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Predictive value of iron store markers in anemia of chronic kidney disease

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ABSTRACT

Anemia is a major manifestation of chronic kidney disease (CKD) and shown to have an impact on the mortality and morbidity. Iron deficiency is considered as the most common cause of inadequate response to Erythropoietin. Body stores of iron are usually assessed by Transferrin saturation (Tsat) levels (normal: 20%-30%) and serum ferritin levels (normal >150ng/ml). Tsat and serum ferritin are extensively used in clinical practice in monitoring iron status of patients with CKD on erythropoietin treatment. Serum ferritin is an acute phase reactant and thus may be elevated in a number of conditions, including infections, inflammation, malignancy, and liver disease. Many of these conditions are common in CKD patients. Similarly, low Tsat may reflect either iron deficiency or intense erythropoiesis, causing disequilibrium between iron stores, the circulation, and the bone marrow. In addition, Tsat is calculated as the ratio of serum iron and TIBC, and TIBC is known to fall during infections and inflammation, both of which are common in dialysis patients. If TIBC falls acutely, Tsat values may be falsely high. Studies have shown that none of the traditional iron indices, such as Tsat and serum ferritin, have a high level of utility i.e. sensitivity and specificity of >80%. In this context the serum ferritin and T Saturation are still being used for the determining the iron stores, to define the type of anemia and guide the management. We in the present study tried to analyze the iron store markers for predicting anemia.

Key words: Anemia, CKD, Serum Iron, Total iron binding capacity (TIBC), Tsat.

INTRODUCTION

The kidneys are a pair of bean-shaped organs; the main function of the kidneys is to remove waste products and excess water from the blood. Activate form of vitamin D (calcitriol or 1, 25 dihydroxy-vitamin D), which regulates absorption of calcium and phosphorus from foods, promoting formation of strong bone [1].

Chronic kidney disease is when one suffers from gradual and usually permanent loss of kidney function over time. This happens gradually over time, usually months to years. Chronic kidney failure is also referred to as end-stage renal disease (5^{th} stage), wherein there is total or near-total loss of kidney function and patients need dialysis or transplantation to stay alive [2].

Anemia is a major factor that limits the quality of life in chronic kidney failure (CKD) patients and may affect their morbidity and mortality. Insufficient production of erythropoietin from the failed kidneys is the major cause of anemia in this population. Body iron stores should be assessed regularly and accurately. Body stores of iron are usually assessed by transferrin saturation (Tsat) levels (normal: 20%-30%) and serum ferritin levels (normal >150ng/ml).serum iron(60-110ng/ml) ,total iron binding capacity (240-300ng/ml) TSAT and serum ferritin are extensively used in clinical practice in monitoring iron status of patients with CKD [3, 4].

Iron markers used in chronic kidney diseases:
Serum iron is a medical laboratory test that measures the amount of circulating iron that is bound to transferrin. Clinicians order this laboratory test when they are concerned about iron deficiency, which can cause anemia and other problems **(**Normal values of Men 65 to 176 µg/dL and Women: 50 to 170 µg/dL) [5].

The ferritin is a protein allowing the storage of iron. It plays a key role in metabolism, which regulate the intestinal absorption of iron depending on the needs of the organization. It thus functions of detoxification and reserves of iron [6]. Its strength is used to evaluate the reserves of iron and thus provide early detection of iron deficiency or the opposite to enjoy a rise in reserves during treatment with iron supplementation (Normal Values of Men 20 to 30 - 250 to 300 µ g / l and Women15 to 20 - 150 µ g / l) [7].

Total iron-binding capacity is a medical laboratory test. The test measures the extent to which iron-binding sites in the serum can be saturated. Because the iron-binding sites in the serum are almost entirely dependent on circulating transferrin, this is really an indirect measurement of the amount of transferrin in the blood (TIBC: 240-450 µg/dL) [8].

