IRON STATUS IN PATIENTS WITH END STAGE RENAL DISEASE ON HAEMODIALYSIS AT KENYATTA NATIONAL HOSPITAL RENAL UNIT.

A DISSERTATION SUBMITTED AS PART FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI.

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DECLARATION

I certify that this dissertation is my own original work and has not been presented for a degree at any other university.

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LIST OF ABBREVIATIONS

ADP - Adenosine diphosphate
ATP - Adenosine triphosphate
BMA - Bone marrow aspirate
BMA - C - Bone marrow aspirate for cytology
CHOIR - Correction of anaemia and outcomes in renal insufficiency trial
CHr - Reticulocyte haemoglobin content
CKD - Chronic kidney disease
CREATE - Cardiovascular risk reduction through early treatment with epoetin beta trial.
CRP - C - Reactive Protein
DNA - Deoxyribonucleic acid
ECF - Extracellular fluid
EDTA - Ethylene diamine tetra-acetic acid
eGFR - Estimated glomerular filtration rate
EPO - Erythropoetin
ESA - Erythropoiesis stimulating agents
ESR - Erythrocyte sedimentation rate
ESRD - End stage renal disease
FBC - Full blood count
g/dl - Grams per decilitre
FE - Iron
HB - Haemoglobin
Hct - Haematocrit
HD - Haemodialysis
IDA - Iron deficiency anaemia
IL - Interleukin
i.v - Intravenous
KNH - Kenyatta National Hospital
m² - Metre squared
mcg/l - Micrograms per litre
min - Minute
ml - Millilitre
ng/ml - Nanograms per millilitre
NKF - DOQI - National kidney foundation disease outcome quality initiative
PBF - Peripheral blood film
QOL - Quality of life
TIBC - Total iron binding capacity
TSAT - Transferrin saturation
RBC - Red blood cells
rHuEPO - Recombinant Human Erythropoetin
SD - Standard deviation
TNF - Tumour necrosis factor
USA - United States of America
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ABSTRACT

Background
Iron deficiency is an established association to the anaemia of chronic kidney disease. In chronic kidney disease, causes of anaemia are diverse; the main causes being the reduced production of erythropoietin as the renal function progressively deteriorates and associated abnormal changes in iron metabolism. In patients with end stage renal disease, anaemia is virtually a universal occurrence. In addition, in patients with end stage renal disease on haemodialysis, iron deficiency is the most frequent cause of anaemia despite treatment with erythropoietin.

There is no local data currently, looking at the iron status of the patients with end stage renal disease on haemodialysis despite evidence that correction of anaemia not only reduces mortality and morbidity but also improves the quality of life. This study seeks to establish the iron status of patients with end stage renal disease on haemodialysis and subsequently will form a basis for future objective clinical and laboratory interventions in patient care.

Objectives
The aim of the study was to determine the iron status, prevalence of anaemia and iron deficiency anaemia in patients with end stage renal disease on haemodialysis at Kenyatta National Hospital renal unit.

Study Design - A descriptive, cross sectional study.
Setting - Renal Unit, Kenyatta National Hospital.
Methodology
A cross sectional survey was carried out involving 165 patients with end stage renal disease on haemodialysis recruited sequentially at the Kenyatta National Hospital renal unit over a period of four months. These patients had their demographics, socio-economic details, as well as clinical history that included: aetiology of chronic kidney disease, date of commencement of haemodialysis, number of haemodialysis weekly sessions as well as use of iron, erythropoietin, blood transfusion or any other medications documented in a study questionnaire. A structured physical examination was carried out.
At enrolment, 8 millilitres of blood was drawn. 5 millilitres was put in a plain vacutainer for measurements of serum iron, ferritin, transferrin and total iron binding capacity. 3 millilitres was put in an ethylene diamine tetra-acetic acid vacutainer for measurement of full haemogram and erythrocyte sedimentation rate as well as for the peripheral blood film.

**Results**

One hundred and sixty five patients on stable maintenance haemodialysis were recruited into the study. Mean age was 45 years ± 16SD. Males were 60.6%(100), females were 39.4%(65). Majority of the patients (81.5%;135) were receiving haemodialysis once per week. Only 53.3%(98) patients were taking erythropoietin while 50.3%(83) were taking iron sucrose. On general examination, 99.4% of the patients were pale.

Mean haemoglobin was 7.7g/dl ± 1.9SD. Notably, 98.2%(162) of the patients had anaemia while 87.9% of the patients had an haemoglobin less than 11g/dl which was the recommended minimum haemoglobin target for end stage renal disease patients on treatment for anaemia. In terms of iron status assessment, 35.2%(58) had low iron status, 36.4% had normal iron status, 26.1%(43) had functional iron deficiency while 2.4%(4) had iron overload. Iron deficiency anemia was observed in 34.5%(54) of the study patients.

Predictors of iron deficiency from this study included not using erythropoietin, low levels of serum iron and transferrin levels as well as no prior history of blood transfusion.

**Conclusion**

Anaemia and iron deficiency are common in patients with end stage renal disease at Kenyatta National Hospital Renal Unit despite the current therapeutic measures.
1.0 INTRODUCTION AND LITERATURE REVIEW

The changes in iron metabolism in chronic kidney disease (CKD) result in altered body iron status. Consequently, anaemia, mainly normocytic normochromic occurs as early as in stage 3 CKD and is almost a universal finding in stage 5 disease. In stage 5 disease anaemia is a common complication and this is mainly due to progressive loss of endogenous erythropoietin (EPO) production by the damaged kidney (1). Notably, following replacement of endogenous erythropoietin with recombinant human erythropoietin (rHuEPO), there is a burst of erythropoietic activity during which most of the body iron is utilized. Consequently, if iron is not replenished, most of the patients develop resistance to rHuEPO which ultimately results to persistence of anaemia (2). Anaemia is an adverse indicator of progression of CKD. Therefore correcting anaemia is considered an important part in slowing or even stopping the progression of CKD (3).

In patients in end stage renal disease (ESRD) on haemodialysis (HD), other than impaired erythropoietin production, iron deficiency significantly contributes to anaemia. Iron deficiency anaemia has been shown to occur in 40% of haemodialysis patients with ESRD (4). In addition, adequate iron stores are vital for anaemic patients to achieve maximum benefit from erythropoiesis stimulating agents (ESA) and decreased iron stores or reduced availability of iron are the most common reasons for poor response to these agents. Besides increased demand for iron driven by the accelerated erythropoiesis that occurs with exogenous rHuEpo administration, iron deficiency and eventually iron deficiency anaemia (IDA) will occur in most patients on haemodialysis because of the ongoing blood losses from dialysis and tubing, frequent blood sampling, gastrointestinal blood losses as well as blood lost at time of needle placement and removal (5, 6). Therefore it is of importance to evaluate ESRD patients on HD for anaemia and iron deficiency concurrently as correction of anaemia has been shown to improve quality of life, reduce mortality and morbidity, reduce incidences and duration of hospitalization and generally improve patients’ survival.

Kalanter et al used monthly serum iron and transferrin saturation (TSAT) to assess iron status in 1283 maintenance haemodialysis patients from 10 haemodialysis centres in Los Angeles, USA (United States of America) from October 2001 to September 2002. Patients with baseline serum iron less than 45mcg/dl had a mortality of 23% (lowest quartile) compared to other quartiles that had a mortality of 2.7% to 12.3%. Likewise low TSAT levels were
associated with a higher mortality of 18.7 \% (lowest quartile) compared to 10.6\% to 13.4\% in other quartiles. Hospitalization was also significantly higher in the lowest serum iron and TSAT quartiles compared to the other quartiles (7).

Fishbane S, et al, used serum ferritin and TSAT to assess iron status in 50 haemodialysis patients. In that study, 31 patients (62\%) were iron deficient. Significantly, mean serum ferritin was 120.1 ± 115.8 ng/ml and mean TSAT was 19.4 ± 11.7\% in the iron deficiency group while in the patients with adequate iron status, mean serum ferritin was 182.4 ± 121.1ng/ml and TSAT was 27.4 ± 19.4\% (8).

In December 2001, Barbara JA et al evaluated iron status in 40 haemodialysis patients who were on intermittent iron administration for correction of iron deficiency anaemia. After six months, haemoglobin was maintained at acceptable level of 12.1g/dl. However, TSAT significantly rose from 25.9\% to 30.4\% and ferritin rose from 350 ng/ml to 486ng/ml. 2 patients were found to have iron overload (TSAT > 50\%) at the end of the study (9).

Victor et al studied 1774 patients treated by maintenance HD in 3 dialysis centres in New York from January 1998 to June 2007. The main aim was to determine the effect on patient survival of administered epoetin alpha, intravenous (i.v) iron and of haemoglobin (HB) and variables related to iron status. The study showed that long term survival of these patients was favourably affected by a relatively high HB level, by moderate i.v iron administration and by indicators of iron sufficiency. Long term survival of these patients was unfavourably influenced by low HB and indicators of iron deficiency. It was therefore concluded that close attention to iron repletion and iron sufficiency is essential for HD patients well being (10).

In a complex stratified random United States population sample of 15387 subjects, anaemia defined as HB <11g/dl, in females and < 12g/dl in males was found in 569 patients (3.9\%). In 179 of these anaemic patients, creatinine clearance was less than 50 ml/min and of these patients less than 33\% were iron sufficient: tranferrin saturation(TSAT) ≥ 20\%, serum ferritin ≥ 100mcg/l (micrograms per litre) (11). Thus iron deficiency and IDA are highly prevalent in renal insufficiency even before dialysis is required and i.v iron replenishment has been showed to slow the deterioration of renal function in CKD (12).
Van Wyck studied iron status in patients receiving EPO (erythropoietin) for dialysis associated anaemia. They evaluated markers of iron storage in 27 patients with normocytic normochromic anaemia undergoing acute rHuEpo (150-300u/kg thrice weekly) treatment for anaemia. 20 patients were found to develop evidence of exhausted iron stores (TSAT < 16%, Ferritin < 30mcg/l (13).

In 1997, the ESRD National Cooperative Anaemia Project mandated by the Health Care Financing Administration examined the cause of haematocrit (Hct) < 25% in a group of HD patients receiving rHuEpo. 60% of the patients were found to be iron deficient (TSAT<20%, Ferritin<100ng/ml (14).

