ANAEMIA AND ITS ASSOCIATED RISK FACTORS AMONG PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS ATTENDING MUHIMBILI NATIONAL HOSPITAL - MEDICAL DEPARTMENT

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By

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A dissertation Submitted in (partial) Fulfillment of the Requirements for the Degree of Master of Medicine (Internal Medicine) of Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: ***Anaemia and its associated risk factors among patients with Human Immunodeficiency Virus attending Muhimbili National Hospital - Medical Department***, in fulfillment of requirements for the degree of Master of Medicine (Internal Medicine) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Avelina Mgasa**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature……..... Date…….....……

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To my dear son Rodgers and to the memory of my late father, Major Paschal Mgasa, who inspired me to become a good doctor.

ABSTRACT

Background: Human Immunodeficiency Virus (HIV) infection is associated with significant haematological abnormalities. In this study anaemia and associated risk factors were evaluated among HIV-infected in-patients and outpatients attending the HIV care and treatment clinic of the Medical department

Methods: A standardised questionnaire was used to obtain information on social-demographic characteristics, clinical history, and information on anti-retroviral therapy. Patients were staged according to WHO guidelines and CD4 counts determined. Anaemia was determined from a complete blood count. Iron status was established using transferrin concentration, serum iron and serum ferritin levels, and serum B12 and folate were analyzed. Univariate and multivariate logistic regression were used to determine the association between anaemia and associated risk factors.

Results: A total of 316 HIV-infected patients were recruited. Anaemia was significantly higher among the no income (63.1%) and low income (54%) compared to the medium income (42.4%) and high income (37.5%) patients, p=0.047. Severity of anaemia increased with advanced stage of HIV infection and low CD4 count (p= 0.0001 and p=0.0001 respectively). Patients who were not on any anti-retroviral therapy were found to have higher prevalence of anaemia compared to those on therapy, 68.1% vs 49.3%: with those on AZT containing regimes having a higher prevalence of anaemia 50.3% compared to those on non-AZT containing regimes 47.4% (p=0.0001). Low serum folate and low iron were found to be associated with anaemia (p=0.002 and 0.0001 respectively). On multivariate analysis history of blood transfusion since HIV diagnosis, thrombocytosis, ,low CD4 count and low serum iron were predictors of anaemia.

Conclusion and recommendation: The risk factors for anaemia among HIV-infected patients are multifactorial. Treatment of anaemia in HIV infection should include initiating anti-retroviral therapy together with administration of iron and folic acid supplements.

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
ART	Antiretroviral therapy
AZT	Zidovudin
CD4	Cluster of differentiation 4
CI	Confidence interval
CPL	Centralized Pathology Laboratory
CTC	Care and treatment clinic
EPO	Erythropoietin
HAART	Highly Active Antiretroviral Therapy
HGB	Haemoglobin concentration
HIV	Human Immunodeficiency Virus
MNH	Muhimbili National Hospital
µL	Microlitre
NACP	National AIDS Control Program
NNRTI	Non nucleoside Reverse Transcriptase Inhibitors
RBC	Red blood cells
SD	Standard deviation
UPT	Urinary Pregnancy Test
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background

Infection with Human Immunodeficiency Virus (HIV) is significantly associated with immunologic, infectious, neoplastic and haematological manifestations. A number of haematological complications associated with Human immunodeficiency virus (HIV) infection have been reported, some may result in significant complications among these patients [1, 2].The Pathophysiology of HIV-associated anaemia involves three basic mechanisms; decreased Red Blood Cells (RBCs) production, increased destruction of RBC, and ineffectiveness of RBC production [3].

The causes of anemia in HIV infection are multifactorial [4]. HIV may directly affect bone marrow stromal cell or cause cytokine secretion, leading to decreased production of RBCs and other bone marrow elements. Tumor necrosis factor and other cytokines inhibit hematopoiesis and cytokine levels are elevated in HIV. Treatment of HIV and reduction of virus load by the use of highly active antiretroviral therapy may improve hematopoiesis [5].

Patients with HIV may also acquire chronic parvovirus B19 infection of bone marrow, resulting in profoundly decreased numbers of RBCs. In addition, anemia may result from the indirect effects of HIV infection, such as adverse reactions to medications such as zidovudine, opportunistic infections, neoplasms, or nutritional abnormalities stemming from anorexia, malabsorption or metabolic disorders. Although many drugs used to treat HIV-related disorders are myelosuppressive, severe anemia is most often related to the use of zidovudine [6].

Any alterations in the components of normal erythropoiesis, which include an adequate supply of iron, folate, and vitamin B12, an intact bone marrow, and the essential hematopoietic growth factor, erythropoietin, may produce anemia [7].

It has been shown that there is an independent association between anemia and decreased survival regardless of Cluster of differentiation (CD4+) T-lymphocyte count and plasma HIV Ribonucleotide analogue (RNA) concentration and that anaemic HIV-infected patients who recover from anemia have better survival rates as compared to those who do not recover [3].

1.2 HIV/AIDS

According to the World Health Organization (WHO) report at the end of 2010, about 34 million people were living with HIV globally; of these 3.4 million were children less than 15 years. 2.7 million New HIV infections were reported in 2010, including 390,000 among children less than 15 years. Globally, the annual number of people newly infected with HIV continues to decline, although there is regional variation. There is still high number of new infection in sub-Saharan Africa, where most of the people newly infected with HIV live, an estimated 1.9 million people became infected in 2010. This was 16% fewer than the estimated 2.2 million people newly infected with HIV in 2001 and 27% fewer than the annual number of people newly infected between 1996 and 1998, when the incidence of HIV in sub-Saharan Africa peaked overall. The number of people dying from AIDS-related causes worldwide is steadily decreasing from a peak of 2.2 million in 2005 to an estimated 1.8 million in 2010. The number of people dying from AIDS-related causes began to decline in 2005–2006 in sub-Saharan Africa, South-East Asia and the Caribbean. The annual number of people newly infected with HIV has risen in the Middle East and North Africa from 43 000 in 2001 to 59 000 in 2010.The incidence of HIV infection in Eastern Europe and Central Asia has been accelerating again since 2008 after slowing drastically in the early 2000s. The trends in deaths due to AIDS-related cause also differ. Sub-Saharan Africa accounts for the vast majority of the averted deaths which is about 1.8 million[8].

In Tanzania current data reveals that there has been a decline in prevalence over years from 11% in 2003 to 7% in 2006 [9]. The current prevalence of HIV infection among sexually active populations (between 15 and 49 years of age) is reported to be 5.7 % more women being infected than men [10].

1.3 ANAEMIA

Reduction in one or more of the major red blood cell (RBC) measurements can result into anaemia. Haemoglobin concentration (HGB) measures the concentration of the major oxygen-carrying pigment in whole blood. Values may be expressed as grams of haemoglobin per 100 mL of whole blood (g/dL) or per liter of blood (g/L). On other hand Haematocrit (HCT) is the percent of a sample of whole blood occupied by intact red blood cells while RBC count is the number of red blood cells contained in a specified volume of whole blood. Anaemia is defined as values more than two standard deviations (SD) below the mean, by using these ranges, a HGB <13.5 g/dL or a HCT <41.0% represents anaemia in men and value <12.0 g/dL or <36.0 %, respectively, represents anaemia in women. Other normal ranges apart from the above have been proposed. World Health Organisation (WHO) defines anaemia in men and women as HGB <13 and <12 g/dL, respectively [2].

1.3.1 The red blood cell life cycle

Erythropoiesis in the adult takes place within the bone marrow under the influence of the stromal framework, cytokines, and the erythroid specific growth factor, erythropoietin (EPO). EPO a true endocrine hormone is produced in the kidney by cells that sense the adequacy of tissue oxygenation relative to the individual's metabolic activity.

EPO enhances the growth and differentiation of the two erythroid progenitors: burst forming units-erythroid (BFU-E) and colony forming units-erythroid (CFU-E) into normoblasts of increasing maturity [11]. The resulting matured RBC circulates for 110 to 120 days, after which it is removed from the circulation by macrophages that detect senescent signals, by mechanisms that are poorly understood. Normally the rate of RBC production equals the rate of RBC loss. If approximately, survival of mature RBC is 100 days, 1 percent of RBCs are removed from the circulation each day. Then to achieve a constant RBC mass, RBC losses must be replaced with an equal number of reticulocytes during the same time period. An imbalance between production and destruction of RBC results into anaemia.