Transferrin saturation, measured as a percentage, is a medical laboratory value. It is the ratio of serum iron and total iron-binding capacity, multiplied by 100 [9]. For an explanation of some clinical situations in which this ratio is important, see Total iron-binding capacity. The three results are usually reported together (Transferrin saturation: 20-50%) [10].

The objective of the study is to evaluate the accuracy of the current tests, TSAT,serum ferritin, serum iron, total iron binding capacity in assessing and monitoring body iron stores, which can influence iron management and treatment in patients with CKD.

EXPERIMENTAL SECTION

Inclusion Criteria:

- Age >18 yrs
- Patients with chronic kidney diseases diagnosed by serum creatine >1.5
- Patients who have all the documented investigations which include creatine, iron markers, Hemoglobin.

Exclusion Criteria:

- Age < 18 yrs.
- Patients with inadequate data
- Patients with Acute or rapidly progressive renal failure.
- Previous history of iv iron therapy and recent (<1 month) blood transfusion

In this retrospective analysis, patients who were attending the nephrology outpatients department with serum creatinine more than 1.5mg/dl and with iron store marker evaluation were included. Total 207 patients were included who satisfied the inclusion and exclusion criteria. The main parameters considered for this study are Serum iron ferritin, total iron binding capacity and transferrin saturation. Patients were grouped in to two based on the hemoglobin level.

All patients are suffering from chronic kidney diseases from stage 1 to 5. And these patients are subjected to test for iron markers (i.e. serum iron, ferritin, total iron binding capacity, and transferring saturation) and creatinine (The total Study has been performed at NIMS, under the guidance of Dr.Gangathar MD. DM).

Materials:

1. Iron Buffer Reagent: Hydroxylamine hydrochloride 220mM in acetate buffer, pH 4.5 with surfactant. 2. Uibc Buffer Reagent: Tris 500 mM, pH 8.1 with surfactant, sodium azide 0.05% (w/v) as preservative. 3. Iron Color Reagent: Ferrozine 16.7mM in hydroxylamine hydrochloride. 4. Iron Calibrator: 500µg/dL): Ferrous chloride in hydroxylamine hydrochloride.

Instruments used for this study:

Use a Visible spectrophotometer calibrated at 560 nm.

Methods

Determination of iron / TIBC (ferrozine) procedure:

Manual procedure:

Serum Iron:

Label the test tubes as "Standard", "Calibrator", "Control", "Sample". Add 2.5 mL of Iron Buffer reagent to all the tubes, then add 0.5 mL sample to respective tubes and mix well (Note: Use iron-free water for "Standard"). Record the absorbance of all the tubes in visible spectrophotometer at 560 nm using the Standard reagent (A1 reading). Add 0.05 mL of iron color reagent to all tubes and mix well. Place all tubes in heating bath at 37º C for 10 minutes. Measure the absorbance in visible spectrophotometer at 560 nm with Standard reagent (A2 reading).

The following equation is used for this calculation

$$\frac{A2test - A1test}{A2cal - A1cal} \times concentration = \text{Total Iron (ug/dL)}$$

Where, A = Absorbance

Cal = Calibrator

UIBC (Unsaturated Iron-Binding Capacity):
Label the test tubes "standard", "Calibrator", "Control" and “Sample". Add 2.0 mL of UIBC Buffer reagent to all test tubes. Add 1.0, 0.5 mL of iron-free water to "standard", "Calibrator" respectively and mix well, to the calibrator add 0.5 mL of iron calibrator. To "Test" add 0.5 mL respective sample plus 0.5 mL iron calibrator and mix well. Record the absorbance values as A1 reading for all the tubes in visible spectrophotometer at 560nm using standard reagent. Add 0.05 mL iron color reagent to all tubes and mix well. Place all tubes in heating bath at 37º C for 10 minutes. Record the absorbances of all tubes as A2 reading.