As part of the wave 1 of the Dialysis Morbidity and Mortality Study, 25% of the United States HD patients were sampled between October and December of 1993. TSAT was less than 20% in 54% of the patients and less than 10 % in 25% of the patients. 36% of the patient had a ferritin level of less than 100ng/ml. Nearly 1/3 of the patients received no iron supplementation during this period (15).

The Health Care Financing Administration Core Indicators Project used data from 7292 HD patients selected from 18 HD networks to profile a variety of outcomes including Hct level. In the fourth ¼ of 1993 only 46% of patient had Hct > 30%. By the fourth ¼ of 1995 this had increased to 63% and a further increase to 72% was seen by the fourth ¼ of 1996. In this study it was noted that the amount of rHuEpo required to achieve these Haematocrits was greater in those patients with a low TSAT (16).

Iron replacement is crucial to the maintenance of normal iron status even in haemodialysis patients with functional iron deficiency. These patients have normal iron stores as depicted by normal serum ferritin levels but TSAT levels less than 20%. The DRIVE STUDY, an open label randomized controlled multicentre trial conducted in 37 centres across USA, objectively showed efficacy for i.v iron therapy among dialysis patients on erythropoietin with ferritin levels between 500-1200ng/ml and TSAT less than 25%. The haemoglobin and TSAT response significantly occurred faster in the i.v iron group than in the no iron group (17).
Hence haemodialysis patients not responding to erythropoietin and who have been diagnosed with absolute or functional IDA should receive iron supplementation and subsequently put on routine follow up.

1.1 FUNCTIONS OF IRON

1.1.1 HAEMATOLOGICAL. Iron is a component of haemoglobin that is involved in transporting oxygen to various tissues in the body. Total body iron is about 4grams, 2/3 of this is in HB. 1/4 of total body iron is stored in the reticuloendothelial cells of the liver, spleen and bone marrow bound to ferritin and hemosiderin.

1.1.2 NON-HEMATOLOGICAL. Iron is a key component of various cellular enzymes: - oxidases, catalases, aconitases, nitric oxide synthetases (18, 19, 20). Iron deficiency adversely affects metabolic processes that include electron transport, catecholamine metabolism, and deoxyribonucleic acid (DNA) synthesis. Iron is an integral part of the requirement of mitochondrial enzymes of electron transport chain and of the cytochrome system as well as iron sulfur proteins required for oxidative phosphorylation of ADP adenosine diphosphate(ADP) to adenosine triphosphate(ATP). Iron deficiency in rats results in reduced performance corrected rapidly by iron but not by blood transfusion (21). In HD patients iron deficiency has been reported and i.v iron repletion was associated with positive effects on serum albumin, muscle mass, hospitalization frequency and duration, blood pressure as well as reduced mortality (7).

1.2 TYPES OF IRON DEFICIENCY ANAEMIA IN HAEMODIALYSIS

1.2.1 Absolute iron deficiency. This occurs when circulating iron and iron stores are depleted; TSAT < 20%, SERUM FERRITIN < 100ng/ml for non-haemodialysis CKD, and TSAT < 20% and serum ferritin < 200ng/ml for haemodialysis – CKD patients (1). Notably about one fifth of patients starting dialysis have absolute iron deficiency.

1.2.2 Functional iron deficiency. This occurs when not enough iron from iron stores is released to support haemoglobin synthesis. It can occur in the presence of adequate iron stores when erythropoiesis is increased during therapy with ESA. Hemodialysis patients may also have co-existing occult infections or other conditions that affect the body’s ability to mobilise iron rapidly. This effect is mainly caused by increased levels of circulating cytokines that induce macrophages of the reticuloendothelial system to avidly take up and hold iron. These cytokines mainly IL (interleukin) – Iβ IL – 6 and TNF (tumour necrosis
factor)-α cause a decrease in endogenous Epo production or a decrease in responsiveness of erythroid precursor cells to endogenous or exogenous Epo (22, 23, 24). TSAT is low (could decrease to levels of absolute iron deficiency) but serum ferritin levels remain normal or slightly elevated.

1.3 MECHANISM OF FUNCTIONAL/ABSOLUTE IRON DEFICIENCY ANAEMIA CAUSED BY EPO ADMINISTRATION.

EPO causes a dramatic increase in RBC (red blood cell) production, HB, and haematocrit. The iron uptake by the erythroid cells is increased to meet the demand of increased RBC production and subsequently reticuloendothelial cells are unable to release stores of iron fast enough to meet demand. Consequently, despite adequate levels of stored iron (ferritin), insufficient iron is available for EPO stimulated RBC production. Iron deficient erythropoiesis develops, RBCs produced are small, have low HB content and a high concentration of protoporphyrin. With time, functional iron deficiency limits response to EPO therapy and higher doses are required to reach target HB and HCT levels. Eventually absolute iron deficiency occurs (low serum ferritin) and HB and HCT fall despite high EPO doses (25).

K/DOQI guidelines recommend maintenance of ferritin > 200ng/ml and TSAT > 20% to ensure adequate iron supply for erythropoiesis for the haemodialysis patients with ESRD. Guidelines also recommend workup for anaemia in CKD when HB < 11g/dl, Hct < 33% for premenopausal women and prepubertal patients and HB<12g/dl, Hct < 37% in adult males and post menopausal females (1).

1.4 STAGES OF IRON DEFICIENCY ANAEMIA

1. DEPLETED IRON STORES – characterised by low ferritin and absent bone marrow iron levels. HB and RBC indices are normal.

2. LATENT IRON DEFICIENCY – characterised by low TSAT, low serum iron, elevated serum transferrin, HB is normal. PBF (peripheral blood film) shows a normocytic normochromic picture, that later changes to microcytic hypochromic picture as severity of anaemia increases.

3. IRON DEFICIENCY ANAEMIA – As in 2 above but HB is low and PBF shows a microcytic hypochromic picture as well changes in RBC indices: mean cell volume(MCV), mean cell haemoglobin(MCH), mean cell haemoglobin concentration(MCHC).
1.5 AETIOLOGY OF IRON DEFI CIENCY AND IRON DEFICIENCY ANAEMIA IN PATIENTS WITH ESRD ON HAEMODIALYSIS.

1.5.1 BLOOD LOSS

a) At each visit to the renal unit, either for haemodialysis or routine reviews, blood samples are taken for the following tests: Haemoglobin, haematocrit, urea, electrolytes, creatinine, albumin, calcium, phosphates, iron studies. About 6mls of blood is drawn on each occasion.

(b) Accidental and surgical blood loss including vascular access complications. (Fixing and removing subclavian, femoral, permanent catheters as well as arteriovenous fistula fashioning for hemodialysis).

(c) Blood retention in the dialyzer and blood lines at the end of each hemodialysis treatment.

(d) Chronic (occult) GI blood loss.

A haemodialysis patient may lose up to 3mg/day of iron as a result of blood loss (up to 20 ml blood) during each treatment. This amount of iron exceeds 1-2 mg of dietary iron intake which inevitably leads to iron stores depletion and IDA (26).

One study showed an average monthly blood loss of 167mls to 226 mls and a monthly iron loss of 57 to 84 mgs among patients on haemodialysis. Blood drawing for laboratory testing resulted in a monthly loss of 127 mls in a group of HD patients receiving haemodialysis for greater than two days in a week. As a result patients on HD can experience an annual loss of up to 3000mg (5).

The anaemia work group of the Dialysis Outcome Quality Initiative suggested that 25 – 100mg of iron would need to be replaced weekly in haemodialysis patients just to offset the iron lost because of the ongoing external blood losses. On the other hand peritoneal dialysis patients suffer much less blood losses related to dialysis procedure and are less likely to have iron deficiency than haemodialysis patients (27).

1.5.2 NUTRITIONAL CAUSES.

In chronic kidney disease, anorexia, as experienced in other chronic illnesses leads to reduced intake of nutrients including iron. There is also reduced intake of heme iron containing feeds (meat) as well as reduced gut absorption of iron due to gut oedema.
1.5.3 HOOKWORM INFECTION

This is a common cause of iron deficiency in the tropics and hence a cause of iron deficiency and IDA in the patients in tropics with chronic kidney disease. The hookworm enters the body through skin (bare feet). It migrates to the small intestines where it attaches itself to the villi. The hookworm damages the villi resulting in blood loss. It feeds on nutrients meant to be absorbed by the host across the villi. It also produces anticoagulants that promote continued bleeding. Whereas an adult *Ancylostoma duodenale* sucks 0.2-0.25 ml/day, an adult *Necator Americana* sucks about 0.04ml/day of blood.

1.5.4 ROLE OF INFLAMMATION

Inflammation is common in CKD and is now recognized as a major contributor to mortality and morbidity. Inflammation results from haemodialysis procedure itself, infection, chronic GI bleeding (28). Qureshi et al detected markers of inflammation in 48% of haemodialysis patients and found that inflammation was an independent risk factor for mortality. Inflammation was more prevalent in the non survivors, 44%, than the survivors, 13% (29). Inflammation appeared to be inter-related with occurrence of malnutrition and cardiovascular disease which is the leading cause of death in haemodialysis patients (30).

In iron deficient or marginally iron sufficient chronic haemodialysis patients, the adverse effects on mortality and morbidity of Epo administration has been attributed to the rise in inflammatory cytokines. Pro inflammatory cytokine TNF was reported to be six times higher in HD patients than in controls; 5.6 ± 0.9 versus 0.9 ± 0.1pg/ml. (31)

In another study, plasma concentration of TNF α, the anti-inflammatory cytokine IL-4 and the total peroxidise concentration were measured in a 90 day randomized controlled trial in patients receiving Epo without or in combination with i.v iron. Patients receiving both i.v iron and Epo had lower pro-inflammatory cytokine TNF α, a lower level of total peroxidise (marker of total radical concentration) and a higher anti inflammatory cytokine IL - 4. These observations suggest a possible role for these cytokines in relation to the adverse effect of iron deficiency on morbidity and mortality in Epo treated CKD and HD patients in whom there was no attention to iron status (32).

Inflammation increases the severity of anaemia by reducing the patient’s ability to respond to treatment. C - reactive protein(CRP), a marker of inflammation is a strong predictor of resistance to erythropoietin in HD patients (33). IL-6 is the main cytokine that stimulates
production of hepcidin in the liver and is the main regulator of extracellular ion concentration through its effect on the ion channel ferroportin. IL-6 is also the main stimulus for the production of CRP, a non-specific but sensitive marker of inflammation. CRP may be used as a surrogate measure of iron status in HD patients.

