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CLINICAL STUDY EVALUATING IRON STATUS AT END-STAGE RENAL DISEASE PATIENTS ON HEMODIALYSIS

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ABSTRACT

Background*:** One of the main defects in the diagnosis of iron deficiency in chronic kidney disease patients is the inaccuracy of commonly used tests. Initial studies have shown that the Reticulocyte hemoglobin content (CHr) test is a promising test in improving the diagnosis of iron deficiency. The purpose of this study was to compare the Reticulocyte hemoglobin content (CHr) test with the well-known tests for the diagnosis of iron deficiency, Transferrin saturation (Tsat), ferritin test, and Hemoglobin concentration to determine which is most accurate and effective. ***Methods: A number of participants are 75, all study participants will undergo laboratory tests twice at baseline and end of the study, laboratory tests are: (Tsat, ferritin, CHr, Hb, BUN, creatinine blood test, Blood Urea concentration, ALT, AST, Serum Iron, and TIBC), participants consist of two groups, (Group1) 44 adult patients, with end-stage renal disease on hemodialysis from Kidney Department of Tanta University Teaching Hospitals, (Group 2) 31 adult healthy participants, were randomly selected from the community. Each patient was followed for four months. Patients whose CHr < 29 pg, were given intravenous sacrofer® 100mg, dose/week for 4 months. ***Results***: There was no significant difference between groups in the final mean Transferrin saturation (Tsat) and Serum ferritin test. The mean CHr remained in the targeted range (30.9 ―32.4 pg) throughout the study period in both groups. There are a statistically significant difference means of CHr and Hb for Group 1 at baseline and final P values were 0.0372, <0.0001 respectively. CHr has a lower level of test variability compared to serum ferritin and transferrin saturation. In this study, hemoglobin concentration was taken as the gold standard. CHr had sensitivity 60.87% and specificity 98.02%. Serum iron had sensitivity of 58.70% and specificity of 84.16%, while transferrin (TIBC) had sensitivity of 73.91% and specificity of 55.45. The variability (%CV) of CHr was found to have significantly less variation than the other tests, with a CV of 8.31% compared to hemoglobin concentration 18.99%, TIBC 29.07%, Serum iron 44.41%, transferrin saturation of 46.19% and serum ferritin of 50.67%

KEYWORDS: chronic kidney daises; Anemia; Reticulocyte Hemoglobin Content, Hemoglobin concentration; Serum ferritin; trans ferritin saturation.

INTRODUCTION

Anemia has been defined by the World Health Organization (WHO) as the condition where the hemoglobin concentration in the blood is lower than normal. Or a condition in which the number of red blood cells is insufficient to meet the physiological needs of the body such as the transportation of oxygen and carbon dioxide to and from body cells, nutrients, etc. Anemia is diagnosed if hemoglobin levels are lower than or equal (12 g/dL) in female, or lower than or equal (13 g/dL) in male. A lack of hemoglobin concentration in the blood leads to the inability of the blood to perform its role as required, which leads to the emergence of a group of symptoms such as fatigue, shortness of breath and lack of physical energy, etc.[1]

Chronic kidney disease is defined as a defect in kidney composition or function present for more than 3 months,[2] The most important criteria that must be present for more than 3 months for the patient to be classified as CKD are summarized in (table.2). High blood pressure and diabetes are the most leading causes of chronic kidney disease in developed and underdeveloped countries; However, glomerulonephritis and other unclear reasons are a cause of chronic kidney disease and this is common in Asia as well as in sub-Saharan Africa countries.[3-5]

Table 2: Criteria for CKD.[6]

- Albuminuria (AER ≥30 mg/24 h; ACR ≥30 mg/g [≥3 mg/mmol]).
- Glomerular Filtration Rate GFR <60 mL/min/1.73 m^2

Anemia is a condition in which the body has fewer red blood cells than normal. Red blood cells carry oxygen to tissues and organs throughout the body and enable them to use energy from food. With anemia, red blood cells carry less oxygen to tissues and organs—particularly the heart and brain—and those tissues and organs may not function as well as they should.[9] Anemia is a common complication that appears in patients with chronic kidney disease (CKD), especially in those whose blood clearance rate is less than 30-40 ml/min. The factors that cause anemia in chronic kidney disease patients are complex, but the main cause is the relative deficiency of erythropoietin. Erythropoietin is produced by cortical peritubular interstitial fibroblast-like cells in the kidneys. The process of producing erythropoietin decreases as the total functioning of kidney mass decreases. Iron deficiency is also common in anemia with chronic kidney disease. Iron is normally metabolized by hepcidin. Inflammations lead to changes in the rates of hepcidin and this leads to a decrease in the availability of iron for erythropoiesis, This, in turn, contributes to anemia in chronic kidney patients.[6]

