SERUM ERYTHROPOIETIN IN PATIENTS WITH ANAEMIA ON HAART ATTENDING THE KENYATTA NATIONAL HOSPITAL COMPREHENSIVE CARE CENTRE.

DR DAVID KANYEKI GATUKUI

JANUARY 2013

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE), UNIVERSITY OF NAIROBI

DECLARATION

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

Signed…….Date……...

DR DAVID KANYEKI GATUKUI, MBChB (UON)

DEDICATION

This book is dedicated to my loving parents Annie and Samson Gatukui, thank you for everything, I love you and God bless you.

ACKNOWLEDGEMENTS

The Almighty God, You are Faithful

My supervisors for your honest guidance, patience and expertise during this study

My colleges for your suggestions and input

Francis Njiiri the statistician for your good work

Laboratory staff at Lancet Kenya Laboratories for conducting the tests

And the rest I have not mentioned

Thank you and God bless you

SUPERVISORS ANNOUNCEMENT

This thesis report has been submitted for examination with our approval as the University supervisors.

Signature…… Date ……..

Dr Omondi Oyoo

Senior lecturer , Department of Clinical Medicine and Therapeutics University of Nairobi

Consultant Rheumatologist Kenyatta National Hospital

Signature……. Date……

Dr Jamilla Rajab

Senior Lecturer, Haematology and Blood Transfusion Unit, Department of Human Pathology, School of Medicine, University of Nairobi.

Consultant Haematologist, Kenyatta National Hospital.

Signature…….. Date……

Prof Joshua Kayima

Associate Professor,Consultant Physician Nephrologist,Department of Clinical Medicine and Therapeutics University of Nairobi.

Signature…….. Date……

Dr Peter Wanzala

Center for Public Health Research KEMRI

Signature……. Date……...

Dr Enoch Omonge

Consultant Physician and Lecturer, University of Nairobi Department of Medicine and Therapeutics, Head, Clinical Pharmacology Unit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTG	Aids Clinical Trial Group
AIDS	Acquired Immunodeficiency Syndrome
ARV	Anti Retro Viral
AZT	Azido-thymidine (zidovudine)
Bcl-x2	B cell Lymphoma x2 gene
BFU-E	Burst Forming Unit- Erythrocyte
BMI	Body Mass Index
CCC	Comprehensive Care Centre
CD4	Cluster of Differentiation 4
CFU-E	Colony Forming Unit- Erythrocyte
CI	Confidence Interval
CMV	Cytomegalovirus
CXR	Chest Radiograph
D4T	Stavudine
ELISA	Enzyme Linked Immunosorbent Assay
EPO	Erythropoietin
eGFR	estimated Glomerular Filtration Rate
FDA	Food and Drug Authority

EuroSIDA STUDY	Europe wide cohort of patients with HIV with or without Aids
FAHI	Functional Assessment in HIV Infection
HAART	Highly Active Antiretroviral Therapy
HB	Haemoglobin
HER Study	Human Immunodeficiency Virus Epidemiology Research Study
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IL-1β	Interleukin – 1 beta
KNH	Kenyatta National Hospital
MACS Study	Multicentre Aids Cohort Study
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
NNRTI	Non Nucleoside Reverse Transcriptase Inhibitor
RNA	Ribonucleic Acid
SIE	Serum Immune Erythropoietin
TNFα	Tumor Necrosis Factor alfa
UoN	University of Nairobi
US	United States
WHO	World Health Organisation
ZDV	Zidovudine

ABSTRACT

Background: The HIV/AIDS pandemic is one of the leading health challenges in the world and Kenya today. Anaemia is the leading hematological abnormality in HIV/AIDS and an independent contributor to morbidity and mortality. HAART has been shown to be effective in reversing anaemia in HIV/AIDS, however a significant proportion of patients remain anaemic despite being on antiretroviral therapy. Deficiency of erythropoietin has been demonstrated as a cause of anaemia in HIV infected HAART naïve patients. The levels of erythropoietin have not been studied in anaemic patients who are on HAART.

Objectives: To determine serum EPO levels of HIV infected anaemic patients who have been on HAART for more than six months.

Study design and setting: Cross sectional descriptive study, carried out at a national hospital HIV treatment and follow-up outpatient facility: Comprehensive Care Centre, Kenyatta National Hospital.

Methods: A total of 196 HIV elisa positive HAART experienced patients with anaemia visiting the Comprehensive Care Centre were consecutively recruited. They were evaluated by total blood counts, CD4 count, documented WHO clinical stage and serum erythropoietin levels. Serum erythropoietin levels were measured by IMMULITE 2000 Elisa method.

Results : A total of 196 HIV positive adult patients with anaemia and who had been on HAART for more than six months were evaluated. A total of 181 (92.3%) were found to have a deficient erythropoietin response to anaemia in HIV, (EPO < 500IU/L). Subjects with a Hb of less than 6.5g/dL were up to 54 times as likely to have elevated EPO levels (OR 54.375 (5.641 - 524.139) p= 0.001) while subjects with a Hb of between 6.5 to 7.9g/dL were 27 times as likely to have elevated EPO levels compared to subjects with Hb of 9.5g/dL and above (OR27.84 (3.322 - 233.312) p=0.002. In this study Hb was the main predictor of erythropoietin response.

Co**nclusion:** Erythropoietin deficiency is nearly universal in anaemic patients on HAART for more than six months.

1.0 INTRODUCTION

Anaemia is the commonest hematologic abnormality in HIV/AIDS (1). The aetiology of this anaemia is multi factorial but it has been shown to characteristically involve a reduced increment of serum erythropoietin levels (2). Studies done in the pre-HAART era confirmed low EPO levels in HIV infected patients with anaemia and demonstrated a blunted response compared to patients with iron deficiency anaemia (3). Furthermore it has been demonstrated that EPO levels in anaemic HIV patients are similar to those seen in EPO deficient chronic kidney disease patients with anaemia (4). This reduction and blunting of the EPO response is secondary to the elevation of inflammatory mediators and cytokines. In HIV elevated levels of IL-1β have been shown to correlate negatively with EPO levels and are thought to cause a reduction of the same (5). At the same time circulating levels of the proinflammatory cytokine TNF-α receptor have been shown to correlate negatively with EPO levels in anaemic HIV infected patients (5) . The use of erythropoietin in such deficient patients has been demonstrated to reduce transfusion requirements in HIV infected patients with anaemia on treatment with zidovudine and increase quality of life (6) (7).

With the onset of the HAART era the prevalence of anaemia has declined. Nonetheless it remains prevalent and moreover it is associated with the same poor outcomes of reduced survival and poor quality of life seen in the pre-HAART era (8). It is unknown whether anaemia among HAART experienced patients is associated with the same depressed erythropoietin levels seen in HAART naïve patients. It is important to have data on EPO levels in anaemic HIV infected patients on HAART since the use of EPO to correct anaemia in HIV HAART naïve patients is a validated strategy which may be similarly effective in HAART experienced patients.

2.0 BACKGROUND

Anaemia in HIV infection has been found in up to70% of patients with advanced HIV (1). In a dissertation by Mwita, (2009, MMed UoN, unpublished Dissertation), looking at the correlates of anaemia in HIV, anaemia was overall the commonest hematologic abnormality at 33% of 411 patients in the study. It has been shown to be a statistically significant predictor of disease progression to AIDS and independently associated with the risk of death (9). Furthermore,

anaemia has been shown to have a negative impact on functional outcomes and quality of life. Fortunately, with the onset of the HAART era there has been a dramatic decline in the prevalence of anaemia in HIV as shown by Semba et al in the HER study where there was a decline of anaemia prevalence from a baseline of 38% to 26% at one year in 188 women taking HAART (10). Moore and Forney demonstrated a decline in the prevalence of anaemia in a mostly male population of 905 HIV infected patients followed up at John Hopkins, Baltimore in 1996 upon initiation of HAART. At 1 year of follow up the percentage of patients with Hb>14g/dl increased from 21% to 42% in the group that received HAART (11).

Anaemia is clinically important because a hemoglobin in the range of 10-14g/dl can be associated with decreased quality of life in measures of functional status and fatigue (12). At lower hemoglobin levels of less than 10g/dl there is association between anaemia and mortality in HIV infection (8) .

The question that remains however is what proportion of anaemia in HIV positive patients on HAART is associated with reduced EPO levels?

2.1 PHYSIOLOGY

Although the pathophysiology of HIV-1-related anaemia is not completely understood, it involves 4 basic mechanisms: a) Blood loss due to coexisting medical conditions; b) Decrease in red blood cell (RBC) production, as a consequence of bone marrow infiltration by neoplasm or infection, use of myelosuppressive medications, HIV infection itself, inadequate production of endogenous erythropoietin or a blunted response to erythropoietin ; c) Increased RBC destruction due to hemolysis; and d) Ineffective RBC production, as a result of nutritional deficiencies, most commonly, deficiencies in iron, folic acid, or vitamin B12.

The causes of HIV-associated anaemia are multi factorial; however, anaemia of chronic disease appears to be the most frequent cause. Anaemia of chronic disease is characterized by a decrease in erythrocyte production and a suppression of the reticulocyte response. A blunted physiologic response to erythropoietin is also observed. Serum erythropoietin levels are inappropriately low

for the degree of anaemia present; however, they are still higher than those of patients who are not anaemic. This type of anaemia is typically moderate, normochromic, and normocytic.