Rathaus et al, Tel Aviv, Israel performed 8332 measurements of CRP in 401 HD patients followed for 3 - 60 months and found that all the parameters of iron balance (serum iron, TSAT, % hypochromic RBCs and HB were proportionately affected by an inflammatory state as measured by CRP. It was concluded that CRP be part of routine haematological assessment of HD patients to allow correct interpretation of data in anaemic patients (34). Inflammation may cause anaemia by decreasing iron transfer into the bone marrow and/or inhibiting the synthesis of transferrin, an iron binding transport protein in the plasma and ECF. This results in iron deficiency and iron deficiency anaemia (35).

1.5.5 IMPAIRED INTERSTINAL ABSORPTION OF IRON

In ESRD patients on hemodialysis, iron deficiency anaemia may result from inadequate intestinal absorption of iron at the duodenum and upper jejunum and or when the need for iron exceeds dietary intake (36). Since iron is necessary for HB production, iron deficiency anaemia ensues and this reduces the patient’s ability to respond to ESAs.

1.6 TESTS USED TO EVALUATE IRON STATUS OF A PATIENT.

1.6.1 SERUM FERRITIN.

This is an intracellular iron storage protein that reflects iron reserves. After absorption in the intestinal mucosal cells of the duodenum and upper jejunum, iron is either transported to the blood stream or combines with apoferritin, a complex binding protein to form ferritin. Ferritin shows the amount of iron stored in the body. Low serum ferritin levels indicate iron deficiency anaemia. It is the most commonly used parameter to assess the amount of iron stored in the body. 1mcg/l = 8-10mg stored iron (37). Reference range is 30 – 200 ng/ml (nanograms per millilitre). Brian et al in journal of family practice 2000 reports systematic overview of 55 studies relevant to the laboratory tests for the diagnosis of IDA in variable patient population and found serum ferritin radioimmunoassay to be the most powerful test. Also, IDA in pregnancy has been defined by the National Academy of Science panel on Nutrition as ferritin levels lower than 12ng/ml. Therefore ferritin levels are
considered gold standard non invasive test in the diagnosis of iron deficiency. Ferritin is an acute phase reactant and is elevated in patients with chronic inflammatory conditions and in acute infections. It is also elevated in iron overload states, e.g. haemochromatosis, in patients with liver disease, in haemoglobinopathies and in malignancies.

1.6.2 TRANSFERRIN
A protein that binds and transports iron to the bone marrow where it is stored and used to make new red blood cells. Each transferrin binds to two ferrous ions. Plasma soluble transferrin receptor concentration rises two to three times the normal when anaemia is present and can also be used to assess anaemia status. But rise occurs only after iron stores become functionally depleted whereas ferritin concentration falls earlier as iron stores fall. Normal levels is 26-47 micromol/l

1.6.3 TOTAL IRON BINDING CAPACITY (TIBC)
Measures the total binding capacity of transferrin. It measures the total amount of iron it takes to completely fill transferrin. It also shows how well transferrin can carry iron to the blood. Range (45 – 66 micromol/l). TIBC is elevated when body iron is low.

1.6.4 SERUM IRON
Measures the amount of iron that is bond to transferrin in the blood. Free, unbound iron is very toxic. It reflects the amount of iron in the blood. It varies widely in healthy individuals and diurnal variation as well as inter- laboratory variation has been reported. Range (11 – 29 micromol/l)

1.6.5 TRANSFERRIN SATURATION (TSAT)
Measures iron occupancy of transferrin. It is the ratio of serum iron to the TIBC expressed as a percentage. It measures how much iron is bound to transferrin and is readily available to make RBCs. Normally, 20 -50 % of available transferrin sites are saturated with iron.

1.6.6 RETICULOCYTE HAEMOGLOBIN CONTENT (CHr)
CHr is now considered a marker of iron status with slightly higher accuracy compared to serum ferritin and TSAT. Reticulocytes are closely related to the cells in the bone marrow that are actively using iron for the synthesis of HB. They stay in the circulation for 24 hours. Therefore the HB content of reticulocytes reflects the amount of iron available during RBC
development. It provides a real time estimate of iron availability. A CHr < 29pg indicates IDA. According to the 2006 K/DOQI guidelines, it can be used instead of TSAT in evaluation of patients with iron deficiency (1). Fishbane et al evaluated iron management in haemodialysis patients using serum ferritin and TSAT to the management guided by CHr and found that there was no significant difference between the two groups in terms of final mean haematocrit and ESAs dosage (38).

1.6.7 BONE MARROW ASPIRATE FOR CYTOLOGY (BMA-C).
Traditionally a definitive diagnosis of iron deficiency anaemia requires a demonstration of depleted iron stores in bone marrow (lack of stainable iron on Prussian staining of bone marrow). However this is an invasive and painful procedure and while a clinical trial of iron supplementation is inexpensive and non traumatic, patients are often treated based on clinical history and ferritin levels without a BMA-C.

Absence of stainable iron in a bone marrow that has particles is diagnostic of IDA without other laboratory tests. But since this is an invasive, painful test, it has been displaced by serum iron, TIBC and serum ferritin.

1.6.8 FULL HAEMOGRAM, ESR AND PERIPHERAL BLOOD FILM.
A full haemogram comprises of the following parameters: white blood cell count and differential counts, RBC counts, RBC indices namely MCH, MCV, mean corpuscular haemoglobin concentration (MCHC) and platelets. These parameters are affected depending on the temporal occurrence and severity of anaemia.

The peripheral blood film (PBF) is used for morphological classification and typing of haematological changes as well as classification of the type of anaemia. From a PBF, one is able to appreciate various blood cell characteristics such as RBC sizes, shapes, pallor, nucleated RBCs, WBC morphology and distribution of WBC subsets such as neutrophils, eosinophils, basophils, monocytes and lymphocytes. Any inclusions such as parasites, for example, malaria parasites or RBC fragments can also be appreciated from a PBF.

In microcytic hypochromic anaemia typical of iron deficiency anaemia, HB is low, RBC counts are relatively high compared to HB, MCV, MCH are low, MCHC is slightly reduced, and on PBF, RBCs are poorly haemoglobinised. Some poikilocytes are present including pencil cells and occasional target cells.
In normocytic normochromic anaemia, HB is low, MCV, MCH, MCHC are normal. On PBF, the RBCs appear adequately haemoglobinised. 
In macrocytic anaemia, HB is low, the MCV, MCH and MCHC are raised, and the RBC count is relatively low compared to the HB. On PBF, RBCs appear large and well haemoglobinised.

The reticulocyte count in anaemia is also an important parameter. It is elevated in haemolysis, haemorrhage, and in nutritional deficiencies responding to treatment. Very low reticulocyte count suggests hypoplasia or aplasia of bone marrow or extensive bone marrow infiltration. The leucocytes are also important; their various parameters and indices change in various types and degrees of anaemia. Neutrophil leucocytosis is found in infection, haemolysis or bleeding. Neutrophil left shift is found in leuco-erythroblastic anaemia, in association with nucleated RBCs. Primitive WBCs for example, blasts, are seen in leukaemia, myeloproliferative disorder or leucoerythroblastic anaemia. Leucopenia is seen in bone marrow failure or infiltration, megaloblastic anaemia, hypersplenism and in immune disorders like feltys syndrome and systemic lupus erythematosus. Hypersegmented neutrophils is a common feature of megaloblastic anaemia.

Platelet counts are also affected to various degrees depending on the type and severity of anaemia. Thrombocytosis is seen in bleeding, haemolysis, myeloproliferative disorders, and iron deficiency with or without bleeding. Thrombocytopenia is seen in bone marrow aplasia or infiltration and in megaloblastic anaemia.

The ESR is an acute phase reactant is used as a surrogate marker of inflammation. It can be positively correlated with C - reactive protein. ESR is increased in various inflammatory conditions like infections and also in chronic kidney disease. However in haematological assessment, ESR is used to correlate and corroborate the cellular changes such as increased WBC, increased platelets and can be an indicator of the next cause of action. For example in renal failure a markedly raised ESR can point to myelomatosis and therefore investigation for the same. In patients with ESRD a dimorphic picture of anaemia is frequently encountered.

1.6.9 STOOL STUDIES (OCCULT BLOOD, OVA AND CYSTS)
Also form an integral part in the evaluation of patients with iron deficiency. When positive for occult blood, it pre-empts gastrointestinal blood loss as a cause of IDA which then should
be followed by endoscopy studies. This is not a routine test for evaluating patients with IDA according to the revised 2006 K/DOQI guidelines (results have many false negatives and false positives due to various confounders). When ova/cysts of hookworm are detected treatment is given accordingly.

1.7 K/DOQI GUIDELINES IN EVALUATING IRON STATUS OF PATIENTS IN CKD WITH ESRD ON HAEMODIALYSIS.

Updated KDOQI guidelines 2006 states that HB, SERUM FERRITIN and TSAT or CHr should be evaluated jointly to provide the relevant information about iron stores and iron available for erythropoiesis (1). Guidelines also recommend evaluation of iron deficiency before starting treatment with ESAs to ascertain the contribution and severity of iron deficiency which may be significant to anaemia.

1.8 RECOMMENDED TARGETS (K/DOQI GUIDELINES).

1.8.1 Haemoglobin.

Revised 2006 K/DOQI Guidelines recommend a target HB of 11g/dl but less than 13g/dl. This is associated with measurable quality of life benefits. Intentionally treating to an HB equal to or more than 13g/dl may increase patients’ risks for adverse effects. The higher HB levels in ESRD have been linked to increased mortality (39). In choosing the upper and lower limits for the HB, the NKF/KDOQI anaemia workgroup in 2006 considered published trials of all cause mortality and adverse cardiovascular events in patients assigned to higher HB targets compared to lower HB targets. Most of the data was derived from the correction of haemoglobin and outcomes in renal insufficiency(CHOIR) study as well as cardiovascular risk reduction by early anaemia treatment with epoetin beta(CREATE) study.(1)

1.8.2 Ferritin levels

Ferritin levels should be maintained above 200ng/ml.

1.8.2 TSAT levels or CHr levels

TSAT should be greater than 20%; CHr should be greater than 29pg/cell (40,41, 42, ).