The following equation is used for this calculation

$$\textbf{UIBC (ug/dL)} = \frac{A2test - A1test}{A2cal - A1cal} \times concentration$$

Where A = Absorbance
Cal = Calibrator

TIBC (Total Iron-Binding Capacity):
Level + UIBC = TIBC (ug/dL)
Conversion ug/dL x 0.179 = umo/L

Stability of final reaction:
The test samples should be read within 15 minutes after color development.

TIBC: The range represents the 95% confidence intervals from a clinically normal population. It is recommended that each laboratory establish its own range of expected values.

Specificity:
A comparison of serum iron procedure with another widely used commercial method resulted in a coefficient of correlation of 0.993 with a regression equation of y = 1.02 x + 7.0. A study performed between procedure and a similar TIBC procedure resulted in a coefficient of correlation of 0.976 with a regression equation of y = 0.92 x + 32.5. (9&10)

Sensitivity:
This IRON / TIBC procedure has a sensitivity of 1.5 Ug/dL per 0.001 absorbance unit.

Quantitative Determination of Ferritin in Human Serum
Materials provided with the test kits:
Antibody coated micro titer plate with 96 wells. Reference standard set; contains 0, 10, 50, 150, 400, and 800 ng/ml lyophilized; (NIBSC-WHO 80/602, human liver standard); Enzyme Conjugate Reagent; Color Reagent A; Color Reagent B; 2N HCl.

Instrumentation:
The following equipment items are required to perform this assay:

- A vortex mixer or equivalent to mix reagents.
- A micro titer plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is required for use in the absorbance measurement.

Assay procedures: Secure the desired number of coated wells in the holder. Dispense 20µl of standard, specimens, and controls into appropriate wells. Dispense 100µl of Enzyme Conjugate Reagent into each well. Thoroughly mix for 30 seconds. It is very important to have completed mixing in this setup. Incubate at room temperature (18-25°C) for 60 minutes. Prepare TMB solution 15 minutes before use. Remove the incubation mixture by flicking plate content into a waste container. Rinse and flick the micro titer wells 5 times with running tap or distilled water. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets. Dispense 200µl of TMB solution into each well. Gently mix for 5 seconds. Incubate at room temperature for 20 minutes without shacking. Stop the reaction by adding 50µl of 2N HCl to each well. Gently mix for 30 seconds to make sure that the blue color changes to yellow color completely. Within 30 minutes, read the optical density at 450nm with a micro titer plate reader.

Test for percent Transferrin saturation: The most specific and sensitive test for iron accumulation is the transferrin Saturation. This is calculated from the serum iron concentration and the total iron-binding capacity (TIBC), which is a measure of transferrin concentration as each transferrin molecule can bind two atoms of iron. The percentage saturation is then calculated (100 x serum iron / TIBC) and a value of greater than 55% (men) or 50% (women) suggest iron accumulation due to HC. The measurement of transferrin saturation should be repeated on a fasting sample to confirm its elevation.

RESULTS

The total number of patients studied was 207.The mean age 47.9±13.65 yrs. Hemoglobin levels----9.80±5.56gm/dl. Serum Creatinine--5.56±7.71mg/dl; Serum Iron---84.31±95.66 ug/dL; Serum Ferritine—272.66±258.53ng/ml; TIBC--260.58±83.42 ug/dL; T.sat----29.1±19.11%. Among the total patients, patients with Iron deficiency were ---76 (37%), Patients with Ferritin deficiency—64(31%); Patients with TIBC deficiency--- 32(15.4%); Patients with T.sat deficiency--- 70(34 %.)

