RECOMBINANT HUMAN ERYTHROPOIETIN (rHu-EPO) RESPONSIVENESS IN CHRONIC KIDNEY DISEASE (CKD) PATIENTS AT THE KENYATTA NATIONAL HOSPITAL RENAL UNIT.

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Declaration

This dissertation is my original work and has not been presented for a degree at any other university.

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Dedication

To God be the Glory

To my wife Zipporah, thank you for your support and encouragement. Ezzy; Ndavi, Joy and Uathimo, thank you for helping me see God's favour even when things are tough. Thank you for speaking into my life.

Tata, I especially want to thank you for how you have been a constant encourager and counsellor. Mum and Watwii for always being in my corner and pushing me on. I am forever grateful.

To Baba, Mama and the Ojiambo home you are truly family. Thank you for the persistent prayer and support.

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I am also thankful to the patients at the renal unit who participated in this study, and for the work done by my research assistants.

List of Abbreviations

AcSDKP - N-acetyl-seryl-aspartyl-lysyl-proline

ACEi- Angiotensin-converting enzyme inhibitor

AIDS-Acquired Immunodeficiency Syndrome

AIHA- Autoimmune hemolyticanemia

ARB- Angiotensin receptor blocker

CKD- Chronic Kidney Disease

CMV-Cytomegalovirus

DNA-Deoxyribonuleic acid

eGFR- Estimated glomerular filtration rate

EPO- Erythropoietin

EPOr- Erythropoietin receptor

ESA-Erythrocyte stimulating agents

ESRD- End Stage Renal Disease

G6PDD- Glucose 6-phosphatase dehydrogenase deficiency

Hobbs's- Sickle cell disease

HIF - Hypoxia-inducible factor

HIV-Human Immuno deficeincy Virus

IFNγ- Interferon gamma

IL1-Interleukin 1

IL6-Interleukin 6

IU-International Units

JAK2-Janus Family tyrosine protein kinase 2

kDa –kilodaltons

KDIGO – Kidney Disease: Improving Global Outcomes

KNH-Kenyatta national Hospital

Kt/V- a marker of dialysis adequacy

NKF-KDOQI-National Kidney Foundation Kidney Disease Outcomes Quality Initiative

mRNA- messenger ribonucleic acid

mU/ml-milli units per milliliter

PRCA- Pure red cell aplasia

rHuEPO- Recombinant human erythropoietin

RAAS-Renin-angiotensin-aldosterone system

TNF-Tumours necrosis factor

TNFα-Tumor necrosis factor alpha

TSAT-Transferrin saturation

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ABSTRACT

Background: In the Kenyan setup, unnecessary blood transfusion in chronic kidney disease patients on haemodialysis exposes them to risks that include iron overload; blood tranfusion related events and increased probability of future renal graft rejection due to alloimmunization.

Objective: We set out to determine adequacy of response to Erythropoietin as given at the renal unit in KNH as per the NHIF coverage.

Methods: This was a prospective study conducted at the renal unit in Kenyatta National Hospital over an 8 week period. The calculated sample size for this study was 15. Thirty eight (38) patients aged 18 years and above with confirmed CKD were recruited. Adequate Response to EPO was determined by an increase in haemoglobin of at least 1 g/dl every 4 weeks for the 8 weeks of follow-up.

Results: Adequate response to EPO treatment was 39% at 4 and 8 weeks, with a mean EPO dosage of 4000 Units(44-88U/kg) a week at entry. The resulting haemoglobin change was from a mean of 7.9g/dl (SD1.1) at entry to 9.4g/dl (SD1.4) at week 8. At the same time, adequate response to EPO ranged from 29% to 60% for different weekly dosages of iron sucrose at different times of follow-up with the highest EPO response with 200 mg at 4 weeks. Adequate response at week 8 was higher for those with a mean ferritin level of 962 µg/l compared to those with a mean ferritin level of 532 µg/l (p-Val 0.033).

Conclusion: The EPO response observed in this study at 39% is markedly lower than that quoted in other studies along with low doses administered, perhaps due to the constraints of NHIF provisions.

Recommendation: Increasing EPO dosage with appropriate iron administration while remaining within safe doses with regard to cardiovascular safety is an option under a more expanded NHIF cover. The other alternative of maintaining the current regime and optimising vitamin D should be

studied in our setup as has been demonstrated within Africa among other factors including adequacy of dialysis.

INTRODUCTION&LITERATURE REVIEW

The reduced life quality that comes with chronic kidney disease (CKD) and the attendant anaemia still remains a challenge in day to day care. Repeated hospitalization, cardiovascular disease, impaired cognition, decreased libido and increased mortality add to the difficulty faced by many a patient with CKD. The anaemia is typically normocytic, normochromic and hypoproliferative. As kidney function deteriorates, a major cause of anaemia is deficiency of erythropoietin (EPO).

A cross-sectional study (Prevalence of Anemia in Early Renal Insufficiency –PAERI-study) co-ncluded that the prevalence of anaemia in predialysis CKD was about 48% [1]. The proportion of those with stage 3 CKD (estimated GFR 30-59 ml/min//1.73m^2) that were anaemic was about 42% while anaemia in stages 4 CKD (estimated GFR 15-29 ml/min/1.73m^2) and 5 CKD (estimated GFR <15ml/min/1.73m^2) was reported in 54% and 75% of the patients respectively. The prevale-nce of anaemia in CKD at KNH was about 67% in a cross-sectional study carried out in 2015[2].Along with EPO deficiency, some other factors responsible for anaemia in CKD include uraemic induced inhibitors of erythropoiesis, acute and chronic infection/inflammation, acute and chroni-c blood loss and non-adherence to erythrocyte stimulating agents (ESA). Iron deficiency, hyper-parathyroidism and bone marrow fibrosis, shortened red blood cell survival, folate and vitamin B12 deficiency, impaired dietary iron absorption (hepcidin-ferroportin axis) and reticulondoendoth-elial cell iron blockade (hepcidin-ferroportin axis) further contribute to the anaemia. These also result in ESA ineffectiveness that contributes to anaemia in CKD [3].

EPO effectiveness is influenced by adequate iron intake and physiological availability of iron. In essence the dose-response relationship in-vitro and to an extent in-vivo can be demonstrated [4]. However, ESA hyporesponsiveness can occur when haemoglobin response is suboptimal for a given dose of ESA. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) attempt to define hyporesponsiveness to EPO as the existence of more than one of the subsequent circumstances [5]:

(1) A less than 2% increase in haemoglobin level at a constant EPO dose,

(2) Up to 50% increase of the EPO dose essential to preserve a particular hemoglobin level

In certain studies, hyporesponsiveness is categorised as acute when it lasts less than 4 months then remits and chronic when it persists permanently or semi permenently beyond 4 months [6].The magnitude of ESA hyporesponsiveness ranges from 5% to about 20% [4, 7] with different criteria used to establish ESA hyporesponsiveness.

This study aims to determine ESA responsiveness in patients with anaemia in CKD within our local population.