Anemia is often diagnosed by determining the hemoglobin concentration in the blood and this is the most popular analysis for diagnosing anemia. However, Anemia can be diagnosed with several other tests, such as blood reticulocyte count, hematocrit (packed cell volume), blood film analysis or hemoglobin electrophoresis and mean cell volume.[1] There are several biomarkers of iron metabolism, uptake, and stores, which include serum ferritin, transferrin saturation, serum iron, and soluble transferrin receptor. Unfortunately, there is a wide variation in the degree of sensitivity and accuracy of these markers, Results differ with different patients' population, as well as with (gold standards) used to determine iron deficiency.[7]

Treating iron deficiency in CKD patients is one of the most important things for their health care. Treatment of iron deficiency in oral doses is often ineffective in patients with hemodialysis. So, the National Kidney Foundation-Kidney Disease Outcome Initiative (NKF-KDOQI) strongly recommends that iron should be given intravenously to these conditions.[8-10] Inaccurate identification of iron deficiency makes it difficult to properly treat anemia, with difficulty in reaching the target hemoglobin and hematocrit levels, as well as determining the required iron dose.[11-13] For this reason, iron diagnostic tests are performed every 3 months.[14] Testing is not only important for the diagnosis of an iron deficiency but also to avoid excessive iron treatment and the side effects it may cause.[15,16] The test should accurately detect patients with iron deficiency and those who need extensive treatment by iron, in addition to excluding those with normal iron levels. Ideally, the results should allow, to maintain the target range of hemoglobin level, without a significant increase in test prices. However, the most commonly used tests by American dialysis units are serum ferritin and transferrin saturation.[10] Both tests have accuracy defects when used in hemodialysis patients.[17] A group of previous studies demonstrated that the use of serum ferritin at a level between 100—200 ng / mL has a sensitivity only between 41—48%, and a specificity between 75—100 for diagnosis iron deficiency.[18,19] A transferrin saturation of 20 — 21% has a sensitivity of 81—88%, but with a specificity of only 63%.[20] Reducing cutoff values improves specificity, but at the same time, it significantly reduces sensitivity in this case.[17]

METHODS

This clinical study was conducted after approval from the Research Ethics Committee at Tanta University, Egypt, under No (2112019), Written approval (Informed consent) was obtained from all study participants. The study was conducted at the Kidney Department of the Tanta University Teaching Hospitals.

Patients

Our study was conducted on 75 participants with two groups:

Group 1 (patients): The criteria in this group included that, the participants were over the age of 18 years, subjected to hemodialysis treatment for more than 3 months, did not undergo a blood transfusion in the previous three months, had no hematologic disease except anemia, not scheduled to receive a kidney transplant, and did not have active bleeding. This group consists of 44 adult patients, with end-stage renal disease on hemodialysis, it consists of 19 women and 25 men, with ages ranging from 21 to 71 years, who attended at the Kidney Department of Tanta University Teaching Hospitals, between January 2019 and May 2019.

Group 2 (control): consisted of 31 adult healthy participants, were selected from the community, it consists of 12 women and 19 men with ages ranging from 23 to 36 years.

All participants were subjected to the following:

- A detailed assessment of history, with a special focus on age, sex, body Weight, Diabetes mellitus, Systolic blood pressure, Diastolic blood pressure, anemia, as well as the duration of the chronic kidney disease.
- All participants undergo laboratory tests twice at baseline and end of the study. Laboratory investigations included: Transferrin saturation (Tsat), Serum ferritin test, Reticulocyte hemoglobin content (CHr), Hemoglobin concentration, Serum Iron, Total Iron-Binding Capacity (TIBC), Blood

Urea Nitrogen (BUN) test, creatinine blood test, Blood Urea concentration, Alanine Aminotransferase (ALT) test, and Aspartate Aminotransferase (AST) test, data summarized in table.3. All laboratory assessments were performed by al mokktabar laboratories®.

- Each participant was followed for four months. Patients whose CHr < 29 pg, were given intravenous sacrofer® 100mg, dose/week for 4 months. Intravenous iron was only administered if the patient met the above criteria. More than one course should be given during the 4-months study period.

