Tropical Journal of Nephrology Vol.15 No. 1, June, 2020 43 - 52

Evaluation of Iron Status amongst Multiple Transfused Chronic Kidney Disease Patients at the Lagos State University Teaching Hospital, (LASUTH) Ikeja and Gbagada General Hospital

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ABSTRACT

Introduction: Anaemia is defined by the World Health Organization (WHO) as a haemoglobin (Hb) concentration of less than 13g/dl in adult males and non-menstruating women and less than 12g/dl in menstruating women. Most end stage renal disease (ESRD) patients in our facility present with severe anaemia requiring multiple blood transfusions. In the course of treatment, especially when on dialysis, these patients are also placed on iron therapy and erythropoietin. Iron studies are not conducted routinely in Nigeria mainly due to the high cost of the tests. It is hypothesized that these patients may be iron overloaded and may require chelation therapy. There is a paucity of literature on iron status evaluation amongst chronic kidney disease (CKD) patients in Nigeria. This research determined the iron profile of this group of patients in our facility and the findings may provide recommendations on their management.

Aims: To determine the levels of commonly used iron indices and the risk of iron overload in multiple transfused chronic kidney disease patients attending the Lagos State University Teaching Hospital (LASUTH) and Gbagada General Hospital all situated in Lagos.

Methodology: This was a cross sectional hospital based comparative study of 83 multiple transfused CKD patients, 46 CKD controls and 36 normal controls. Recruitment was carried out for 3 months. Blood samples were analyzed for common iron indices using standard laboratory techniques.

Results: A total of 83 multiple transfused (MT) patients were studied. There were 43(52%) males and 40(48%) females. The non- transfused CKD control group (NT CKD) were 46 with 27(59%) males and 19(41%) females, while the normal control group were 36 with 16(44%) males and 20 (56%) females. The mean ferritin in the MT was 198.44±145.92ng/ml while it was 128.51±155.23ng/ml and 60.56±48.49ng/ml in the NT CKD and normal controls respectively ($p<0.001$). The mean transferrin saturation (TSAT) was as follows: MT = 38.84±13.91%, NT CKD= 43.87±10.32%, normal controls= 40.58± 6.73% ($p=0.069$). Only 1(1.2%) of the study group had functional iron deficiency, and 1 (2.2%) of the CKD control had an iron overload. Despite multiple blood transfusions, none of the patients in the study group had an iron overload.

Conclusion: The risk of iron overload was found to be low in the study group.

INTRODUCTION

Anaemia is a common complication of chronic kidney disease (CKD) resulting in significant morbidity and mortality. [1-3] The World Health Organization (WHO) has defined anemia as a haemoglobin (Hb) concentration of less than 13g/dl in men (15 years of age and above) and less than 12g/dl in non pregnant women (15 years of age and above).[4] Anaemia in chronic kidney disease (CKD) was defined by the

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European Renal Best Practice Guidelines (ERBP) [5] in 2009 as Hb below 11.5g/dl and 13.5g/dl (< 12g/dl in those aged >70yrs) in women and men respectively. The KDIGO guidelines suggest a diagnosis of anaemia in adults with CKD when Hb concentration is <13g/dl in males and <12g/dl in females.[6] Blood transfusions may be required in patients with severe symptomatic anaemia. Vincent *et al* [7] in a multi-center study carried out on critically ill patients, reported that anaemia was common and usually required blood transfusion. It was also observed that patients who received transfusions had a higher mortality rate.[7] Chronic blood transfusions, though essential in many patients with chronic anaemia such as ß-thalassemia, myelodysplastic syndrome and sickle cell disease (SCD), may cause iron overload. This is because every unit of transfused blood contains 200-250 mg of iron and the human body has no active mechanism to excrete excess iron.[8,9] Since patients with anaemia of chronic kidney disease are multiple transfused in this environment, the possibility of iron overload occurring in them also exists.