Physiologically, decreasing blood hemoglobin concentrations are followed by increasing levels of erythropoietin . EPO, in turn, stimulates the growth of BFU-E and CFU-E and increases the release of reticulocytes into peripheral blood. However this natural feedback mechanism appears to be impaired in pathological conditions including HIV, cancer and other chronic illnesses (13). When compared with patients with uncomplicated iron-deficiency anaemia, inadequately low concentrations of erythropoietin have been repeatedly reported in HIV patients (3) (5) (2). In addition, treatment with recombinant erythropoietin had a profoundly beneficial effect in a high number of anemic HIV-infected individuals (14) . This led to the hypothesis that HIV-associated anaemia may be at least partly due to relative hypoerythropoietinemia. Although the underlying pathophysiological mechanism is not yet fully understood, it is postulated that proinflammatory cytokines, i.e., TNFα and IL-1β, possibly lead to reduced renal erythropoietin synthesis.

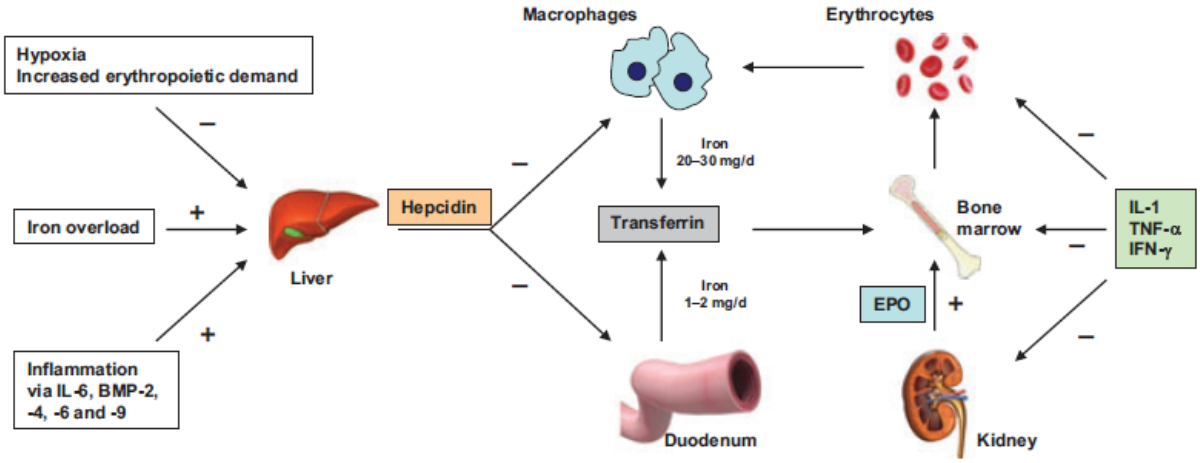

Fig 1: Effects of inflammation on erythropoiesis; Key + = stimulatory effect, - = inhibitory effect (15).

LITERATURE REVIEW

2.2 PREVALENCE OF ANEMIA IN HIV PATIENTS ON HAART

It has been noted in various studies that while the prevalence of anaemia declines with the use of HAART, mild to moderate anaemia remains common. In a study by Moore, Richard D.; Forney Darrell on the prevalence of anaemia in HIV patients taking HAART in John Hopkins School of Medicine,38% of patients had hemoglobin levels below 12g/dl despite being on HAART for one year (11). Using data from the EuroSIDA Study Group Mocroft A, et al in a study of 6725 HIV positive anaemic patients, found that of 1624 patients who initiated HAART, anaemia prevalence was 53% and 46% at six and twelve months respectively (8).

In a study by Muita et al on "The Correlates of Anemia in HIV infected patients at the Kenyatta National Hospital" , overall prevalence of anaemia in HAART experienced patients was 17.5% out of 212 patients on HAART for 6 months or greater. The predominant red cell morphology in this study for anaemic patients both HAART naïve and experienced was normocytic normochromic at 67.2%. This finding represents anaemia of chronic disease which is usually due to erythropoietin deficiency or impaired response (2). A study for MMed (Path) dissertation by Oduor et al on "Normochromic Normocytic Anaemia and Associated Diseases" showed normocytic normochromic anemia to be the commonest peripheral blood film feature in HIV infected patients in KNH (23).

Omoregie et al in a study of anaemia prevalence among HIV patients in Benin City, Nigeria, found that while non-use of HAART was associated with anaemia prevalence rates of up to 60%, patients on HAART had anaemia prevalence rates of up to 51% nonetheless(n=217) (16).

HAART has contributed to survival and overall health of patients with HIV infection, and anaemia has not been perceived to be a major clinical issue. Sharpe and colleagues conducted a retrospective study to assess the frequency of anaemia and its correlation if any with HAART. The records of 1410 patients treated at a large HIV clinic in 1998 were analysed, 13% of patients had low hemoglobin levels (<11g/dl). Low hemoglobin was associated with a low CD4 count (<200/uL). Thus contrary to current perceptions anaemia continues to be a common occurrence in the HAART era (11).

2.3 SERUM ERYTHROPOIETIN LEVELS IN HIV

Multiple pathophysiologic mechanisms are known to operate in the development of anaemia in HIV. It has been shown that erythropoietin responses are inadequate for the degree of anaemia experienced and that there is a blunted response to erythropoietin. A study by Spivak et al in 1989 measured serum immunoreactive erythropoietin in 152 HIV infected patients to determine whether anaemia occurring in HIV infection was associated with erythropoietin lack. They found that while the ability to produce erythropoietin in these patients was intact, the serum levels of erythropoietin were inappropriately low for the degree of anaemia (2).

Anaemia was present in 18% of patients who were HIV positive, 50% of patients with an AIDS related condition and 75% of patients with clinical AIDS. The mean SIE level for untreated AIDS patients (26.2\m=+-\2.4mU/mL) was greater than for patients who tested positive for HIV or patients with an AIDS-related condition but not outside the normal range for SIE (4 to 26mU/mL), and the incremental increase in SIE level for a given decline in hemoglobin level was much less in AIDS patients than in patients with uncomplicated iron deficiency anaemia (2).

Autoimmune mechanisms involving erythropoietin may also be involved in anaemia causation. In a prospective study by Aristotelis Tsiakalos et al, it was demonstrated that high titers of circulating anti-EPO autoantibody was an independent predictor of developing anaemia in HIV after controlling for other variables (17).

In a previous retrospective study they have shown that in HIV infected patients, circulating anti-EPO autoantibodies were independently associated with lower hemoglobin and higher erythropoietin levels .N. V Sipsas et al (18).

Various studies done in the pre-HAART era demonstrated the efficacy of epoetin alfa for treatment anaemia in HIV infected patients. It increased and maintained hemoglobin levels (mean ↑ 2.5g/dl) independent of change in CD4 count. There was also a reduction in transfusion requirements from 20% to 5% of patients. The mean quality of life score by the FAHI scale improved significantly (7).

HIV-infected adults with ZDV-related anemia have been shown to benefit from exogenous recombinant human erythropoietin (rHuEpo) if their serum immunoreactive erythropoietin (SIE)

levels are below 500 IU/l (12). Recombinant human EPO has been approved by the FDA for the treatment of AZT-induced anemia in AIDS patients. However, the beneficial response of EPO was observed only in those patients whose serum EPO levels were less than 500 IU/L (6). Since the serum EPO levels in some AIDS patients receiving AZT therapy are significantly higher (3000IU/L) (6), it suggested a relative resistance to the proliferation and differentiating action of EPO on bone marrow progenitor cells. Indeed, AZT was shown to cause a concentration-dependent down- regulation of EPO receptor expression as monitored by the number of EPO receptors and the levels of EPO receptor mRNA in bone marrow progenitor cells (19).Erythropoietin is a glycoprotein synthesized by the kidney in response to an oxygen-sensitive control mechanism . It binds to specific receptors in the bone marrow, stimulating the proliferation of red cell precursors and leads to an increased number of mature erythrocytes. Data on erythropoietin levels in HIV-infected adults on HAART are limited. Several factors may affect SIE levels in HIV, including anaemia, HAART and ZDV therapy.

In a study by the Canadian Pediatric AIDS research group on the serum immune erythropoietin levels and related factors amongst HIV infected children, erythropoietin levels were found to be affected by HIV infection, use of zidovudine and absence or presence of anaemia. Serum erythropoietin levels among HIV infected children were generally lower than 200IU/L. The median serum erythropoietin levels amongst HIV infected subjects with a Hb of less than 10g/dl were 98 and 31 IU/L for zidovudine treated and zidovudine naïve subjects respectively ($p=0.002$). Although SIE levels amongst HIV-infected subjects were higher than levels among children with renal failure and healthy subjects, the median levels amongst anaemic HIV-infected children were lower than those reported for children with various types of anaemia, including iron deficiency anaemia (4).

It is likely that the treatment of anaemia in HIV infection will continue to be a significant supportive strategy in the future. As we enter a new era of treatment for HIV infection, we are likely to see the need for supportive measures aimed at managing the outcomes related to drug therapy or longstanding disease. In a recent study of transfusion practices amongst US AIDS clinical trials units and North American regional hemophilia centers, 46% of centers used rHuEpo as initial treatment for anemia amongst HIV-infected patients who were not receiving

ZDV, whereas 52% used the drug as initial therapy for ZDV-induced anaemia (20). Transfusion may represent special risks for the HIV- infected patients, including virus activation with possible disease progression and de novo cytomegalovirus (CMV) infection or reinfection. Because of an underlying immunodeficiency and predisposition to infection and malignancy in HIV-infected patients, identification of those transfusion options that minimize further immunosuppression is particularly important for these patients (21).

There is emerging evidence that epoetin alfa has effects beyond erythropoiesis that may be beneficial in HIV. Silva M, Benito A & Sanz C et al have shown that EPO can induce the expression of the anti-apoptotic bcl-x2 in EPO dependent progenitor cell lines (22).

EPO has also been shown to have neuroprotective effects. In animal studies it has been shown to cross the blood brain barrier and protect neurons and astrocytes from injury (23) .