Iron status tests

Serum ferritin, Transferrin saturation, Hemoglobin concentration, Serum Iron, and Total Iron-Binding Capacity tests were determined twice at baseline and end of the study by standard methods. Reticulocyte hemoglobin content (CHr) was determined twice at baseline and end of the study, using the ADVIA® 120 hematology system (Bayer Corporation, Tarrytown, NY, USA). CHr is derived from the simultaneous measurement of volume and hemoglobin concentration in reticulocytes. The CHr value is an indication of the hemoglobin content of each reticulocyte.

Statistical analysis

Primary outcome measures were Transferrin saturation (Tsat), Serum ferritin test, Reticulocyte hemoglobin content (CHr), Hemoglobin concentration, Serum Iron, Total Iron-Binding Capacity (TIBC), Blood Urea Nitrogen (BUN) test, creatinine blood test, Blood Urea concentration, Alanine Aminotransferase (ALT) test, and Aspartate Aminotransferase (AST) test. The "Mann Whitney test" was used to analyze differences between any two groups of study. The correlation analysis between CHr and other parameters (Table 7) was also carried out by Spearman's correlation coefficient (r). At 95% confidence interval, p-value of < 0.05 was regarded to indicate statistical significance. Variation in test results was expressed as coefficient of variation. All results are reported as mean ± standard deviation. P-value (<0.05) were considered statistically significant. This analysis aimed to find out whether the use of sacrofer® had increased Baseline values of (Tsat, Serum ferritin, CHr, and Hemoglobin concentration) for the whole patient group, and the patient is given sacrofer® only, during the study. In addition to determining the relationship between CHr response and response marker category (table 7). The relationship was analyzed for each test separately using logistic regression. All statistical analyses were conducted using GraphPad Prism 8.0.1 software.

RESULTS

The total number of participants 75. (2 patients) were withdrawn before the end of the study period due to death. Table 3 shows the characteristics of the study participants. Specifically, factors other than iron that could impact on anemia management, such as BUN, Creatinine, Urea, AL, AST were significantly different between the groups at baseline. Also, the difference in age and systolic blood pressure was statistically significant for the two groups. There were no significant difference between the two groups as to means Tsat and serum ferritin at any time point.

The 2007 KDOQI Anemia guidelines recommended the use of CHr < 29pg as an indicator of iron deficiency.[9] Treatment of iron deficiency in oral doses is often ineffective in patients with hemodialysis. So, the National Kidney Foundation-Kidney Disease Outcome Initiative (NKF-KDOQI) strongly recommends that, iron should be given intravenously to these conditions.[8,9,10]

CHr was tested for patients at baseline, resulting in 16 (36.3%) Patients receiving 256 doses of intravenous sacrofer® 100mg. IV iron was given only to those whose CHr was less than 29 pg. The mean CHr remained in the targeted range (30.9 ―32.4 pg) throughout the study period in both groups (Table 3, Table 4). There are a statistically significant difference means of CHr and Hb for Group 1 at baseline and final (Table 4). In group 1, administration of intravenous sacrofer® 100mg, increased the values of all diagnostic tests for iron deficiency, but the increase was not statistically significant for Tsat and Serum Ferritin test, as for CHr, Hemoglobin concentration tests, Serum Iron, and Total Iron-Binding Capacity (TIBC) the differences (between the baseline and final) were statistically significant, P values were 0.0393, <0.0001, <0.0001, and <0.0001 respectively.

Table 3: Participants characteristics.

	Baseline			
	Group 1 (N = 44)	**Group 2 (N = 31)**	**P value**	**P value summary**
Age (years), mean ± SD	49.1 ±14.4	29.7 ±4.8	<0.0001	****
Men, n, (%)	25 (56.8)	19 (61.3)	0.8129	**ns**
Duration of hemodialysis (months)	64.4 ±42.4	0 (0)	<0.0001	****
BMI (kg/m^2), mean ± SD	26.6 ±5.5	28.9 ±2.9	0.0208	*
Diabetes mellitus, n, (%)	5 (11.4)	0 (0)	0.0728	**ns**
Systolic BP (mmHg), median ± SD	130.0 ±16.2	123.2 ± 8.7	0.0062	**
Diastolic BP (mmHg median ± SD	77.7 ±13.3	71.2 ± 11.7	0.034	*
Cause of CRF (n)				
Hypertension	26			