For a patient on treatment for anaemia with an ESA, the guidelines recommend evaluating patient for IDA every month till anaemia is corrected and every three months thereafter.
2.0 STUDY JUSTIFICATION

Chronic kidney disease is associated with changes in body iron status. In addition, anaemia is a common complication of CKD and it is a universal finding in patients in ESRD. In patients with ESRD on haemodialysis, iron deficiency anaemia is a major aetiologic factor with a prevalence of 40% (3). When present, anaemia is an independent risk factor for cardiovascular mortality and morbidity and hence the need for evaluation and follow up of patients with anaemia.

Currently, there are no studies, both locally and regionally to assess iron status in patients with ESRD on haemodialysis. In various renal care centres including KNH renal unit, patients in ESRD on haemodialysis are empirically started on haematinsics that include oral or intravenous iron and erythropoietin without initial or subsequent objective assessment of the extent and magnitude of their iron deficiency or recovery from IDA. This study will provide an objective overview of the iron status of the patients on haemodialysis at KNH renal unit. It will also provide the rationale for early evaluation of iron status of anaemic patients in ESRD on haemodialysis with a view for optimising treatment for better outcomes.

3.0 RESEARCH QUESTION

What is the iron status in patients with ESRD on haemodialysis at KNH renal unit?

4.0 OBJECTIVES

4.1 Broad objective

To determine serum iron status in patients with ESRD on haemodialysis at KNH renal unit.

4.2 Specific objectives

1. To determine the prevalence of anaemia in patients with ESRD on haemodialysis at KNH renal unit using HB.
2. To determine the iron status of patients with ESRD on haemodialysis at KNH renal unit by use of serum ferritin and TSAT.
3. To determine the prevalence of iron deficiency anaemia in ESRD patients on haemodialysis by use of serum ferritin, TSAT, HB and PBF.
4. To document socio-demographic factors of patients with IDA in comparison with those with no IDA.
4.3 Secondary objective
1. To document the modalities used in treatment of anaemia in patients with ESRD on haemodialysis: - erythropoietin, iron, blood transfusion.

5.0 METHODOLOGY
5.1 STUDY DESIGN
This was a descriptive cross sectional study.

5.2 STUDY SITE
The designated area of the study was the renal unit of Kenyatta National Hospital, a teaching and referral hospital in Nairobi, Kenya. The renal unit had a wide catchment area of patients not only from all parts of Kenya but also from the entire East African region. As at 29th September 2010, there were 152 patients on maintenance haemodialysis, though one to two patients joined the unit weekly for haemodialysis. Each patient was dialysed for a minimum of 1 or 2 sessions every week. The renal unit was in operation 24 hours in a day, the entire seven days in a week.

5.3 STUDY POPULATION
The study population consisted of consenting adult individuals and assenting adolescents (with consent from parents or guardians) from ages 13 years in ESRD on haemodialysis for 3 or more months.

5.4 STUDY DURATION
Study duration was from November 2010 to February 2011.

5.5 SAMPLE SIZE
A total of 165 patients in ESRD on haemodialysis was studied, calculated using the formula below.

\[
N = \frac{Z^2 P(1-P)}{d^2}
\]

Where: \(N\) is the required sample size.
- \(Z\) is the confidence interval at 95% (standard value of 1.96)
- \(P\) is the estimated prevalence of IDA in ESRD (30%)
- \(d\) is the margin of error at 7% (standard value of 0.07)
5.6  PATIENT SELECTION

5.6.1 Inclusion criteria
1. Patients with ESRD on haemodialysis for 3 or more months.
2. Consenting adults above 18 years of age and assenting persons over 13 years of age with consent from parents or guardians.
3. Written informed consent and assent for patients above 18 years and below 18 years respectively. (Consent for those less than 18 years was got from the parents/guardians)

5.6.2 Exclusion criteria
1. Patients on haemodialysis due to other causes other than ESRD.
2. Patients with liver disease, malignancy or any known haemoglobinopathy.

5.7  CASE DEFINITIONS.

End stage renal disease
Patients with an estimate GFR < 15 ml/min/1.73 m² prior to the onset of haemodialysis having demonstrated progressive deterioration in renal function over the previous 3 months.

Iron status definitions

Normal iron status
Defined as: 1. Serum ferritin $\geq$ 200ng/ml
2. Transferrin saturation $\geq$ 20%

Iron deficiency (low iron status)
Defined as: 1. Serum ferritin < 200ng/ml
2. Transferrin saturation < 20%

Iron deficiency anaemia
Defined as: 1. Serum ferritin < 200ng/ml
2. Transferrin saturation < 20%
3. HB < 13.5g/dl for males and < 12g/dl females

Iron overload
Defined as: 1. Serum iron > 29mcmol/l
2. Serum ferritin > 500ng/ml
3. Transferrin saturation > 50%

No documented values for HB in iron overload (pathological states).
**Functional iron deficiency**
Defined as: 1. Serum ferritin > 200ng/ml
   2. transferrin saturation < 20%

### 5.8 Sampling method
All patients with end stage renal disease on haemodialysis at KNH renal unit who fitted the inclusion criteria and consented/assented to the study were studied.

### 5.9 Study feasibility
At the beginning of the study, there were 152 patients on maintenance haemodialysis at KNH - Renal unit with one to two patients joining the unit for dialysis every week. Haemodialysis at the renal unit was in operation 24 hours a day, 7 days in a week.

### 6.0 METHODS
#### 6.1 Screening and recruitment
A study assistant was stationed at the renal unit patient’s registration desk, who screened all the patients files. All those in established end stage renal disease and met the inclusion criteria were informed of the study and requested to fill the consent/assent form.
Upon filling the consent/assent form, a study registration number was issued and noted by the study assistant, alongside the patient’s full names and the patient’s hospital file number. This avoided double participants’ registration in the subsequent visits.
6.2 Patients’ flow

173 Patients on haemodialysis

With ESRD

No (none) → Excluded (0)

Yes

Eligible for study

Consent to study

No (3) → Excluded (3)

Yes

Questionnaire

Hx and P/E

165 patients: FBC + PBF + ESR / serum iron / ferritin / transferrin/TIBC/TSAT

Exclude: Malignancies, liver disease, Haemoglobinopathies
1-sickle cell disease-excluded
4-hepatitis B- excluded
6.3 Clinical methods

The principal investigator administered study questionnaires that were used to collect data from the recruited patients. Data consisting of socio-demographics which include age, gender, marital status, occupation and place of residence was documented. Data on the date of commencement of haemodialysis, and number of haemodialysis weekly sessions, was also obtained through the interview.

Information on aetiology of the end stage renal disease, use of erythropoietin, iron and other medications as well as any previous blood transfusions was recorded.

A detailed physical examination was conducted by the principal investigator.

6.4 Laboratory methods

Just before start of a HD session, about 8mls of blood was drawn from the cubital vein and 5mls was put in a sterile plain vacutainer for serum iron, ferritin, transferrin and TIBC. These blood samples were immediately transferred to the laboratory where the serum samples were frozen at –20 degrees Celsius until the time of the analysis at LANCET- KENYA laboratories.

Serum ferritin and transferrin was measured by electrochemiluminescence immunoassay (ECLIA) using COBAS E 411 immunoassay analyzer. Serum iron and total iron binding capacity (TIBC) was measured using COBAS INTEGRA 400/800 (based on the Ferro Zinc method without deproteination). Transferrin saturation (TSAT) was then derived from the measurements of serum iron and TIBC.

$$\text{TSAT is } \frac{\text{serum iron}}{\text{TIBC}} \times 100\%$$

The other 3 ml of blood was put in an EDTA (ethylene diamine tetra acetic acid) vacutainer for FBC, ESR and PBF. Full haemogram was done at KNH haematology department by the department’s HUMAN ANALYZER- CELLDYNE 3200

Erythrocyte Sedimentation Rate (ESR) was done at KNH haematology department using the WINTROBES method. The peripheral blood films reporting by direct visualization on a microscope at various powers of magnification after staining with MAYGRUNWALD/GIEMSA stains was done at KNH haematology department by a haematologist, who was one of the supervisors for this study.
All patients recruited in this study underwent the standard care as offered at KNH - Renal unit.

6.5 Quality control measures.
The standard operating procedures in all aspects of this study was adhered to at all times.
The recommended procedure for specimen collection was adhered to at all times. This included proper phlebotomy site cleaning and the use of appropriate vacutainers.
Proper labelling of the specimens and storage was adhered to at all times to minimise pre-analytical sources of errors.
The LANCET Kenya laboratory and the KNH haematology laboratory run external and internal quality controls.

7.0 DATA MANAGEMENT AND ANALYSIS
Data collected from the study proforma was entered and managed in a predesigned Microsoft Access database and statistical analysis was done using statistical package for social scientists, SPSS version 17.0 after cleaning and verification.
Characteristics of the study data were described using counts, means, medians and standard deviations for continuous variables and proportions for categorical variables
Associations between the patients’ socio-demographic data, iron status, serum ferritin, transferrin, transferrin saturation and haemoglobin levels were analyzed using chi-square test.
For continuous variables, differences in mean values in patients with or without IDA was analyzed by student’s t-test (normally distributed) or Mann-Whitney U test (skewed data).
Associations were considered significant when p value was equal to or less than 0.05. The data was presented using descriptive statistics then presented using tables, pie charts and bar charts

8.0 ETHICAL CONSIDERATIONS
The study was undertaken after approval by the department of internal medicine, university of Nairobi and the KNH scientific and ethical committee.
Patients eligible to participate in the study were included only after providing consent/assent following the process as outlined below.
1. The patients were informed that the project involved local research.
2. The patients were told the purpose of the research.
3. The procedures of the study were explained clearly with full details of all the tests to be done.
4. The patients were assured that participation was voluntary and no medical attention would be denied should they decline to participate.
5. The patients were informed of the medical benefits and also physical and psychological harms to their satisfaction prior to being included in the study.
6. The patients were assured of full and free access to their results and that therapeutic interventions would be recommended where need arises, according to the accepted standards of practice.
7. It was asserted that confidentiality would be strictly maintained and all data would be securely stored and only revealed upon a need-to-know basis and that all costs regarding investigations in this study would be borne by the principal investigator.