2.4 ERYTHROPOIETIN AS A TREATMENT MODALITY OF ANEMIA IN HIV

Recombinant human erythropoietin has proven beneficial in the treatment of anaemia in HIV. A large prospective study involved over 1900 HIV-infected anemic patients with serum EPO levels $\leq$ 500 U/L . Mean hematocrits increased from 28 to 33 percent after 24 weeks of rEPO (4000 to 8000 U subcutaneously six times per week) and the rate of transfusion fell significantly from 40 percent in the six weeks before the study to 29 percent after six weeks, 22 percent after 12 weeks, and 13 percent after 48 weeks (14).

Based on clinical studies EPO therapy should be reserved for patients with anaemia in whom serum EPO concentrations are below 500U/L as data from these studies suggested that patients with endogenous serum erythropoietin levels greater than 500 U/L do not generally respond to EPO treatment (6).

In a study by Abrams et al on the impact of EPO therapy on the quality of life in anaemic patients with HIV/AIDS found that EPO therapy increased and maintained haemoglobin levels (mean increase=2.5g/dl). Transfusion requirements were also significantly reduced from 20% to 5% of patients (P<0.01). Mean total QOL score measured by the Functional Assessment of HIV Infection (FAHI) scale and Physical Well-Being subscale score improved significantly (P<0.05).

QOL improvements associated with increases in haemoglobin were independent of changes in CD4+ counts and baseline anaemia severity. This study also demonstrated that such benefit was not limited to HAART naïve patients only as approximately 40 percent of the study population was on HAART. The mean baseline haemoglobin level was 9.6g/dl, indicating that these patients, on average, had WHO grade 1 anaemia at study entry (7).

2.5 ANAEMIA AND SURVIVAL IN HIV DISEASE.

Most studies examining anaemia in HIV have found that anaemia has consistently been found to be associated with mortality even after controlling for other variants known to affect survival (CD4 count, viral load). In a prospective study by Wamalwa D.C, Obimbo E.M, et al on the predictors of mortality in HIV-1 infected children on ARV treatment at the Kenyatta National Hospital, anaemia was found to be a strong predictor of mortality over a course of twenty one months after initiation of HAART. In this study 135 HIV-1 infected children aged between 18 months to 12 years were followed up between 2004 to 2008 after initiation of NNRTI based HAART . Only Hb< 9g/dl HR 3.00(95% CI 1.21-7.39) p=0.02 was predictive of mortality on both univariate and multivariate analysis. In this study mortality was highest in the first four months after initiation of HAART (24).

Data collected during the pre-HAART era from 6725 patients in the EuroSIDA cohort showed that 60% were anaemic (Hb <14g/dl for men and<12g/dl for women).Comparative data during the HAART era showed that anaemia had declined to 40%. Anaemia therefore remains a common problem despite advances in ARV therapy. Data from EuroSIDA correlating anaemia and prognosis in HIV, confirmed that even the presence of mild to moderate anaemia was associated with significantly increased risk of mortality. At recruitment to the study, 40.4% had normal levels of hemoglobin, 58.2% had mild anemia and 1.4% had severe anemia. At 12 months after recruitment, the proportion of patients estimated to have died was 3.1% [95% confidence interval (CI) 2.3–3.9] for patients without anaemia, 15.9% for patients with mild anaemia (95% CI 14.5–17.2) and 40.8% for patients with severe anaemia (95% CI 27.9–53.6; P < 0.0001)(8). In a multivariate, time-updated Cox proportional hazards model, adjusted for demographic factors, AIDS status and each antiretroviral treatment as time-dependent covariates, a 1 g/dl decrease in the latest haemoglobin level increased the hazard of death by 57% [relative

hazard (RH) 1.57; 95% CI 1.41–1.75; P < 0.0001], a 50% drop in the most recent CD4 lymphocyte count increased the hazard by 51% (RH 1.51; 95% CI 1.35–1.70; P < 0.0001) and a log increase in the latest viral load increased the hazard by 37% (RH 1.37; 95% CI 1.15–1.63; P = 0.0005) (8).

Lundgren et al for the EuroSIDA study group showed that the risk of death associated with a low haemoglobin is similar to the risk associated with a low CD4 or a high HIV1 RNA and is independent of both. Both factors were found to be independently associated with the risk of death in a multivariate model adjusted for demographic factors such as age, sex and risk group, treatment, and AIDS status. For each 1-g/dL decrease in Hb level, the relative hazard of death (RHD) was 1.39 (95% CI, 1.34–1.43; *P* < .0001). The RHD associated with a 50% decrease in $CD4^+$ cell count was 1.36 (95% CI, 1.31–1.41; *P* < .0001). The relative hazard of death rates varied between 90 fold for patients with severe anaemia and CD4<50cells/uL to 2 fold for patients with mild anaemia and CD4 count of >200c/uL compared to patients with no anaemia and CD4> 200c/uL (25).

2.6 ANAEMIA AND QUALITY OF LIFE IN HIV

The Anaemia in HIV Working group reported that 32.4%of patients on ARVs reported experiencing daily fatigue or several times per week and 50% of the patients ranked fatigue as among one of the worst side effects they experienced. In the same survey physicians reported that fatigue was one of the least significant consequences of HIV and HIV therapy. Fatigue is a primary marker of anaemia (26).

A growing body of evidence indicates that a Hb of approximately 12g/dl is associated with significant declines in functional status and quality of life. As HIV infection has become a chronic disease with the use of HAART, what was previously considered a hemoglobin level of little clinical consequence is now considered a degree of anaemia that can compromise a patients quality of life (27).

2.7 IMPACT OF ANAEMIA ON HIV PROGRESSION IN PATIENTS ON HAART

Various studies done in the pre-HAART era demonstrated that anaemia was a predictor of disease progression. In the MACS study the impact of anaemia was not the primary focus, however the baseline Hb was found to be the most significant prognostic indicator of early death (28).

In the era of HAART a similar relationship has been shown to exist between anaemia and disease progression in patients on HAART. In a case control retrospective study to examine the association of HIV/AIDS progression with the development or treatment of anaemia, the risk of disease progression was 5.2 times more likely in patients with anaemia compared to matched patients without anaemia. 75% of the study population was on HAART. Retrospective chart reviews of over 500 patients seen in a large urban infectious clinic were carried out. HIV positive patients with anaemia (cases) were matched to HIV positive patients who did not have anaemia (controls). Cases and controls were matched for age, gender, HAART use and CD4 count, anaemia was defined as hemoglobin less than11g/dl. Disease progression was defined as any HIV related opportunistic infection or death. The prevalence of anaemia in this study was 21% of the study population (29).

In a EuroSIDA study cohort Lundgern JD, Mocroft A, et al examined the possibility of a clinically prognostic scoring system for patients receiving HAART. The study cohort involved 2027 HIV infected patients and involved patients who were anaemic and those with normal Hb >12g/dl for women and>14g/dl for men. The relative hazard of clinical progression was 2.2 among men with hemoglobin levels 8–14 g/dL and women with levels of 8–12 g/dL. The relative hazard of clinical progression was 7.1 in men and women with hemoglobin levels of <8 g/dL (9).

3.0 JUSTIFICATION

Mild to moderate anaemia remains common even with the use of HAART in HIV infected patients. Anaemia in patients on HAART has been shown to be associated with disease progression, increased morbidity and mortality and poor quality of life. HIV infection is

characterized by a chronic and systemic inflammatory process and recurrent infections, settings in which serum EPO levels have been shown to be characteristically low.

Data from various studies done in the pre-HAART era demonstrated that a blunted erythropoietin response, compared to other non-HIV infected anaemic patients, to be an underlying mechanism of anaemia of chronic disease in HIV. These studies led to the use of EPO in the treatment of anaemia due to zidovudine initially and then in patients with AIDS in general. This use has been associated with a decline in the transfusion requirements and an increase in the quality of life in HAART naïve patients. At the same time efficacy of using human recombinant erythropoietin for correction of this anaemia has been demonstrated.

It has been demonstrated in various studies that HAART is effective in the reversal of anaemia and further is protective against the development of anaemia depending on the duration of use. While the mechanisms involved in anaemia reversal are not fully understood, it is known that HIV infection is associated with abnormal growth of committed hematopoietic progenitor cells. Further, HIV-1 infection of marrow stromal cells is sufficient to result in anaemia and other cytopenias. Additionally marrow suppression by opportunistic infections, tumors, or various medications may also contribute to the anaemia commonly observed in HIV-infected persons. HAART may ameliorate many of these effects in an indirect manner simply by decreasing the HIV-1 viral burden.

Locally a high prevalence of anaemia has been demonstrated in HIV infected HAART experienced patients and erythropoietin may be effective in treatment and reversal of this, if EPO deficiency is demonstrated in this population. Locally erythropoietin has been shown to be available and affordable and in common use for example in the treatment of anaemia of chronic kidney disease, furthermore it may be a cheaper, more convenient and safer alternative in the management of mild to moderate anaemia compared to blood transfusion which requires hospital admission, causes immune activation with a risk of enhanced viral replication and exposes the patient to transfusion related infection such as hepatitis B and C amongst others.

There exists a knowledge gap whether erythropoietin deficiency underlies the anemia seen in HAART experienced patients. If present, it will allow further studies to be done on the use of EPO to correct for anaemia in HAART experienced patients.

4.0 OBJECTIVES

Broad objective

1. To determine EPO profile of anaemic patients on HAART at the comprehensive care clinic in Kenyatta National Hospital

Primary objective

1. To determine the serum EPO levels in HIV patients on HAART with anemia and thereby determine the proportion of patients with EPO deficiency in the study population.

2. To document sociodemographics, hemoglobin levels, CD4 count and WHO clinical stage in the study population.

Secondary objectives.