Diabetes	6					
PCKD	4					
Others	8					
CHr < 29 pg, n, (%)	16 (36.4)		7 (22.6)		0.2182	**ns**
WHO anemia, n, (%)	22 (50.0)		2 (6.5)		0.0001	***
Tsat (%), mean ± SD	38.6	20.3	42.8	16.3	0.1933	**ns**
Serum Ferritin (ng/mL), mean ± SD	345.3	160.8	411.8	173.1	0.1494	**ns**
CHr (pg) mean ± SD	30.9	3.5	31.9	2.4	0.0446	*
Hb (g/dL) mean ± SD	11.1	3.1	14.5	1.6	<0.0001	****
Serum Iron (µg/dL) median ± SD	57.9	24.6	95.0	30.2	<0.0001	****
TIBC (µg/dL) median ± SD	181.7	39.9	302.7	61.6	<0.0001	****
BUN mg/dL median ± SD	56.8	14.9	14.7	7.5	<0.0001	****
Creatinine (mg/dL), median ± SD	8.1	2.2	1.4	1.6	<0.0001	****
Urea mg/dL median ± SD	123.4	28.9	14.9	3.6	<0.0001	****
ALT(IU/L) median ± SD	20.5	7.2	15.8	3.8	0.0025	**
AST (IU/L) median ± SD	21.3	10.4	17.0	4.5	0.0102	*

± SD: Standard Deviation.	Hb: Hemoglobin concentration.
AST: Aspartate Aminotransferase test.	PCKD: Polycystic kidney disease.
ALT: Alanine Aminotransferase test.	TIBC: Total iron-binding capacity.
BUN: Blood Urea Nitrogen test.	Tsat: Transferrin saturation.
BP: Blood Pressure.	Urea: Blood Urea concentration.
CHr: Reticulocyte hemoglobin content test.	WHO: World Health Organization.
Creatinine: Creatinine blood concentration.	

Table 4: Iron parameters of Group 1 at Baseline and Final of study.

	Group 1			
	Baseline (N = 44)	**Final (N = 41)**	**P value**	**P value summary**
WHO Anemia, n, (%)	22 (50.0)	6 (14.6)	0.0006	***
CHr < 29, n (%)	16 (36.4)	4 (9.8)	0.0047	**
Tsat (%), Mean, (±SD)	38.6 ±20.29	42.1 (±24.2)	0.6256	**ns**
Serum Ferritin (ng/mL), Mean, (±SD)	345.3 (±160.8)	405.2 (±258.7)	0.3919	**ns**
CHr (pg), Mean, (±SD)	30.9 (±3.541)	32.4 (±2.2)	0.0393	*
Hb (g/dL), Mean, (±SD)	11.1 (±3.07)	14.1 (±1.6)	<0.0001	****
Serum Iron (µg/dL), Mean, (±SD)	57.9 (±24.59)	115.7 (±48.0)	<0.0001	****
TIBC (µg/dL), Mean, (±SD)	181.7 (±39.86)	234.3 (±49.8)	<0.0001	****
BUN (mg/dL), Mean, (±SD)	56.82 (±14.86)	55.44 (±14.74)	0.7576	**ns**
Creatinine (mg/dL), Mean, (±SD)	8.127 (±2.215)	8.154 (±1.621)	0.8869	**ns**
Urea (mg/dL), Mean, (±SD)	123.4 (28.86)	119.2 (±29.87)	0.5043	**ns**
ALT(IU/L), Mean, (±SD)	20.52 (±7.235)	19.22 (±6.646)	0.4032	**ns**
AST (IU/L), Mean, (±SD)	21.27 (±10.38)	22.24 (±5.704)	0.1329	**ns**

Table 5 shows a comparison between patients and control groups. All tests of diagnosis iron deficiency, did not show a statistically significant difference between the two groups (patients and control) at the end of the study. This can be explained by giving iron to the group of patients, which led to raising their iron levels. It can also be observed that there are fundamental differences in (BUN, Creatinine, Urea, ALT, and AST) levels, because group 1 suffers from chronic kidney failure and group 2 is healthy young adults.

Table 5: Iron and other parameters by patient and control groups.