ERYTHROPOIETIN

This glycoprotein that is 30.4-kDapromotes the survival, differentiation and proliferation of red blood cell progenitors within the bone marrow. The gene that encrypts EPO is on chromosome7. With approximately 165 amino acids, the mature molecule has 4 carbohydrate chains with each chain having 10 molecules of sialic acid (giving it a low isoelectric pH of 4.4). The carbohydrate moieties are essential in preventing degradation and slowing down the clearance of EPO and bio-synthesis and secretion [8]. In the foetus, the liver is the major producer of erythropoietin and subsequently the kidney becomes the

major source of erythropoietin. Within the kidney hypoxia drives erythropoietin production via the DNA binding hypoxia-induced-factor [9]. Minor quantities of EPO mRNA have as well been detected in the spleen, liver, lung, testis and brain [10]. In certain di-seased states extra renal EPO is synthesised by the liver in hepatitis with ESRD or hepatomas, and other paraneoplastic sources of EPO [11, 12].EPO creation is controlled by delivery of oxygen related to oxygen requirements through a hypoxia-inducible factor (HIF)[13] that ctivates the EPO gene [14**]**.The states that lead to reduced oxygen, sensed at the kidney, will lead to EPO production. Other regulators of EPO production include androgenic steroids [15, 16], anabolic steroi-ds and cobaltous chloride. Protein deprivation and inflammatory cytokines (IL-6 and TNF-α) de-crease EPO production [17].EPO is secreted into plasma and within the bone marrow binds to its receptors (EPO-R) on erythroid progenitors [18]. This receptor is a high-affinity receptor expressed at relatively low levels typically between the burst-forming unit erythroid (BFU-E) and normo-blast stages, though is not present from mature RBCs[18].Biological activity is conferred to the EPO-R through the physical association of Janus Family tyrosine protein kinase 2 (JAK2) with its mem-brane –proximal region of the EPO-R cytoplasmic domain[19].

Serum level of EPO

The normal EPO levels range in human serum or plasma is about 5 - 25 mU/mL[20]. This can be augmented 100- to 1000-times in reaction to hypoxia or haemorrhage. In individuals who are healthy and anaemic patients instigated by loss of blood, haemolysis, deficiency in iron, aplastic bone marro-w or deficiencies nutritionally, levels of EPO are inversely proportional with haematocrit and hemo-globin levels[21]. In CKD, rheumatoid arthritis, AIDS and cancer the anemiais characteri-sed by low EPO levels. The IL1 and TNF levels

are noted to have a contributory role in such instances of anaemia of chronic disease apart from other complex pathological processes[17].

RECOMBINANT EPO EFFECTIVENESS AND ITS RISKS

Various forms of recombinant EPO exist with differences arising from the carbohydrate moieties. Recombinant EPO that arises from cells of mammals is completely glycosylated. Most common forms in practice are Epoietin-alpha and Epoietin-beta that are produced in Chinese hamster ovary cell lines [22]. These two differ in structure and pharmacological traits but not in therapeutic efficacies.

Differences in ESAs production have been linked to production of excess immunogenic proteins that cause production of antibody and lead to pure red cells aplasia [23]. This is a problem that can impact on dialysis patients leading to blood transfusion dependence, with attendant risks of transfusions and sensitization, whence antibody production leading to alloimmunization to potential donors' results, directly attenuating the benefits of a future kidney transplant[24].

The other major issue in ESAs is the adverse effects that lead to a prothrombotic, arterial hypertension, ischaemic heart disease and convulsions. This has led to studies that attempted to shed light on whether increased cardiovascular risks would be associated with high doses of ESAs. The landmark trials that attempted to address the risk-benefit balance of ESAs are:

1. The Normal Haematocrit study(NHS)
2. Hemoglobin correction and Outcomes in Renal Insufficiency (CHOIR) studies
3. Trial to minimize Cardiovascular Events with Aranesp Therapy(TREAT)

4. The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE).

For recombinant EPO therapy to be effective supplemental iron is required for all patients with serum ferritin less than 100μg/L or serum transferring less than 20%.Epoietin-beta dosing frequently begins at 20 to 50 IU/kg body weight 3 times a week. The dosage may be increased every 4 weeks by a weekly dose of20 IU/kg for each administration if the Hb increase is not enough [5]. For patients with CKD on dialysis the Food and Drug Administration in the USA suggests initiation of ESA treatment in those individuals with haemoglobin less than 10g/dl and as this haemoglobin approaches 11g/dl it recommends the ESA being withheld or reduced. It is noteworthy that haematocrit changes lag behind dose adjustments by 2-6 weeks. This directs the rationale of haemoglobin being assessed twice every week for 2-6 weeks after adjustment of a dose [25].

The approach to poorly controlled hypertension while on ESA therapy for the dialysis patient with CKD includes increasing antihypertensive therapy, increasing ultrafiltration or reducing the ESA dose to reduce the haematocrit response. Thromboembolic events that include migratory thrombophlebitis, microvascular thrombosis, pulmonary embolism and thrombosis of the renal artery, temporal vein and renal vein should be prevented by anticoagulation [25].This has to be balanced against the dysfunctional platelet activity in uraemia.

ERYTHROCYTE STIMULATING AGENT (ESA) HYPORESPONSIVENESS

The frequency of ESA hyporesponsiveness varies in different settings and according to the crite-ria used for its establishment [4-6]. In the Netherlands cohort study the cumulative incidence of ESA hyporesponsiveness was about 3% based on hemoglobin less than 9.7g/dL and EPO dose greater than or equal to 14,000 IU/week[26].Studies have quoted prevalence of ESAs hyporespo-nsiveness to range from 5–20%[4,7] of chronic kidney disease patients depending on the criteria for its diagnosis. The aetiopathology of ESA hyporesponsiveness include poor adherence, iron deficiency (absolute or functional)[3], inflammation[17,27,28,29,30], as well as inadequate dialysis[30], nutrient deficiencies (vitamin B12, folate, vitamin C, and carnitine)[30] and angio-

Tensin-converting enzyme inhibitors [31] among others summarized in table1.

Table 1: The aetiopathological basis of EPO hyporesponsiveness in CKD

Factors in the aetiology of ESA Hyporesponsiveness	Pathophysiological basis
Absolute iron deficiency[3] = a ferritin concentration less than 100 µg/L with or without reduced transferrin saturation (TSAT)	Haemolysis Gastrointestinal bleeding Frequent phlebotomy for laboratory studies
Functional iron deficiency[3] = a ferritin concentration greater than 100 µg/L associated with a TSAT <20%	Inflammatory state of CKD Reticuloendothelial cell iron blockade Impaired gastrointestinal absorption
Chronic Inflammation and infection[27,28,29,30]	IFN- γ, TNF- α,IL-6 result in rHuEPO erythroid progenitor cell resistance[27] Impair the release of stored iron in the reticuloendothelial system for the production of hemoglobin[28]; Enhanced hepatocyte synthesis of hepcidin especially by interleukin-6 [29].
Inadequate dialysis[30]	Uraemic toxins impair erythropoiesis and ESA responsiveness .
Hyperparathyroidism	Renal osteodystrophy resulting in osteitis fibrosa[30] has been associated with EPO hypo responsiveness secondary to increased erythrocyte fragility, inhibited EPO synthesis, erythropoiesis and bone marrow fibrosis.
ACE & ARBs	AcSDKP inhibits the entry of hematopoietic stem cells in the S phase of the cell cycle [31],
Nutrient deficiencies (vitamin B12, folate, vitamin C, carnitine)	Protein energy malnutrition[32] Nutrient depletion by dialysis and oxidative stress low intake, atrophy due to increased protein catabolism and decreased synthesis Insulin resistance. Micronutrient and vitamin deficiencies especially folic acid and B12 that lead to erythropoietin resistance increased levels of homocysteine leads to malnutrition-inflammation-atherosclerosis syndrome[33] Vitamin D deficiency[34,50]
Antibody-mediated pure red cell aplasia	Development of antibodies to endogenous EPO and the recombinant forms results in pure red cell aplasia (PRCA) whose features include resistance to recombinant human erythropoietin therapy and tralizing antibodies against erythropoietin[35]. Other causes of PRCA include autoimmune disorders, thymomas, systemic lupus erythematosus, haematologic malignancies, solid tumors, HIV, infectious mononucleosis, parvovirus B19[36], and viral hepatitis[37].The time intervals between starting EPO and developing PRCA ranges from 2 to 90 months[38].
Noncompliance	EPO noncompliance defined as use below 90%(35-55% of dialysis patients)[39]
Haemogloginopathy[40]	Thalassemia;HbSS;G6PD deficiency AIHA