In Group I (Anemia) N= 118: Age--- 47.91±13.65yrs, HB----9.8±2.2gm/dl,Serum Creatinine—5.56±7.71 mg/dl, Serum Iron---84.31±95.66, Ferritine—272.66±258.53, TIBC----260.58±83.42, T.sat----29.1±19.11. Patients with iron deficiency (<60mcg/dl) were 44(37%). The results were shown in **Table-1**

Patients with Ferritin deficiency (<100 mcg/dl) were ---37(31%); Patients with TIBC (total iron binding capacity)(<200 mcg/dl)—21(18%); Patients with TIBC (total iron binding capacity) (≥200 mcg/dl)—97(82%); Patients with T.Sat (Transferrin saturation) (<20%)- 34(28%) ; Patients with T.Sat (Transferrin saturation) (≥20%)--84(72%).

In Group II (Noraml hemoglobin) N= 89. Age--- 50.56±13.30 yrs, HB-11.84±1.56gm/dl, Creatinine --- 4.83±10.68 mg/dl, Iron- 85.05±110.70 ug/dL, Ferritin—242.71±237.91 ng/ml, TIBC---276.38±84.78 ug/dL, T.sat---26.77±16.33%.Among these patients with Iron deficiency (<60 mcg/dl) -32(35%); Ferritin deficiency patients (<100 mcg/dl) ----27(39%); TIBC (total iron binding capacity) (≥200 mcg/dl)--78(88%); As shown in **figure 1 and 2**

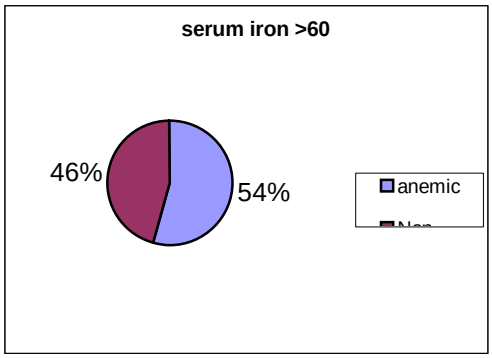
TIBC (total iron binding capacity) (<200 mcg/dl)—11(12%); T.Sat (Transferrin saturation) (<20 %) ----36(40%); T.Sat (Transferrin saturation) (≥20 mcg/dl) ----53(60%).

Table: 1 Comparative results between Anemic and non Anemic patients

	Group I Anemia	Group II non Anemia
Number of patients	118	89
Age	47.91±13.65	50.56±13.30
Sex (M:F)	75:43	74:15
Serum creatinine	5.56±7.71	4.83±10.68
Hemoglobin	9.8±2.2	11.84±1.56
Serum Iron <60mcg/dl	44(37%)	32(35%)
Serum Iron > 60 mcg /dl	74(63%)	57(64%)
Serum Ferritin <100ng/ml	37(31%)	27(39%)
Serum Ferritin >100ng/ml	81(68%)	62(70%)
TIBC <200 mcg/dl	21(18%)	11(12%)
TIBC >200mcg/dl	97(82%)	78(88%)
Tsat <20%	34(28%)	36(40%)
Tsat >20%	84(72%)	53(60%)

From total number of patients:

Figure 1 and 2 Serum iron as a marker of anemia & non-anemia:



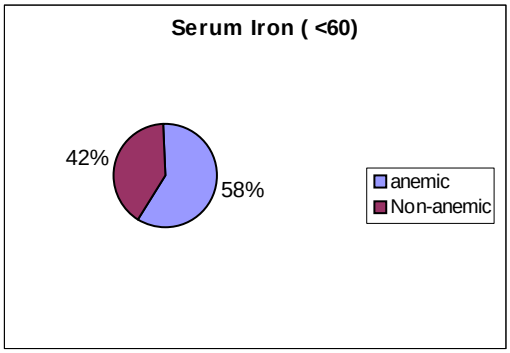


Table 2 statistical analysis data for serum markers

sensitivity	38.26087
specificity	65.21739
PPV	57.89474
NPV	45.80153
OR	1.161972
RR	0.619718
AR	-61.3636

PPV: Positive Predictive Value, NPV: Negetive Predictive Value, OR: Optimum risk, RR: Relative risk, AR:Atributed risk

Ferritin as a marker of anemia:

Among the ferritin deficiency group 37 were anemic and 27 were not anemic. In normal ferritin group 79 were anemic and 64 were non anemic.