The symptoms and signs of anaemia are dependent upon the degree of anaemia and the rate at which it has evolved and the oxygen demands of the patient. Anaemia that has evolved slowly has fewer symptoms because there is time for multiple adaptive homeostatic forces to adjust to a reduced oxygen carrying capacity of blood. These symptoms can result from two factors: decreased oxygen delivery to tissues, and resultant hypovolemia in patients with acute and marked bleeding. The symptoms of impaired oxygen delivery reflect the fall in haemoglobin concentration. The primary symptoms include dyspnea, fatigue, palpitations and dizziness and severe anaemia may lead to lethargy and confusion and potentially complications such as congestive heart failure, angina, arrhythmia, and/or myocardial infarction and increased risk of stroke. Anemia can be caused by one or more of three independent mechanisms which include decreased RBC production, increased RBC destruction, and blood loss as described in the following sections.

1.3.2 Decreased RBC production

The common causes for reduced RBC production include lack of nutrients, such as iron, B12, or folate which can be due to inadequate diet, malabsorption as in pernicious anaemia or sprue, blood loss which cause iron deficiency, Bone marrow disorders (including aplastic anaemia, pure RBC aplasia, myelodysplasia, tumor infiltration), Bone marrow suppression (caused by drugs, chemotherapy and irradiation).Low levels of Erythropoietin as seen in chronic renal failure, hypothyroidism and hypogonadism can also lead to ineffective RBC production. A rare cause of anaemia due to reduced EPO production has been described in patients with autonomic dysfunction and orthostatic hypotension [12, 13]. In chronic disease/inflammation, anaemia is associated with reduced availability of iron due to decreased absorption from the gastrointestinal tract and decreased release from macrophages, a relative reduction in erythropoietin levels, and a mild reduction in RBC lifespan.

1.3.3 Increased RBC destruction

RBC life span below 100 days is the operational definition of hemolysis. Hemolytic anaemia will ensue when the bone marrow is unable to keep up with the need to replace

more than about 5 percent of the RBC mass per day, corresponding to a RBC survival of about 20 days.

1.3.4 Blood loss

Blood loss is the most common cause of anaemia and may take any one of a number of forms including obvious bleeding such as seen in trauma, melena, hematemesis, menometrorrhagia or occult bleeding as seen in slowly bleeding ulcer or carcinoma.

Iron deficiency usually occurs in males and females after losses of ≥ 1200 mL and ≥ 600 mL, respectively. Because about 25 percent of menstruant females have absent iron stores, any amount of bleeding will result in anaemia in this subpopulation. Availability of iron is normally rate-limiting for RBC production. Iron deficiency following chronic bleeding will lead to a reduced marrow response hence worsening the degree of anaemia.

1.3.5 Morphologic approach

Anaemia can also be classified according to measurement of RBC size, from blood smear and as reported by automatic cell counter indices. The mean cell volume (MCV) indicates the volume of the average red cell in a sample, normal range being 80-97fL and Mean cell haemoglobin (MCH) is the average amount of hemoglobin in the average red cell ranging 27.0-31.2pg. The Mean cell haemoglobin concentration (MCHC) is the average concentration of hemoglobin in a given volume of blood and it ranges from 31.8-35.4.g/dL. Therefore, RBCs larger than the nucleus of a small lymphocyte on a peripheral smear are considered macrocytic, while those that appear smaller are considered microcytic.

Macrocytic anaemia

Macrocytic anaemias are characterized by an MCV above 100 fL. An increased MCV is a normal finding of reticulocytes. Causes of macrocytic anaemia due to abnormal nucleic acid metabolism of erythroid precursors includes folate or cobalamin deficiency and drugs interfering with nucleic acid synthesis, such as zidovudine and hydroxyurea.

Abnormal RBC maturation as seen in myelodysplastic syndrome and acute leukemia can also present with macrocytosis. Other causes include alcohol abuse, liver disease, and hypothyroidism. A report from a family practice group found macrocytosis in 2 to 4 percent of patients [14], while a study of 1,784 randomly selected elderly people living at home found macrocytosis in 6.3 percent of men and 3.3 percent of women [15].

Microcytic anaemia

The term microcytic anaemias refers to presence of RBCs with MCV below 80 fL usually accompanied by a decreased hemoglobin content within the RBC, accompanied with reductions in MCV and MCH, producing a hypochromic as well as a microcytic appearance on the blood smear. Pathologic processes leading to the production of hypochromic microcytic RBC includes reduced iron availability as see in severe iron deficiency, the anaemia of chronic disease, and copper deficiency. The most common causes of microcytosis in clinical practice are iron deficiency, alpha or beta thalassemia minor and the anaemia of chronic disease (anaemia of chronic inflammation). To identify the cause of iron deficiency anaemia the following investigation should be perfomed serum ferritin concentration, total iron binding capacity, serum transferrin and serum iron concentration. In chronic disease (inflammation) the hallmarks of anaemia include a low serum iron, low total iron binding capacity (transferrin), and a normal to increased serum ferritin concentration.

Normocytic anaemia

In normocytic anaemia, the mean RBC volume is normal (MCV between 80 and 100 fL).

1.3.6 Systemic disorders

In systemic disorder anaemia may be the first manifestation along with other nonspecific complaints such as fever, weight loss, anorexia, and malaise. Simple laboratory tests may give additional clues toward the underlying disease process. Anemia is also a common complication of renal disease, and may be multifactorial.

1.3.7 Evaluation of the Patient

Anaemia is one of the major signs of many diseases. It is never normal and its cause(s) should always be looked for. Evaluation of the anaemic patient must include history, physical examination, and simple laboratory testing. The medications use, both prescribed and over-the-counter, should be examined. Specific questions should be asked about the use of alcohol, aspirin, and nonsteroidal antiinflammatory drugs. A past medical history of blood transfusions, liver disease, treatment of the patient with iron or other hematinics, herbal preparations, and exposure to toxic chemicals in the workplace or environment should be obtained. Assessment of nutritional status is important especially in the elderly and alcoholics.

Physical examination

The aim of physical examination is to find signs of organ or multisystem involvement and to assess the severity of the patient's condition. Therefore the presence or absence of tachycardia, dyspnea, fever, or postural hypotension, pallor, jaundice, lymphadenopathy, hepatosplenomegally, bone tenderness and echymosis should be noted.

Laboratory Evaluation

Initial testing of the anaemic patient should include a Full blood count (FBP). The FBP from an anaemic HIV infected patient that reveals macrocytosis may suggest AZT toxicity or folate/cyanocobalamine deficiency[6]. Microcytosis may suggest iron deficiency while thrombocytopenia and leucopenia suggest bone marrow infiltration by HIV itself or malignancy associated with HIV [7]. Presence of thrombocytosis is associated with reactive bone marrow due to HIV infection. Peripheral blood smear that shows reticlocytosis may suggest hemolysis or blood loss. Other features of hemolysis like increased total and direct bilirbin should be looked to make a diagnosis of hemolytic anemia. Stable anaemia with a low reticulocyte count is strong evidence for deficient production of RBCs. When there is low reticulocyte percentage accompanied by pancytopenia is possible diagnosis is aplastic anaemia, while a reticulocyte percentage of zero with normal white blood cell and platelet counts suggests a diagnosis of pure red cell aplasia.

White blood cell count and differential - In FBP a low total white blood cell (WBC) count in a patient with anaemia should lead to consideration of bone marrow suppression or replacement, hypersplenism, or deficiencies of cobalamin or folate. In the other hand a high total WBC count may reflect the presence of inflammation, infection or a haematologic malignancy, increased absolute neutrophil count in infection, an increased absolute monocyte count in myelodysplasia, an increased absolute eosinophil count in certain infections, a decreased absolute neutrophil count following chemotherapy, a decreased absolute lymphocyte count in HIV infection or following treatment with corticosteroids.

Pancytopenia – This is combination of anaemia, thrombocytopenia, and neutropenia. The presence of severe pancytopenia narrows the differential diagnosis to disorders such as aplastic anemia, folate or cobalamin deficiency, or hematologic malignancy.