In developing countries, patients with CKD commonly present in end-stage renal disease (ESRD) with life threatening symptomatic anaemia, necessitating urgent blood transfusions. A study was done in Nigeria by Ulasi *et al*, [10] and they reported a mean haematocrit concentration of 22% amongst patients in stage 4 and 5 CKD. This severe anemia reported in developing countries is due to late referrals and the poor socio-economic status of most patients making it difficult for them to purchase erythropoietin (EPO) at the early stages of anaemia. Multiple co-morbidities such as parasitoses, malnutrition, and haemoglobinopathies may also contribute to anaemia severity. Most patients are referred to nephrology unit late, and many of them would have had multiple transfusions. Most centers in Nigeria do not check iron status routinely as this would mean additional cost to the patients and so anemic patients continue to have blood transfusions, a situation which can lead to haemosiderosis or haemochromatosis. Some of these patients may require chelation to prevent iron overload.

There is a dearth of research on iron status evaluation amongst CKD patients in Nigeria. This research determined the iron profile of this group of patients in our facility and the findings may provide recommendations on their management. This work also provides useful information on iron haemodynamics in non - CKD 'healthy' Nigerians. It is hoped that this work will stimulate interest in this area of research.

PATIENTS, MATERIALS AND METHOD

This was a cross sectional hospital based comparative study. All patients aged 18years and above in stage 3 to 5 CKD managed in the medical outpatient (MOP), medical wards and dialysis units who satisfy the inclusion criteria were recruited. A convenience sampling was employed to enrol participants until the desired sample size was achieved. Subjects with haemoglobinopathies were excluded after haemoglobin electrophoresis. Subjects who had recent transfusions were included after 72hours.

Controls: Two control groups were used:

a. Group 1 was made up of all chronic kidney disease patients in stages 3 to 5 with no history of blood transfusion seen in MOP and medical wards during the study period.
b. Group 2 was made up of healthy volunteers with no history of CKD. Absence of kidney injury in normal controls was determined using MICROALBU strips manufactured by Erba Lachema: all those with urinary albumin of greater than 30mg/g were excluded. Controls with haemoglobinopathies were excluded after Hb electrophoresis. Recruitment was done until desired sample size was achieved.

Elevated C-reactive protein (CRP) and leucocytosis were used to exclude patients with inflammation in this study.

An interviewer-administered questionnaire was used to record socio-demographic data, risk factors for CKD, number of blood transfusions, use of ESA and iron therapy, physical examination findings and laboratory findings. The haemoglobin and haematocrit values at initial evaluation were obtained from their case records. Serum iron, TIBC, CRP, electrolytes, urea and creatinine were analysed using the CX5 Beckman/Coulter automated machine.

Serum ferritin was analysed using the Beckman/Coulter access machine.

Transferrin saturation (TSAT) was calculated using the formula:

TSAT = SERUM IRON/TIBC * 100.

Normal values[11]
WBC count=4000-11,000cells/cmm
Serum iron=50-150microgram/dl
TIBC =240-450µg/dl

Ferritin
Male adults=25-250ng/ml
Female adults=20-200ng/ml
TSAT=25-50%
CRP= <10mg/L

Operational definitions [12]
Absolute iron deficiency= serum ferritin<100ng/ml with TSAT < 20%.
Functional iron deficiency= normal ferritin levels with TSAT <20%.

Reticulo-endothelial blockade or anaemia of chronic disease=TSAT<20% and ferritin>800ng/ml. Risk of iron overload=TSAT> 50% and ferritin> 800ng/ml [13]

Data were analyzed using SPSS version 17.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill), a statistical computer software.

Descriptive statistics (minimum, maximum, mean, and standard deviation) were determined for all indices and other appropriate variables. Proportions and percentages were calculated for categorical variables.

Student's t-test, one-way analysis of variance (ANOVA), and parametric inferential statistical procedure were used to compare the means of groups of patients. *P*-values less than 0.05 were considered to be statistically significant (95% confidence level).