- To explore the association between serum EPO deficiency and:
    1. *CD4 count*
    2. *Hemoglobin*
    3. *WHO clinical stage*

5.0 METHODOLOGY

5.1 STUDY SITE

The study was carried out at the Kenyatta National Hospital Comprehensive Care Centre.

5.2 STUDY POPULATION

The study population was HIV infected patients aged 18 years and above attending the comprehensive care centre at Kenyatta National Hospital who had been on HAART for more than six months.

5.3 STUDY DESIGN

This study was a cross-sectional study.

5.4 CASE DEFINITION

A case was defined as any patient aged 18 years and above with a laboratory diagnosis of HIV infection using HIV ELISA, Bioline or Western Blot who has been on HAART for 6 months or more.

5.5 DEFINITION OF TERMS

Anaemia was defined as a haemoglobin concentration of less than 11g/dl classified as per WHO criteria (30) (31).

HAART- was defined as any one of the following combinations of antiretroviral medications; 1) one protease inhibitor plus two nucleoside analogues 2) one protease inhibitor plus one nucleoside analogue plus one non-nucleoside analogue 3) 2 nucleoside analogues plus one non-nucleoside reverse transcriptase inhibitor.

CD4 count- the current CD4 count was defined as the value done at the time of the study.

Duration of treatment-was defined as the cumulative duration of treatment of an individual up to the time of the recruitment day

WHO stage- was considered as the stage documented in the patients case file on the day of recruitment into the study.

6.0 PATIENT SELECTION

Inclusion Criteria

-Patients with a diagnosis of HIV older than 18 years attending the comprehensive care centre at Kenyatta National Hospital.

-Patients who had been on HAART for more than 6 months

-Patients who had given informed consent

-Patients with anaemia as per the defined hemoglobin concentration

Exclusion Criteria

-Patients with a serum creatinine greater than 130mmol/L or eGFR of below 90ml/min

-Pregnant women.

-Patients with documented poor adherence to HAART

-patients who refused to give consent

6.1 STUDY VARIABLES

Dependent variables

1. Haemoglobin level
2. HIV staging by WHO criteria
3. CD4 count
4. Demographic data: age, gender, marital status, employment and education level.

Independent variable

1. Seum erythropoietin level

6.2 SAMPLING METHOD

Screening and recruitment

The principle investigator with the help of the study assistant went through the files of patients booked for a particular day. Files for patients who had been on HAART for more than six months were selected, patients serial three monthly total blood counts were reviewed and those found to be anaemic were further screened according to the inclusion and exclusion criteria to achieve the specified sample size. Patients were sampled consecutively until the specified sample size was achieved.The study assistant was a registered clinical officer.

Patients who satisfied the inclusion and exclusion criteria were then interviewed in the examination room where the study was explained in a language they understood. Consent was sought for and if given, the process proceeded as explained below.

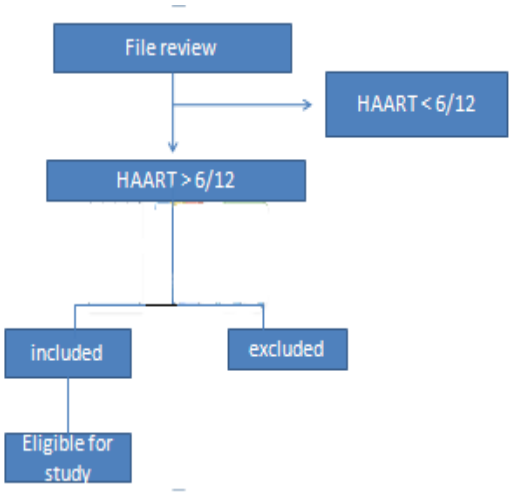

Fig 2: Sampling frame for patient accrual

6.3 SAMPLE SIZE DETERMINATION

The sample size was determined using the following formulae (3).

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where n is the required sample size

Z is the critical value of a standard normal distribution at 95% confidence = 1.96

P Prevalence – we used a value of 50% there being no previous prevalence studies on erythropoietin profile

D is the margin of error approximately 7%= 0.07

Substituting in the above formula we got

N = 196

6.4 CLINICAL METHODS

The sociodemographic profile and clinical data was obtained according to a study proforma and was completed by ticking the appropriate response in the box provided. For patients who were unable to read the principle investigator or study assistant interpreted the questions in Kiswahili.

6.5 LABORATORY METHODS

From each patient, five milliliters of blood was drawn for CD4, total blood count, serum creatinine and serum erythropoietin level determination. All blood samples were collected at between 9am and 12pm and transported at ambient temperatures to the designated laboratories. TBC was done at the CCC laboratory while serum erythropoietin levels were done at Lancet Laboratories. TBC was determined by coulter counter method which is a quantitative automated hematology analyser that calculates WBC count, hemoglobin concentration, haematocrit, platelet count, mean corpuscular hemoglobin concentration. CD4 count was determined by the Partec Cytoflow method described in appendix 2.Serum erythropoietin levels were determined using the IMMULITE 2000 EPO test done at Lancet Laboratories Kenya described in appendix 1.

7.0 DATA MANAGEMENT AND ANALYSIS

The collected data was verified, cleaned and entered into a computer data base and analysis ~done using SPSS-Version 17.0 programme. Chi square test was used in the analysis of categorical variables such as association between WHO stage and HAART regimen with erythropoietin levels.

Students T-test was used to test whether there was correlation between continuous variables such as age, Hb and erythropoietin levels. 95% confidence interval was used to determine the factors that could explain the outcome variables. P value of less than 0.05 was considered significant

8.0 ETHICAL CONSIDERATIONS

1. Permission to carry out the study was sought from the Kenyatta National Hospital Scientific and Ethical Review Committee.

2. Patients were enrolled into the study after giving informed written consent.

3. Results of the investigations were communicated to the primary health care providers at the Comprehensive Care Centre to facilitate HIV/AIDS care as required.

4. Those that decline to give consent were not discriminated upon.

5. Confidentiality for each client was maintained.

RESULTS

Between June 3rd 2012 and August 28th 2012 a total of 651 patients were evaluated for inclusion into the study, 455 were excluded for various reasons as shown in the flow chart below leaving 196 whose data was analysed.

Figure 2: Recruitment flow chart

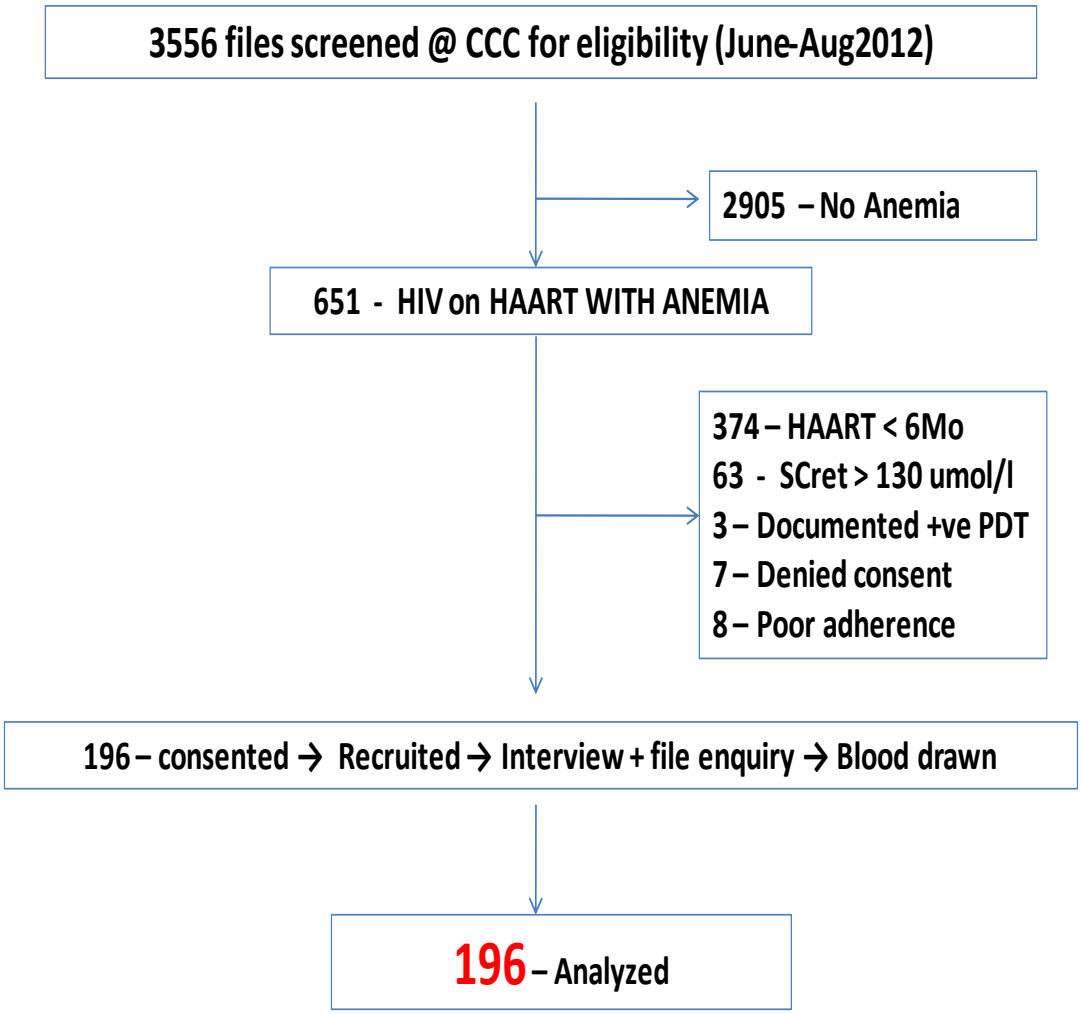

Table 1: Sociodemographic characteristics of the patients

VARIABLE	F (%) N = 196	95% CI
GENDER Female Male	 128 (65.3%) 68(34.7%)	 58.64% to 71.96% 28.04% to 41.36%
AGE in yrs Mean (SD) Median (IQR) Min-Max	 39.70 39 (28-50) 20-72	
MARITAL STATUS Single Married Separated Widowed Divorced	 38 (19.4%) 116 (59.2%) 10 (5.1%) 26 (13.3%) 6 (3.1%)	 13.86% to 24.94% 52.32% to 66.08% 2.02% to 8.18% 8.55% to 18.05% 0.67% to 5.53%
EDUCATION None Primary Secondary Tertiary	 2 (1%) 41 (20.9%) 117 (59.7%) 36 (18.4%)	 -0.39% to 2.39% 15.21% to 26.59% 52.83% to 66.57% 12.98% to 23.82%
EMPLOYMENT STATUS Employed Unemployed Retired Student	 162 (82.6%) 28 (14.3%) 5 (2.6%) 1 (0.5%)	 9.4% to 19.2% 0.37% to 4.83% -0.49% to 1.49%
RESIDENCE **Eastern block**(Nairobi, Central, Eastern, N.Eastern & Coast) **Western block** (Rift Valley, Nyanza, Western)	 161 (82.1%) 35 (17.9%)	