CHAPTER TWO

2.0 LITERATURE REVIEW, PROBLEM STATEMENT, RATIONALE AND OBJECTIVES

2.1 LITERATURE REVIEW

2.1 Anaemia in HIV/AIDS

Anaemia is an important clinical problem in patients with HIV infection and those with AIDS. In 1998, the Anaemia in HIV Working Group issued a consensus statement addressing the impact of anaemia on HIV-infected individuals, as well as treatment strategies and future research directions. The Anaemia in HIV Working Group reconvened in 2002 to evaluate the then available data and to determine the implications of those data for patient management [16].

2.1.1 Pathogenesis of Anaemia in HIV/AIDS

The etiology of anaemia in HIV infection is multifactorial [17]. Several attempts have been made to elucidate the mechanisms leading to HIV-associated anaemia. Direct infection of erythroid progenitors have been suggested but have not been proven. Soluble factors like HIV proteins and cytokines have been suggested to inhibit growth of haematopoietic cells in the bone marrow of patients with HIV-infection [18]. Other implicated factors include changes in cytokine production with subsequent effects on haematopoiesis [19], opportunistic infectious agents such as Mycobacterium avium complex through effect on bone marrow [20], administration of therapeutic agents such as zidovudine, ganciclovir and trimethoprim-sulfamethoxazole and myelophthisis caused by cancers such as lymphosarcoma. Deficiency of vitamin B12, folate and iron are frequently reported in HIV patients [21].

Many studies around the world have documented persistently high occurrence of anaemia in HIV-infected persons, especially as disease progresses [1, 22]. Anaemia in HIV

infected patients has also been linked to poor dietary intake, effect of chronic inflammation, and opportunistic infections [20] .

Alani et al however reported that after specific investigations appropriate storage amounts of these micronutrients were revealed. Therefore supplementation may be beneficial in some patients, but often fails to reverse anaemia in this population. In anaemic HIV patients reticulocytopenia is a consistent finding. Additionally, inadequately low endogenous erythropoietin concentrations have been reported. Thus, it is speculated that a blunted erythropoietin feedback mechanism contributes substantially to the pathogenesis of anaemia in HIV patients [18].

In study by Voth R.S et al they reported the main immunological complication and hallmark of HIV infection is cellular CD4 T-lymphocyte depletion for which various mechanisms are involved: HIV induced cytolysis; dysregulation of cytokines; cytotoxic T-lymphocyte responses and HIV induced autoimmune reactions which are not mutually exclusive have been suggested[23].

2.1.2 Causes of Anaemia in HIV Infected Persons

Volberding et al in his study on Anemia in HIV Infection reported an obvious cause of anemia in patients with HIV infection is blood loss [16]. Blood loss may be associated with such conditions as neoplastic disease as seen with Kaposi sarcoma in the gastrointestinal tract or gastrointestinal lesions that accompany opportunistic cytomegalovirus infection. Other than blood loss, through HIV associated neoplasms in the gastrointestinal tract (GIT) the pathophysiology of HIV-associated anemia may involve 3 basic mechanisms: decreased RBC production, increased RBC destruction, and ineffective RBC production [16].

Decreased RBC production

Decreased RBC production may be a consequence of infiltration of the bone marrow by neoplasm or infection, use of myelosuppressive medications, HIV infection itself, a decreased production of endogenous erythropoietin, a blunted response to erythropoietin , or hypogonadism [16] .

Increased RBC destruction

Increased or premature RBC destruction in the spleen or the circulatory system may occur in patients with HIV infection [24]. Hemolytic anaemia may result from RBC autoantibodies, hemophagocytic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, or glucose-6-phosphate dehydrogenase deficiency [16]. Hemolysis may also develop as a consequence of the use of various medications [25].

Ineffective RBC production

Anaemia may result from nutritional deficiencies which include deficiencies in iron, folic acid, or vitamin B_{12}. In patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology [16]. Vitamin B_{12} deficiency may result from malabsorption in the ileum or from gastric pathology caused by an array of infections or other conditions that affect the gastric mucosa in HIV-infected patients [25].

2.1.3. Factors Associated with Anaemia in HIV-Infected Persons

Individuals with HIV infection who are significantly more likely to develop anaemia include women and African American persons. There is also evidence of increased risk of anaemia in association with zidovudine use, CD4 cell counts of <200 cells/µL and high viral load [26] .

Sex

In the Anaemia Prevalence Study Group[16], which involved nearly 10,000 patients with HIV infection, there was a 71% greater prevalence of anaemia among women than among men when anaemia was defined as a hemoglobin level of <12 g/dL in women and of <13 g/dL in men. Both the Women's Interagency HIV Study (WIHS) [26] of 2625 women and the Human Immunodeficiency Virus Epidemiology Research (HER) Study [27] of 1186 women corroborate these findings. The higher prevalence of anaemia in female HIV-infected persons, compared with male individuals, presumably reflects the overall higher prevalence of anaemia in female persons, which may be largely attributed to monthly menstrual blood loss and to the drains on iron stores that occur with pregnancy and delivery [16].

Race

In anaemia Prevalence Study, the WIHS [26], and the HER study [27], it was found that HIV-positive patients who were African American demonstrated a higher prevalence of anaemia than that found in other races. However in the most recent report from the Anaemia Prevalence Study Group [28], the prevalence of anaemia in HIV-infected persons was 39% among African American women, 19% among white women, 31% among African American men, and 12% among white men. African American individuals with HIV infection may be at particular risk for the development of anaemia, in part as a result of the presence of inherited hematologic disorders, such as sickle cell disease and thalassemia. Dietary factors may also be involved [29].

Zidovudine treatment

Zidovudine treatment is associated with bone marrow suppression and an increased risk of developing anaemia [32, 33]. The HER study [27] demonstrated that, although the use of zidovudine was associated with an increased risk of anaemia (defined as a hemoglobin level of <12 g/dL) in the pre-HAART era (1993–1996), use of zidovudine during the HAART era (1996–2000) was not significantly associated with anaemia. In contrast, the

WIHS recorded the presence of anaemia (defined as a hemoglobin level of <12 g/dL or a physician's diagnosis) in 41.6% of subjects receiving zidovudine therapy, compared with 34.3% of those not receiving zidovudine, P value < 0.01 [26].

Worsening HIV disease parameters

Low CD4 cell counts (<200 cells/µL) and higher HIV-1 RNA levels in plasma have each been independently associated with an increased risk of anaemia in multivariate analyses [28, 31]. Although no causal relationship has been documented, retrospective analyses have found an association between anaemia at baseline, decreased survival, and increased disease progression in patients with HIV infection [30, 38]. In the EuroSIDA study [30], the 12-month survival rate was 96.9% among non anaemic patients, compared with 84.1% among patients with anaemia at baseline (defined as a hemoglobin level of <12 g/dL for women and of <14 g/dL for men, and it was 59.2% among those with severe anaemia (defined as a hemoglobin level of <8 g/dL for both men and women. In the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project, Sullivan et al [22] analyzed the medical records of 32,867 individuals with HIV infection. The median duration of survival was significantly shorter in persons with anaemia (defined as a hemoglobin level of <10 g/dL) than in those without anaemia, regardless of baseline CD4 cell count. Among individuals with CD4 cell counts of 200cells/µL, the relative risk of death was 148% higher in those who developed anaemia. Among patients whose baseline CD4 cell counts were <200 cells/µL, the risk of death was increased by 56% in the presence of anaemia. Survival rates improved markedly among subjects recovering from anaemia.