RESULTS

Characteristics of the Study Groups

A total of 90 multiple transfused subjects were recruited into the study. However, only 83 were eligible for data analysis as seven of them were excluded for various reasons such as incompletely filled questionnaires, incomplete laboratory results, presence of leukocytosis and elevated C- reactive protein. Their mean age was 48.2± 14.6 years (range 19 – 80 years) with a male to female ratio of 1.08: 1.

A total of 50 CKD controls were recruited but 46 were eligible for data analysis. Four were excluded due to incomplete laboratory results. The mean age was 52.63± 15.5 years (range 27- 84 years) with male: female of 1.42:1. A total of 40 normal controls were recruited into the study but 4 were excluded for incomplete laboratory results. Only 36 were eligible for data analysis. They had a mean age

Table 1: The demographic characteristics of the subjects and the controls

CHARACTERISTICS		MULTIPLY-TRANSFUSED	CKD CONTROLS	NORMAL CONTROLS	
Mean Age (Yrs)	M	39.94±12.70	47.93±14.57	52.56±16.02	F=3.685 p=0.0293
	F	48.48±14.74	52.74±15.12	36.25±11.41	F=7.557 p=0.0010
Mean Hb Concentration	M	5.06±1.03	9.67±2.91	12.36±0.8	F=113.3 p=<0.0001
	F	5.05±1.31	9.7±2.2	10.39±0.94	F=107.9 p=<0.0001
Mean PCV	M	17.95±3.54	32.03±8.74	42.24±3.19	F=123.4 p=<0.0001
	F	17.92±4.33	31.0±6.68	34.93±2.74	F=105.1 p=<0.0001
Mean serum creatinine		8.51±5.35	4.90±4.43	0.77±0.13	p<0.001*
Mean WBC		5.73±2.04	5.93±2.25	5.19±1.22	p = 0.223
Mean CRP		2.49±2.25	2.00±1.99	2.89±2.79	p = 0.210

**ANOVA test statistically significant at 95% confidence level*

Table 2: Transfusion pattern in multiple transfussed participants and their CKD stage

No. of transfusions (3 months)	STAGE 3 CKD	STAGE 4 CKD	STAGE 5 CKD	Total
2 – 5	3	8	47	58
6 – 10	1	0	13	14
> 10	0	1	10	11
Total	**4**	**9**	**70**	**83**

Most of the study subjects were in stage 5 CKD and were transfused with 2 to 5 pints of blood only.

Table 3: Comparison of iron usage between multiple transfused CKD patients and non transfused patients

	N	Min	Max	Mean±SD	Confidence Interval Lower	Upper	p-value
Parental Iron (mg) (3 months):							
Multiple transfused	83	100	1100	225.30±307.96	3.64	232.50	0.057
CKD Control	46	100	2100	110.87±352.91			
Total dose of oral iron (3 months):							
Multiple transfused	83	400	3400	1177.11±831.88	101.58	776.81	0.131
CKD Control	46	100	5880	839.48±1689.41			

**Student's t-test statistically significant at 95% confidence level*

Table 4: Prevalence of iron deficiency and iron overload in the study subjects and CKD controls

	Multiple transfused	CKD Control
Prevalence of absolute iron deficiency (Ferritin <100mgl/ml and TSAT <20%)	None	None
Prevalence of functional Iron deficiency (Ferritin >100mgl/ml and TSAT <20%)	1(1.2%)	None
Prevalence of iron overload (ferritin >1000ng/ml and TSAT >50%	None	1(2.2%)

By definition (KDOQI), only 1.2% of the study subjects had functional iron deficiency while none of the CKD controls had iron deficiency. One (2.2%) CKD control had an iron overload but none of the study subjects had iron overload.

Table 5: Comparison of transferrin saturation and ferritin levels between multiple transfused and control groups

	Multiple transfused CKD patients	Non transfused CKD patients	Normal controls	
Transferrin Saturation (%)	38.84±13.91	43.87±10.32	40.58±6.73	0.069
Ferritin (ng/ml)	198.44±145.92	128.51±155.23	60.56±48.49	<0.001*

The multiple transfused group had ~~a~~ higher mean ferritin compared to normal controls. The mean transferrin saturation was however similar in both groups.