	Final			
	Group 1 (N = 44)	**Group 2 (N = 31)**	**P value**	**P value summary**
WHO Anemia, n, (%)	6 (14.6)	2 (6.45)	0.4523	**ns**
CHr < 29, n (%)	4 (9.8)	3 (9.67)	>0.9999	**ns**
Tsat (%), Mean, (±SD)	42.1 (±24.2)	40.9 (±9.577)	0.3913	**ns**
Serum Ferritin (ng/mL), Mean, (±SD)	405.2 (±258.7)	387.2 (±158.8)	0.881	**ns**
CHr (pg), Mean, (±SD)	32.4 (±2.2)	32.2 (±1.408)	0.7494	**ns**
Hb (g/dL), Mean, (±SD)	14.1 (±1.6)	14.4 (±1.198)	0.2465	**ns**
Serum Iron (µg/dL), Mean, (±SD)	115.7 (±48.0)	96.8 (±21.07)	0.1835	**ns**
TIBC (µg/dL), Mean, (±SD)	234.3 (±49.8)	311.9 (±46.45)	<0.0001	********
BUN (mg/dL), Mean, (±SD)	55.4 (±14.7)	14.7 (±4.193)	<0.0001	********
Creatinine (mg/dL), Mean, (±SD)	8.2 (±1.6)	1.3 (±0.9175)	<0.0001	********
Urea (mg/dL), Mean, (±SD)	119.2 (±29.9)	15.0 (±2.412)	<0.0001	********
ALT(IU/L), Mean, (±SD)	19.2 (±6.6)	15.6 (±2.254)	0.0581	**ns**
AST (IU/L), Mean, (±SD)	22.2 (±5.7)	17.0 (±1.91)	<0.0001	********

The results of the group that received iron courses (N=16) were analyzed, Tsat and Serum Ferritin results showed a noticeable increase but were not statistically significant. The difference was significant only for CHr and Hemoglobin concentration CHr, Hemoglobin concentration tests, Serum Iron, and Total Iron-Binding Capacity (TIBC) the differences with a P-value <0.0001 for each test (table6).

Table 6: Iron parameters for patients with CHr < 29 pg, before and after IV iron administration.

	Patients Given IV Sacrofer (N = 16)			
	Baseline	**Final**	**P value**	**P value summary**
WHO Anemia, n, (%)	16 (100)	3 (18.8)	<0.0001	********
CHr < 29, n (%)	16 (100)	1 (6.3)	<0.0001	********
Tsat (%), Mean, (±SD)	31.6 (±21.2)	39.2 (±27.4)	0.5391	**ns**
Serum Ferritin (ng/mL), Mean, (±SD)	291.9 (±170.6)	392.6 (±284.0)	0.445	**ns**
CHr (pg), Mean, (±SD)	26.8 (±2.2)	32.2 (±2.3)	<0.0001	********
Hb (g/dL), Mean, (±SD)	7.7 (±0.6)	13.7 (±1.6)	<0.0001	********
Serum Iron (µg/dL), Mean, (±SD)	37.0 (±13.7)	110.9 (±54.2)	<0.0001	********
TIBC (µg/dL), Mean, (±SD)	147.5 (±27.2)	231.0 (±48.9)	<0.0001	********
BUN (mg/dL), Mean, (±SD)	52.9 (±11.8)	58.5 (±16.0)	0.3363	**ns**
Creatinine (mg/dL), Mean, (±SD)	8.0 (±2.1)	8.0 (±1.5)	0.6619	**ns**
Urea (mg/dL), Mean, (±SD)	119.1 (±27.4)	122.9 (±34.3)	0.9778	**ns**
ALT(IU/L), Mean, (±SD)	21.3 (±6.5)	19.9 (±5.9)	0.5439	**ns**
AST (IU/L), Mean, (±SD)	20.4 (±4.9)	21.8 (±5.4)	0.5685	**ns**

CHr change of response markers

Table 7 summarizes the impact of a one-unit change in Tsat, Serum Ferritin, Hb, Serum Iron, TIBC, BUN, Creatinine, Blood Urea concentration, ALT, AST, Age, Duration of Dialysis, Body Mass Index, Systolic BP, Diastolic BP (referred as response markers) on CHr change. From the table, it can be observed that, Serum Ferritin has no significant correlation with the change in CHr, (P = 0.5863).

All Response Markers showed no statistically significant correlation with CHr except (WHO Anemia, CHr < 29, Tsat, Hb, Serum Iron, and TIBC), this can be explained by the high sensitivity of the CHr test to diagnose iron deficiency. In Tsat, Hb, Serum Iron, TIBC increase influenced CHr change regardless of treatment. For every 1% increase in Tsat, CHr increased by only 0.2231 pg. Every 1g/dL increase in Hb, CHr increased by only 0.5941pg. Every 1 µg/dL increase in Serum Iron, CHr increased by only 0.3973 pg. Every 1 µg/dL increase in TIBC, CHr increased by only 0.3223 pg. All other Response markers had no statistically significant correlation with the change in CHr. 95% confidence interval for each response marker showed in Table 7.

Table 7: Impact of a one-unit change in each response marker on change in Reticulocyte hemoglobin content test.