2.1.4 Impact of correction of anaemia

Abrams et al reported correction of anaemia in patients with HIV infection has been associated with meaningful improvements in quality of life and physical functioning [31] they evaluated epoetin alfa treatment (100–300 U/kg 3 times per week) in 221 HIV-positive patients with anaemia (defined as a hemoglobin level of 11 g/dL). Of these patients, 207 subjects for whom both baseline and follow-up measurements were

available demonstrated significant and sustained improvement in haemoglobin levels (mean increase, 2.5 g/dL). Small increases in the haemoglobin level (up to 2 g/dL) were associated with a beneficial effect on total quality of life (as measured by the Functional Assessment of HIV Infection scale), whereas increases of 2g/dL were significantly associated with greater improvement in quality of life score. Grossman et al in their study[24] they randomly assigned 269 HIV-infected patients with anaemia (defined as a haemoglobin level of <12 g/dL) to receive 16 weeks of treatment with epoetin alfa at either 100 U/kg 3 times per week or 40,000 U once per week. Quality of life was measured using 2 instruments: the Linear Analog Scale Assessment (LASA) and the validated Medical Outcomes Study HIV Health Survey (MOS-HIV). Significant increases from the baseline level in mean hemoglobin levels and in quality of life scores were demonstrated for both dosage regimens. Although the prevalence of severe anaemia has decreased since the introduction of HAART, mild-to-moderate anemia continues to be common [32].

A subset of 1624 patients was evaluated as part of the EuroSIDA study. Before HAART was initiated, mild anaemia (defined as a haemoglobin level of <12 g/dL for women and of <14 g/dL for men) was present in 64% of subjects, and severe anaemia (defined as a hemoglobin level of <8 g/dL for both women and men) was present in 1.5% of subjects. After 6 months of HAART therapy, mild anaemia was present in 52% of subjects, and severe anaemia was present in 1.2%. After 12 months, further improvements were recorded, with 45.6% of patients demonstrating mild anaemia and 0.6% demonstrating severe anaemia [30].

Use of HAART

HAART use may result in improvement of existing anaemia. A multivariate analysis of the WIHS study found that HAART was significantly associated with correction of anaemia; improvement was noted within 6 months, and a greater resolution occurred after a longer duration of HAART use [26].

Use of epoetin alfa

In multiple controlled and uncontrolled studies, epoetin alfa has been proven safe and effective for the treatment of anaemia in HIV infection [31]. In an early, combined analysis of four 12-week, randomized, double-blind, multicenter, controlled clinical trials [33], epoetin alfa (100–200 U/kg 3 times per week) significantly improved hematocrit levels (P 0.05) in patients with AIDS who were receiving zidovudine, with endogenous erythropoietin levels of 500 IU/L. An increase of >1 g was seen by week 2, with furthers increases of >2 g by week 4. Treatment was further associated with significant reductions in transfusion requirements and improvements in overall quality of life. More recent clinical studies of anemic patients with HIV infection have found that epoetin alfa may also be administered once per week (40,000 U), resulting in improvements comparable to those associated with thrice-weekly administration [16].

2.2 PROBLEM STATEMENT

Anaemia is common in persons with HIV infection and is associated with poor prognosis [34]. It has been known to be the commonest haematological abnormality in patients with HIV [35]. In addition to causing reduced physical functioning and quality of life, also anaemia at the time of ART initiation have been reported to be associated with HIV disease progression and mortality and the risk of mortality increases with the severity of anaemia[23].

The revised World Health Organisation (WHO) clinical staging of HIV disease in adults and adolescents, put severe anaemia with haemoglobin values below 8.0 g/dl in a WHO HIV clinical stage three[36]. A study by Makubi et al on risk factors for anaemia among HIV infected children attending care and treatment clinic at Muhimbili National Hospital in Dar ea Salaam, Tanzania concluded that anaemia in HIV infected children has multiple causes [37].

Anaemia is among causes of admission in patients with HIV infection, raising morbidity and mortality due to anaemia among HIV/AIDS patients is a new challenge in management of HIV/AIDS.

2.3 RATIONALE

Anaemia in HIV-infected patients can have serious implications, which vary from functional and quality-of-life decrements to an association with disease progression and decreased survival [16].

As anaemia burden on morbidity and mortality among HIV patients increase, there is justified need to understand risk factors for development of anaemia. This study will establish risks for development of anaemia among patients with HIV infection. The results of this study will provide knowledge regarding risk factors and provide insight towards designing appropriate preventive measures and effective interventions.

This knowledge is expected to improve care of HIV infected patients, particularly those with increased risk of developing morbidity and mortality due to anaemia. This study will also form a baseline for further intervention studies to modify risk factors for anaemia among HIV patients and shed light on management guidline.

2.4 OBJECTIVES

2.4.1 Broad objectives

Assessment of anaemia and its associated risk factors among patients with human immunodeficiency virus infection attending Muhimbili National Hospital(MNH) Medical department.

2.4.2 Specific objective

1. To determine prevalence of anemia by sociodemographic, clinical and laboratory characteristics among HIV positive patients attending MNH Medical department.
2. To determine association between anaemia and WHO clinical stage of HIV among HIV positive patients attending MNH Medical department.
3. To determine association between anaemia and CD4 count among HIV positive patients attending MNH Medical department.
4. To determine association between anaemia and ARV regimen among HIV positive patients attending MNH Medical department.
5. To determine association between anaemia and micronutrient levels (Folic acid, Vitamin B12, Iron) among HIV positive patients attending MNH Medical department.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a hospital based descriptive cross-sectional study.

3.2 Study Area

This study was conducted at Muhimbili National Hospital (MNH) Medical department (medical wards and care and treatment clinic (CTC)). MNH is Tanzania's largest tertiary level referral hospital. It receives referred patients from district hospitals in Dar es Salaam and from other parties of the country. It is located in the city of Dar es salaam which is also the largest commercial city in Tanzania with a population of about 4.36 million [38]. MNH Medical wards admit patients daily. Patients with diagnosis of HIV are admitted in infectious unit and to other units if primary reason for admission is not HIV disease. About five to six HIV infected patients are admitted per day. The CTC has a total of 6,300 regular patients of these 3911 were aged 15 years and above. Patients attend clinic once in a month for clinical evaluation and refill of ARV for those on ARV. The clinic runs from Monday to Friday and patients attend on their scheduled date or at any time when a compelling need arises.

3.3 Study duration

The study was conducted between July 2012 and September 2012.

3.4 Study participants

Participants in this study were patients aged 18 years or older with the diagnosis of HIV who attended MNH medical department during the study period.

3.5 Inclusion criteria - All consented HIV infected patients aged 18 years and older admitted at MNH medical wards and patients attending MNH CTC at the time of study were included in the study.

3.6 Exclusion criteria

Pregnant and lactating women, patients under 18 years, as well as patients known to have chronic kidney disease**,** patients with known sickle cell disease and thalassaemia and patients who received blood transfusion within three month before the study were excluded from the study.

3.7 Sample size

Sample size calculations was done using Kish and Lislie formular

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

n=Minimum sample size,

Z=Standard normal deviate corresponding to two sided specified significant level. This is 1.96 (at 95% confidence interval)

e=Margin of error 5%

P= Proportional of patients

A study done in Iran involving similar cohort on Prevalence,severity and related factors of anaemia in HIV/AIDS patients[39] ,reported prevalence of anaemia was 71% therefore

N = $\underline{1.96^2 \text{ x } 0.\ 71 \text{ x } (1\text{-}0.71)=}$ 316

0.05 x0.0 5

Calculated sample size was (N) = 316

3.8 Sampling techniques and study procedure

Study subjects were recruited from the medical wards and from care and treatment clinic during five working days Monday to Friday.

For patients in the wards they were recruited on Monday, Wednesday and Friday. A maximum of 5 patients were recruited per day by sequential sampling.

At CTC patients were enrolled into the study every Tuesday and Thursday, by systematic sampling using daily attendance register as a sampling frame. Every 10^{th} patient was enrolled using different starting number from the daily attendance register. If a patient refused to participate the next immediate patient (in the sampling frame was selected) upon their consent. A maximum of 7 patients were recruited per day.

All the information regarding the study objectives and procedures were explained to the patients and they were provided with information sheet. Patients were required to provide written or oral informed witnessed consent before enrollement into the study. To ensure confidentiality of the collected information, participants were given unique identification codes, and names were not used. To avoid double enrollment, patient files of those enrolled were labeled using stickers for easy identification.