Table 6: Serum ferritin in study participants with respect to age and gender

Age Groups	Sex	Multiple Transfused	CKD Controls	Normal Controls	P-value
<40	M	255.83±168.54	253.19±310.75	78.57±34.0	0.165
	F	148.33±101.55	52.5±33.29	40.17±47.49	0.007*
40-59	M	211.0±165.54	105.79±75.48	94.29±46.85	0.076
	F	173.0±142.81	119.94±133.05	47.5±48.53	0.067
60 and above	M	231.6±121.08	121.27±66.62	54.0±65.05	0.018*
	F	176.0±140.71	76.40±44.91	—-	0.156

**ANOVA test statistically significant at 95% confidence level*

of 37.9± 12.0 years (range 18- 60 years) with a male to female ratio of 0.8: 1.

The mean ferritin was lower in females compared to males in all the groups. The mean was highest in the males less than 40 years compared to older males as opposed to what is seen in females: with the older females having higher mean ferritin than the younger ones.

DISCUSSION

Patients with chronic kidney disease most often present with anaemia of chronic disease due to reduced erythropoietin production. A few of them present with iron deficiency anaemia due to anorexia, vomiting, upper GI bleeding, blood losses from frequent blood sampling and during the haemodialysis procedure. Hemodialysis patients are also predisposed to iron deficiency because they are often on erythropoietin therapy which increases iron requirements in them. Since iron deficiency is the most common cause of erythropoietin (EPO) resistance, the detection and correction of iron deficiency are essential in managing renal anaemia.

The study subjects were managed with multiple blood transfusions because they presented with uraemia and severe symptomatic anaemia. They required urgent haemodialysis and correction of haemodynamic instability with blood transfusions. Severe anaemia is reported in this study with a mean Hb of 5.06 ±1.17 before blood transfusion. In contrast, the CKD controls who were predominantly predialysis patients had moderate anaemia with a mean Hb of 9.67±2.63. This severe anaemia reported in this study is similar to what was reported in other Nigerian studies. Ulasi, *et al* [10] reported a mean haematocrit of 22% amongst patients in stage 4 and 5 CKD while Akinsola *et al* [14] reported anaemia in predialysis patients with mean haematocrit of 24.1± 6.7%. Oluboyede *et al* [15] also described moderately severe anaemia in chronic renal failure with a mean Hb of 7.4%.

It was hypothesized that the study subjects may be iron overloaded because of frequent blood transfusions since iron overload was reported in the pre-erythropoeitin era when blood transfusions were used for the treatment of renal anaemia[13].

It was however noted that most of our study subjects had 2 to 5 pints of blood in 3 months, without optimal correction of their anaemia. Iron overload was also found to be low in this study group.

The mean TSAT were within normal limits in all the groups and there were no age or sex variation. Transferrin saturation (TSAT) measures the iron available in blood circulation but has the limitation of significant diurnal variation due to variation in serum iron concentration. It may vary from 15% to 70% based on the time of sampling [16]. Transferrin saturation (TSAT) is the most commonly used measure of iron available for erythropoiesis. The KDIGO guidelines recommend percentage hypochromic red blood cells or reticulocyte haemoglobin content in the evaluation of iron status but a combination of TSAT and serum ferritin may be used if the above two are not available. The results of mean TSAT in the study subjects was within the normal limits and similar to the findings in both control groups. This normal mean TSAT value may be explained by the administration of iron (RBC) in the multiple transfused group and iron therapy in the CKD controls resulting in partial correction of iron deficiency. This finding concurs with that reported

by Kaneko *et al* [17] in Japanese haemodialysis patients.