ACEi, angiotensin-converting enzyme inhibitor; AcSDKP, N-acetyl-seryl-aspartyl-lysyl-proline; AIHA, autoimmune hemolyticanemia; ARB, angiotensin receptor blocker; EPO, erythropoietin; G6PDD, glucose 6-phosphatase dehydrogenase deficiency; HbSS Sickle cell disease; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

Effectiveness of ESA, according to FDA guidelines, targets haemoglobin between 11 and 12 g/d. Two landmark trials have addressed the debateable upper limit issue for concentration of haemoglobin in terms of cardiovascular safety. The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoietin Beta (CREATE) and the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) studies established the optimum haemoglobin levels at 11-12 g/dl [41, 42].

The KDIGO guidelines recommend that ESA therapy in adult CKD (non-dialysis) patients with Hb more than10 g/dl should not be initiated; those with Hb less than 10g/dl must be viewed as individuals pegged on the clinical scenario(s) be it the rate of Hb concentration fall, response to prior iron therapy, transfusion risk, associated ESA risks and the presence of anaemia symptoms [5].Of note, stage 5 CKD patients require ESA therapy to avoid Hb below 9 g/dl.

The EPO Unit

The EPO activity International Unit (U) is founded on measurements of EPO gotten utilising the exhypoxicpolycythemic mouse assay or alike in vivo assays, and is defined as the EPO amount which gives similar erythroid stimulation amount as five cobalt micromoles

Renal function.

Renal function is measured by getting the serum creatinine level of the patient and computing the Creatinine clearance utilising the Cock Croft Gault equation with serum creatinine in μmol/l (43):

$$EstimatedGFR = \frac{(140 - ageinyears)xMassinkgxK}{Serumcreatininein\ \mu mol/l}$$

K- Constant (1.23 Male;1.04 female).

CHRONIC KIDNEY DISEASE STAGES

Stage 1 disease - normal eGFR (greater than 90 mL/min per 1.73 m^2

Stage 2 disease - eGFR between 60 to 89 mL/min per 1.73 m^2

Stage 3 disease - eGFR between 30 and 59 mL/min per 1.73 m^2

Stage 4 disease - eGFR between 15 and 29 mL/min per 1.73 m^2

Stage 5 disease - eGFR of less than 15 mL/min per 1.73 m^2or ESRD

JUSTIFICATION

Transformation of the anaemia management with recombinant human erythropoietin (rHu-EPO) in patients with chronic kidney disease has improved their life quality with reduced morbidity and mortality. Response to EPO resulting from many factors has received attention and needs to be documented in Kenya especially with the National Hospital Insurance Fund covering for twice weekly dosing of EPO and twice a week of dialysis, which may not be adequate according to international guidelines. The study purpose is to examine the realities of renal anaemia care and whether it is sufficient or requires improved EPO dosing for the patients. This is a prospective study and will be carried out at the KNH renal unit.

<u>Study question</u>

How do CKD patients on dialysis in KNH renal unit respond to EPO ?

<u>Broad Objective:</u>

To determine the pattern of EPO responsiveness in CKD patients on haemodialysis.

Specific objectives:

To determine the EPO responsiveness in CKD patients on haemodialysis by assessing haemoglobin change.

Secondary objectives:

To compare those who adequately respond with those who do not in relation to these selected factors:

1. Mean EPO and iron sucrose dosage.
2. Dialysis access
3. Selected comorbidities: Diabetes Mellitus, Hypertension
4. Ferritin levels
5. Sociodemographic characteristics: age and gender

Methodology

Study design:

This was a prospective study comprising 38 patients with anaemia and CKD on haemodialysis, who qualified to be on EPO (Hb less than 9g/dl) and with 31 followed up for 4 weeks and 28 for 8 weeks during treatment with EPO. The study participants were given EPO according to what is supported by NHIF in the renal unit at 2000U twice weekly and the participant's ability to pay for additional EPO guided by inadequate response. Iron supplementation was determined by the renal unit practitioners, except for those with ferritin above 800µg/L who had their dose withheld. Responsiveness to EPO was determined by changes in haemoglobin at 4 and 8 weeks of follow up.

Study site:

KNH RENAL UNIT

Kenyatta National hospital is the largest public referral hospital in Kenya situated at its capital city. It serves people from various parts of the nation. The renal unit serves 150 patients who require dialysis and renal related health services in a month. These services are available 24 hours daily. The unit provides for 2 sessions per week of haemodialysis for those on maintenance haemodialysis. The unit manages approximately 60 patients daily under the care of nephrologists, nephrology fellows, registrars, medical officers and nurses.

Study participants

Records of all patients with CKD on haemodialysis were used to identify and recruit a total of 38patients from the renal unit who fulfilled the inclusion criteria. The study participants were adult aged 18 years and above with CKD on haemodialysis who were commencing on EPO according to the outlined criteria. 31 followed for 4 weeks and 28 for 8 weeks. Patients EPO response was based on patterns of EPO dose and haemoglobin levels over the respective follow up periods. Iron storage in the study participants was assessed using the serum ferritin during the routine total blood count, blood urea, electrolytes and creatinine estimation at the beginning of recruitment. At 4 weeks and 8 weeks haemoglobin levels were determined for each study participant.

Inclusion criteria:

1. All patients with CKD on chronic haemodialysis twice weekly
2. Those who were to commence EPO for the first time at a dose of 2000 U twice weekly as supported by NHIF
3. At least 1 month follow-up before the study

4. Those who gave informed consent
5. Haemoglobin below 9g/dl

Exclusion criteria:

1. Patients on haemodialysis diagnosed with acute kidney injury
2. Those who did not consent to the study
3. Those who required trasfusion

Data variables

EPO responsiveness:

Adequate EPO responsiveness was defined as a haemoglobin change of minimum 1g/dl in 4 weeks in this study [5].

Social and demographic characteristics:

- Age
- Gender
- Marital status
- Residence

Ferritin levels

Normal ferritin levels 40-160µg/L.

For this study serum ferritin level below 100µg/L was considered to represent absolute iron deficiency as per KDIGO [5].

Data collection

The data obtained from the patient records included the following: baseline sociodemographic characteristics, treatment history parameters and clinical characteristics. The frequency and total EPO dosage per week and unit dose changes during the 8 week follow up were charted. Ferritin levels were determined upon entry in to the study. The haemoglobin levels were noted at the start of the study, at 4and 8 weeks for 38, 31 and 28 participants respectively.