3.9.0 Data Collection Technique

A structured questionnaire was used to collect socio-demographic information, medical and past medical history including detailed drug use apart from ARV. Information on ARV types and duration was obtained from patients CTC card number 2 for patients on ARV. Gynaecological history was taken for all enrolled women. Suspected pregnancy i.e last normal menstrual period (LNMP) longer than 1 month had UPT done. Patients income was assessed by asking patients approximate income per month and they were categorized into no income if patient had no source of income, low income if patient had income of less than 200,000/= Tanzanian shillings, medium income if between Tsh.200,000/= and 500,000/= and high income if monthly income was more than 500,000/= Tsh. WHO clinical staging was done to all recruited patients after a thorough history and clinical examination according to standard clinical examination methods. Revised WHO clinical staging of HIV for resource-constrained settings was used. It was developed by the WHO in 1990 and revised in 2007. Staging is based on clinical findings that guide the diagnosis, evaluation, and management of HIV/AIDS, and does not require a CD4 cell count. This staging system is used in many countries to determine eligibility for antiretroviral therapy. Clinical stages are categorized as 1 to 4, progressing from primary HIV infection to advanced HIV/AIDS (Appendix I) [39].

3.9.1 Clinical Examination

Patients were examined for pallor, jaundice, cyanosis, Respiratory rate, Pulse rate, body temperature, blood pressure, mouth lesions, gum bleeding, Lymphadenopathy, skin and nails changes, Spleen and Liver size were recorded. Mid Upper Arm Circumference (MUAC) was measured from left upper arm at the mid point between tip of shoulder and elbow by using a tape measure to the nearest centimeter, MUAC of <18.5 was taken as acute severe malnutrition, 18.5-<22 acute moderate malnutrition and ≥ 22 normal nutrition. Weight was measured using Secca weighing scale and the reading recorded to the nearest 0.5 kilograms. Height was measured using a height measuring rod. Patients had to remove their shoes and cap and the height was recorded to the nearest 0.5 centimeters. BMI was calculated by dividing weight in kilograms by height in meters squared BMI of <18. 5 Kg/m^2 was taken as under weight,18.5-24.9 kg/m^2 normal, 25-29.9 kg/m^2 over weight and ≥30 kg/m^2 obesity. Waist circumference was measured by using tape measure to the nearest centimeter, patients were instructed to stand, top of hip bone was located tape measure was placed horizontally parallel to the floor measurements was taken just above the iliac crest at the end of normal expiration in a relaxed abdominal muscles. Normal value for male was 102cm while for females was 80cm.

3.9.2 Laboratory investigations

Patients were aseptically cleaned on the antecubital fossa of the right or left upper limb using cotton swab and methylated spirit. A total of 10mls of blood was drawn.Three mls of blood was collected into a sterile vacutainer with EDTA anticoagulants for determination of complete blood count using automated counter i.e cell dyne system 1200 (Abbott Diagnostic Division). A second EDTA containing vacutainer was used to collect 3mls of blood for determination of CD4 count, which was done using FACS caliber. Blood samples for Serum ferritin, serum B12 and folate were placed in 4ml empty sterile vacutainer. These were analyzed using Abbott automated AxSym™ Chemistry analyzer system (Abbott, USA) whilst Serum iron and transferrin concentration were analyzed using architect c8000 analyzer system (Abbott, USA).

3.10 Definition of terms

3.10.1 Anaemia

Anaemia was defined using WHO criteria as haemoglobin level less than 13mg/dL for men and less than 12g/dL for women.Haemoglobin 11 - 12.9 g/dL for men and 11 - 11.9 g/dL for women was used to define mild anemia, hemoglobin 8 - 10.9 g/dL for both genders defined moderate anemia and hemoglobin < 8 g/dL for both genders defined severe anemia[40]. In this study patients were grouped into three categories, normal haemoglobin 13mg/dl and above for men and 12mg/dL and above for women, non severe anaemia Haemoglobin 8 – 12.9g/dL for men and Haemoglobin 8 – 11.9g/dL for women, severe anaemia Haemoglobin less than 8g/dL for both gender.

3.10.2 Iron status

A serum iron level in the range 4.5 – 27.5micromoles/liter (umol/L) was considered normal. Transferrin saturation percentage was obtained by using the following formula:- Serum iron x 100 / TIBC where TIBC =Transferrin level x 25. Normal values of TIBC ranged from (43-80.6 umol/L). Transferrin saturation percentages normally range from 20-50%. Absolute iron deficiency was defined with serum ferritin of <100ng/ml and transferrin saturation of < 20%, Functional iron deficiency was defined as serum ferritin of $\geq$ 100ng/ml and transferrin saturation < 20%, Adequate iron store was defined with serum ferritin of $\geq$ 100-800ng/ml and transferrin saturation of 20-50%. Iron overload was considered when patient had serum ferritin of > 800ng/ml transferrin saturation of > 50%[41].

3.10.3. Serum Folate

Normal values were regarded to be 7.2-15.4ng/mL as per Axysm folate test kit package insert, where serum folate of 3- 7.2ng/ml was defined as recent inadequate dietary intake of folate and serum folate < 3ng/ml was defined as serum folate deficiency[42].

3.10.4. Serum Vitamin B12

In this study, serum B12 level was interpreted as per Axysm B12 test kit package insert where serum B12 level 19.1 – 119.3pmol/L was defined as normal range, serum B12 level <19.1pmol/L below normal range and was defined as serum B12 deficiency and serum B12 level >119.3pmol/L above normal range and was considered as over supplementation of B12 [43].

3.10.5. CD4 Count

HIV associated immunodeficiency was defined as insignificant immunosuppression when CD4 levels was ≥500 cells/µl, 349 – 499 cells/µl as mild immunosuppression, 200 – 349 cells/µl as advanced immunosuppression, <200 cells/µl as severe immunosuppression [9].

3.11. Ethical consideration

Ethical clearance to conduct the study was granted by Muhimbili University of Health and Allied Sciences Ethical Review Board. Permission to do the study was obtained from Muhimbili National Hospital management with research clearance number 253 2012/2013. Informed consent was sought from potential study participants . All patients who were diagnosed to have HIV by provider initiative of Testing and Counseling for HIV (PITC) were given proper pre test and post test counseling and confirmation by determine before result of HIV were disclosed. Patients names were not used on patients' questionnaires but laboratory investigation forms. Identification codes were used in the questionnaires to maintain confidentiality. Patients who were found to have anaemia were treated appropriately based on type and severity of anaemia, when Zidovudin was implicated it was replaced by another ARV as per guidline. All patients were given feedback of their results and these results were kept in patient's file for subsequent use by their attending physicians.