Figure 3& 4 Ferritin as a marker of anemia & non-anemia:

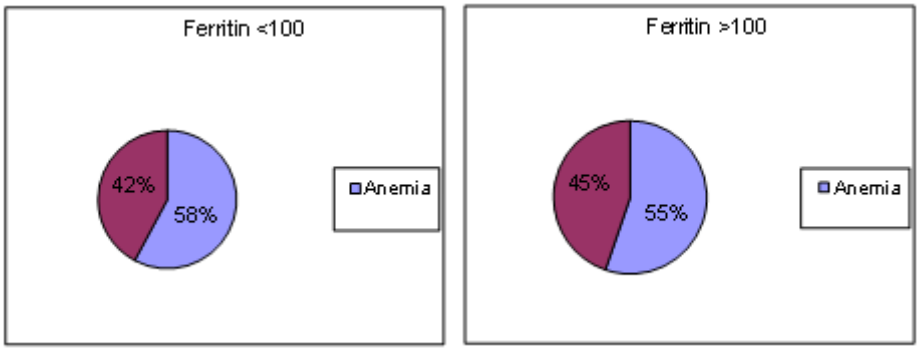


Table 4 statistical analysis data for ferritin markers

sensitivity	31.89655
specificity	70.32967
PPV	57.8125
NPV	44.75524
OR	1.110173
RR	0.468354
AR	-113.514

TIBC as a marker of anemia:

Among the high TIBC group (175) 97 patients were anemic and 78 patents were non anemic. Among the low TIBC (32) 21 patients were anemic and 11 patients were nonanemic.

Figure 5& 6 TIBC as a marker of anemia & non-anemia:

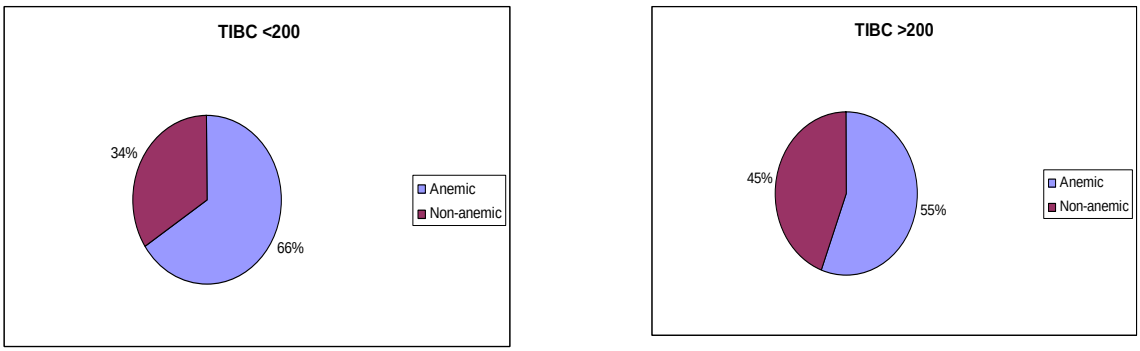


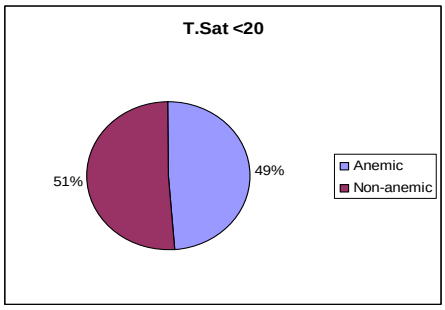
Table 5 statistical analysis data for TIBC

sensitivity	17.79661
specificity	87.64045
PPV	65.625
NPV	44.57143
OR	1.535145
RR	0.216495
AR	-361.905

T.sat as a marker of anemia:

Among the Tsat <20% group(70), 34 were anemic and 36 were non anemic. Among the Tsat >20% group (138) , 81patienst were anemic and 57 patients were non anemic.