Sample size determination

The formula below was used for minimum sample size determination with the aim of demonstrating a significant difference [44] :

$$\frac{(u+v)^2\sigma^2}{(\mu-\mu_0)^2}$$

Where

-$\mu-\mu_0$ difference between mean,μ, and the null hypothesis value,μ_0;corresponding to the minimum change in haemoglobin per month=1g/dl[5]

-σ standard deviation= 1.2g/dl [45]

- u=1.28 the value of $Z_{1-\beta}$ with power of 90%

-v=1.96 the standard normal deviate at 95% confidence level

Thus, the minimum sample of 15 to be followed up to 8 weeks was used in this study. Thirty eight patients on chronic haemodialysis were recruited from July 2018 to January 2019 with 31 followed for 4 weeks and 28 for the entire 8 weeks.

Sampling technique of study participants

Screening and Recruitment

Consecutive sampling of patients on maintenance haemodialysis each week and commencing EPO was carried out. Subsequently, with consent, a questionnaire was filled and blood samples (mini-mum of 4ml) collected at entry. A history, clinical check-up and file perusal for to obtain the following data: estimated GFR, County, Contact mobile number, date of birth, marital status, gender, weight and medication.

LABORATORY

A sample of blood (about 4ml) was collected from each participant and 2 ml dispensed into a plain vacutainer for biochemistry for ferritin estimation while the other2 ml was placed in an EDTA vacutainer for total blood count (TBC) analysis with levels of haemoglobin in g/dl being the measure of interest. The containers were labelled with the study number of the participant. The collected blood sample for chemistry was centrifuged and the serum kept in the deep freezer at -20^0C until the time of analysis, while full haemogram was carried immediately. Specimen collection was carried out by the principal investigator and research assistants while handling, processing and analysis was carried out by the laboratory technologists in the Renal and Biochemistry laboratories at Kenyatta National Hospital.

At week 4 and 8 of follow-up, 2 ml of blood was collected and placed in an EDTA

vacutainer for haemoglobin levels in g/dl.

Equipment

The machines that were used for the biochemistry analysis were the Sysmex® Blood cell Counter from Hass Scientific for Full Haemogram and Muglia immunochemistry equipment for ferritin levels.

Principles of biochemical analysis

a) Ferritin measurement

The Ferritin Quantitative Test Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) The system uses one anti-ferritin antibody for solid immobilization phase and a mouse monoclonal anti-ferritin antibody within the antibody-enzyme horseradish peroxidase-conjugate solution. The test sample can react concurrently with the antibodies and sandwich the ferritin molecules from the solid phase to the enzyme-related anticorps. A tetramethylbenzidine solution is added and incubated after incubation and washing, resulting in the development of a blue colour. With the addition of 2N HCl, the color development is halted and spectrophotometrically measured at 450 nm [46]. For this study serum ferritin level below 100µg/L will be considered to represent absolute iron deficiency due to the inflammatory state of most CKD patients [3].

b) Blood cell count principles

Complete blood count was accomplished by an automated analyzer. It enunciates a very minor quantity of the sample through thin tubing with sensors that count the cells number going through it. Flow cytometry then measures and differentiates cell types in whole blood sample. The parameter of interest in this study to be measured was the Hb concentration in g/dl and determined its changes over the period of follow up as described above (Pp13).

Quality Control and Assurance

Quality control was carried out every morning to assess the functionality of the analyser and the reagents stability using the Levey-Jennings (LJ) chart. Renal laboratory runs quality control (QC) materials every day to ensure the quality of results is guaranteed at all times. Quality control ma-terials are usually analyzed in parallel with the blood specimens to ensure reliable results for all the parameters. Renal Laboratory also actively participates in monthly external quality assurance (EQA) programme provided by Riqas ® Company. Standard operating procedures were followed for specimen collection, storage and transportation. Lab- Internal QA/QC measures were adhered to. Once entry of data was done, there was a comparison of the entries in the database with manual records to guarantee accuracy. Checks were performed for data completion and inconsistencies resolved with a review of manual records before data analysis.

Data management

Data were collected by the PI and two research assistants. All filled consent and data collection forms were stored in a lockable cabinet, accessed only by the principal investigator and the statistician. Data were entered weekly into a password-protected MS Access database managed by the statistician with analysis carried out using SPSS version 23. EPO responsiveness was analysed and presented as means and percentages. Univariate analysis provided measures of central tendency and dispersion was carried out along with bivariate analysis of the association between EPO response (dependent variable) and sociodemographic and clinical characteristics.

Ethical Considerations:

The study was undertaken after approval by the DoCMT, UoN and the KNH/ UoN ERC. All eligible patients were clarified in detail the study purpose, in simple terms, with conversant written consents or assent gotten from every participant in the study. There was assurance of confidentiality every time. Patients had the freedom to stop participating at any time and there was no discrimination against them. Laboratory outcomes were conversed to their primary clinicians and a results copy reserved in the patients file on a need-to-know basis. All the patients with severe anaemia received blood transfusions and haematinics with standard review of anaemia in chronic kidney disease. Management of all aspects of CKD patients was strictly adhered to throughout the study.

The consenting process was as follows:

1. The patients were informed that the project is local research.

2. The purpose of the study was clarified to the patients.

3. All the tests have been clearly explained by the procedures of the study with full details.

4. The patients were guaranteed that taking part in study was voluntary and that they were not denied medical attention if they refused to take part.

5. Participants were fully informed about the medical benefits as well as physical and mental damage before their inclusion in the study.

6. The patients were guaranteed of full and unlimited access and recommended therapeutic interventions where necessary, in accordance with accepted professional practice.

7. Confidentiality was kept strictly and all data were securely stored, only revealed on a necessary basis, and the lead researcher was responsible for all investigation costs.

Following the full explanation and acceptance by the patient of the above, the participants signed the consent form upon request.

The funding for this study was provided by the KNH Research and Programs Department.

RESULTS

A total of 38 patients with chronic kidney disease, who were starting dialysis, were recruited between July and December 2018. Thirty one (31) patients were followed up to 4 weeks while 28 completed follow-up at 8 weeks. The numbers followed at 4 and 8 weeks were more than the minimum required sample size of 15. Those lost to follow up had moved to other facilities.