Response Marker	r *(95% confidence interval)*	P value	P value summary
Age (years)	0.01833 *(-0.1440 to 0.1797)*	0.8256	**ns**
Gender	0.1144 *(-0.04837 to 0.2713)*	0.1676	**ns**
Body Mass Index (kg/m2)	0.02317 *(-0.1392 to 0.1844)*	0.7806	**ns**
Diabetes mellitus	0.08988 *(-0.07307 to 0.2482)*	0.279	**ns**
Systolic BP (mmHg)	-0.1205 *(-0.2770 to 0.04223)*	0.146	**ns**
Diastolic BP (mmHg)	-0.1156 *(-0.2724 to 0.04716)*	0.1632	**ns**
Duration of hemodialysis (months)	-0.07329 *(-0.2324 to 0.08966)*	0.3776	**ns**
WHO Anemia	-0.5335 *(-0.6401 to -0.4067)*	<0.0001	********
CHr < 29 (n)	-0.6911 *(-0.7671 to -0.5958)*	<0.0001	********
Tsat (%)	0.2231 *(0.06348 to 0.3716)*	0.0066	******
Serum Ferritin (ng/mL)	0.04525 *(-0.1175 to 0.2056)*	0.5863	**ns**
Hb (g/dL)	0.5941 *(0.4783 to 0.6897)*	<0.0001	********
Serum Iron (µg/dL)	0.3973 *(0.2516 to 0.5254)*	<0.0001	********
TIBC (µg/dL)	0.3223 *(0.1692 to 0.4601)*	<0.0001	********
BUN (mg/dL)	-0.02436 *(-0.1855 to 0.1381)*	0.7696	**ns**
Creatinine (mg/dL),	-0.05546 *(-0.2154 to 0.1074)*	0.5047	**ns**
Urea (mg/dL)	-0.06273 *(-0.2224 to 0.1002)*	0.4504	**ns**
ALT(IU/L)	-0.1275 *(-0.2836 to 0.03508)*	0.1237	**ns**
AST (IU/L)	0.00411 *(-0.1579 to 0.1659)*	0.9606	**ns**

Table 8: Sensitivity, specificity, PPV, NPV, likelihood ratio and Accuracy of (Tsat, Serum Ferritin, CHr, Serum Iron, TIBC).

	Tsat	Serum Ferritin	CHr	Serum Iron	TIBC
Sensitivity	23.91%	10.87%	60.87%	58.70%	73.91%
Specificity	92.08%	93.07%	98.02%	84.16%	55.45%
Positive Likelihood Ratio	3.02	1.57	30.74	3.71	1.66
Negative Likelihood Ratio	0.83	0.96	0.4	0.49	0.47
Disease prevalence (*)	31.29%	31.29%	31.29%	31.29%	31.29%
Positive Predictive Value (*)	57.89%	41.67%	93.33%	62.79%	43.04%
Negative Predictive Value (*)	72.66%	69.63%	84.62%	81.73%	82.35%
Accuracy (*)	70.75%	67.35%	86.39%	76.19%	61.22%

In iron deficiency anemia, hemoglobin, ferritin and transferrin saturation become abnormal, and decrease in iron stores is reflected by falling serum ferritin. Decrease in serum iron and increase in transferrin (TIBC) are difficult to correlate and document. From Table 8, serum iron and TIBC had lack specificity and are time consuming, expensive and these add no additional help in diagnosis. As well as Tsat and serum ferritin have the low sensitivity compered to CHr, Serum Iron, TIBC. Serum iron, although routinely used for diagnosis of iron deficiency along with transferrin (TIBC) and transferrin saturation, lacks correlation with the disease. Likewise, transferrin (TIBC) levels display variations in disorders of iron metabolism and are decreased in chronic inflammatory disorders and malignancies.[21] In this study, hemoglobin concentration was taken as the gold standard. CHr had sensitivity 60.87% and specificity 98.02%. Serum iron had sensitivity of 58.70% and specificity of 84.16%, while transferrin (TIBC) had sensitivity of 73.91% and specificity of 55.45. However, in study by Peter et al. in Canada showed a relatively good correlation between ferritin and TIBC.[22] while a weak correlation was seen between ferritin and TIBC in a study by Schuepbach et al.[23] This study indicated that serum iron, transferrin (TIBC), and transferrin saturation had poor sensitivity and specificity for the diagnosis of iron deficiency state. Moreover, owing to more time on the analysis of these parameters plus expenses incurred on their reagent purchase are strong evidences against their utility for diagnosis of iron deficiency state. Limitations of serum ferritin in chronic inflammation, malignancy or chronic infection can be catered for by combing ferritin with other hematological parameters like CHr, and other RBC indices.