Following the full explanation and acceptance by the patient of the above, the subject was requested to sign the consent form.
9.0 RESULTS

Characteristics of the study population

Demographic characteristics

A total of 165 clients visiting KNH haemodialysis centre were selected and studied. Mean age was 45 years ± 16 SD ranging between 14 and 80 years. Age distribution was comparable across the different age categories with the highest proportion (22.4%; 37) aged <30 years and the lowest (15.2%; 25) being 41 – 50 years old as shown in figure 1 below.

![Age distribution of respondents.](image)

Gender distribution revealed high proportion of males (60.6%; 100) than females (39.4%; 65). Almost three-quarter of the participants (70.3%; 116) had attained at least a secondary education while a very small proportion (3.0%; 5) had no formal education as shown in figure 2 below.
Figure 2: Level of education of respondents.

Over two-thirds of the participants (63.0%; 116) were not receiving any form of earnings; 19.4% due to retirement and the rest due to unemployment as shown in figure 3 below.

Figure 3: Occupation status of the respondents.

A high proportion of the respondents came from Central province (41.2%; 68) followed by Eastern province (21.2%; 35). Rift valley and Nyanza provinces had comparable proportion of clients (13.3%; 22), as shown in figure 4 below.
A majority of the patients (81.8%; 135) had only one session of haemodialysis per week. The other patients (18.2%; 30) had two sessions of dialysis per week.

Most of the patients complained of body malaise (64.2%; 106) and difficulty in breathing (18.8%; 31). Other specific complaint(s) were present in less than 10% of the patients. Forty-seven patients (28.5%) did not present with any complaint(s).

Most of the patients (43.0%; 71) had been on haemodialysis for less than six months as shown in figure 5 below.
With regard to general examination, the greater majority of the participants were pale (99.4%; 164) while only (46.7%; 77) had oedema. Systematic examination revealed an equal number of Abdominal (7.3%; 12) and Cardiovascular (7.3%; 12) abnormalities. The study population had comparable distribution of Respiratory (2.4%; 4) and Musculoskeletal (1.8%; 3) abnormalities.

Approximately half of the study participants (52.1%; 86) had a history of blood transfusion. Out of 86 participants with history of blood transfusion, a small proportion (17.4%; 15) had not been transfused in the previous 3 months. Out of 71 with history of blood transfusion in the last 3 months, 70.4% (50) had been transfused once while the rest more than once.
Figure 6: Sessions of blood transfusion previous 3 months per patient

Among the 71 that had been transfused in the previous 3 months, majority (40.8%; 29) had been transfused 2 units of blood while 36.6 %; (26) had been transfused only one unit as shown in figure 7 below.

Figure 7: Number of unit B blood transfusion used previous 3 months
Regarding anaemia treatment medications, approximately half of that study participants (53.3%; 88) were using Erythropoietin. Similarly, half of participants (50.3%; 83) were using intravenous iron. A small proportion of the participants (7.3%; 12) were using oral iron.

HAEMOGLOBIN LEVELS

As shown in figure 8 below, haemoglobin levels observed a Gaussian distribution with two peaks, one at HB 6.5 - 7.0 g/dl and the other at 7.5 - 8.5 g/dl.

![Haemoglobin level (g/dL) distribution](image)

**Figure 8: Distribution of HB (g/dl)**

Mean haemoglobin level was 7.9 g/dl (+ 1.9 SD) g/dl ranging from 3.8 to 13.2 g/dl. Low haemoglobin (Hb<13.5 for males, Hb<12.0 for females), indicating the presence of anaemia was observed in 98.2% (162) of all the participants.

Of the 98.2% (162) patients with anaemia, 60.6% (100) were males and 37.7% (62) were females. Majority of the study participants (87.9%; 145) did not achieve minimum NKF/KDOQI haemoglobin target expected while anaemia was being corrected. The 145 patients had haemoglobin less than the target 11g/dl. Males were 52.1% (86) and females were 35.8% (59).
The table below shows the haematological and biochemical parameters of the respondents.

**Table 1: Haematological and biochemical parameters**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>165</td>
<td>7.9 ± 1.9</td>
<td>3.8</td>
<td>13.2</td>
</tr>
<tr>
<td>PCV</td>
<td>165</td>
<td>26.1 ± 8.3</td>
<td>12.4</td>
<td>39.4</td>
</tr>
<tr>
<td>RBC</td>
<td>165</td>
<td>3.0 ± 0.7</td>
<td>1.4</td>
<td>5.4</td>
</tr>
<tr>
<td>WBC</td>
<td>165</td>
<td>6.8 ± 3.5</td>
<td>2.2</td>
<td>22.2</td>
</tr>
<tr>
<td>MCV</td>
<td>165</td>
<td>85.0 ± 5.9</td>
<td>66.6</td>
<td>102.0</td>
</tr>
<tr>
<td>MCH</td>
<td>165</td>
<td>27.1 ± 5.1</td>
<td>17.8</td>
<td>84.7</td>
</tr>
<tr>
<td>MCHC</td>
<td>165</td>
<td>31.2 ± 2.4</td>
<td>22.6</td>
<td>39.4</td>
</tr>
<tr>
<td>Platelets</td>
<td>165</td>
<td>272.3 ± 124.3</td>
<td>67.8</td>
<td>682.0</td>
</tr>
<tr>
<td>ESR</td>
<td>165</td>
<td>51.0 ± 17.2</td>
<td>2.0</td>
<td>85.0</td>
</tr>
<tr>
<td>Serum iron</td>
<td>165</td>
<td>11.1 ± 7.0</td>
<td>1.7</td>
<td>48.2</td>
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<tr>
<td>TIBC</td>
<td>165</td>
<td>51.8 ± 14.3</td>
<td>24.8</td>
<td>112.3</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>165</td>
<td>23.6 ± 16.6</td>
<td>3.7</td>
<td>100</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>165</td>
<td>676.8 ± 665.7</td>
<td>12.3</td>
<td>3033.4</td>
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<td>Transferrin</td>
<td>165</td>
<td>25.9 ± 7.3</td>
<td>6.5</td>
<td>60.4</td>
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</table>

The table below shows the respective proportions of the biochemical and haematological parameter of the respondents.
<table>
<thead>
<tr>
<th>Variables</th>
<th>N=165</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (M: 13.5-17.5g/dl, F: 12-15.5g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>162</td>
<td>98.2</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>1.8</td>
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<tr>
<td>PCV (M: 40-52%, F: 36-48%)</td>
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<td></td>
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<tr>
<td>Low</td>
<td>161</td>
<td>97.6</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>2.4</td>
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<tr>
<td>RBC (M: 4.4-6.1X10^12 /L, F: 4.2-5.6X10^12/L)</td>
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<td>94.5</td>
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<td>Normal</td>
<td>9</td>
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<td>Normal</td>
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<td>ESR (0-15mm/hr)</td>
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<td>4.2</td>
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<tr>
<td>High</td>
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<td>95.8</td>
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<td>Serum iron (11-29micromol/l)</td>
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<td>TIBC (45-66micromol/l)</td>
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<td>Low</td>
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<td>TSAT (20-50%)</td>
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<td>97</td>
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<td>High</td>
<td>11</td>
<td>6.7</td>
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<tr>
<td>Serum ferritin (200-500ng/ml)</td>
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</tr>
<tr>
<td>Low</td>
<td>58</td>
<td>35.2</td>
</tr>
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<td>31.5</td>
</tr>
<tr>
<td>High</td>
<td>55</td>
<td>33.3</td>
</tr>
<tr>
<td>Transferrin (26-47micromol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>87</td>
<td>52.7</td>
</tr>
<tr>
<td>Normal</td>
<td>76</td>
<td>46.1</td>
</tr>
<tr>
<td>High</td>
<td>2</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Iron status assessment
Assessment of iron status was done using serum ferritin (SF) and TSAT levels. Analysis of TSAT and ferritin levels revealed that 35.2% (58) had low iron status (ferritin < 200ng/ml, TSAT < 50%). 36.4% of the patients had normal iron status (TSAT ≥ 20%, ferritin ≥ 200ng/ml). Only 2.4%(4) had iron overload (serum iron > 29mcml/l, TSAT > 50%, ferritin > 500ng/ml while 26.1% had functional iron deficiency(TSAT < 20%, Ferritin ≥ 200ng/ml)

![Iron status among the study participants](image)

**Figure 9**: Iron status among the study participants

Prevalence of iron deficiency anaemia
This was done by getting the proportion of patients who had anaemia (HB<13.5g/dl males, HB< 12g/dl females) and with low iron status (ferritin < 200ng/ml, TSAT< 20%). Using these parameters 34.5 % (54) of the study participants had iron deficiency anaemia.

Peripheral blood film
Assessment of peripheral blood film (PBF) was done in order to see the respective haematological characteristics. Among other observed features, the most commonly occurring cases include; Rouleaux formation (72.1%; 119) and Normocytic normochromic (66.7%; 110) as shown in figure 10 below.
Figure 10: Outcome of the analysis of peripheral blood films among the study respondents.

Iron deficiency anaemia in relation to other patient characteristics.

Only one out of four selected demographic characteristics was significantly associated with iron deficiency anaemia. Occurrence of iron deficiency anaemia (50.0%; 18) was significantly associated with age category 30 – 40 years (OR=3.71; 95%CI: 1.29 – 10.72; P=0.015).

There was a significant association between iron deficiency anaemia and duration on haemodialysis in months. High occurrence of iron deficiency anaemia (43.7%; 31) was significantly associated with less than 6 months of haemodialysis (OR=2.23; 95% CI: 1.07 – 4.66; P=0.033) compared to one year or more on haemodialysis (25.8%; 16).