3.12. Data processing and analysis

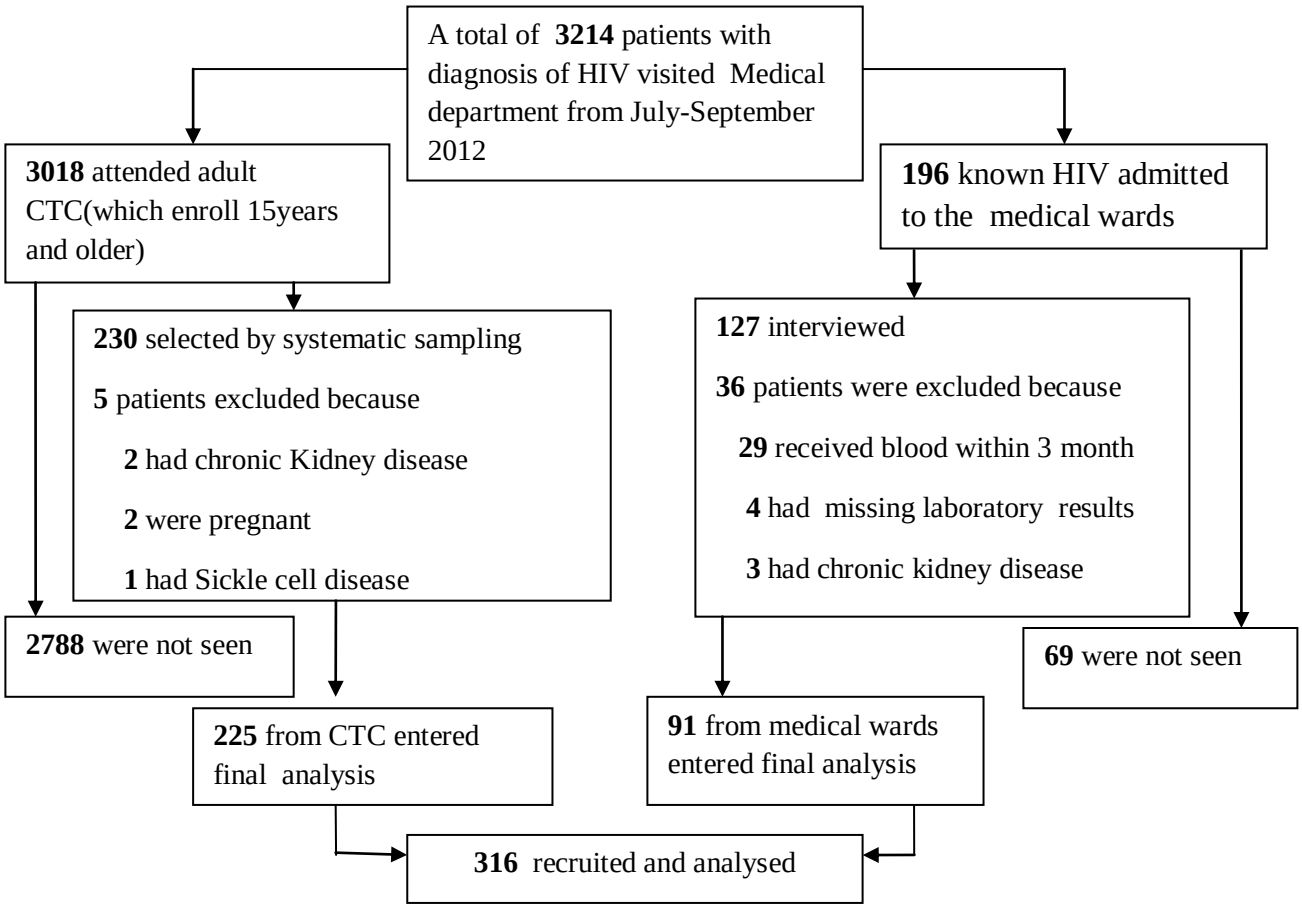
All questionnaires were checked for completeness by the investigator. Data was entered into the computer and cleaned. Analysis was done using statistical package for social sciences (SPSS) version 16.0. Continuous data were summarized using means and standard deviation (SD). Chi-square test was used to determine an association between categorical variables. Student t - test was used to compare mean Haemoglobin of two groups of patients from medical wards and HIV clinic. Multivariate logistic regression models was used to assess the association between anaemia and individual risk factors which were significant on univariate analysis all factors with P value of 0.2 and below were considered for multivariate analysis. Difference was considered significant if p value was less than 0.05.

CHAPTER FOUR

4.0 RESULTS

From July 2012 to September 2012 a total of 3214 HIV infected patients visited Muhimbili National Hospital medical department. 3018 patients aged 15 years and above attended adult CTC and 196 were admitted to the Medical wards. Of the 3018 patients at CTC 230 were selected by systematic sampling and 2788 were not seen.Of the 230 patients, 5 patients were excluded from the study because 2 had Chronic kidney disease(CKD), 2 were pregnant and 1 had sickle cell disease therefore from CTC 225 patients entered final analysis. Of the 196 admitted patients 69 were not interviewed because either were admitted over the weekend or investigator was collecting data at CTC. 127 were interviewed 29 had blood transfusion within 3 month,4 had missing laboratory results and 3 had CKD therefore 91 patients entered final analysis,making a total of 316 patients refer figure 1 below.

Figure 1:Patient flow



4.1. Sociodermographic characteristics of HIV patients attending MNH Medical Department

A total of 316 HIV infected patients were recruited. Mean (±SD) age was 44.1(±10.1), median age was 44 years and interquantile range 44 years. Majority of the patients 236(75%) were in age group 25-49 years.

Female patients comprised 232(73%) of study population. Seventy nine percent(251/315) of participants were employed,160(50.6%) were married, 199(63%) attained primary education and 215(64.1%) had low income or lack source of income (Table 1).

Table 1. Sociodemographic characteristics of the study population (N = 316)

Characteristics	Categories	Frequency(N)	%
Age(years)	18-24	4	1.3
	25-49	236	74.7
	50+	76	24.0
Sex	Females	232	73.4
Marital status	Married	160	50.6
	Single	41	13.0
	Widow/Widower	75	23.7
	Divorced	40	12.7
Level of education	No formal education	21	6.6
	Primary	199	63.0
	Secondary	76	24.1
	College/University	20	6.3
Ocupation	Unemployed	65	20.6
	Employed	251	79.4
Approximate income	No income	65	20.6
	Low income	150	47.6
	Medium income	85	26.9
	High income	16	5.0

4.2. Prevalence of anemia by sociodemographic, clinical and laboratory characteristics among HIV infected Patients attending MNH Medical Department

The mean (±SD) HGB was 11.4(± 2.55)g/dl. More than half of the patients 164(51.9%) had anaemia, with 33/164(20.1%) of anaemic patients presenting with severe anaemia. More than half of the female 121/232(52.2%) were anaemic, so were male patients,43/84(51.2%).

Anaemia was common among the unemployed patients 41/65 (63.1%) than the employed ones 123/251(49%), the difference seen approached significancy level, P= 0.051. Anemia was significantly more prevalent in participants with no income 41/65 than in those with low, medium and high income P=0.047.

Admitted patients had significantly higher anaemia prevalence 69(75.8%) compared to 95(42.2%) of patients who were recruited from the CTC, P value 0.001 (Table 2).

Patients who reported use of herbal medication in this study had higher prevalence of anaemia, P value 0.04 (Table 3A).

Of ARV naive patients 30(68.2%) were anaemic while of those on ARV despite of the regimen 134(49.7%) were anaemic,P value was 0.02.

On physical examination presence of jaundice, silky hair, blackening of nails, lymphadenopathy, skin changes and fever were significantly associated with anaemia (Table 3A).

Mid upper arm circumference assessment revealed anaemia in all patients with Severe acute malnutrition and 78.9% of patients with Moderate acute malnutrition,P value was 0.01. Body mass index assessment revealed 30(75%) of patients with underweight had anaemia while those with normal weight 85(55.6%) had anaemia P value 0.001.

Waist circumference assessment revealed anaemia in 60.4% of patients with values below normal,P value 0.01 (Table 3A).

On laboratory characteristics Leucopenia and Leucocytosis were associated with anaemia as it was seen in 53(60.2%) and 53(60.2%) respectively, P value 0.04. Lymphopenia,erythropenia, Thrombocytopenia and Thrombocytosis were significantly associated with anaemia, P value 0.001 (Table 3B).

Table 2: Prevalence of Anemia by sociodemographic characteristics among HIV Patients attending MNH Medical Department (N=316)

Characteristics	Attribute	Total patients N=316	Patients with anaemia N=164(51.9%)	P value

Age(years)	18-24	4(100%)	1(25%)	0.431
	25-49	236(100%)	126(53.4%)	
	50+	76(100%)	37(48.7%)	
Sex	Female	232(100%)	121(52.2%)	0.490
Marital status	Married	160(100%)	89(55.6%)	0.263
	Single	41(100%)	16(39%)	
	Widow/Widower	75(100%)	40(53.3%)	
	Divorced	40(100%)	19(47.5%)	
Level of education	No formal education	21(100%)	10(47.6%)	0.234
	Primary	199(100%)	111(55.8%)	
	Secondary	76(100%)	36(47.4%)	
	College/University	20(100%)	7(35%)	
Ocupation	Unemployed	65(100%)	41(63.1%)	0.051
	Employed	251(100%)	123(49%)	
Approximate income	No income	65(100%)	41(63.1%)	0.047
	Low income	150(100%)	81(54%)	
	Midium income	85(100%)	36(42.4%)	
	High income	16(100%)	6(37.5%)	

Table 3A: Prevalence of anemia by clinical characteristics among HIV infected patients attending MNH Medical Department (N=316)

Characteristics	Total patients	Patients with anemia N=164(51.9%)	P value
Place of recruitment			