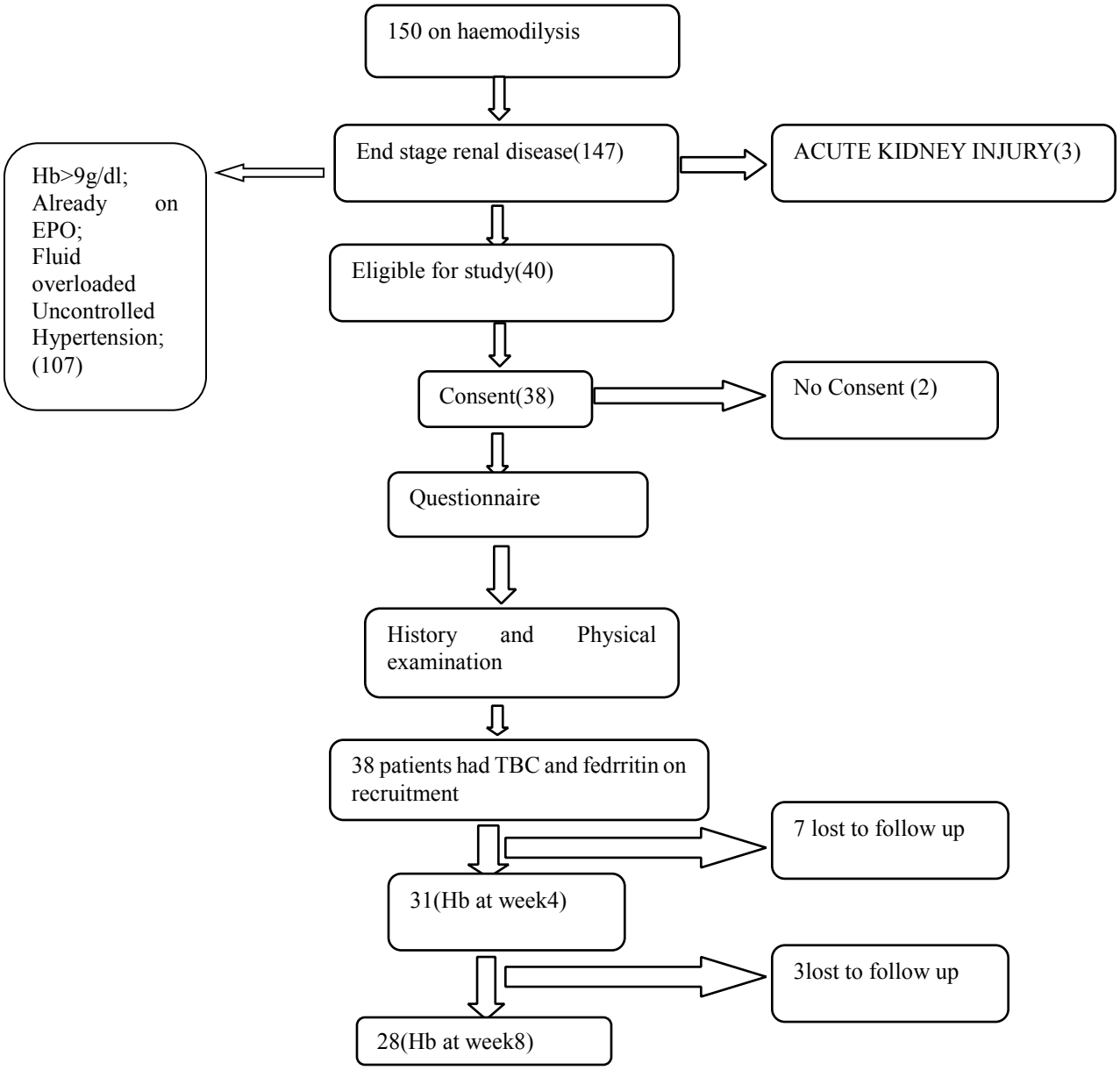

Figure 1: Flow Chart of patient flow from recruitment to completion of follow-up.

1. SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

At recruitment the study participants were predominantly residents of Nairobi City (55%) followed by neighbouring Kiambu (32%), Muranga, Nyeri and Kajiado counties. In the cohort about 37% were male, 63% female and 66% were married (table 2). The mean age was 45 years (range 19 to 74 years).

Table 2: Socio-demographic characteristics of the study participants

Variable		**N**
County of residence	Nairobi	21
	Kiambu	12
	Nyeri	2
	Muranga	2
	Kajiado	1
Gender	Female	24
	Male	14
Marital status	Married	25
	Single	9
	Other	4

2. COMORBIDITIES AND CLINICAL CHARACTERISTICS

The distribution of comorbidities among the CKD patients on dialysis at recruitment are as shown in table 3. The majority (76%) had hypertension followed by diabetes mellitus

(16%), HIV (8%) and the rest with less frequency of occurrence. Some of the patients had multiple comorbidities. Seventy nine percent had 1 comorbidity, 13% had 2 comorbidities while 8% had 3 comorbidities.

Table 3: Frequency distribution of comorbidities among the study participants.

Comorbidity	**N**	**%**
Hypertension	29	76.3
DM	6	15.8
Others	6	7.9

The mean ferritin was 734 ng/ml at recruitment with a mean eGFR 9 ml/minute/1.73m^2 (range of 2 to 16 ml/minute/1.73m^2). The number with ferritin below 100 µg/L were 3.

Of the participants 30% and 27% received iron sucrose at a dosage of 100mg and 200mg per week respectively while 43% did not receive iron sucrose due to elevated serum ferritin.

The mean EPO dosage administered twice weekly during the study at entry was 2000U twice weekly (48-88U/kg weekly) the average weight was 60kg (range 45 to 83 kg)

Table 4: Clinical and laboratory characteristics of study participants with CKD on haemodialysis

Variable	**Mean (SD)**	**Median**	**Minimum**	**Maximum**
Ferritin ng/ml	734(582)	539	22	2024
eGFR ml/min/1.73m^2	9(4)	4	2	16
Hb9(g/dl) at entry	7.9(1.1)	8	5.3	9
Weight(kg)	60(10)	60	45	83

Of note 45% had tunnelled cuffed catheters, 39% had acute catheters while 16% had an AV fistula for haemodialysis access (fig1).

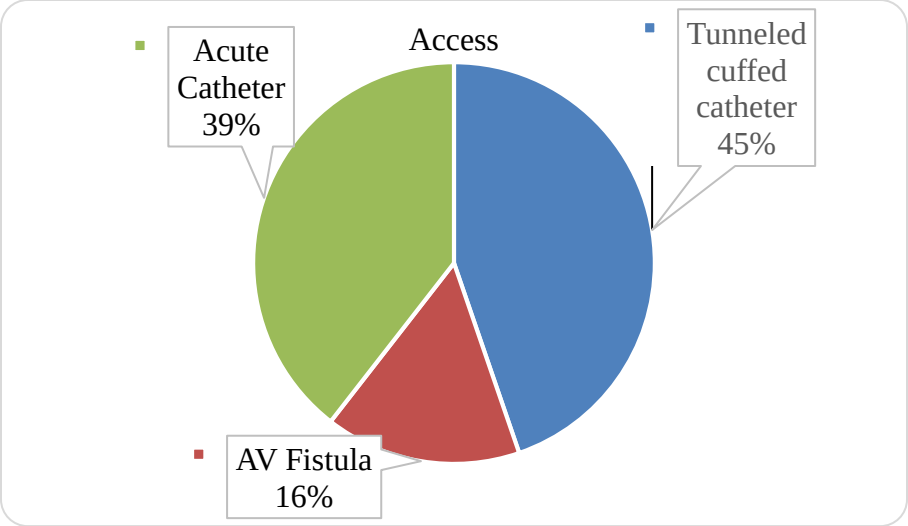

Figure 2: Distribution of haemodialysis access options

3. EPO RESPONSIVENESS

Adequate Response to EPO was determined by an increase in haemoglobin by at least 1 g/dl at every 4 weeks for the 8 weeks of follow-up. In table 5 adequate response to EPO treatment was 39% at 4 and 8 weeks.

Table 5: Responsiveness to EPO in CKD patients on haemodialysis

Variable category	**Adequate response**		**Inadequate Response**	
	No.	**%**	**No.**	**%**
Response to treatment at week 4 (n=31)	12	39	19	61
Response to treatment at week 8 (n=28)	11	39	17	61

mean of 7.9g/dl at entry to 9.4g/dl at week 8 (table 6). Iron sucrose supplementation was not given to 11 participants at 4 and 8 weeks resulting in 20 and 17 receiving iron sucrose at 4 and 8 weeks respectively. At weeks 4 and 8, 27% and 54% respectively among those who did not get iron sucrose due to high ferritin levels responded adequately to EPO. At

the same time, response to EPO ranged from 29% to 60% for different weekly dosages of iron sucrose at different times of follow-up. A higher EPO response with 200 mg than 100 mg of iron sucrose supplementation was observed at 4 weeks with no much difference at 8 weeks.