The mean ferritin was highest in the male study subjects below the age of 40 years compared to the older males. This pattern is seen in the controls as well though the normal controls had relatively lower values which are within normal in healthy individuals. Conversely, the female subjects had lower mean ferritin compared to the older females and this pattern is also seen in the controls. Normal ferritin concentration varies by age and sex [18,19] and starting from adolescence, males have higher values than females and this persists into late adulthood. Values in men peak between 30 and 39 years and tend to remain constant until about 70 years. Among women, serum ferritin values remain relatively low until menopause and then rise. Our observation in the CKD patients is similar to the finding by Casale *et al* [20] who reported that serum ferritin was higher in males than females in every age and they also noted an age related tendency to rise as a result of activation of the reticuloendothelial system and due to the increase of iron storage with ageing.

Ferritin is a reliable guide to body iron stores[13]. Aljama *et al* [21] found serum ferritin useful in detecting iron overload with the observation that all patients with serum ferritin above 300ng/ml had increased iron stores. The elevated ferritin values among the multiple transfused group compared to the low values among the controls in this study is not likely to be due to inflammation as the observed mean CRP values in all the groups were within the normal values stated for this study[11]. It may therefore be inferred that iron infusions (RBC and iron therapy) were responsible for the increased iron stores observed in both CKD groups compared to the normal controls. The normal mean TSAT and higher ferritin values in the study subjects and CKD controls compared to the normal control further confirms the effect of iron supplementation on the iron haemodynamics in the CKD patients studied. Similarly, Kirchbaum [22] in a retrospective evaluation of iron parameters in patients on chronic haemodialysis receiving intravenous iron therapy and erythropoietin found a 125% increase in ferritin levels over 4 years.

The mean ferritin values of patients in the multiple transfused group and CKD control males fell within the recommended ferritin targets by EBPG [23] and KDOQI [13] however the mean values for females in both groups however fell short of the target. This finding may be due to physiological iron losses peculiar to females (menstrual losses and pregnancy) even before the onset of chronic kidney disease.

A study by Nakanishi *et al* [24] in Japan shows that the relationship between ferritin body iron stores may be altered in inflammatory states but it is still a good measure of the amount of iron stored in the body. Fernandez- Rodriguez *et al* [25] found serum ferritin to be the most effective means of detecting depleted iron stores in stable chronic renal failure patients not receiving erythropoietin although they did not find TSAT to be a reliable marker of iron deficiency in them. Kalantar- Zadeh *et al* [26] reported that the determination of both serum ferritin concentration and TSAT could achieve high sensitivity and specificity.

Kalantar- Zadeh *et al* [27,28] found serum ferritin of greater than 500ng/ml in half of all maintenance haemodialysis patients at a large center which is a departure from the relatively lower ferritin values in this study. The lower values in this study may be due to the poor socioeconomic condition prevalent among our patients preventing proper nutrition and regular procurement of intravenous iron: or perhaps the cutoffs may be lower in this environment as their premorbid iron status is not known. Further studies, such as nutritional status assessment are required to shed more light in this area.

Using a TSAT of 50% as cutoff and serum ferritin >800mg, none of the multiple-transfused patients in the present study was at risk of iron overload and only one CKD control was at risk of iron overload with serum ferritin of 1000.5ng/ml and TSAT of 65%. These figures are much lower than that reported by Maiz *et al* [3], who observed iron overload in 14 (6.14%) of 228 patients on chronic haemodialysis; 13 of whom were on either iron therapy or blood transfusion. The study by Maiz stated that most of the patients required regular transfusion over 49.6 months, however, the total dose of iron received by the patient was not reported. In contrast, the patients in this study were followed up for only 3 months and the average iron infusion in them was 400mg in that period. Only 1.2% of the multiple transfused patients in this study had functional iron deficiency. The results in this study are quite different from those of Post *et al* [29] who found a

29% prevalence of absolute iron deficiency among predialysis patients on erythropoietin and 26% of patients without erythropoietin entering a new dialysis unit. The patients were however not on iron infusions as were some of the predialysis patients in this study. The iron deficiency may be explained by the erythropoiesis stimulating effect of erythropoietin in the erythropoietin group. The low prevalence of iron deficiency in this study may be due to the alteration in TSAT due to iron supplementation and so presents a picture of partial correction.