The variability of the tests was compared, by measured longitudinally for the six iron deficiency diagnosis tests, and it was expressed as coefficient of variation (%CV). CHr was found to have significantly less variation than the other tests, with a CV of 8.31% compared to hemoglobin concentration 18.99%, TIBC 29.07%, Serum iron 44.41%, transferrin saturation of 46.19% and serum ferritin of 50.67% (figure 1). Recalculation of CV, excluding post-intravenous iron treatment results, yielded 8.451% for CHr, compared to hemoglobin concentration 19.74%, TIBC 29.68%, Serum iron 42.72 %, transferrin saturation at 43.1% and serum ferritin at 47.6%.

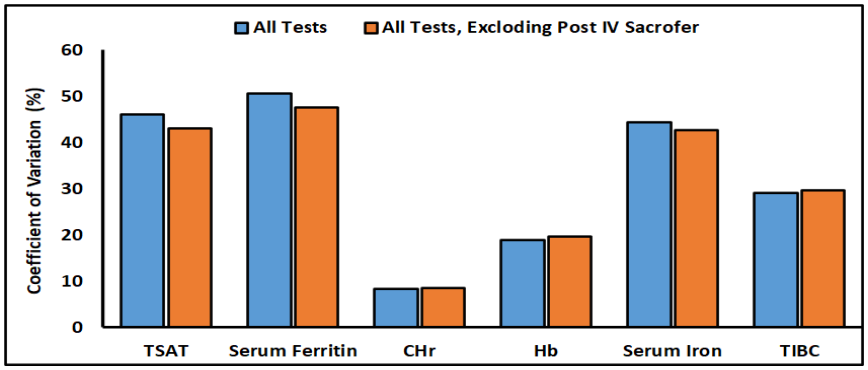

Figure 1: Coefficient of variation (CV) for the six tests. Blue bars represent tests of all Groups at baseline and final, orange bars represent tests of all Groups at baseline and final with excluding tests performed after intravenous iron treatment (Sacrofer).

Figure 2 shows the mean and standard deviation of the four-iron deficiency diagnostic tests, for group 1 at baseline and final, in addition control group. The difference in the error bar for each test is clear, Since CHr has the smallest error bar, and then, in the second place, the hemoglobin concentration test. TSAT and Ferritin both have the biggest error bars, this is an indication that Tsat and Serum Ferritin tests are less accurate than CHr.

