Departments of Pathology and Medicine (Division of Nephrology), University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi from November 2013 to February 2015. It was cross-sectional, prospective study. Seventy-seven clinically diagnosed patients of CKD (Stage 3, 4, and 5) of either sex, age >18 years with Hemoglobin (Hb) <11 g/dL were included in the study. Patients on iron supplementation (oral, i.v. or i.m.) or ESAs during the last three weeks, coexisting hemoglobinopathies (thalassemia, sickle cell anemia, etc.), any active bleeding, previously diagnosed to have nonrenal cause of anemia, chronic infections or inflammation such as tuberculosis and collagen vascular diseases, malignancy or end-stage liver disease or intake of immunosuppressive agents and blood transfusion within the last four weeks were excluded from the study. The diagnosis of CKD was made on the basis of K/DOQI guidelines of the National Kidney Foundation, USA.[5] GFR was calculated by using the abbreviated Modification of Diet in Renal Diseases (MDRD) study equation:

Estimated GFR (mL/min/1.73 m^2) = $1.86 \times (Pcr)^{-1.154} \times (age)^{-0.203}$

(multiply by 0.742 for women and 1.212 if African-American)

Pcr: Plasma creatinine

Complete clinical history and physical examination were done. A fasting venous blood sample (12 mL) was collected from patients in ethylenediamine tetraacetic acid and plain iron-free vials/containers for the study and following blood parameters were assessed:

Hemogram: Hb, hematocrit (Hct), RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration (MCHC) was determined by

Bechman Coulter LH 500. Peripheral smear (Wright's stain) and reticulocyte count (supravital staining) was done by microscopy. Biochemical markers of iron: SI and TIBC were determined using the standard technique (ICSH 1978). TSAT was calculated using the above parameters as follows: (SI/TIBC) × 100. SF and sTfR were determined by enzyme linked immunosorbent assay (ELISA) (Calbiotech, Inc. and BioVendor, respectively). Markers of inflammation, namely, hsCRP were determined by ELISA (Diagnostics Biochem Canada Inc.); IL-6 levels were assayed using the IL-6 enzyme immunoassay (Diaclone); erythrocyte sedimentation rate (ESR) was determined by the Westergren method.

Informed consent was taken from all cases before blood collection. Approval from the Institutional Ethics Committee for Human Research (IEC-HR) was obtained.

Statistical analysis was performed using MS EXCEL and Statistical Package for the Social Sciences program (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). All the quantitative data were compared between males and females by unpaired *t*-test. Wherever the variables were not following normal distribution, log transformation has been applied to normalize those parameters. Qualitative parameters were compared by Chi-square test/Fisher's exact test. Sensitivity and specificity of SF at various cut-offs, TSAT <16%, TIBC >450 µg/dL with sTfR/log ferritin as gold standard was done by 2/2 table test. Receiver operating characteristic (ROC) curve was obtained for SF <70 µg/L. *P* value <0.05 was considered statistically significant.

Results

Clinical profile

Seventy-seven CKD patients with anemia were enrolled in the study. The mean age [± standard deviation (SD)] of patients with CKD was 49.6 (±14.0) years. The mean duration (±SD) of CKD was 20.6 (±18) months. Out of 77 patients, 65 patients had various associated comorbid conditions. Majority had only hypertension (32.4%) followed by only diabetes mellitus (DM) (23.3%). 24.6% (19/77) patients had both hypertension and DM. Few patients had lupus nephritis (3.8%, 3/77). None of the patients were smokers. History of alcohol ingestion was present in 5.2% (4/77) patients. The male-to-female ratio was 0.57:1 with 36.4% (28/77) men and 63.6% (49/77) women. There was no significant difference in the clinical profile of men and women. CKD patients were classified into various stages based on their eGFR values (Table 1). Twelve patients were in HD while 65 patients had not received any dialysis. As the range was high, these values were log transformed for analysis.

Hematological and iron profile

All the patients enrolled in our study were anemic and had Hb <11 g/dL. Eleven (14.28%) patients had microcytic hypochromic anemia and three (3.9%) patients had macrocytic anemia. Rest of the patients had predominantly normocytic normochromic anemia (Table 2).

Detection of functional iron deficiency using conventional iron parameters

There were 31/77 (40.25%) patients with iron-deficient erythropoiesis using cut-off >1 of sTfR/log ferritin ratio (taken as the gold standard). Out of these 31 patients, 12 (38.70%) patients, had SF <12 µg/L indicating (absolute iron deficiency) absent iron stores. Rest of the 19/31 (61.29%) patients had FID. This was compared with the known standard methods for assessment of FID. Combination of SF >100 µg/L and TSAT <20% is used in CKD patients for detection of FID.[5] In these 19 cases; it detected FID in only two patients.

Table 1. Mean eGFR values in CKD patients in different stages (*n*=77).

Stage	eGFR (mL/min/1.73 m^2)	Mean log eGFR	Number (n)	(%)
Stage 3	30–59	1.63±0.09	31	40.3
Stage 4	15–29	1.32±0.08	17	22.1
Stage 5	<15	0.87±0.23	29	37.7

eGFR: Estimated glomerular filtration rate.