Low occurrence of iron deficiency anaemia (23.3%; 31) was significantly associated with history of blood transfusion (OR=0.34; 95% CI: 0.18 – 0.67; P=0.001) compared to no blood transfusion (46.8%; 37).
Table 3: Iron deficiency anaemia in relation to other patient characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No IDA (n=57)</th>
<th>IDA (n=108)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Duration on haemodialysis in months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>31</td>
<td>43.7</td>
<td>40</td>
<td>56.3</td>
</tr>
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<td>6 - 11 months</td>
<td>10</td>
<td>31.3</td>
<td>22</td>
<td>68.8</td>
</tr>
<tr>
<td>≥ 12 months</td>
<td>16</td>
<td>25.8</td>
<td>46</td>
<td>74.2</td>
</tr>
<tr>
<td>Number of haemodialysis sessions per week</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47</td>
<td>34.8</td>
<td>88</td>
<td>65.2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>33.3</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>History of blood transfusion</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>20</td>
<td>23.3</td>
<td>66</td>
<td>76.7</td>
</tr>
<tr>
<td>No</td>
<td>37</td>
<td>46.8</td>
<td>42</td>
<td>53.2</td>
</tr>
<tr>
<td>History of blood loss not haemodialysis related</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>40.0</td>
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<td>60.0</td>
</tr>
<tr>
<td>No</td>
<td>47</td>
<td>33.6</td>
<td>93</td>
<td>66.4</td>
</tr>
</tbody>
</table>

In this study, low occurrence of iron deficiency anaemia (21.6%; 19) was significantly associated with use of **Erythropoietin** (OR=0.28; 95%CI: 0.14 – 0.56; P < 0.001) compared to non-use (49.4%; 38). Particular dose regimen for erythropoietin had different dose effect in reducing occurrence of iron deficiency anaemia. More than or equal to 2000 iu per week was more effective (OR=0.14; 95%CI: 0.06 – 0.56; P < 0.001) compared to < 2000 iu per week (OR=0.68; 95%CI: 0.26 – 1.61; P=0.385) the former having significantly low occurrence of iron deficiency anaemia (12.1%; 7) while the later was not (40.0%; 12) as compared to the reference group (Not taking).

Also noted was that low occurrence of iron deficiency anaemia (20%; 17) was significantly associated with use of **parenteral iron** (OR=0.27; 95%CI: 0.14 – 0.54; P < 0.001) compared to no use of parenteral iron (48.8%; 40). Like use of erythropoietin, iron more than or equal to 100 mg per week was more effective (OR=0.06; 95%CI: 0.02 – 0.23; P < 0.001) compared to iron less than100 mg per week (OR=0.77; 95%CI: 0.34 – 1.75; P=0.537) the former having
significantly low occurrence of iron deficiency anaemia (6.0%; 3) while the later was not (42.4%; 14) as compared to the reference group (not taking).

Table 4: Iron deficiency anaemia in relation to use of medication

<table>
<thead>
<tr>
<th>Variables</th>
<th>IDA (n=57)</th>
<th>No IDA (108)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  %</td>
<td>N  %</td>
<td>OR</td>
<td>Lower</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking</td>
<td>19 21.6</td>
<td>69 78.4</td>
<td>0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>Not taking</td>
<td>38 49.4</td>
<td>39 50.6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Erythropoietin dosage per week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2000 iu weekly</td>
<td>7 12.1</td>
<td>51 87.9</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>&lt;2000 iu weekly</td>
<td>12 40.0</td>
<td>18 60.0</td>
<td>0.68</td>
<td>0.29</td>
</tr>
<tr>
<td>Not taking</td>
<td>38 49.4</td>
<td>39 50.6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Parenteral Iron (i.v)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venofar</td>
<td>17 20.5</td>
<td>66 79.5</td>
<td>0.27</td>
<td>0.14</td>
</tr>
<tr>
<td>Not taking</td>
<td>40 48.8</td>
<td>42 51.2</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Parenteral Iron (i.v) dosage per week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 100 mg weekly</td>
<td>3 6.0</td>
<td>47 94.0</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>&lt;100 mg weekly</td>
<td>14 42.4</td>
<td>19 57.6</td>
<td>0.77</td>
<td>0.34</td>
</tr>
<tr>
<td>Not taking</td>
<td>40 48.8</td>
<td>42 51.2</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

Iron deficiency anaemia and peripheral blood film features.

Two out of the four peripheral blood film features were significantly associated with occurrence of iron deficiency anaemia (table 5). High occurrence of iron deficiency anaemia (53.1%; 17) was significantly associated with presence of microcytic hypochromic picture on the PBF (OR=2.64; 95%CI: 1.20 – 5.79; P=0.014) compared to absence of the same (30.1%; 40). Conversely, low occurrence of iron deficiency anaemia (14.3%; 3) was significantly associated with presence of macrocytic normochromic picture on PBF (OR=0.28; 95%CI: 0.08 – 0.99; P=0.037) compared to absence of the same (37.5%; 54).
Table 5: Iron deficiency anaemia in relation to peripheral blood film features.

<table>
<thead>
<tr>
<th>Variables</th>
<th>IDA (n=57)</th>
<th>No IDA (108)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rouleaux formation</td>
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<tr>
<td>Present</td>
<td>42</td>
<td>35.3</td>
<td>77</td>
<td>64.7</td>
<td>1.13</td>
</tr>
<tr>
<td>Absent</td>
<td>15</td>
<td>32.6</td>
<td>31</td>
<td>67.4</td>
<td>Reference</td>
</tr>
<tr>
<td>Microcytic hypochromic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17</td>
<td>53.1</td>
<td>15</td>
<td>46.9</td>
<td>2.64</td>
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<tr>
<td>Absent</td>
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<td>Normocytic normochromic</td>
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<tr>
<td>Present</td>
<td>37</td>
<td>33.6</td>
<td>73</td>
<td>66.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Absent</td>
<td>20</td>
<td>36.4</td>
<td>35</td>
<td>63.6</td>
<td>Reference</td>
</tr>
<tr>
<td>Macrocytic normochromic</td>
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<td></td>
<td></td>
</tr>
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<td>Present</td>
<td>3</td>
<td>14.3</td>
<td>18</td>
<td>85.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Absent</td>
<td>54</td>
<td>37.5</td>
<td>90</td>
<td>62.5</td>
<td>Reference</td>
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</tbody>
</table>

Table 6 shows the relationship between occurrence of iron deficiency anaemia and selected laboratory parameters. Occurrence of iron deficiency anaemia was significantly high (43.2%; 41) among patients with a deficiency in serum iron (OR=2.56; 95% CI: 1.29 – 5.11) compared to those with normal/high serum iron (22.9%; 16).

It was observed in this study that occurrence of iron deficiency anaemia was associated with high levels of TIBC. A patient with a high TIBC was 16.65 (95% CI: 5.04 – 54.99) times more likely to have iron deficiency anaemia (73.9%; 17) compared to one that had low TIBC (14.5%; 8). The likelihood of occurrence of iron deficiency anaemia decreased to 3.42 (95% CI: 1.44 – 8.14) for a patient with a normal level of TIBC (36.8%; 32).

As depicted in table 6 below, occurrence of iron deficiency anaemia was associated with high transferrin levels (59.0%; 46) compared to low transferrin levels (12.6%; 11). A patient with normal/high transferrin levels was 10.00 (95% CI: 4.55 – 20.00) times more likely to have iron deficiency anaemia compared to one who had low transferrin levels.
Table 6: Iron deficiency anaemia in relation to selected laboratory parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>IDA (n=57)</th>
<th>No IDA (108)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>Lower</td>
</tr>
<tr>
<td>RBC (M: 4.4-6.1X10^12 /L, F: 4.2-5.6X10^12/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>52</td>
<td>33.3</td>
<td>104</td>
<td>66.7</td>
<td>0.40</td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>55.6</td>
<td>4</td>
<td>44.4</td>
<td>Reference</td>
</tr>
<tr>
<td>PCV (M: 40-52%, F: 36-48%)</td>
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<td></td>
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</tr>
<tr>
<td>Low</td>
<td>56</td>
<td>34.8</td>
<td>105</td>
<td>65.2</td>
<td>1.60</td>
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<tr>
<td>Normal</td>
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<td>25.0</td>
<td>3</td>
<td>75.0</td>
<td>Reference</td>
</tr>
<tr>
<td>WBC (4-11X10^9/L)</td>
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</tr>
<tr>
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<td>0.0</td>
<td>7</td>
<td>100.0</td>
<td>UD</td>
</tr>
<tr>
<td>Normal</td>
<td>52</td>
<td>36.4</td>
<td>91</td>
<td>63.6</td>
<td>1.58</td>
</tr>
<tr>
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<td>33.3</td>
<td>10</td>
<td>66.7</td>
<td>Reference</td>
</tr>
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<td>MCV (77-97FL)</td>
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<td>50.0</td>
<td>6</td>
<td>50.0</td>
<td>2.00</td>
</tr>
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<td>Normal</td>
<td>51</td>
<td>33.3</td>
<td>102</td>
<td>66.7</td>
<td>Reference</td>
</tr>
<tr>
<td>MCH (26-32pg)</td>
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</tr>
<tr>
<td>Low</td>
<td>16</td>
<td>37.2</td>
<td>27</td>
<td>62.8</td>
<td>1.17</td>
</tr>
<tr>
<td>Normal/high</td>
<td>41</td>
<td>33.6</td>
<td>81</td>
<td>66.4</td>
<td>Reference</td>
</tr>
<tr>
<td>MCHC (32-36g/dl)</td>
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<td>59</td>
<td>67.0</td>
<td>0.86</td>
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<td>36.4</td>
<td>49</td>
<td>63.6</td>
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</tr>
<tr>
<td>Platelets (150-400x10^9/l)</td>
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</tr>
<tr>
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<td>83</td>
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<td>0.7</td>
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<td>High</td>
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<td>42.1</td>
<td>11</td>
<td>57.9</td>
<td>Reference</td>
</tr>
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<td>28.6</td>
<td>5</td>
<td>71.4</td>
<td>0.75</td>
</tr>
<tr>
<td>High</td>
<td>55</td>
<td>34.8</td>
<td>103</td>
<td>65.2</td>
<td>Reference</td>
</tr>
<tr>
<td>Serum iron (11-29micromol/l)</td>
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<td></td>
<td></td>
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<td>Low</td>
<td>41</td>
<td>43.2</td>
<td>54</td>
<td>56.8</td>
<td>2.56</td>
</tr>
<tr>
<td>Normal/high</td>
<td>16</td>
<td>22.9</td>
<td>54</td>
<td>77.1</td>
<td>Reference</td>
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<tr>
<td>TIBC (45-66micromol/l)</td>
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<td></td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>14.5</td>
<td>47</td>
<td>85.5</td>
<td>Reference</td>
</tr>
<tr>
<td>Normal</td>
<td>32</td>
<td>36.8</td>
<td>55</td>
<td>63.2</td>
<td>3.42</td>
</tr>
<tr>
<td>High</td>
<td>17</td>
<td>73.9</td>
<td>6</td>
<td>26.1</td>
<td>16.65</td>
</tr>
<tr>
<td>Transferrin (26-47micromol/l)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>12.6</td>
<td>76</td>
<td>87.4</td>
<td>Reference</td>
</tr>
<tr>
<td>Normal/high</td>
<td>46</td>
<td>59.0</td>
<td>32</td>
<td>41.0</td>
<td>10.00</td>
</tr>
</tbody>
</table>
4.3 Multivariate analysis

Binary logistic regression was used to model occurrence of iron deficiency anaemia (1=present 0=absent). Twelve significant factors identified at bivariate analysis were considered for multivariate analysis. The ‘backward conditional method’ was specified in order to eliminate confounders and effect modifiers. After ten successful iterations, 4 independent predictors of iron deficiency anaemia were determined as shown in table 7 below.