Table 6: Iron sucrose dosage and Hb response

Variable		Response to treatment at week 4				p-value
		Responsive		Inadequate response		
		N	%	n	%	
Iron sucrose dosage(mg)	0	3	27	8	73	0.242
	100	3	30	7	70	
	200	6	60	4	40	
Variable		Response to treatment at week 8				p-value
		Responsive		Inadequate response		
		N	%	n	%	
Iron sucrose dosage(mg)	0	6	55	5	45	0.412
	100	3	30	7	70	
	200	2	29	5	71	

Adequate response ranged between 17 % for participants with AV fistula and 67% for those with acute catheters at week 4 while at week 8 there was a reversal of these findings (table 7). Although the association between gender and marital status and EPO response was not statistically significant, the males were slightly more responsive to EPO than females at week 4 with the trend remaining the same at week 8. For marital status the slightly better response among the married compared to the single/divorced/widowed study participants was observed at week 4.

Table 7:EPO response and selected characteristics at week 4 and 8

Variable		Response to treatment at week 4				
		Responsive		Inadequate Response		P value
		N	%	N	%	
Access	Permanent Catheter	5	31	11	69	0.102
	AV Fistula	1	17	5	83	
	Acute Catheter	6	67	3	33	
Gender	Female	8	40	12	60	0.842
	Male	4	36	7	64	
Marital Status	Married	9	43	12	57	0.131
	Single/Widowed/Divorced	3	30	7	70	
		Response to treatment at week 8				
		N	%	N	%	
Access	Permanent Catheter	6	38	10	62	0.202
	AV Fistula	4	67	2	33	
	Acute Catheter	1	17	5	83	
Gender	Female	6	32	13	68	0.225
	Male	5	56	4	44	
Marital Status	Married	7	39	11	61	0.531
	Single/Widowed/Divorced	4	40	6	60	

In table 8 there was not much difference in the mean age and weight among those with adequate response compared to those with inadequate response at both week 4 and 8. However, those with adequate response at week 8 had a higher mean ferritin level of 962 µg/l than those with inadequate response of 532 µg/l (p-val 0.033) which difference was not observed at week 4. The diabetics in this study were found to be responsive in week 4(p-val 0.012) but in week 8 noted to be inadequately responsive (p-val 0.330). The mean estimated GFR among responders and inadequate responders was the same at the two periods of follow-up.

Table 8: EPO response and age, weight, ferritin levels and selected comorbidities

Variables				**4 weeks**	
			n	Mean (SD)	P value
Age (yrs)		Responsive	12	49 (14)	0.209
		Inadequate Response	19	42 (14)	
		Total	31	45 (14)	
Weight (kg)		Responsive	12	61 (11)	0.731
		Inadequate Response	19	60 (11)	
		Total	31	60 (11)	
Ferritin(µg/l)		Responsive	12	544 (565)	0.331
		Inadequate Response	19	735 (496)	
		Total	31	661 (524)	
HTN	Yes	Responsive	10	N/A	0.531
		Inadequate Response	14		
	No	Responsive	2		
		Inadequate Response	5		
DM	Yes	Responsive	5	N/A	0.012
		Inadequate Response	1		
	No	Responsive	7		
		Inadequate Response	18		
				8 weeks	
Age (yrs)		Responsive	11	46(12)	0.854
		Inadequate Response	17	45(15)	
		Total	28	45(14)	
Weight (kg)		Responsive	11	62(15)	0.385
		Inadequate Response	17	58(9)	
		Total	28	60(12)	
Ferritin(µg/l)		Responsive	11	962(666)	0.033
		Inadequate Response	17	532(347)	
		Total	28	701(530)	
HTN	Yes	Responsive	9	N/A	0.736
		Inadequate Response	13		
	No	Responsive	2		
		Inadequate Response	4		
DM	Yes	Responsive	1	N/A	0.330
		Inadequate Response	4		
	No	Responsive	10		
		Inadequate Response	13		

DISCUSSION

EPO RESPONSIVENESS

This was a study of responsiveness among patients with anaemia in CKD on haemodialysis and standard doses of EPO as per the NHIF provision in the expanded program of haemodialysis country wide. Baseline investigations were carried out among 38 patients who met the inclusion criteria. Upon receiving EPO at entry into the study, 31 were followed for 4 weeks while 28 completed 8 weeks of follow up. This number surpassed the minimum required number (15) of study participants to complete 8 weeks of follow up.

The participants were predominantly young females from Nairobi and its neighbouring counties. A local crossectional study carried out in the same unit had 50 % of the study participants being male. It is also important to keep this in mind as they were within child bearing ages and the fact that testosterone has some role to play in erythropoiesis.

The percentage of the participants with adequate response according to the study here at KNH study was 39 %. The current practice in the KNH renal unit provides for two doses of EPO weekly for the patients covered under the National Hospital Insurance Fund (NHIF). This limitation may explain the low level of EPO response in the study. In contrast other studies document adequate response between 80 and 96% using different criteria and higher doses of EPO [6,26]. In the NECOSAD study [26] one of its inclusion criteria was that the weekly dosage be 200U/kg/week with 96% were noted to adequately respond. In this study the mean EPO dose ranged between 44 and 88 units per kg body weight on entry while other studies have used a wide range of EPO between 15 to 500 units per kg body weight [4,6,26].Sibbel et al carried out a retrospective cohort study and noted that 89% were adequately responsive with a mean age of 62yrs and serum ferritin of 757µg/L with

a wide range of EPO doses from 2000U to over 16000U per session of dialysis[6]. Of note, the adequate responders were predominantly female at 55%; with 53% of chronic hyporesponders being female.

In Egypt, study participants on a similar dosage of EPO as those in this study with a standard 4000U of EPO weekly while commencing them on active oral vitamin D (α-calcidol) with each dialysis session. The lowered EPO resistance that was demonstrated raised the discussion of the anti-inflammatory effects of vitamin D and its contribution to the multifactorial response of anaemia in CKD in this cohort of patients on maintenance haemodialysis in Africa. This study was assessing the Erythropoietin resistive index (ERI) that is determined by the EPO dosage divided by weight and then divide by the average haemoglobin pre administration of vitamin D and compared to that post vitamin D administration. The results was a drop in ERI from 7.39 to 6.61 which is an indicator of improved responsiveness. The study population had 30 on maintenance haemodialysis three times weekly with a 1:1 ratio of male to female and mean serum ferritin of 761µg/L compared to the 734µg/L in this study.

However, the cardiovascular risks of high EPO dosage revealed in the CREATE and TREAT studies on attempts to normalise haemoglobin must be taken into consideration. A strategy to improve the response of CKD patients is to administer intravenous iron while monitoring the iron status. The average haemoglobin improved from 7.9g/dl to 9. 4g/dl during the 8 week period of follow up. In light of this, the prospective study in Egypt illustrated how with EPO dosage being restricted with the addition of oral vitamin D, the possibility that dialysis units in resource limited settings can improve the responsiveness; based on the haemoglobin increase on average by 1.14 g/dl though over a 3 month period

from 8.34g/dl to 9.48g/dl. Though this is beyond the scope of this study, it is useful to have in mind.