Table 2. Hematological and iron parameters in CKD patients (*n*=77).

Parameter	Mean±SD	Range
Hb (g/dL)	8.9±1.8	4.1–10.9
PCV (%)	27.7±6.0	11.9–41.0
RBC ($\times10^{12}$/L)	3.2±0.78	1.6–6.5
MCV (fL)	87.1±7.8	67.0–106.0
MCH (pg)	28.6±2.7	21.9–34.4
MCHC (g/dL)	32.5±1.7	28.6–38.9
TLC ($\times10^{9}$/L)	8.3±28.3	2.2–15.3
Platelet count ($\times10^{9}$/L)	245.7±142.2	26.0–772.0
SF (µg/L)	150.06±184.87	3.0–1000.0
TIBC (µg/dL)	283.62±84.70	104.0–506.0
TSAT (%)	28.18±15.80	7.5–81.1

Hb: Hemoglobin, PCV: Packed cell volume, RBC: Red blood cell count, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, TLC: Total leukocyte count, TIBC: Total iron binding capacity, TSAT: Percentage transferrin saturation, SF: Serum ferritin, sTfR: Soluble transferrin receptors, SI: Serum iron.

It showed a sensitivity of only 6.45% in detection of FID. However, the specificity was 82.60, positive predictive value, and the negative predictive value was 20% and 56.71%, respectively. Different cut-offs of SF were further tested for detection of FID and are shown in Table 3. SF at a cut-off <70 µg/L showed the best sensitivity and specificity in this cohort. ROC curve analysis using SF <70 µg/L showed area under curve (AUC) of 0.778 with 95% confidence interval (CI) of 0.67–0.88 which was statistically significant (P = 0.001) (Figure 1). Out of 19 cases of FID, 14 patients were identified by this criterion.

Role of high sensitivity C-reactive protein and interleukin-6 in detection of functional iron deficiency

Table 4 shows the inflammatory parameters in this cohort. All the patients had raised IL-6 (normal <2.97 mg/L). HsCRP was raised (>6 mg/L) in 77.92% (60/77) patients while 20.07% (17/77) patients had hsCRP <6 mg/L. Only five patients had a normal ESR.

SF <70 µg/L showed a sensitivity of 83.87% and a specificity of 73.91%. We studied this parameter in the presence and absence of raised hsCRP, a specific marker of inflammation (Table 5). Addition of hsCRP increased the diagnostic power of SF <70. It showed a higher sensitivity in patients without inflammation, though the results were statistically not significant (Z = 1.32, P >0.05). Hence, in the absence of raised hsCRP, cut-off of SF <70 µg/L had very good sensitivity (100%). However, in the presence of raised hsCRP sensitivity was reduced (79.16%). Out of 19 FID cases detected by the gold standard, 14 were detected by SF <70 µg/L. The five FID cases that were missed belonged to the group with raised hsCRP.

Table 3. Different cut-off of SF for detection of FID in CKD.

Parameter (µg/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SF <20	41.93	97.82	16.88	71.42
SF <30	61.29	91.30	82.60	91.30
SF <40	74.19	86.95	79.31	83.33
SF <50	77.41	86.95	80.0	85.10
SF <60	77.41	82.60	75.0	84.44
SF <70	**83.87**	**73.91**	**68.42**	**87.17**
SF <100	93.54	65.21	64.44	93.75

SF: Serum ferritin, PPV: Positive predictive value, NPV: Negative predictive value.

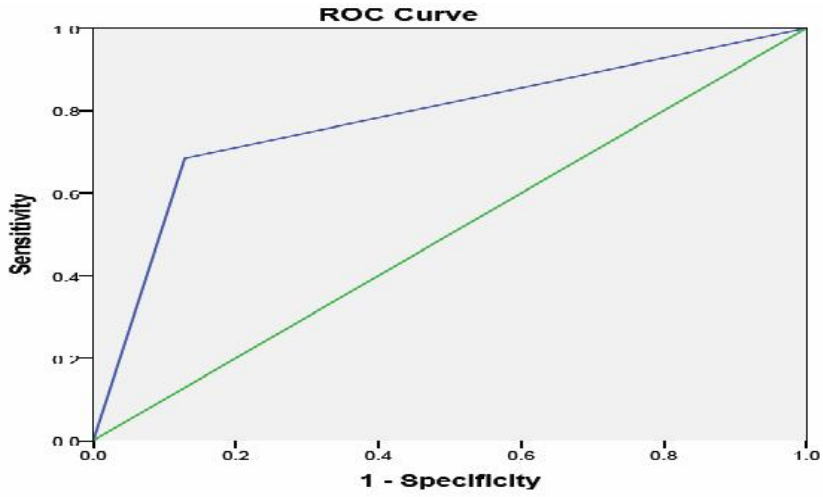

Figure 1. Receiver operating characteristic curve of serum ferritin <70 µg/L (area under curve 0.778; 95% confidence interval 0.67-0.88) (*P* = 0.000).