Wards	91(100%)	69(75.8%)	0.001
Malaria infection within past 3 month	68(100%)	41(60.3%)	0.10
History of blood transfusion since HIV diagnosis	28(100%)	21(75%)	0.01
Cotrimoxazole chemoprophylaxis	234(100%)	127(54.3%)	0.15
Herbal medication use	17(100%)	13(76.5%)	0.04
Patients on ARV	272(100%)	134(49.7%)	0.02
Presence of Jaundice	3(100%)	3(100%)	0.09
Hair changes	21(100%)	16(76.2%)	0.02
Nail changes	35(100%)	22(62.9%)	0.17
Presence of Lymphadenopathy	7(100%)	6(85.7%)	0.07
Presence of Tachypnoea (breath per minutes)	12(100%)	11(91.7%)	0.01
MUAC(cm)			
Severe acute malnutrition	3(100%)	3(100%)	0.01
Moderate acute malnutrition	19(100%)	15(78.9%)	
Normal nutrition status	294(100%)	146(49.7%)	
Body mass index(Kg/m^2)			
underweight	40(100%)	30(75%)	0.001
Normal weight	153(100%)	85(55.6%)	
Overweight and obese	123(100%)	49(39.8%)	

Table 3B: Prevalence of anemia by laboratory characteristics among HIV infected patients attending MNH Medical Department (N=316)

Characteristics	Total patients	Patients with anemia N=164(51.9%)	P value
Leucocytes			
Leucopenia	88(100%)	53(60.2%)	0.04
Normal count	219(100%)	104(47.8%)	
Leucocytosis	9(100%)	7(77.8%)	
Lymphocyte			
Lymphopenia	16(100%)	15(93.8%)	0.001
Normal count	285(100%)	143(50.2%)	
Lymphocytosis	15(100%)	6(40%)	
Red blood Cells			
Erythropenia	190(100%)	116(61.1%)	0.001
Normal level	126(100%)	48(38.1%)	
MCV(fL)			
Microcytosis	44(100%)	40(90.9%)	0.001
Normocytosis	130(100%)	68(52.3%)	
Macrocytosis	142(100%)	56(39.4%)	
MCH(pg)			
Hypochromasia	40(100%)	40(88.9%)	0.001
Normochromasia	117(100%)	65(55.6%)	
Hyperchromasia	154(100%)	59(38.3%)	
Platelates			
Thrombocytopenia	22(100%)	16(72.7%)	0.001
Normal count	279(100%)	134(48%)	
Thrombocytosis	15(100%)	14(93.3%)	

4.3. Association between anaemia WHO clinical stage of HIV, CD4 count and type of ARV regimen among HIV infected patients attending MNH Medical department

Majority of stage 1 patients had no anaemia (103) 60.6% while only 29.2% of stage 4 patients had no anaemia. Among patients with stage 4 disease 33.3% had severe anaemia while severe anaemia was seen in only 1.2% of stage 1 patients. Severity of anaemia increased with advanced stage of HIV infection and this was statistically significant with P value of 0.0001(Table 4).

CD4 count ranged from 5 to 1288 cells/microlitre,with mean CD4 of 77.84 ± 243.9 cells/microlitre. Out of 316 patients 80(25.3%) had CD4 less than 200 cells/microlitre. Anaemia was seen in all levels of CD4, of the patients with CD4 above 500cell/µL 31(36.5%) had anaemia while 64(80%) of patients with CD4 of less than 200cells/µL had anaemia. 28(35%) of patients with CD4 of less than 200 cells/µL had severe anaemia while only 2(2.4%) of patients with CD4 above 500 cells/µL had severe anaemia. Low CD4 count was significantly associated with anaemia P value 0.0001(Table 4).

Anaemia was more prevalent in ARV inexperienced patients 30/44 (68.2%) than in those on ARV, regardless of the type. Anemic patients constituted 89/177 (50.3%) of patients on AZT containing regimen while there were 45/95 (47.4%) of anemic patients among patients on non AZT containing regimen. Non severe (27.5%) and severe (25%) anemia were more prevalent in ARV inexperienced patients than it was for patients on ARV treatment. Among patients on ARV treatment, severe anemia was more prevalent among patients on non AZT containing regimen (13.7%) than among patients on AZT contaning regimen (5.1%) P value = 0.0001(Table 4).

Table 4: Association between anaemia and WHO clinical stage of HIV, CD4 count and type of ARV regimen (N=316)

Variable	Anaemia			Total	P value
Clinical stage of HIV	Normal HGB	Non severe anaemia	Severe anaemia		
Stage I	103(60.6%)	65(38.2%)	2(1.2%)	170(100%)	0.0001
Stage II	23(47.9%)	23(47.9%)	2(4.2%)	48(100%)	
Stage III	12(24%)	25(50%)	13(26%)	50(100%)	
Stage IV	14(29.2%)	18(37.5%)	16(33.3%)	48(100%)	
CD4 count					
500+	54(63.5%)	29(34.1%)	2(2.4%)	85(100%)	0.0001
349-499	41(64.1%)	21(320.8%)	2(3.1%)	64(100%)	
200-<349	41(47.1%)	45(51.7%)	1(1.1%)	87(100%)	
<200	16(20%)	36(45%)	28(35%)	80(100%)	
Types of ARV regimen					
Not on ARV	14(31.8%)	19(43.2%)	11(25.0%)	44(100%)	0.0001
AZT containing regimen	88(49.7%)	80(45.2%)	9(5.1%)	177(100%)	
Non AZT containing regimen	50(52.6%)	32(33.7%)	13(13.7%)	95(100%)	

4.4. Association between Anaemia and micronutrient levels (Vitamin B12, Folic acid and Iron)

Serum vitamin B12 levels ranged from 4.9 to 128,with mean(±SD) of 69.27(± 34.323). Vitamin B12 deficiency was seen in 2.5% of study population. With regard to serum levels of vitamin B12 and anaemia 3(37.5%) patients with B12 deficiency had anaemia, while severe anaemia was present in 1(12.5%) patient. Patients with hypervitaminosis B12, 21(45.7%) were anaemic and 5(10.9%) had severe anaemia.There was no association between serum levels of B12 and anaemia P value 0.72 (Table 5)

Folate levels ranged from 2.7 to 20 with mean of 8.42 ± 3.68 out of 316 study participats 128 (40.5%) had subnormal folate levels while normal levels were seen in 174(55.1%). Patients with folate levels less than 7.2 were likely to be anaemic and as it was seen in 72(56.2%) patients. Severe anaemia was seen in 5(35.7%) with serum folate levels more than 15.4 this was statistically significant with P value 0.001(Table 5)

Serum Iron levels ranged from 1 to 38.8 with mean serum iron levels of 12.21 ± 7.06. Out of 316 patients 261(82.6%) had normal serum iron levels with 48(15.2%) having low levels of serum iron. Patients with serum Iron levels of less than 4.5 were likely to be anaemic and anaemia was seen in 41(85.4%) and severe anaemia was present in 18(37.5%) of patients this trend was similary seen in patients with serum Iron levels of more than 27. On assessing iron status out of 316 patients only 46(14.6%) patients had adequate iron store, absolute Iron deficiency was seen in 85(26.9%) patients and functional Iron deficiency was seen in 36(11.4%) patients, iron overload was present in 2(0.6%) patients(Table 5).

Table 5: Association between anaemia and micronutrient levels (Vitamin B12, Folic acid and Iron) N=316

Variable	Anaemia			Total	P value
	Normal HGB	Non severe anaemia	Severe anaemia		
B12 levels					
<19.1	5(62.5%)	2(25%)	1(12.5%)	8(100%)	0.72
19.1-119.3	122(46.6%)	113(43.1%)	27(10.3%)	262(100%)	
>119.3	25(54.3%)	16(34.8%)	5(10.9%)	46(100%)	
Serum folate					
<7.2	56(43.8%)	65(50.8%)	7(5.5%)	128(100%)	0.001
7.2-15.4	92(52.9%)	61(35.1%)	21(12.1%)	174(100%)	
>15.4	4(28.6%)	5(35.7%)	5(35.7%)	14(100%)	
Serum Iron					
<4.5	7(14.6%)	23(47.9%)	18(37.5%)	48(100%)	0.0001
4.5-27.5	144(55.2%)	105(40.2%)	12(4.6%)	261(100%)	
>27.5	1(14.3%)	3(42.9%)	3(42.9%)	7(100%)	

4.5.Comparison of mean Haemoglobin of the study participants by place of recruitment

Patients recruited from medical wards had significantly lower mean hemoglobin (9.67±3.00) than were patients recruited from the HIV clinic who had mean hemoglobin of 12.11 ± 1.95, P value <0.0001 (Table 6).