Majority of the study cases were of the female gender (65.3%) with a male to female ratio of 1:1.8. The study population was young with a median of 39 and a mean age of 39 years

minimum age was 22 years with a maximum of 72 years. Majority of the study cases 116 (59.2%) were married. Most of the study population 162 (82%) was engaged in gainful employment. Most of the study population, 161 (82.1%) came from Nairobi, Eastern, Central and Coast provinces.

Table 2: Clinical characteristics of the study cases (n=196)

VARIABLE	FREQUENCY % N=196	95% CI
WHO STAGE		
1	11 (5.6%)	2.38%- 8.82%
2	32 (16.3%)	11.13%-21.47%
3	99 (50.5%)	43.5%-57.5%
4	54 (27.6%)	21.34%-33.86%
CD4		
>500	21 (10.7%)	
350-499	42 (21.3%)	
200-349	65 (32.75%)	
<200	69 (35.25%)	
HAEMOGLOBIN		
9.5-11 G/DL	88 (44.9%)	37.94%-51.86%
8.0-9.4G/DL	62 (31.6%)	25.09%-38.11%
6.5-7.9 G/DL	33 (16.8%)	11.57%-22.03%
<6.5 G/DL	13 (6.6%)	3.12%-10.08%
DURATION OF HAART (months)		
MEAN	22	
MEDIAN	13	
MIN-MAX	6-116	

Majority of our patients had WHO stage 3 and 4 HIV disease as was documented in the patients file on the day of study inclusion. 50% had stage 3 HIV disease and 27% had HIV stage 4 disease by WHO classification. Combined patients with WHO stage 3 and 4 were the majority at 163 (78.1%)

Similarly, the larger proportion of patients was observed to have moderate to severe immunosuppression. Majority 134(68%) had a CD4 count of less than 350c/µL while only 69 (35%) of patients had CD4 cell count of less than 200c/µL.

Majority of the study population had mild to moderately severe anaemia by WHO/ACTG grading of anaemia severity. (44.9%) and (31%) of the study participants had grade 1 and grade 2 anaemia respectively. Only 6% of the study participants experienced severe grade 4 anaemia.

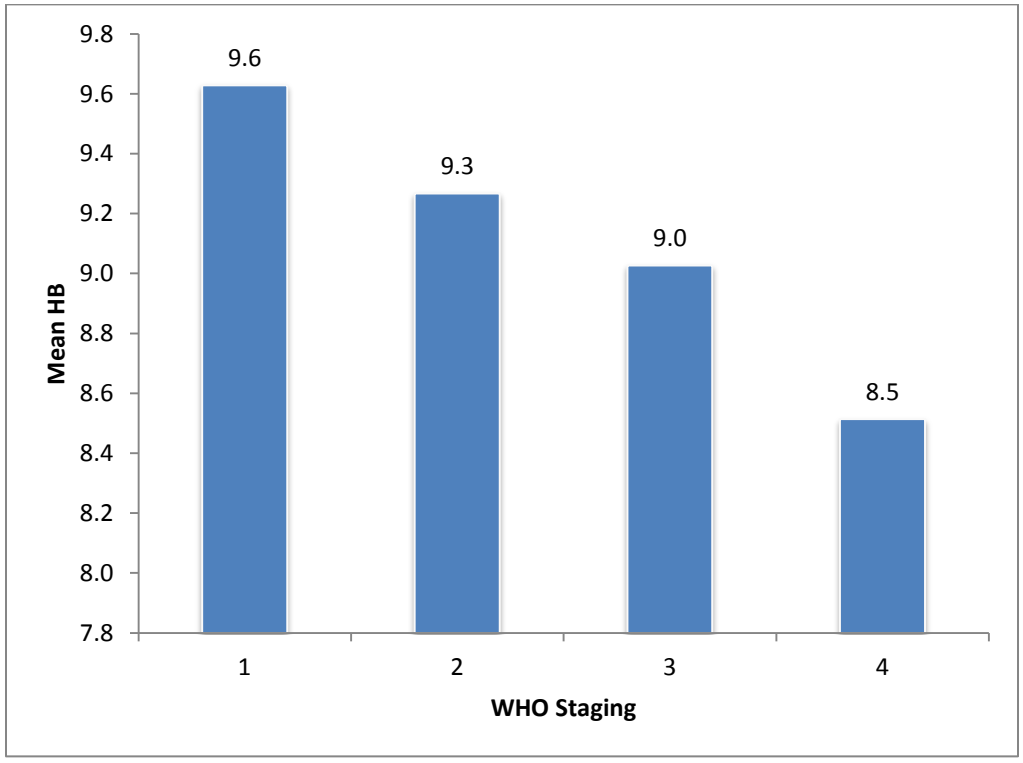

Figure 3: Mean haemoglobin for each WHO stage

Mean haemoglobin levels were observed to be lower with advancing WHO stage, in study participants with stage 4 HIV disease mean Hb was 8.5g/dL compared to a mean of 9.6g/dL for patients with stage 1 disease.

Table 3: Distribution of the HAART regimens in the study population

NRTI backbone	N=169	%
TDF Based	163	83%
AZT Based	18	9%
D4T Based	5	3%
Others	10	5%

Majority of the study cases 163 (83%) were on first line HAART which was Tenofovir (TDF) based, 18 (9%) of the study cases were on AZT based regimens. All of the study cases were on a backbone of HAART that included Lamivudine (3TC) as the second nucleoside reverse transcriptase inhibitor.

Table 4: Comparison of who staging and cd4 count in the study population (N=196)

WHO	MEAN CD4 COUNT	MEDIAN CD4 COUNT	95% CI	WHO CD4 COUNT RANGE
STAGE 1	346	344	328- 363	>500
STAGE 2	313	295	295-330	350-499
STAGE 3	295	250	272- 317	200- 349
STAGE 4	171	117	149-192	<200

The mean CD4 counts in stage 1 of 346 and in stage 2 of 313 were lower than expected from the WHO value of 500 and 350- 499 respectively. The mean CD4 count in stage 3 and 4 correlated with WHO values.

SERUM ERYTHROPOIETIN LEVELS

We then went ahead to assay blood samples in order to determine the serum EPO levels in the study cases. For descriptive and correlation purposes we took EPO deficiency as serum level of less than 500IU/L as described in literature for anaemic populations with HIV.

Table 5: Prevalence of Erythropoietin deficiency in the study cases (n=196)

VARIABLE	F (%) N-=196	95% CI
SERUM EPO MEAN (SD) MEDIAN (IQR) MIN-MAX	 137.77 67.55 4- 850	
ERYTHROPOIETIN STRATA <500 IU/L >500IU/L	 181 (92.3%) 15 (7.7%)	 88.57%-96.03% 3.97%-11.43%

Erythropoietin deficiency was almost universal in the study cases with 181 (92.3%) having levels below 500 IU/L and only 15 (7.7%) having levels above 500IU/L. Mean Serum EPO was 137 IU/L with a median of 67IU/L.

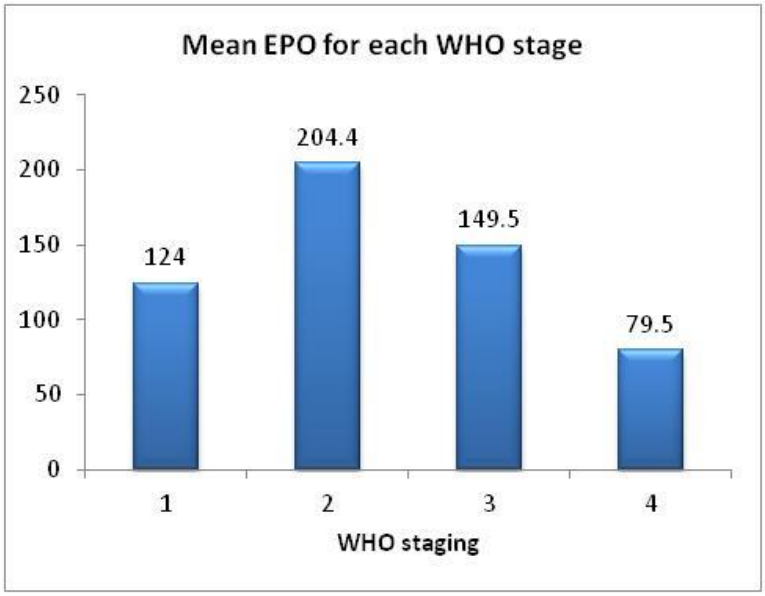

Figure 4: Mean EPO levels at each WHO stage

Patients in WHO stage 4 were observed to have the lowest mean EPO levels of 79.5IU/L. There was a general trend to have declining EPO levels with advancing HIV disease as seen by higher EPO levels of 204IU/L and 149IU/L in HIV stage 2 and 3 respectively.