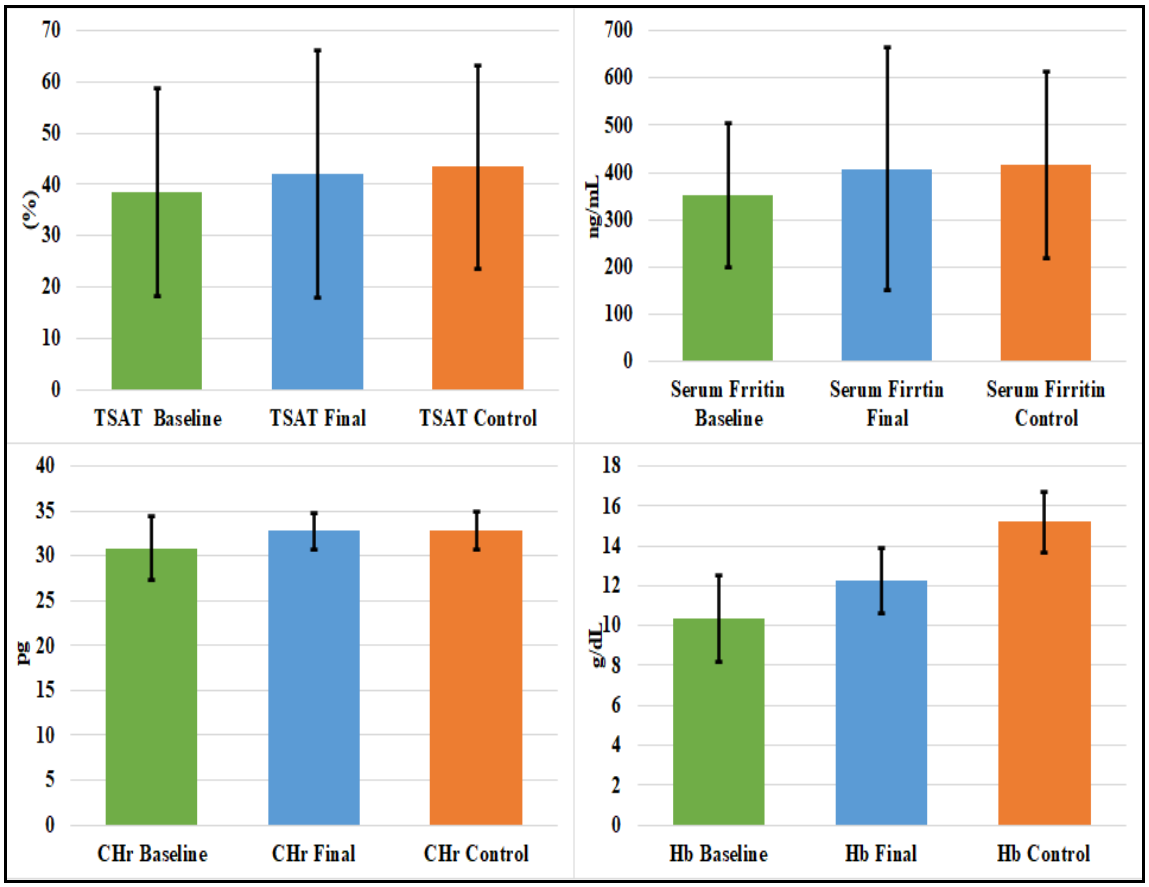

Figure 2: Mean and standard deviation of Transferrin saturation (Tsat), ferritin test, Reticulocyte hemoglobin content (CHr), Hemoglobin concentration for group 1 at baseline, final, and control group.

DISCUSSION

Reticulocyte hemoglobin content (CHr) may be the most accurate iron deficiency test.[24,25] In several recent studies, conducted on hemodialysis patients with iron deficiency, suggested that using the CHr test is the most accurate and effective test in this field.[26-28] Fishbane et al found that, the CHr test had a sensitivity of 100% and a specificity of 80%, the study was conducted on 32 hemodialysis patients.[19] These studies and others have demonstrated the promising advantages of using the CHr test in diagnosing iron deficiency in hemodialysis patients.

Our study aims to compare the CHr test with the serum ferritin, Transferrin saturation (Tsat), Hemoglobin concentration, Serum Iron, and Total Iron-Binding Capacity (TIBC) tests, as a guide to iron management in hemodialysis patients.

The variability (%CV) of CHr was found to have significantly less variation than the other tests, with a CV of 8.31% compared to hemoglobin concentration 18.99%, TIBC 29.07%, Serum iron 44.41%, transferrin saturation of 46.19% and serum ferritin of 50.67%. Recalculation of CV, excluding post-intravenous iron treatment results, yielded 8.451% for CHr, compared to hemoglobin concentration 19.74%, TIBC 29.68%, Serum iron 42.72 %, transferrin saturation at 43.1% and serum ferritin at 47.6%.

CHr had sensitivity 60.87% and specificity 98.02%. Serum iron had sensitivity of 58.70% and specificity of 84.16%, while transferrin (TIBC) had sensitivity of 73.91% and specificity of 55.45.

The main findings of this study are that the: (1) CHr test has a lower variability than hemoglobin concentration, Transferrin saturation, and Serum ferritin; (2) CHr test showed higher specificity and higher accuracy compared to other tests Table 8; (3) The diagnosis of iron deficiency using CHr, led to less use of intravenous iron compared to TSAT and Serum ferritin tests. The goal of administering sacrofer® 100mg to hemodialysis patients is to maintain the levels of CHr at the targeted range (30.9 — 32.4 pg). We found that the CHr test is much more stable than the Tsat, Serum Ferritin, and hemoglobin concentration tests. The coefficient of variation for CHr was on the order of 90% lower than for the other tests. The high variability value in the TSAT and Serum ferritin tests indicates a significant problem in the diagnosis of iron deficiency for hemodialysis patients.

We expect that iron management based on the CHr test will reduce the costs of purchasing iron doses, and most importantly it reduces the possibility of misdiagnosis and thus reduces the incidence of side effects of iron overload. In conclusion, we have found that, iron deficiency management based on CHr is simple and practical to perform. The test may be performed during routine blood testing hemoglobin and hematocrit, by the same hematology analyzer, at a little incremental cost. Compared to management based on serum ferritin and transferrin saturation. The superior performance of CHr might be explained by a far lower level of test variability compared to serum ferritin and transferrin saturation.

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