Supplementation with iron sucrose at 200 mg weekly resulted in clinically observable increase in EPO response compared to 100 mg weekly at 4 weeks. The PIVOTAL study provides strong evidence about proactive iron supplementation with median dose of iron at 264 mg per month, observing the safety cut-off, compared to those who got median dose of iron at 145 mg per month reactively with ferritin concentration of less than 200 micrograms/ml or the TSAT less than 20%[47]. In this study the safety cut-off limit for administration of iron sucrose was at 800 µg/l that is higher than the 700µg/l in the PIVOTAL trial. The PIVOTAL trial adds to the knowledge gap on how much iron can be given even with high ferritin levels. It also illustrated improved EPO response while maintaining lower EPO dosage in the proactive arm of the study. In this study those with high (962µg/l) ferritin levels at 8 weeks had a statistically significant better response than those with ferritin levels of 532 µg/l.

It is important to note that the iron status in CKD is better monitored with the TSAT levels due to the significant inflammation which results to an increase in ferritin as an acute inflammatory marker. The assessment of iron status in the study vis a vis ferritin is a limitation though not a constriction to the effort to tease out the impact of iron on EPO responsiveness.

The association between most characteristics and EPO responsiveness in this study was not statistically significant. This could be explained by the small sample size and the design of the study. However, a clinical difference was observed among males at 8 weeks follow-up. Those using acute catheters at 4 weeks and AV fistulas at 8 weeks were responsive.

Adequacy of dialysis was studied by Ndinya et al concluded that the central venous catheters which were mainly non-cuffed non-tunneled delivered a poor dialysis dose in 21% to 24% of the patients based on the Kt/V and Urea reduction Ratio. Based on the mean blood flow rates about 88% had a poor dialysis dose in this cross sectional study [49]. These observations point towards possible impacts on EPO responsiveness with respect to adequacy of dialysis in the renal unit at KNH. The study on fluid overload in a setting of adequate dialysis being a predictor of poor response to EPO in Japan [45] adds more evidence to the need for adequate dialysis of CKD patients on maintenance dialysis in the local setup.

CONCLUSION

In conclusion, studies have demonstrated the factors that influence EPO response. These include factors such as poor adherence[40], iron deficiency (absolute or functional)[3], inflammation[17,27,29,30],as well as inadequate dialysis[30],vitamin D[34,50],vitamin B12, folate, vitamin C, and carnitine[29], protein energy malnutrition [33], antibody mediated pure red cell aplasia[35,36,37.38] among others.

A poor rate of responsiveness of about 2 out of 5 patients with CKD on dialysis that is lower than in other studies with doses being lower than recommended with predominantly female patients were predominant. Adequate iron stores seem to be associated with better response which is an avenue for intervention

RECOMMENDATIONS

This study has documented that the EPO dosage given weekly in the unit under NHIF should be increased. This is an area that can be immediately addressed by ensuring that the one size fits all dosage does not continue to be practised. Commencing at a minimum of 100U per kg per week may be beneficial and safe as the average dosage in this study was averaging 44-88U per kg per week.

Additionally, a prospective interventional study on vitamin D supplementation in our setup with the current EPO in view of the current limitations of consistent and adequate EPO administration should be carried out [50].

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APPENDIX 1: CONSENT EXPLANATION.

My name is **Dr WILLIAM MUIA NDAVI**, a post graduate student in the department of medicine and Therapeutics, of the University Of Nairobi. I am conducting research on patients with end stage renal disease, undergoing dialysis, at the Kenyatta National

Hospital Renal Unit.

Purpose of the study.

This is a non interventional study aiming at determining the responsiveness to erythropoietin in patients with end stage renal disease and describe the associated socio-demographic, clinical and laboratory characteristics.

End stage renal disease is defined as a state in which your kidney is not able to adequately filter blood and remove waste products and extra fluid .

Erythropoietin(EPO) is a hormone produced by the kidney that promotes the formation of red blood cells by the bone marrow. In end stage renal disease erythropoietin is deficient and that is the reason the hormone is a necessary addition to care.

Procedures.

If you agree to participate in this study there will be a request that:

1. The patient or answers questions relating to their socio – demographics, past and present medical history.

2. The patient undergoes a physical examination inclusive of measurements of height and weight.

3. The patient has 6mls of venous blood drawn for the determination of your blood counts, ferritin and kidney function at the start. Then at 4weeks andthe end of 8 weeks of follow up 2ml for blood count will be drawn.

4.The patient will be followed up over 8 weeks with the weekly EPO dosage and iron sucrose level being tabulated.

Risks.

There will be minimal pain while drawing the blood sample for laboratory tests. Swelling at the site of venupuncture may appear. If it occurs feel free to get contact Dr. William Muia Ndavi for examination and management.

Blood pressure increase in kidney disease is common with a known adverse effect of EPO administration being an increase in blood pressure. This will be managed by increasing the antihypertensive with modification of the filtration of dialysis or reducing the EPO dosage. Increased blood clotting is also another concern and the standard of care in the renal unit is to provide a blood thinners (heparin) to prevent it from occurring while ensuring the risk of bleeding is kept low.

Benefits.

1. All the above examination and procedures shall be done free of charge. (The principal investigator shall bear the cost of the laboratory investigations).
2. The results of these investigations will be explained to you(the patient) a copy retained in their medical file for access by the primary doctor at the facility.
3. For those with anaemia, they stand to benefit from the care.

Confidentiality.

Strict confidentiality will be maintained and all the data obtained will be securely stored and used for purposes of this study only.

Conclusion

Participation in this study is voluntary and you (the patient) are free to withdraw at any time during the course of this study period. Your refusal to participate or withdrawal from the study will not in any way affect the quality of your treatment.

Kindly note that apart from the benefits outlined above, there will be no monetary compensation for your participation in the study. If you have any questions concerning the study kindly contact any of the following:

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Through the secretary
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APPENDIX2:CONSENT FORM

I,..... agree to participate in the study Recombinant Human Erythropoietin(EPO) responsiveness in Chronic Kidney Disease patients at KNH. I do this with the full understanding of the purpose of the study and the procedures involved. These procedures include filling in a study form, receiving Erythropoietin and having 6millilitres of blood withdrawn from my cubital fossa for laboratory tests, namely: a full blood count, serum ferritin and urea/electrolytes and creatinine on entering the study. The study follow up period is 8 weeks. Then 2ml for a full blood count on each of weeks 4 and 8 of the study period while under the standard care of EPO and haematinic management. 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 WILLIAM MUIA NDAVI/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. WILLIAM MUIA NDAVI,

DEPARTMENTOF CLINICALMEDICINE AND THERAPUTICS,

UNIVERSITY OF NAIROBI.

Mobile: 0722381549

OR

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CHAIRPERSON, KNH/UON ETHICAL REVIEW COMMITTEE,

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

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

APPENDIX 3:MAELEZO YA IDHINI

Jina langu ni Dr. WILLIAM MUIA NDAVI, mwanafunzi wa shahada ya Uzamili katika Idara ya Magonjwa ya Ndani(Internal Medicine) ya Chuo Kikuu cha Nairobi. Nafanya utafiti kwa watu walio na ugonjwa wa figo unaohitaji usafishaji wa damu katika hospitali kuu ya Kenyatta.