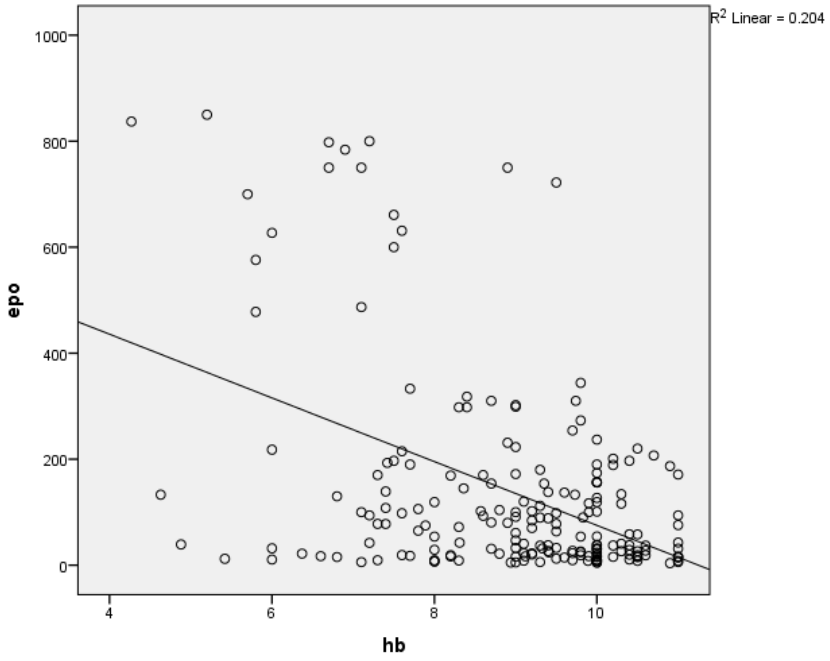

Figure 5: Linear regression analysis EPO against Hb

On linear regression analysis, there was a trend towards high erythropoietin levels but this was typically observed when haemoglobin was below 6g/dL. Majority of the study population also demonstrated rising erythropoietin levels with anaemia but these were typically below 500IU/L.

ASSOCIATIONS BETWEEN VARIABLES.

Bivariate analysis was then performed to determine associations between serum EPO levels and various patient and disease characteristics of interest. These were WHO staging of HIV, anaemia severity by WHO/ACTG grading and the CD4 count.These associations are illustrated in the table below.

		epo.groups2						
		<500 IU/L		>/=500 IU/L				
		n	%	n	%	Chi-square	P value	OR (95%OR)
Anaemia	9.5 - 11.0 g/dl	87	98.9%	1	1.1%	38.798	1	1
	8.0 - 9.4 g/dl	61	98.4%	1	1.6%		.803	1.426 (0.088 - 23.245)
	6.5 - 7.9 g/dl	25	75.8%	8	24.2%		.002	27.84 (3.322 - 233.312)
	<6.5g/dl	8	61.5%	5	38.5%		.001	54.375 (5.641 - 524.139)
WHO group	1-2	37	86.4%	6	13.9%		1	1
	3-4	144	94.2%	9	5.8%		0.088	0.385 (0.129 - 1.151)
cd4.groups1	<200 cells/uL	67	97.1%	2	2.9%	4.473	1	1
	200-499 cells/uL	94	88.7%	12	11.3%		.063	4.277 (0.927 - 19.738)
	>/=500 cells/uL	20	95.2%	1	4.8%		.680	1.675 (0.144 - 19.447)

Table 6: Univariate analysis of factors associated with erythropoietin levels

Patients with a haemoglobin of less than 6.5g/dL were up to 50 times more likely of having elevated serum EPO levels compared to patients with a haemoglobin of greater than 9.5g/dL (OR 54 (5.6- 524) p= 0.001), likewise study cases with a haemoglobin of between 6.5- 7.9 g/dL were up to 27 times more likely of having elevated EPO levels compared to cases with a hb of greater than 9.5g/dL (OR 27 3.3- 233) p= 0.002).

Patients who were WHO class three and four were seven times less likely to have elevated EPO levels compared to patients who were WHO class one and two (OR 0.385 (0.129 – 1.151) however this was not statistically significant p value 0.088

DISCUSSION

Erythropoietin deficiency has been demonstrated in anaemic HIV positive patients in the pre HAART era due to the effects of profound immunosuppression and inflammation. It has not been conclusively demonstrated whether HAART, due to its immune reconstitutive ability, is associated with reversal of EPO deficiency in patients with anaemia. This study therefore sought to determine whether the same deficiency occurs in HAART experienced patients with anaemia in an ambulatory care setting in Kenyatta National Hospital. Furthermore these levels are likely to be lower with advanced HIV disease, we therefore went ahead to explore associations between erythropoietin deficiency and current documented WHO AIDS stage, current CD4 count and haemoglobin level of the study population.

The study population was young with a mean age of 39 years and was observed to have advanced HIV disease supported by the proportions of patients in WHO stage three and four disease. This generally mirrors the findings of a similar study by Mwita et al in a study on the Correlates of Anaemia in HIV where he found a mean age of 38.9 years (n=411). The mean CD4 counts of our study cases correlated well for the WHO stage of disease especially for stage three and four.

Females were 128 (65.3%) of the study participants compared to males who were 68 (34.7%) of the study population with a male to female ratio of 1:1.8 which correlates well with that found by Mwita (M:F,1:1.6) who carried out a study on the Correlates of Anaemia in HIV in the same setting. The Kenya AIDS Indicator Survey 2007 showed that 3 out of 5 or 60% of HIV infected Kenyans are females and our study though limited to a single outpatient setting captured this finding (33).

Ninety-nine (99) (50.5%) of the study cases and 54 (27.6%) of the study cases were WHO class three and four respectively. This observation shows that a large proportion of the study population had advanced HIV disease. This finding was replicated in the study by Mwita et al whereby he studied the Correlates of Anaemia in HIV, majority of the patients with anaemia (29 (78.4%) n=37) six months or more after being on HAART were WHO class three and four.

However this finding is consistent with the observation that anaemia is a marker of disease progression. This has been demonstrated in the EuroSIDA prospective study cohort in Europe where it was shown that patients with anaemia six months after initiation of HAART had a relative hazard of disease progression of 2.2 – 7.1 for mild to severe anaemia respectively compared to HAART experienced patients with no anaemia (25).

Mean serum EPO level in the study population was 137 IU/L (110 – 164 IU/L) 95% CI) (normal response to anaemia > 500 IU/L).The study population demonstrated near universal erythropoietin deficiency with 92% having levels below 500IU/L despite varying degrees of anaemia. The cutoff for EPO response has been derived from well validated EPO replacement studies in anaemic HIV positive patients in the pre-HAART era. Patients with levels below 500IU/L have been shown to benefit from EPO replacement and are thus deemed deficient (6)

Similarly a high prevalence of insufficient EPO response in the face of anaemia has been demonstrated in similar studies all which were carried out before the widespread availability of HAART. In a study by Abrams et al carried out in San Francisco, California, USA, EPO deficiency was prevalent in 82% of 269 participants who took part in an EPO replacement study. Of note is that only 40% of this study population was HAART experienced (7). Mean Hb in Abrams' study was 9.6g/dL compared to a mean of 8.96g/dL in our study, mean CD4 count was 118cells/μL and the mean age was 40years compared to means of 270cells/μL and 39 years in the study by Abrams and this study respectively. This shows that the two studies were well matched and despite being on HAART, both populations were found to have severe immunosuppression.

Likewise, in a Canadian paediatric study by Allen et al EPO deficiency was universal in a paediatric population with HIV and anaemia that was HAART naïve, this study found a similar EPO response in the study population when compared to a subset of patients with anaemia secondary to chronic kidney disease. In this study HIV infected children invariably had EPO levels below 200IU/L, despite being anaemic with a mean Hb of 10.6g/dL.(4)

On bivariate analysis haemoglobin was the main predictor of the serum erythropoietin profile with lower haemoglobins being significantly associated with higher erythropoietin levels. This finding was similar to that of Allen et al (4).

The mechanisms of anaemia in the setting of chronic inflammation are complex and multi factorial. Erythropoiesis is impaired by among other factors reduced iron recycling by macrophages, elevated levels of tumor necrosis factor and interferon which inhibit erythropoiesis and by reduced increment in the levels of erythropoietin towards anaemia (2) (5). In our study we examined the erythropoietin levels in anaemic patients who were on HAART at various stages of HIV. The severity of anaemia was found to increase with the severity of the HIV infection but the level of EPO failed to increase commensurately, suggesting that one of the reasons for anaemia in this population was erythropoietin deficiency. Likewise anaemia in other chronic inflammatory disorders has been demonstrated to be associated with inadequate erythropoietin response. By using anaemia as our inclusion criteria in patients who were already HAART experienced, it is likely that we had a selection bias for patients who were progressing in HIV. HIV disease progression has conclusively been linked to anaemia particularly in patients who are HAART experienced (9) (28) (29). This may be demonstrated by our finding of majority patients being in WHO stage 3 and 4 with CD4 count levels consistent with this clinical stage. This finding may account for the inflammatory drivers of inadequate EPO response in our study population.

Our study did not show any significant association between EPO levels and the WHO stage of the patient or between EPO level and the CD4 count. Similarly Allen et al was not able to demonstrate any associations between the CD4 count of the study participants and the EPO level (4).