An important observation in this study was that majority of the patients did not receive excessive iron through blood transfusion. With a mode of 2 pints of blood which is approximately 400mg of iron, the risk of iron overload is not expected to be high. The amount of iron required to develop iron overload is not known, however it is estimated that the storage limit of the reticuloendothelial system may be exceeded after the accumulation of 5g of iron[30]. The KDOQI guidelines recommend 1000mg loading dose of iron before the commencement of erythropoietin and maintenance doses depend on results of the iron status evaluation and should be individualized [13]. It is therefore obvious that our severely anaemic patients are not adequately infused with iron and are more likely to become iron deficient with erythropoeitin and haemodialysis therapy. The management of anaemia is still very sub-optimal in them and is a likely major contributor to their poor outcomes. Urgent attention is required by all stakeholders to tackle this problem in other to move our practice to acceptable international standards.

LIMITATIONS OF THE STUDY

1. The study was cross- sectional and clinic based and so may not adequately assess the dynamics of iron status in the study participants.
2. Newer methods of assessing iron status such as reticulocyte haemoglobin content (CHr) and percentage hypochromic cells which are more sensitive and specific would have provided more information on the iron status of the participants, but were not assessed in this study.

CONCLUSION

Severe anaemia is common in the study group and its correction was inadequate. The risk of iron overload is also low in them.

Anaemia treatment should be commenced early with iron therapy rather than blood transfusions in patients with asymptomatic anaemia, since the recommended ferritin targets are better met and also to reduce the risks associated with frequent blood transfusions.

Future studies should be conducted in patients who have received at least 20 pints of blood since the risk of iron overload is more likely in them.

Collaborative studies with the nephrology community in other centers in Nigeria which will give results that will be more representative for this environment is recommended.

REFERENCES

1. Fishbane S. Anaemia in chronic kidney disease: status of new therapies. Current opinion in nephrology and hypertension. 2009; 18(2): 112-115.
2. Jurkovits CT, Abramson JL, Vaccarino LV, Weintraub WS, McClellan WM. Association of high serum creatinine and anaemia increases the risk of coronary events: results from the prospective community – based atherosclerosis risk in communities (ARIC study) J Am soc Nephrol. 2003; 14: 2919-2925.
3. Maiz HH, Abderrahim E, Zouaghi K. Anaemia and end-stage renal disease in developing world. Art organs. 2002; 26(9): 760-764.
4. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1) (http://www.who.int/vmnis/indicators/haemoglobin.pdf, accessed [9/16/2020]).
5. Locatelli F, Coric A, Eckardt K, Wiecek A, Vanholder R. Anaemia management in patients with Chronic Kidney Disease: a position statement by the anaemia working group of European Renal Best practice