Madhumuniyautafiti

Utafiti huu si wa kupeana tiba lolote ilani wakufuatilia idadi ya watu walio na ugonjwa wa figo wanaotumia erythropoietini na kuonyesha vile erythropietini huongeza kiwango cha damu. Nia ingine ni kuhusiana shida hili na hali ya kijamii na uchumi; vipimo vya lebu na ulemavu wa mgonjwa.

Kushindwa kwa figo kwa ziada humaanisha kutokua na uwezo wa kusafisha damu.

Erythropoietini ni homoni inayozalishwa kutoka figo ilikukuza utengenezaji wa damu katika uboho wa mfupa. Kwa hivyo figo ikishindwa kutekeleza usafaishaji wa damu huwa na upungufu wa erythropoietini na inabidi wale wako na madhara hii kuongeze wa hii homoni katika matibabu.

Utaratibu

Kama unakubali kushiriki katika utafiti huu utaombwa:

1. Kujibu maswali kadhaa ya kijamii na ya kuhusu ugonjwa wako
2. Kufanyiwa uchunguzi wa kimwili na kupimwa ratili.
3. Kutolewa mililita 4 za damu tupeleke kupima kiwango cha damu na ferritin mwanzo wa utafiti.Ikifika wiki ya 4 pamoja na mwisho wa utafiti,yaani wiki ya 8 mililita 2 ya damu itatolewa kujua kiwango cha damu.

Hatari

Kwa kushiriki katika utafiti huu, mgonjwa hatakuwa kwenye hatari yoyote ila tu kutakuwa na maumivu madogo wakati wa kutoa damu. Ikiwa mahali damu itatolewa itafura usiwe na hofu kuitisha matibabu kutoka DR.WILLIAM MUIA NDAVI

FaidayaKushiriki:

1. Uchunguzi wote utafanywa bila malipo yoyote kutoka kwako. Mpelelezi mkuu ndiye

   atakaye gharamia uchunguzi.
2. Matokeo ya uchunguzi huu yatawekwa katika faili yako ya matibabu kwa ajili ya kutazamwa na daktari msingi katika hospitali.
3. Kwa wale walio na upungufu wadamu ,matibabu ya kuongeza damu ikihitajika itapatiwa bila vikwazo.
4. Mgonjwa atafuatiliwa kwa hiyo wiki8 akitumia Erythropoietini na kuorodhesha kiwango

cha dawa pamoja na ile madini ya chuma (kwa kimombo iron) inayoongezewa

Usiri.

Majibu/nakala yoyote itakayotokana na huu uchunguzi itahifadhiwa kwa usiri na kutumiwa Kwa ajili ya utafiti huu tu.

Hitimisho.

Kushiriki kwako katika utafiti huu ni kwa hiari na uko huru kutoka wakati wowote ,katika kipindi hiki cha utafiti. Ukikataa kushiriki au utake kuondolewa kutokana na utafiti, haitaadhiri kwa njia yoyote ubora wa matibabu yako.

Kwa maelezo au maswali yoyote kuhusu utafiti huu, unaweza kuuliza:

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Nambari ya simu 0722381549

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Dr.Wambugu Maranga
Nambari ya simu 0722554692
SLP 21070-002-02
Nairobi

Mwenyekiti – Kamati ya maadili ya utafiti.

Kupitia ofisi ya Katibu

Hospitali kuu ya Kenyatta

Nambari ya simu :020 02726300 Ext 44102

APPENDIX 4: IDHINI

Mimi .....Natoa idhini mwenyewe bilaa nia yoyote ya kushurutishwa au kulazimishwa kushiriki katika utafiti uliotajwa hapa kuhusu utafiti wa Kiwango cha kuongeza damu cha homoni ya erythropietini kwa wagonjwa wa figo wanaooshwa damu kwenye hospitali kuu ya Kenyatta.

Nimeelezewa kikamilifu kwamba habari za kibinafsi kama vile zitachukuliwa na vile mililita sita za damu zitachukuliwa kwenye mkono yangu kwa madhumuni ya vipimo.Nitafuatiliwakwa wiki nane nikitumia dawa ya erythropoietini ikiwa orodha ya madawa yanayo tumika kwa shida la figo. Vipimo vingine wakati wa wiki ya nne na ya nane vitarudiwa na kila kipimo kita kuwa mililita 2 ili kupima kiwango cha damu.

Nimeelezewa kuwa ninaweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihiya mshiriki.....

Sahihiya shahidi.....

Tarehe .....

Ukiwa na swali au jambo lolote unahitaji kuelezwa zaidi, tafadhali wasiliana na

DKT.WILLIAM MUIA NDAVI

NAMBARI YA SIMU: 0722381549.

AMA

DKT.ANTHONY OMOLLO WERE MAKAMU WA MKURUGENZI WA KITUO CHA EAST AFRICAN KIDNEY INSTITUTE KATIKA CHUO KIKUU CHA NAIROBI

NAMBARI YA SIMU:0722711444

MWENYE KITI WA CHAMA CHA UTAFITI NA MAADILI.

HOSPITALI KUU YA KENYATTA/CHUO KIKUU CHA NAIROBI.

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

APPENDIX 5: MEDICAL HISTORY AND SCREENING FORM

General Information

Participant:

Residential address

County

Contact mobile number

Birth date

Dialysis facility/facilities

Marital status:

□ Single □ Married □Divorced □Widowed

Sex

□Male □Female

Are you on any of these drugs?

Enalapril, lisinopril, ramipril, captopril, perindopril, benazapril or olmesartan,

irbesatan, candesartan, telmisartan, valsartan, eprosartan.

APPENDIX 6: MASWALI KUHUSU AFYA

Majina:

Mahali unapoishi

County

Simu ya mkono

Tarehe ya kuzaliwa

Pahali unapoosha figo:

Kijinsia

□ Mwanamume □ Mwanamke

Katika orodha ya madawa haya kuna lolote unalotumia:

Enalapril, lisinopril, ramipril, captopril, perindopril, benazapril au olmesartan, irbesatan, candesartan, telmisartan, valsartan, eprosartan.

APPENDIX 7: DATA ENTRY TABLE

Table2: Data Entry table

Age **Sex** **Weight(kg)**		
Date of commencing EPO **Ferritin at entry** **U/E/Cr at entry** **Estimated GFR**		
Hbat week	**Hb change g/dl**	**EPO Dosage** **Iron Sucrose Dosage** **Weekly** **Weekly**
0 **1** **2** **3** **4** **5** **6** **7** **8**		
Cormobidity		
Cause of CKD **Average Hours of dialysis each week** **Access : is it a permanent catheter/AV fistula /Acute catheter**		

APPENDIX 8: BUDGET

Lab investigations	**150000**
Stationary and printing	**20000**
Ethics	**2000**
Research assisstants	**40000**
Statistician	**30000**
Contingencies	**10000**
Total	**252000**

APPENDIX 9: TIMELINES

PROJECT MONTHS	Aug - Oct 2017	Nov 2017	Dec 2017	Jan 2018	Feb-Mar 2018	Apr - June 2018	July 2018	March 2019	April 2019	May 2019	Sept 2019
Proposal Development											
Departmental presentation of proposal											
Corrections											
Review by Supervisors											
Ethical review and corrections											
Application for research funding and approval											
Data collection											
Data analysis											
Write up results presentation/ & Corrections											
Completed and submitted											