Patients with advanced HIV disease were found more likely to be EPO deficient in the presence of anaemia. Mean levels of EPO were found to be lowest in patients with WHO stage 4 disease (79.5IU/L). On univariate analysis patients in WHO stage 3 and 4 of AIDS were up to seven times less likely to have elevated EPO levels despite having the lowest mean Hb levels (9.0g/dL and 8.5g/dL for stage 3 and 4 respectively). This finding is similar to that of Spivak et al (2) who found that patients with advanced clinical AIDS less increment of EPO levels for a given decline

in Hb levels compared to patients with early HIV disease. Similarly Camacho et al found lower EPO levels in anaemic patients with advanced HIV disease in CDC class III compared to class I and II (3). This has previously been attributed to high levels of inflammatory mediators and the HIV virus itself. (2) (3).

LIMITATIONS

1. In this study the erythropoietin measurements taken were static and did not provide any information about the long term catabolism or production of the hormone over time in response to anaemia.

RECOMMENDATIONS

1. Evaluation of the erythropoietin status should be incorporated into the workup of anaemic patients with HIV whose anaemia is not responding to antiretroviral therapy.
2. Prospective studies should be carried out to determine the benefit of erythropoietin replacement in HAART experienced patients with anaemia and concurrent EPO deficiency.

REFERENCES

1. Leornard, I.Z, Charles,A. Haematologic manifest ations of the Human Immune Deficiency Virus (HIV). *British Journal of Haematology. 1987;66:251-256.*
2. Spivak, J.L, Barnes, D.C, Fuchs E, Quinn, T.C. Serum immunoreactive erythropoietin in HIV-infected patients. *JAMA 1989 Jun 2;261(21):3104-7.*
3. Camacho J, Poveda F, Zamorano F.Serum erythropoietin levels in anaemic patients with advanced Human Immunodeficiency Virus infection. *British Journal of Haematology. 1992 Nov;82(3):608-14.*
4. Upton, D.A, Susan,M. K, Martin P. G. Serum immunoreactive erythropoietin levels and associated factors amongst HIV-infected children. *AIDS. 1998;12:1785-1791.*
5. Kreuzer, K.A, Rockstroh, J.K, Jelkmann W, Inadequate erythropoietin response to anaemia in HIV patients: relationship to serum levels of tumour necrosis factor-alpha, interleukin-6 and their soluble receptors. *British Journal of Haematology. 1997 Feb;96(2):235-9*
6. Fischl, M, Galpin, J.E., Levine, J.D. Recombinant human erythropoietin for patients with AIDS treated with zidovudine. *New England Journal of Medicine. 1990;322(21):1488-1493.*
7. Abrams, D.I, Steinhart, C., Frascino, R. Epoetin alfa therapy for anaemia in HIV-infected patients: impact on quality of life. *International Journal of STD & AIDS. 2000 Oct;11(10):659-65*
8. Mocroft, A, Kirk, O, Barton, S.E. Dietrich. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. *AIDS 1999;13(8):943-950*
9. Lundgren, J.D., Mocroft, A., Gatell, J.M., Ledergerber A Clinically Prognostic Scoring System for Patients Receiving Highly Active Antiretroviral Therapy: Results from the EuroSIDA Study. *The Journal of Infectious Diseases. 2002;185:178-187*
10. Semba, R.D., Shah,N., Klein, R.S. Highly Active Antiretroviral Therapy Associated with Improved Anemia Among HIV-Infected Women. *AIDS Patient Care and STDs. 2001;15(9).*

11. Moore, R.D.; Forney D. Anemia in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy. *Journal of Acquired Immune Deficiency Syndromes. 29(1):pp 54-57.*
12. Henry, D.H., Beall, G.N., Benson, C.A., Recombinant Human Erythropoietin in the Treatment of Anemia Associated with Human Immunodeficiency Virus (HIV) Infection and Zidovudine Therapy. *Ann Intern Med. 1992;117:739-748.*
13. Carole, B.M., Richard, J.J., Steven P., Decreased Erythropoietin Response in Patients with the Anemia of Cancer. *New England Journal of Medicine. 1990;322(:June 14):1689-1692.*
14. John, P. P., Robert, I.A., Michele, V.M., Recombinant Human Erythropoietin Treatment: Investigational New Drug Protocol for the Anemia of the Acquired Immunodeficiency Syndrome. *Archives of Internal Medicine. 1993;153(23):2669-75.*
15. Jonathan O. C., Diagnosis and management of anaemia of chronic disease:current status.*British Journal of Hematology Review2011: 154, 289–300*
16. Omoregie, R., Omokaro, E.U., Palmer, O.,Prevalence of anaemia among HIV-infected patients in Benin City, Nigeria. *Tanzania Journal of Health Research. 2009 Jan;11(1):1-4.*
17. Tsiakalos, A., Kordossis, T., Ziakas, P.D., Circulating antibodies to endogenous erythropoietin and risk for HIV-1-related anemia. *The Journal of Infection. 2010 Mar ;60(3):238-43*
18. Sipsas, N.V., Kokori, S.I., Ioannidis, J.P.A.,Circulating Autoantibodies to Erythropoietin Are Associated with Human Immunodeficiency Virus Type 1 – Related Anemia. *Journal of Infectious Diseases. 1999;180:2044-2047.*
19. Gogu, S.R., Malter, J.S., Agrawal, K.C., Zidovudine-induced blockade of the expression and function of the erythropoietin receptor. *Biochemical Pharmacology. 1992;44(6):1009-1012.*
20. Popovsky, M.A., Benson, K., Glassman, A.B., Hume H, Oberman H a, Pisciotto PT, et al. Transfusion practices in human immunodeficiency. *Transfusion. 1995 Jul;35(7):612-6.*

21. Sloand, E., Kumar, P., Klein, H.G., Transfusion of blood components to persons infected with Human Immunodeficiency Virus type 1: relationship to opportunistic infection. *Transfusion 1994 Jan;34(1):48-53.*
22. Silva, M., Benito, A., Sanz, C., Erythropoietin can induce the expression of bcl-x(L) through Stat5 in erythropoietin-dependent progenitor cell lines. *The Journal of Biological Chemistry. 1999 Aug 6;274(32):22165-9.*
23. Brines, M.L., Ghezzi, P., Keenan, S.,Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proceedings of the National Academy of Sciences of the United States of America. 2000 Sep 12;97(19):10526-31.*
24. Wamalwa, D.C., Obimbo, E.M., Farquhar, C.Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort. *BMC Pediatrics. 2010;:4-11.*
25. Lundgren, J.D., Mocroft, A., Anemia and survival in Human Immunodeficiency Virus. Clinical infectious diseases: *an official publication of the Infectious Diseases Society of America. 2003 Jan;37 Suppl 4(Suppl 4):S297-303.*
26. Volberding, P.A, Levine, A.M., Dieterich, D. Anemia in HIV infection: clinical impact and evidence-based management strategies. Clinical Infectious Diseases: *an official publication of the Infectious Diseases Society of America . 2004 May 15;38(10):1454-63*
27. Volberding, P. Consensus statement: anemia in HIV infection--current trends, treatment options, and practice strategies. Anemia in HIV Working Group. In: *Clinical Therapeutics. 2000. p. 1004-1020; discussion 1003*
28. .Graham, N.M., Piantadosi,H.S., Park, Lawrence P; Phair, John P.; Rinaldo, Charles R.; Fahey JL. CD4+ Lymphocyte Response to Zidovudine as a Predictor of AIDS-Free Time and Survival Time. 1993;6(11):1258-1273.
29. Creagh, T., Mildvan, D., Bohn, H. A case-control study to examine the association of HIV/AIDS progression with anemia in the era of highly active antiretroviral therapy (HAART). In: *International Conference on AIDS. p. abstract no. C10936.*
30. Division of AIDS. Table for grading severity of adult adverse experiences. Bethesda, MD: *National Institute of Allergy and Infectious Diseases, Division of AIDS, August 1992.*

31. National Comprehensive Cancer Network. Anemia: *clinical practice guidelines in oncology. JNCCN 2003;1(suppl 1):2-13.*
32. Daniel WW (1999). Biostatistics: *A Foundation for Analysis in the Health Sciences. 7th edition. New York: John Wiley & Sons.*
33. Kenya AIDS Indicator Survey 2007 (KAIS 2007) Final report, *National AIDS Control Council.*

APPENDIX 1: IMMULITE 2000 EPO

Principle of the procedure

The method utilizes a solid phase enzyme labeled chemilluminescent immunometric assay. The solid phase (bead) is coated with anti-ligand derived from streptavidin. The liquid phase consists of ligand labeled monoclonal murine anti EPO antibody and alkaline phosphatase conjugated to monoclonal murine anti-EPO antibody.

The patient sample and the reagent are incubated together with the coated bead for 30 minutes. During this time the EPO in the sample binds to the ligand labeled monoclonal murine anti-EPO antibody and to the enzyme conjugated murine monoclonal anti-EPO forming a sandwich complex. The immune complex is in turn captured by the streptavidin on the bead via the biotinylated anti-EPO. Chemiluminiscent substrate is added to the reaction tube containing the bead and the signal is generated in proportion to the bound enzyme.

Specimen collection

1. The samples will be collected at a consistent time of the day, between 7.30am to 12noon to avoid diurnal variation
2. Samples will be collected into labeled serum bottles and clotted at room temperature (15-28°C) to avoid variation in values.
3. Haemolyzed samples will be rejected.

APPENDIX 2: PARTEC CYTOFLOW PROCEEDURE

1. Blood is collected in EDTA (anticoagulated bottle) and delivered to the laboratory within 6 hours
2. 20µl of whole blood is added to the Partec test tube
3. Monoclonal antibodies, mouse monoclonal antibodies against CD4 (CD4 mAb PE) and against CD8 (CD45 mAb PE) are added to the whole blood in the partec test tube, mixed gently and incubated for 15 minutes at room temperature shielded from light.