Table 7: Predictors of iron deficiency anaemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>AOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Use of erythropoietin</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Taking</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not taking</td>
<td>2.94</td>
<td>1.28 – 6.67</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>History of blood transfusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Reference</td>
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</tr>
<tr>
<td>No</td>
<td>3.70</td>
<td>1.56 – 8.33</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Level of Serum iron (micromol/l)</strong></td>
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</tr>
<tr>
<td>Low</td>
<td>3.14</td>
<td>1.31 – 7.51</td>
<td>0.010</td>
</tr>
<tr>
<td>Normal</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Transferrin (micromol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/high</td>
<td>14.29</td>
<td>5.26 – 33.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AOR-Adjusted odds ratio

Adjusting for other factors, not putting a patient on erythropoietin predisposed them to iron deficiency anaemia 2.94 (95% CI: 1.28 – 6.67) times. Likewise, no history of blood transfusion was significantly associated with occurrence of iron deficiency anaemia (AOR=3.70; 95% CI: 1.56 – 8.33). Also occurrence of iron deficiency anaemia was significantly associated with low serum iron levels (AOR=3.14; 95% CI: 1.31 – 7.51). High levels of transferrin were also associated with occurrence of iron deficiency anaemia. A patient with normal/high transferrin levels was 14.29 (95% CI: 5.26 – 33.33) times more likely to have iron deficiency anaemia compared to one with low transferrin.
**10.0 DISCUSSION**

This is one of the first studies looking at iron parameters in patients with ESRD on haemodialysis both locally and regionally. There were 165 patients studied. Males were 60.6%, and females were 39.4% with a mean age of 45 years ± 16sd and a range 14-80 years. Other than the larger sample size in this study, the gender characteristics were comparable to other local studies whose target population was patients with ESRD on haemodialysis. Awiki had a sample size 50 patients, males(62%), females(38%), mean age 47.18 years ± 15.3sd, range 18 – 78 yrs (43). Ngigi had a sample size 57 patients, males (60%), females(40%), mean age 46.32 years ± 20sd, range 15- 80 years (44).

Majority of the patients were males. The observed gender disparity was probably because males had stronger economical endowment that made them access dialysis services more readily compared to the females but it has also been documented that chronic renal insufficiency is more common in males than females, hence the more males in ESRD requiring haemodialysis (45).

Notably, majority of the patient population came from central province. This could be attributed to patients easy accessibility of KNH renal unit, the high population of people in that region as depicted by population counts as well as high prevalence of diabetes in the same area so that the representative population in ESRD is more than for each of other regions.

There were no patients from North Eastern. This could be due to various reasons, possibly inability to access the haemodialysis facilities due to the distance factor to major towns with haemodialysis facilities or inability to afford the maintenance haemodialysis due lack of economic empowerment. It could also be that the core population there has very low risk factors for chronic kidney disease so that the ESRD cases are negligible, or, on the other hand they could be well endowed economically so that those who need maintenance haemodialysis were able to access the private dialysis centres in major towns in Kenya. This could be an area for a study in future to unravel the reasons why.

In this study, 44.2 % of the study patients were aged 40 years and below. This could be a selection bias since renal replacement therapy preference is generally given to younger
people rather than the older people. These are young people at their peak of productivity in the society hence the need for close surveillance and monitoring to adequately address all measures that help halt the progression of renal disease to ESRD in the community.

Majority of the patients were not getting adequate haemodialysis. Only 18.8% of the patients were able to dialyse twice per week and the remaining 81.2% dialysed once per week. This was because of the growing ESRD population yet the dialysis facilities, both structural and manpower had remained static. In the study by Awiki in 2005, all the 90 patients on haemodialysis at KNH renal unit, on average, were able to dialyse twice every week (43). Later in 2007, according to the study by Ngigi only 84% of the 105 haemodialysis population at KNH renal unit were able to dialyse twice in a week, 14% dialysed only once per week and 2% had more than three sessions per week (44). The growing haemodialysis population could be a reciprocal of the annual population growth but also by a breakdown of the measures put in place to retard progression of renal disease in the community from a public health perspective.

The prevalence of anaemia as defined the haemoglobin levels was 98.2%. It needs to be noted strongly that all the males (100%) in this study were anaemic (100 males, HB less than 13.5g/dl). Out of the 65 females, 95.4% (62) were anaemic (HB less than 12g/dl). Anaemia has been identified consistently as an independent risk factor for left ventricular hypertrophy and for hospitalisations related to cardiac and non cardiac causes as well as adverse effect on the quality of life (46).

The mean HB in this study was 7.9 g/dl ± 1.9 SD with a range of 3.8 to 13.2g/dl. This was comparable to the studies done earlier at the KNH renal unit. Awiki in 2005 found a mean HB of 8.55g/dl ± 1.35SD (43) while Ngigi in 2007 found a mean HB of 8.43g/dl ± 1.82SD (44). However, in related studies in United States of America, Fishbane et al found a mean HB of 10.9g/dl ± 1.3SD (38) while Victor et al found a HB of 11.0g/dl ± 1.03 SD(10).

According to the study by Fishbane et al, and another one by Victor et al, anaemia is also significantly prevalent in patients with ESRD on haemodialysis in United States of America (38, 10). However unlike KNH unit where anaemia is moderate to severe, the degree of anaemia in these centres is of mild severity. This is largely because these centres have adequate, sustainable resources and functioning infrastructure to effect corrective measures.
early in terms of erythropoietin and parenteral iron use. In my study, only 57.5% of the patients were on iron supplementation (50.3% i.v iron, 7.2% p.o iron). Use of erythropoietin was in only 53.3% of the study population. That showed suboptimal use of the two anaemia treatment medications that could explain the high prevalence of anaemia (98.2%) as well as the significant use of whole blood transfusions. 71 patients had been transfused in the previous three months and majority (40.8%; 29) had been transfused two units of blood.

It was not also surprising, therefore, that majority of the patients (87.9%) in this study were not able to meet the minimum target HB recommended while anaemia is being corrected according the revised 2006, NKF/KDOQI guidelines (minimum target HB of 11 g/dl). The same was reflected by an earlier study in 2010 at the KNH renal unit by Kamau who found that 87.9% of the haemodialysis study patients had HB less than 11g/dl (47).

In this study mean serum iron level was 11.1 mcmol/l ± 7.0 SD, TIBC was 51.8mcmol/l ± 14.3SD, TSAT(%) was 23.6% ± 16.6SD, serum ferritin was 676.6ng/ml ± 665.7SD, while transferrin was 25.9mcmol/l ± 7.3SD. In the study by Victor et al serum iron was 15.29mcmol ± 5.5SD, TSAT (%) was 30.6% ± 9.39SD, TIBC was 44.05mcmol/l ± 10.41SD and serum ferritin was 593.9ng/ml ± 426.76SD. (10) This showed that parameters used in iron status assessment were lower in our study population compared to the one by Victor et al (10).

Also, in this study, iron deficiency was found in 35.2% of study population, normal iron status was found in 36.4% of the study population, functional iron deficiency in 26.1% and iron overload in 2.4% of the patients. Iron deficiency anaemia was found in 34.5% of the study patients. Whereas the functional iron deficiency could be explained by the chronic inflammatory state that characterise CKD and hence ESRD, as well as unbalanced use of iron and erythropoietin, the iron deficiency status was mainly due to suboptimal use of iron and erythropoietin. Only 50.3% of the patients were using parenteral iron. A small percentage of the patients (7.2%) were using oral iron although oral iron is not recommended for treatment of anaemia in ESRD.

Assessment of the Peripheral blood films showed 19.4% of patients with microcytic hypochromic picture and all these patients had iron deficiency anaemia. Hypochromic microcytic picture on peripheral blood film was significantly associated with iron deficiency anaemia (OR 2.64; 95%CI: 1.20-5.79; P = 0.014). Majority of the patients had normocytic
normochromic picture on peripheral blood films, an expected finding reflecting the anaemia of chronic disease.

Iron deficiency anaemia was significantly associated with the 30-40 year age group (OR 3.71; 95%CI: 0.88 - 7.32; P = 0.015) and less than 6 months of haemodialysis. This was probably because it was within that age bracket most of the patients entered the renal unit for dialysis and most had not started erythropoietin and iron supplementation.

A previous history of blood transfusion was associated with low occurrence iron deficiency anaemia (OR 0.34; 95%CI: 0.18 – 0.67; P = 0.001). This may however not be a true association because these patients could have been severely anaemic to deserve the blood transfusion since blood products are routinely given to correct low HB levels and not iron levels. It is also known that repeated transfusions are associated with positive iron balance in the body and eventual iron overload.

Predictably, in this study, low occurrence of iron deficiency anaemia was significantly associated with use of erythropoietin (OR 0.28, 95%CI: 0.14 - 0.56; P < 0.001), erythropoietin dose ≥ 2000iu weekly (OR 0.14, 95%CI: 0.06 – 0.56; P < 0.001), use of parenteral iron (OR 0.27; 95%CI: 0.14 – 0.54; P < 0.001) and parenteral iron dose > 100mg weekly (OR 0.06 95% CI: 0.02 – 0.23; P < 0.001). None of the other medication use was significantly associated with the occurrence of iron deficiency anaemia. Other parameters associated with iron deficiency anaemia include low serum iron levels (OR 2.56; 95%CI: 1.29 – 5.11; P = 0.007), elevated TIBC (OR 3.42 95%CI: 5.04 – 54.99; P < 0.001), and normal or high transferrin levels (OR 10.00 95%CI: 4.55 – 20.00; P < 0.001). Never the less, these are the usual laboratory changes that are routinely observed in patients with iron deficiency anaemia. However, from this study, a low MCV level normally found in iron deficiency states was not significantly associated with iron deficiency anaemia (OR 2.00; 95%CI: 0.61- 6.51; P = 0.344).