Figure 7& 8 Tsat as a marker of anemia & non-anemia:



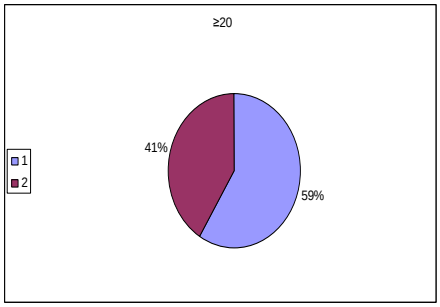


Table 6 statistical analysis data for Tsat

sensitivity	29.56522
specificity	61.29032
PPV	48.57143
NPV	41.30435
OR	0.664609
RR	0.419753
AR	-138.235

Correlation between serum iron and other markers of iron stores:

Iron deficiency (<60mcg) was observed in 76 patients. In this group serum ferritin was low in 33 and normal in 43 patients.

Table 7 Correlation between serum iron and other markers

Comparision between serum iron and other markers	Serum Iron <60mcg/dl (N=76)	Serum Iron >60mcg/dl (N=131)
Serum ferritin<100ng/ml	33	31
Serum ferritin >100ng/ml	43	100
TIBC<200 mcg/dl	45	15
TIBC >200 mcg/dl	31	116
Tsat<20%	59	9
Tsat >20%	17	122

Serum Iron in predicting Ferritin

Table 8 statistical data for Serum Iron in predicting Ferritin

sensitivity	51.5625
specificity	69.93007
PPV	43.42105
NPV	76.33588
OR	2.475619
RR	1.064516
AR	6.060606

Serum Iron predicting Tsat

Table 9 statistical data for Serum Iron in predicting Tsat

sensitivity	86.76471
specificity	87.76978
PPV	77.63158
NPV	93.12977
OR	47.04575
RR	6.555556
AR	84.74576

Table 10 Correlation between Ferritin and other markers:

Correlation between Ferritin and other markers	Serum Ferritin <100ng/ml (N=64)	Serum Ferritin >100ng/ml (N=143)
Serum Iron< 60mcg/ml	33	41
Serum Iron >60mcg/ml	31	102
TIBC<200 mcg/dl	3	29
TIBC >200 mcg/dl	61	114
Tsat<20%	33	36
Tsat >20%	31	107

Table 11 Statistical data Ferritin in predicting serum iron

sensitivity	44.59459
specificity	76.69173
PPV	51.5625
NPV	71.32867
OR	2.648308
RR	0.804878
AR	-24.2424

Table 12 Statistical data Ferritin in predicting Tsat:

sensitivity	47.82609
specificity	77.53623
PPV	51.5625
NPV	74.82517
OR	3.163978
RR	0.916667
AR	-9.09091

Table 13 Correlation between T.sat and other markers:

Correlation between T.sat and other markers	Serum Tsat <20% (N=70)	Serum Tsat >20% (N=138)
Serum Iron< 60mcg/ml	30	14
Serum Iron >60mcg/ml	40	124
TIBC<200 mcg/dl	11	20
TIBC >200 mcg/dl	59	118
Serum ferritin<100ng/ml	33	60

Serum ferritin >100ng/ml	37	78

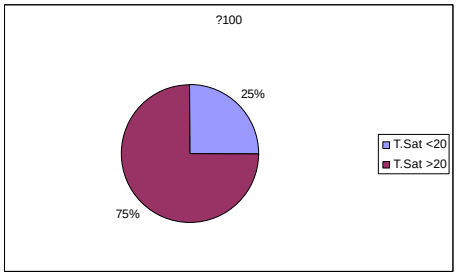
Table 14 Statistical data Tsat in predicting Ferritin:

Sensitivity	35.48387
Specificity	67.82609
PPV	47.14286
NPV	56.52174
OR	1.159459
RR	0.55
AR	-81.8182

Table 15 Correlation between TIBC and other markers:

Correlation between TIBC and other markers	Serum TIBC < 200 mcg/dl (N=32)	Serum TIBC >200mcg/dl (N=175)
Serum Iron< 60mcg/ml	14	60
Serum Iron >60mcg/ml	18	115
Tsat<20%	11	25
Tsat >20%	21	150
Serum ferritin<100ng/ml	3	30
Serum ferritin >100ng/ml	29	145

Among the low ferritin group (64) Tsat was < 20 % in 33 patients and Tsat >20% in 31 patients. Among the normal ferritin group (143) Tsat was less than 20% in 36 patients and Tsat >20% in 107 patients.



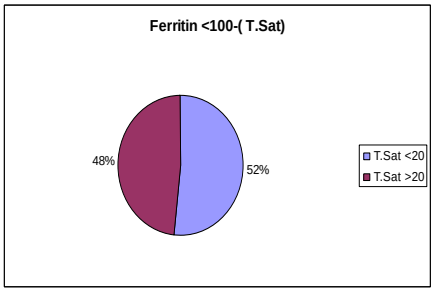


Figure 9& 10 TIBC and other markers:

Table 16 Tsat in predicting Serum Iron:

Sensitivity	68.18182
Specificity	75.60976
PPV	42.85714
NPV	89.85507
OR	6.642857
RR	2.142857
AR	53.33333

DISCUSSION

In the present study we have analyzed different iron store markers for the assessment of iron store status and correlated with the anemia. The Results shown that there is a week correlation of

these markers with iron stores and also with the anemia. In the group of anemia the iron store markers with features of iron deficiency is shown as low serum iron (<60 mcg/dl) in 37%; Low Serum Ferritin (<100ng/ml) in 31%; Low Tsat (<20%) in 28% of cases. Overall iron deficiency anemia in chronic kidney disease as per the iron store markers is 30%. This indicates either iron deficiency is not the major cause of anemia of chronic kidney disease or the iron store markers are of less utility in predicting the iron deficiency. The other factors which can contribute to the anemia include erythropoitin deficiency secondary to kidney disease, vitamin deficiency (Vit B12, Folic acid etc) or other factors such as hyperparathyroidism ,which are known to cause resistance to erythropitin or inhibit the erythropoisis. These factors were not evaluated in the present study.

The correlation between the iron store markers has shown a good correlation between the serum iron and transferring saturation with sensitivity of 86%; specificity of 87%; positive predictive value of 77%; negative predictive value of 93%. Serum ferritin had a poor correlation with other iron store markers such as serum iron and transferring saturation, which may suggest that the ferritin levels may not be a reliable maker of iron store status. The ferritin in previous studies also shown to be poor marker of iron stores as its level is influenced by non–Fe-related conditions, including inflammation, malnutrition, liver disease, infection, and malignancy. Although ferritin is a fascinating molecule, moderate hyperferritinemia is a misleading marker of Fe stores in patients with CKD. It may be time to revisit the utility of serum ferritin in CKD and ask ourselves whether its measurement has helped us or has caused more confusion and controversy. The frequent paradox of high serum ferritin and low Tsat has made it desirable to seek alternative iron markers to predict better whether subsets of patients will respond to iron therapy. Further study is needed to determine the role of hepcidin in assessing iron pathophysiology and determining the presence or absence of an RE blockade situation. For the foreseeable future, the alternative tests will prove most useful not as screens or primary diagnostic markers for iron deficiency but to evaluate better the challenging cases.

CONCLUSION

The study has shown the high prevalence of anemia in kidney disease population. The prevalence of iron deficiency anemia in kidney disease is 30%. There is a good correlation between the serum iron and transferring saturation. Serum ferrtin did not show a good correlation with other iron store markers.

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