4. Two buffers are added, one to stop the reaction and another to hemolyse red cells.
5. Analysis is done within 10 minutes of adding the second buffer using the Partec Cytoflow device.

Quality control

Internal controls

1.After each assay, the Partec Cytoflow analyser is washed by running 1.6ml of partec detergent and cleaned by running 1.6ml of sterile water.
2.A normal fresh blood sample (healthy donor EDTA fixed) is prepared for calibration. Lower and upper level threshold settings are checked and confirmed as per protocol.

External controls

1. External quality control is done at the National HIV Reference Laboratory which is under the Ministry of Medical Services department of diagnostic and forensic services. The reference laboratory has the mandate of running quality assurance activities in monitoring of ART and in particular CD4 testing. It analyses CD4 data from randomly selected CD4 samples from the CCC lab and gives feedback in 14 days.
2.The CCC carries out further independent external quality control using laboratories belonging to Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (German United Society of Clinical Chemistry and LaboratoryMedicine) (DGKL) in Bonn Germany.

APPENDIX 3: WHO CLINICAL STAGING OF HIV/AIDS FOR HIV INFECTED ADULTS AND ADOLESCENTS

PRIMARY HIV INFECTION

Asymptomatic

Acute retroviral syndrome

STAGE 1

Asymptomatic

Persistent generalized lymphadenopathy

STAGE 2

Moderate unexplained weight loss (<10% of presumed or measured body weight)

Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulceration

Papular pruritic eruptions

Seborrheic dermatitis

Fungal nail infections

STAGE 3

Unexplained severe weight loss (>10% of presumed or measured body weight)

Unexplained chronic diarrhea for >1 month

Unexplained persistent fever for >1 month (>37.6ºC, intermittent or constant)

Persistent oral candidiasis (thrush)

Oral hairy leukoplakia

Pulmonary tuberculosis (current)

Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)

Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis

Unexplained anemia (hemoglobin <8 g/dL)

Neutropenia (neutrophils $<0.5x10^9$/L)

Chronic thrombocytopenia (platelets $<50x10^9$/L)

STAGE 4

HIV wasting syndrome

Pneumocystis pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site)

Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)

Extrapulmonary tuberculosis

Kaposi sarcoma

Cytomegalovirus infection (retinitis or infection of other organs)

Central nervous system toxoplasmosis

HIV encephalopathy

Cryptococcosis, extrapulmonary (including meningitis)

Disseminated nontuberculosis mycobacteria infection

Progressive multifocal leukoencephalopathy

Candida of the trachea, bronchi, or lungs

Chronic cryptosporidiosis (with diarrhea)

Chronic isosporiasis

Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)

Recurrent nontyphoidal *Salmonella* bacteremia

Lymphoma (cerebral or B-cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV-associated nephropathy

Symptomatic HIV-associated cardiomyopathy

Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

APPENDIX 4: WHO GRADING OF ANAEMIA SEVERITY IN HIV

Haemoglobin level	Anaemia severity
9.5-11 g/dL	mild
8.0-9.4g/dL	moderate
6.5-7.9g/dL	severe
< 6.5g/dL	life threatening

APPENDIX 5: STUDY PROFORMA

Study No……..

Date……..

IP No……

DOB……..

Age (years)…….

DEMOGRAPHICS

1 Gender Male……. Female……..

2 Marital status single ……. married …….. divorced…... … widowed……. Separated……..

3 Usual residence

4 Occupation self employed…. Employed….. unemployed…. Retired….. student…….

5 Level of education none…. Primary….. secondary….. tertiary……..

6 Duration of HAART use (months)……..

Physical examination

1 weight (kg)……..

2 height (m)……

3 BMI (kg?m²)……

4 Documented WHO staging……..

Laboratory Measures

1.Hb (g/dl)……..
2.MCV (fl)……
3.WBC (cellsx10^9/L)……
4.Lymphocytes(cellsx10^9/L)…….
5.CD4 count(c/µL)……
6.Serum erythropoietin levels IU/L……

APPENDIX 6- CONSENT EXPLANATION

My names are Dr David Kanyeki Gatukui,a postgraduate student in internal medicine. I am conducting a research study on patients attending the Kenyatta National Hospital Comprehensive Care Centre.

Purpose of study

To determine the levels of erythropoietin in anaemic patients who are receving HAART and the correlates of these.

Proceedures

If you agree to join this study you will be requested to;

1.Undergo a general physical examination including measurement of your weight and height
2.Have 5mls of blood taken for determination of your full blood count and your erythropoietin level

Participation in this study is voluntary and you can choose to decline or withdraw from this study without any penalty.

Risks

A mildly unpleasant sensation may be experienced during blood withdrawal for the above laboratory tests

Benefits

1.All the above examinations and procedures will be done free of charge.
2.A copy of the results of these tests shall be availed to your file and the doctor informed of these results
3.Those with anaemia and erythropoietin deficiency will be advised on modalities of management as per accepted standards of practice
4.You will be offered an opportunity to ask questions;to be provided with further information or to undergo certain examinations as per your desire

Confidentiality

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

If you have no objections to participating in the study you will be required to sign an informed consent form

Dr David Kanyeki
Principal investigator,Telephone 0721951406

Deparment of Clinical Medicine and Therapeutics,University of Nairobi

Dr Omondi-Oyoo

Co-principal Investigator, Telephone 0722522359

Department of Clinical Medicine and Therapeutics, University of Nairobi

APPENDIX 6: MAELEZO YA IDHINI.

Kwa majina naitwa **Dr DAVID KANYEKI GATUKUI**, 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 moyo, na wanaohuduria kliniki katika Hospitali kuu ya Kenyatta.

Nia ya Utafiti.

Utafiti huu si wa kupeana tiba lolote ila ni wa kuangalia idadi ya watu walio na shida ya ukosefu wa damu ambao wanatumia dawa za kuzuia virusi vya ukimwi zaidi ya miezi sita katika kliniki ya CCC, hospitali kuu ya Kenyatta.

Taratibu.
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 na urefu.
3. Kutolewa mililita 6 za damu tupeleke kupina kiwango cha damu.

Hatari.

Kwa kushiriki katika utafiti huu, mgonjwa hatakuwa kwenye hatari yoyote ila tu kutakuwa na maumivu madogo wakati wa kutoa damu.

Faida ya Kushiriki:

1. Uchunguzi wote utafanywa bila malipo yoyote kutoka kwako. Mpelelezi mkuu ndiye atakayegharamia uchunguzi wa maabara
2. Matokeo ya uchunguzi huu yatafafanuliwa kwako na nakala iwekwe katika faili yako , ya matibabu kwa ajili ya kutazamwa na daktari msingi katika kliniki.
3. Kwa wale walio na upungufu wa damu , daktari wa kliniki ataelezewa ili aanze matibabu

Usiri.

Nakala yoyote itakayotokana na huu uchunguzi itahifadhiwa kwa usiri na kutumiwa kwa ajili ya utafiti huu tu

Hitimisho.

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

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

Dr David Kanyeki
Mchunguzi mkuu,

Nambari ya simu 0721951406

Idara ya Magonjwa ya Ndani(Internal Medicine)
Chuo kikuu Cha Nairobi.

Dr Omondi-Oyoo

Mchunguzi msaidizi,

Nambari ya simu 0722522359

Idara ya Magonjwa ya Ndani(Internal Medicine)

Chuo kikuu Cha Nairobi.

APPENDIX 7- CONSENT FORM

I……after reading the consent explanation form and having been explained to by Dr David Kanyeki (principal investigator) do voluntarily agree to take part in this study of **ERYTHROPOIETIN PROFILE IN PATIENTS WITH ANAEMA TAKING HAART ATTENDING THE CCC AT KENYATTA NATIONAL HOSPITAL.** I am also aware that I can withdraw from this study without losing any benefits or quality of management of my medical problem being affected.

SIGNED……

THUMB PRINT……

I confirm that I have explained to the patient the details of the consent explanation form.

Signed…….Date……(interviewer)

Dr David Kanyeki
Principal investigator,Telephone 0721951406

Deparment of Clinical Medicine and Therapeutics,University of Nairobi

Dr Omondi-Oyoo

Co-principal Investigator, Telephone 0722522359

Department of Clinical Medicine and Therapeutics, University of Nairobi

KNH/UON- ERC

Email: uonknh_erc@uonbi.ac.ke

APPENDIX 7: FOMU YA IDHINI

Mimi……

Baada ya kusoma maelezo ya idhini na baada ya kuelezewa na Dr David Kanyeki, na kawa hiari yangu, nakubali kushiriki katika utafiti wa **ERYTHROPOIETIN PROFILE IN PATIENTS WITH ANAEMA TAKING HAART ATTENDING THE CCC AT KENYATTA NATIONAL HOSPITAL.** Najua ya kwamba naweza kujiondoa kutoka utafiti huu wakati wowote bila kudhuru kiwango cha matibabu ya ugonjwa wangu.

Sahihi/ alama ya kidole gumba…….. …….

Tarehe……

Nambari ya simu ……..... Ninakoishi……

Nimeelezea mgonjwa yaliyomo katika fomu ya maelezo ya idhini

Sahihi……..

Tarehe ……(interviewer)

Dr David Kanyeki
Mchunguzi mkuu,

Nambari ya simu 0721951406

Idara ya Magonjwa ya Ndani(Internal Medicine)
Chuo kikuu Cha Nairobi.

Dr Omondi-Oyoo

Mchunguzi msaidizi,

Nambari ya simu 0722522359

Idara ya Magonjwa ya Ndani(Internal Medicine)
Chuo kikuu Cha Nairobi.

KNH/UON- ERC

Barua Pepe: uonknh_erc@uonbi.ac.ke