(ERBP). *Nephrol. Dial. Transplant*. (2009); 24 (2):348 -359.
6. KDIGO clinical practice guidelines for anemia in Chronic Kidney Disease. *Kidney International Supplements* (2012) 2, 331-335; doi:10.1038/kisup.2012.45
7. Vincent JL, Baron JF, Reinhart K, Galtioni L, Thijs L, Webb A, et al. Anaemia and blood transfusion in critically ill patients. *JAMA.* 2002; 288(12):1499-1507.
8. Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato T. Body iron metabolism and pathophysiology of iron overload. *Int J Hematol.* 2008; 88 (1):7-15.
9. Cappellini MD. Exjade® (deferasirox, ICL 670) in the treatment of chronic iron overload associated with blood transfusion. *Ther Clin Risk Manag*. 2007; 3(2):291-299.
10. Ulasi II, Arodiwe EB, Ijoma CK. Left ventricular hypertrophy in African black patients with chronic renal failure at first evaluation. *Ethnicity and disease*. 2006; 16: 859-864.
11. Alagappan R. Manual of Practical Medicine. 3rd ed. New Delhi: Jaypee Brothers; 2007. p 760-761.
12. Adil M, Rubin RJ. Anaemia of chronic kidney disease. In: Christopher S. Wilcox, C. Craig Tisher, Editors. *Handbook of Nephrology and Hypertension.* 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2009. p 340-341.
13. KDOQI. National Kidney Foundation. II: Clinical practice guidelines and clinical practice recommendations for anaemia in chronic kidney disease in adults. Am J Kidney Dis. 2006, 47(5 suppl.3):S16- 85.
14. Akinsola A, Durosimi MO, Akinola NO. The haematological profile of Nigerians with chronic renal failure. *Afr J Med Sci*. 2000; 29(1):13-16.
15. Oluboyede OA, Williams AI. Serum ferritin and other iron indices in adult Nigerians with chronic renal failure-review of management of anaemia. *Afr J Med Sci*.1995; 24(3):231-237.
16. Macdougall IC: CERA (Continuous Erythropoietin Receptor Activator): A new erythropoiesis-stimulating agent for the treatment of anemia. Curr Hematol Rep 2005; 4:436– 440.
17. Kaneko Y, Shigeru S, Hirasawa Y, Gejyo F, Suzuki M. Transferrin saturation versus reticulocyte hemoglobin content for iron deficiency in Japanese hemodialysis patients. *Kidney International.* 2003; 63:1086–1093.
18. WHO. Serum ferritin Concentration for the assessement of iron status and iron deficiency in populations. Vitamin and mineral nutrition information system. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.2). (http:/www.who.int/vinnis/ indicators /serum ferritin. Pdf, accessed [22/7/12]).
19. Vincente C, Porto G, de sousa M. Method for establishing serum ferritin reference values depending on sex and age. *J Lab Clin Med*. 1990; 116(6): 779-784.
20. Casale G, Bonora C, Miglia vacca A, Zurita IE, Nicola P. Serum ferritin and Ageing. *Age Ageing* 1981; 10(2):119-122.
21. Aljama P, Ward MK, Pierides AM, East EJ, Ellis HA, Feest TG et al. Serum ferritin concentration: a reliable guide to iron overload in uremic and haemodialysis patients. *Clin Nephrol.* 1978; 10: 101"104.
22. Kirchbaum B. Serial ferritin concentration in haemodialysis patients receiving intravenous iron. *Clin Nephrol.* 2002; 57(6):452-456.
23. Locatelli F, Aljama P, Canaud B, Carrera F, Eckart KU, Horl WH et al. European Best Practice Working Group. Revised guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant. 2004; 19(2):1- 47.
24. Nakanishi T, Kuragano T, Nanami M, Otaki Y, Nonoguchi H, Hasuike Y. Importance of ferritin for optimizing anemia therapy in chronic kidney disease. *Am J Nephrol.* 2010; 32(5):439-446.
25. *Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Dominguez-Cabrera C, Hortal-Case L,*

Perez-Borges P et al. Diagnosis of iron deficiency in chronic renal failure. Am J Kidney Dis. 1999; 34:508– 513.

26. Kalantar - Zadeh K, Hoffken B, Wunsch H, Fink H, Kleiner M, Luft FC : Diagnosis of iron deficiency anemia in renal failure patients during the post – erythropoietin era. Am J Kidney Dis 1995; 26:292-299.
27. Kalantar - Zadeh K, Kalantar - Zadeh K, Lee GH. The fascinating but Deceptive Ferritin: To measure It or Not to measure It in Chronic Kidney Disease? CJASN 2006; 1(1): 509 – 518.
28. Kalantar - Zadeh K, Regider DL, McAllister CJ, Michael B, Warnock DG. Time - dependent associations between iron and Mortality in Haemodialysis Patients. JASN 2005; 16(10): 3070-3080.
29. Post JB, Wilkes BM, Michelis MF. Iron deficiency in patients with chronic kidney disease. Potential role for intravenous iron therapy. International Urology and Nephrology. 2006; 38: 719-723.
30. Esbach JW, Adamson JW. Iron overload in renal failure patients: changes since the introduction of erythropoietin therapy. Kidney International 1999; 55:s55-s43.