From multivariate analysis, predictors of iron deficiency in this study included not using erythropoietin (AOR 2.94; 95%CI: 1.28 – 6.67; P = 0.01), low serum iron levels (AOR 3.14 95%CI 1.31 – 7.51; P = 0.01), normal or elevated transferrin (AOR 14.29 95%CI: 5.26 – 33.33; P < 0.001) as well no prior history of blood transfusion (AOR 3.70; 95%CI: 1.56 – 8.33; P = 0.003).
11.0 Conclusions

1. Anaemia is highly prevalent in our patients with ESRD undergoing haemodialysis.

2. Iron deficiency contributes a significant portion of the anaemia in ESRD. This is in spite of the current measures in place at the KNH renal unit to treat anaemia.

3. Iron deficiency anaemia is also common in the patients with ESRD undergoing haemodialysis.

4. Majority of the patients on haemodialysis come from Central and Eastern provinces. Majority of the patients are aged 50 years and below with a male sex predominance.

5. Blood transfusion, erythropoietin and intravenous iron use are the main modalities used to treat anaemia in the patients with ESRD on haemodialysis at KNH renal unit.

12.0 Study limitations

1. Ferritin is one of the parameters that were used to define iron deficiency in this study. However, ferritin is an acute phase reactant. It varies depending on the body’s physiologic state. Ferritin increases in various inflammatory disorders e.g. infections. It also increases in liver diseases, malignancies and haemoglobinopathies.

2. C- Reactive Protien(CRP) levels were not done to access the extent of inflammation among these patients. Therefore it was not possible to objectively determine the extent to which the chronic inflammatory process that characterises ESRD contributed to iron deficiency in this study.

3. The study was carried out at a single site (KNH- renal unit). The results therefore may not be generalizable to all the patients in end stage renal disease on haemodialysis at other sites.

4. Bone marrow iron studies, considered as the gold standard test for assessment of iron status were not done in this study.
**13.0 Recommendations**

1. There is need to scale up the anaemia management measures at KNH renal unit by increasing the dosages of parenteral iron and erythropoietin.

2. Iron and erythropoietin need to be initiated much earlier in the management of anaemia in CKD preferably in stage 3 or 4 disease.

3. There is need to strictly and promptly implement the revised 2006 KDOQI anaemia management guidelines in end stage renal disease at KNH renal unit. For patients in ESRD, the guidelines recommend evaluation of iron deficiency and IDA before starting ESAs jointly using HB, ferritin and TSAT. This provides relevant information about iron stores and iron available for erythropoiesis.

4. A study needs to be carried out to establish the aetiology of iron deficiency anaemia among patients on haemodialysis at KNH renal unit as well as to establish the other causes of anaemia in the anaemic patients found to have adequate iron stores.
14. REFERENCES


15.0 APPENDICES

APPENDIX 1

Consent explanation form
I DR. M’NTHAKA GITARI am undertaking a study on iron status in patients with end stage renal disease on haemodialysis at KNH-Renal Unit. Iron deficiency and iron deficiency anaemia is one of the common complications of patients with ESRD on haemodialysis. The study is being conducted at KNH, renal unit with cooperation from the staff and permission from the hospital administration.

Procedures
You are being asked to participate in a survey that will take 30 to 45 minutes. If you agree to participate, I will ask you questions and note your responses in writing. I will then examine you and withdraw about 8mls of blood for tests. These tests that will enable me to determine your iron status include: a full haemogram, erythrocyte sedimentation rate, a peripheral blood film, serum iron, serum ferritin, and a total iron binding capacity. I will inform you of the test results. I will then enrol you into clinical care the same day. All the results will remain confidential. The purpose of the consent form is to ask you permission to do so. If you agree to participate, I shall ask you to sign the consent form. However this form will not be linked to your answer. Your individual responses will be seen only by researcher and will be stored in a locked place under his control.

The risk to you as a participant in this study include:

Pain at the cubital region on your arm upon venepuncture.

Swelling at the venepuncture site may appear which is collection of blood under the skin (haematoma).

NB: should any of the above happen to you. Feel free to contact Dr. M’Nthaka Gitari for examination and management.

The benefit to you as a participant include:

Free evaluation of your haemoglobin level, serum iron level, TIBC, TSAT and serum ferritin.

Free copy of your results will be availed to you on request.
The findings of this study will assist in patient care as far as the management of anaemia in patients with ESRD on hemodialysis is concerned.

**Right to refuse or withdraw.**

Your participation in this research is voluntary. You do not have to participate. If you choose to participate, but prefer not to answer certain question, you are free to do so. You are also free to terminate the interview from the study at any time. You are free to ask questions before signing the consent form. If you agree to participate in the study, please sign the consent form.
APPENDIX 2

CONSENT FORM

I,................................................................agree to participate in the study on iron status in patients with end stage renal disease on haemodialysis at KNH renal unit.

I do this with the full understanding of the purpose of the study and the procedures involved. These procedures include filling in a study questionnaire and having 8 millilitres of blood withdrawn from my cubital fossa for laboratory tests, namely: a full blood count, erythrocyte sedimentation rate, peripheral blood film, serum iron, ferritin and total iron binding capacity. All the procedures involved in the study as well as the benefits and the risks to me as a participant have been explained to me by Dr M’NTHAKA GITARI/HIS ASSISTANT

Signature of patient: ........................................

Signature of witness: .....................................

Date: ..........................................................

If you have any question during the course of the study, you may contact the following:

DR. M’NTHAKA GITARI,

DEPARTMENT OF CLINICAL MEDICINE AND THERAPUTICS,

UNIVERSITY OF NAIROBI.

Mobile: 0721 563829

OR

CHAIRPERSON, KNH/UON ETHICAL REVIEW COMMITTEE,

TEL: 020-2726300/0722829500/0733606400/EXT 44102.

Investigator’s statement. I the investigator have educated the research participant on the purpose and implication of this study.

Signed: ............................................. Date: ......................................................
IDHINI

Mimi ............................................... Natoa idhini mwenyewe bila aina yoyote ya kushurutishwa au kulazinishwa kushiriki katika utafiti uliotajwa hapa kuhusu utafiti wa kwango cha madini ya mwili ya chuma (kwa kimwombo, iron) kwa wagonjwa wa figo wanaooshwa damu kwenye hospitali kuu ya Kenyatta.

Nimeelezera kikamilifu kwamba habari za kibinafsi kama vile makao yangu zitachukuliwa na vile vile mililita nane za damu zitachukuliwa kwenye mkono yangu kwa madhumuni ya vipimo.

Nimeelezera kuwa naweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihi ya mshiriki..............................................................

Sahihi ya shahidi..............................................................

Tarehe ..............................................................


Ama:

MWENYE KITI WA CHAMA CHA UTAFITI NA MAADILI.

HOSPITALI KUU YA KENYATTA/CHUO KIKUU CHA NAIROBI.

SIMU: 020-2726300/0722829500/0733606400 EXT 44102.

INVESTIGATORS STATEMENT

I, the investigator have educated the research participant on the purpose and implications of this study.

Signed ..............................................................Date..............................................................
APPENDIX 3

STUDY QUESTIONNAIRE
IRON STATUS IN PATIENTS WITH ESRD ON HAEMO-DIALYSIS AT KNH – RENAL UNIT

PATIENT RECORD FORM

(A) 1. SOCIO-DEMOGRAPHIC DATA

Name ___________________________ Hospital No. ___________________
Age ___________________________ Study No. ___________________
Sex ___________________________ Study Date ___________________
1 = male  2 = Female
Occupation
1 = employed  2 = self employed  3 = retired  4 = Others
Residence
Education attainment
1 = No formal Education  2 = Primary  3 = High School
4 = College/University

2. AETIOLOGY OF CHRONIC KIDNEY DISEASE

(a) ________________________________
(b) ________________________________
(c) ________________________________

3. DATE OF COMMENCEMENT OF HAEMODIALYSIS
_________________________________

4. NO. OF HAEMODIALYSIS SESSIONS PER WEEK.
________________________________________

5. PRESENTING COMPLAINT(S) ON THE DATE OF INTERVIEW

Palpitations, dizziness, difficulty in breathing, body swelling, bleeding, malaise.

6 (a) HISTORY OF BLOOD TRANSFUSION.

1 = Yes  2 = No

(b) HOW MANY TIMES OF TRANSFUSION IN THE PREVIOUS 3 MONTHS.

1 = Yes  2 = No
7(a) HISTORY OF BLOOD LOSS NOT HAEMODIALYSIS RELATED.

1 = Yes  2 = No

(b) IF YES INDICATE SITE OF BLEEDING (SKIN, GIT-malaena stool, haematochezia, bloody/blood stained vomitus, NOSE, DUB-menorrhagia).

8. MEDICATION USE.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade name</th>
<th>Date of commencement</th>
<th>Dose</th>
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<tbody>
<tr>
<td>Erythropoietin</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Iron (i.v)</td>
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<tr>
<td>Iron (p.o)</td>
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<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. OTHER MEDICAL CONDITIONS.

(a) Malignancies

1 = Yes  2 = No  if yes, the actual malignancy

(b) Infections

1 = yes  2 = No  if yes, indicate the actual infection

(c) Others ________________________________________________________________

(B) PHYSICAL EXAMINATION

Weight Kg  Height (cm)

Vital signs

SBP (mmHg)  DBP mmHg

PR /min  RR /min

Temperature  °C

Pallor  1 = present  2 = Absent

Koilonychia  1 = present  2 = Absent

Jaundice  1 = present  2 = Absent

Oedema  1 = present  2 = Absent

Any stigmata of blood loss  1 = present  2 = absent
C) SYSTEMIC EXAMINATION

<table>
<thead>
<tr>
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<th>Abnormality</th>
<th>Describe abnormality</th>
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<tr>
<td></td>
<td>No = 2</td>
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<tr>
<td>Musculoskeletal</td>
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</table>

Interviewers Name____________________________________________

Signature______________________________________________________
## APPENDIX 4

### LABORATORY PARAMETERS

<table>
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<tr>
<th>LAB TEST</th>
<th>RESULTS</th>
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