Table 4. Inflammatory parameters in CKD patients (*n*=77).

Parameter	Mean±SD	Range
hsCRP (mg/L)	63.78±84.26	1.0–254.0
IL-6 (pg/mL)	33.80±32.30	3.5–145.0
ESR (mm in 1st hr)	74.86±39.24	5.0–160.0
SF (µg/L)	150.06±184.87	3.0–1000.0

hsCRP: High sensitivity C-reactive protein, IL-6: Interleukin-6, ESR: Erythrocyte sedimentation rate.

Table 5. SF <70 µg/L in presence and absence of inflammation.

Parameter		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SF <70 (µg/L)	hsCRP >6 mg/L	79.16	77.77	90.47	84.84
	hsCRP <6 mg/L	100	60	63.63	100

SF: Serum ferritin, hsCRP: High Sensitivity C-reactive protein, PPV: Positive predictive value, NPV: Negative predictive value.

Discussion

FID is a major component of anemia in CKD and often is a cause of ineffective treatment. CKD patients are prone to develop FID due to the presence of persistent low-grade inflammation. The level of pro-inflammatory cytokine IL-6 is increased which induces Hepcidin and thereby mediates RE cell block. Moreover, the levels are further increased due to its impaired urinary excretion in CKD.[10,11] Hence with increasing inflammation and deteriorating renal function, hepcidin levels, and iron stores (SF) increase. This results in a vicious cycle making CKD patients prone to developing FID.[12]

CKD patients are frequently given ESA to treat anemia which is primarily due to EPO deficiency. In addition to accelerating erythropoiesis, EPO reduces the levels of circulating pro-inflammatory cytokines, namely, TNF-α, IL-6 and IL-1 as well as Hepcidin thus improving anemia by increasing the availability of both iron and EPO.[10,13-15] However, these patients on ESAs frequently develop FID as the rate of iron supply is unable to fulfil the needs of increased erythropoiesis leading to ESA resistance/hyporesponsiveness.[2]

ESA responsiveness can be maintained by concominant use of i.v. iron therapy. The patients on parenteral iron do improve as iron is now available for erythropoiesis being pro-

vided externally bypassing the RE cell block. However, judicious use is critical as they simultaneously develop iron overload due to retention of iron in RE cells. This further exacerbates FID. In addition, various studies have shown that giving parenteral iron itself can increase the morbidity and mortality by inducing a pro-inflammatory state due to increased oxidative stress (increased IL-6 and TNF-α).[16-20] Furthermore, iron overload has been associated with an increased incidence and severity of infections, related to the growth-promoting effect of iron on microbial pathogens due to inhibition of phagocytosis[21] and saturation of iron binding sites on transferrin and anti-microbial molecule Lactoferrin, which function to sequester iron from microbes and hence prevent their growth.[22,23] Under-treatment of iron results in ESAs hypo responsiveness.

However, it is important to detect FID in CKD as it is easily treatable and is treated with concomitant administration of appropriate doses of ESAs and parenteral iron. The therapy is guided by the response to treatment as monitored by Hb levels and serum TSAT and SF levels.

To guide the adequate treatment, the current criteria for the diagnosis of FID in CKD is TSAT <20% and SF >100 ng/mL.[6,24]

However, these criteria are insufficient as both the values are affected by inflammation. These criteria have shown variable sensitivity and specificity in different studies. The cut-off of ferritin tends to miss-out patients who may still respond to parenteral iron therapy.[6] High TIBC >450 µg/dL, TSAT <16%, and SF <12 µg/L which are used conventionally for detection of iron-deficient erythropoiesis are also insufficient. None of these parameters were effective in detecting FID in the present study. The current gold standard, i.e., TSAT <20% and SF >100 µg/L showed a sensitivity of only 6.45% in detection of FID in our study group. This could be because ours is a small study population (n = 77). The utility of SF at different cut-offs was further tested in the detection of FID (Table 3). SF <70 µg/L showed a sensitivity of 83.87% and a specificity of 73.91%. It correctly identified 14/19 FID patients. Hence, the effect of inflammation on this parameter was studied (Table 5). Inflammation with hsCRP >6 mg/L was present in 77.92% (60/77) patients while 20.07% (17/77) patients had hsCRP <6 mg/L. SF <70 µg/L showed a higher sensitivity in the detection of iron-deficient erythropoiesis in patients without inflammation. Hence, hsCRP could be used to stratify the CKD group in which FID could be detected with high sensitivity and specificity.

To conclude, the detection of FID in CKD patients remains a difficult diagnostic task. The present study attempted to identify laboratory parameters that would increase the diagnostic ability of conventional parameters in detecting FID. TSAT <20% and SF >100 µg/L had a sensitivity of only 6.45%. However, SF <70 µg/L emerged as the most sensitive and specific in identification of iron deficient erythropoiesis. SF >12 µg/L - SF <70 µg/L was able to identify 14/19 cases of FID. Furthermore, hsCRP could be used to stratify the CKD group in which FID could be detected with high sensitivity and specificity. This is a valuable observation, however needs to be validated in larger sample size.

Conflict of interest: None declared.

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