Table 6: Comparison of mean Haemoglobin of the study participants by place of recruitment (N=316)

Place of recruitment	Number (%)	Haemoglobin Mean(±SD)	*P value
Medical wards	91(28.8%)	9.67(±3.001)	<0.0001
HIV clinic	225(71.2%)	12.11(±1.952)	

*** P value calculated by t-test**

4.6.Predictors of anaemia among HIV patients attending MNH Medical department

Factors with P-value ≤0.2 on univariate analysis were considered for multivariate analysis so as to control for confounder risk factors. Table 7 shows factors associated with anaemia on multivariate logistic regression analysis, factors which did not show significant have not been shown; History of blood transfusion since HIV diagnosis, thrombocytosis, low CD4 count, and low serum iron were predictors of anaemia. Other factors could not maintain statistical significance on multivariate logistic regression. It was revealed that anaemia was more likely to occur 4.3 times in patients with history of blood transfusion since HIV diagnosis (aOR=4.27, 95%CI= 1.47-12.39, P value 0.01)

Patients who were found to have thrombocytosis were 15.8 likely to be anaemic compared to patients with normal platelates counts (aOR=15.81, 95%CI=1.72-145.5, P value 0.02).

Anemia was about 2 times more likely to occur in patients with CD4 counts 200-349 cells/µl when compared to patients with CD4 ≥ 349 cells/µl (aOR= 2.09, 95%CI=1.01-4.35, P value 0.05).

Low serum iron levels were associated with a 3.8 times increased likelihood of anemia (aOR=3.84, 95%CI=1.32-11.14, P value 0.01) than those with normal serum iron (Table 7).

Table 7: Multivariate analysis of factors associated with anaemia among study participants (N=316)

Characteristics	Crude OR(95%CI)	P value for crude OR	aOR(95%CI)	P valu for aO
Occupation				
Employed	Reference		Reference	
Unemployed	1.78(1.01-3.12)	0.05	0.45(0.10-1.89)	0.27
Approximate income				
No income	0.69(0.38-1.25)	0.22	1.53(0.62-2.29)	0.46
Low income	0.43(0.22-0.83)	0.01	1.45(0.37-5.67)	0.59
Medium income	0.35(0.11-1.09)	0.07	1.26(0.31-5.12)	0.74
High income	Reference		Reference	
Malaria infection within past 3 month				
Yes	1.54(0.89-2.66)	1.12	1.38(0.68-2.79)	0.37
No	Reference		Reference	
History of Blood transfusion				
Yes	3.03(1.25-7.38)	0.01	4.27(1.47-12.39)	0.01
No	Reference		Reference	
Cotrimoxazole chemoprophylaxis				
Yes	0.69(0.42-1.15)	0.15	1.30(0.69-2.45)	0.42
No	Reference		Reference	
Patients on ARV				
Yes	Reference		Reference	
No	0.45(0.23-0.89)	0.02	1.83(0.67-4.97)	0.24

Table 7: Continues

Characteristics	Crude OR(95%CI)	P value for crude OR	aOR(95%CI)	P value for aOR
Presence of Tachypnoea(breath per minute)				
No	Reference		Reference	
Yes	10.86(1.38-85.13)	0.02	6.55(0.53-80.48)	0.14
Body mass index(Kg/m^2)				
Underweight	4.53(2.03-10.10)	0.001	1.16(0.39-3.45)	0.79
Normal weight	1.89(1.17-3.06)	0.01	1.37(0.76-2.46)	0.29
Overweight and obese	Reference		Reference	
Clinical stage				
Stage one	Reference		Reference	
Stage two	1.67(0.88- 3.18)	0.12	1.29(0.56 – 2.96)	0.55
Stage three	4.87(2.37- 9.980.42)	<0.001	1.26(0.35 – 4.52)	0.72
Stage four	3.73(1.87- 7.48)	<0.001	0.73(0.24 - 1.20)	0.13
Leucocytes				
Leucopenia	1.67(1.01-2.77)	0.04	1.10(0.58-2.09)	0.76
Normal count	Reference		Reference	
Leucocytosis	3.87(0.79-19.05)	0.09	5.39(0.55-53.22)	0.15
Lymphocytes				
Lymphopenia	14.89(1.94-114.27)	0.01	3.67(0.35-39.05)	0.28
Normal count	Reference		Reference	
Lymphocytosis	0.66(0.23-1.91)	0.45	0.41(0.09-1.82)	0.24

Table 7: Continues

Characteristics	Crude OR(95%CI)	P value for crude OR	aOR(95%CI)	P value for aOR
Thrombocytes				
Thrombopenia	2.89(1.09-7.59)	0.03	2.03(0.59-6.98)	0.26
Normal count	Reference		Reference	
Thrombocytosis	15.15(1.97-116.78)	0.01	15.81(1.72-145.5)	0.02
CD4 count(cells/µL)				
500 +	Reference		Reference	
349-499	0.98(0.49 – 1.92)	0.95	1.03(0.47- 2.29)	0.94
200-<349	1.95(1.06 – 3.59)	0.31	2.09(1.01-4.35)	0.05
<200	6.97(3.45-14.08)	<0.001	2.30(0.88 – 6.03)	0.09
Folate(nmol/L)				
<7.2	1.44(0.91-2.28)	0.12	1.67(0.94-2.97)	0.08
7.2 -15.4	Reference		Reference	
>15.4	2.81 (0.85-9.29)	0.09	0.76(0.17- 3.39)	0.72
Iron (mmol/L)				
<4.5	7.21(3.12-16.66)	0.001	3.84(1.32-11.14)	0.01
4.5-27.5	Reference		Reference	
>27.5	7.39(0.88-62.20)	0.07	3.99(0.39-40.94)	0.24

aOR = adjusted odds ratio

CHAPTER FIVE

DISCUSSION

The overall prevalence of anaemia in this study was 164(51.9%). Severe anemia was seen in 10.4% of patients. Anemia prevalence was comparable to both sexes, being slightly more than 50% for each sex. These results were similar to study by Akanmu A et al which reported anaemia in 52.8% of HIV-positive subjects[44]. Higher prevalence has been reported up to 84% cases in study by Parinitha SS and Kulkarni MH [45]. Higher prevalence in this study could be explained by the fact that majority of partcipants (70%) were WHO stage four HIV,and advanced stage is associated with anaemia[28].

Sociodermographic characteristics

The study population in this study had a mean age of 44 years. A similar study in Mulago hospital in Uganda had a mean age of 40.7 years [46] and 38± 2.3 years was the mean age in another study of folate levels in HIV infected patients in Lagos university teaching hospital in Nigeria [44], depicting the fact that HIV affects more the youths in the reproductive age.

The majority of study subjects in the present study were females (73.0%), in line with several other studies emulating this fact [47] overall, women are more likely to be HIV infected than men. In Tanzania in both rounds of population-based HIV testing (2003 and 2007), women were overall more likely to be HIV positive. An extremely high female:male prevalence ratio was found in Kigoma region in 2007 (15:1) [48]. A study on pharmacovigilance of adults on highly active antiretroviral therapy in South Africa showed the overall ratio of males to females of 1:2.3[49]. A study in Uganda by Japhet E Mukaya reported 60.0% of study participants were female [46].

In the present study most participants had attained primary education only, the finding similar to the latest population-based HIV surveillance survey (THMIS 2007-08) which showed, for the first time at population level, that HIV prevalence was lower amongst educated than uneducated or persons with lower education[48] . Majority of study participants were employed and much as employment is linked to income, only a few participants had no income at all.

Married group comprised 50.6% this is similar to other reports which shows HIV prevalence is highest amongst those who are currently or formerly married [48]. AIDS case reporting confirms the observation that more HIV prevalence is amongst married than unmarried persons (NACP, 2006)[7].

Prevalence of Anemia by sociodemographic characteristics among HIV Patients

Fifty three percent (53.4%) of the patients aged 25 to 49 years were anemic. Anemia prevalence was comparable in both males and females. Many studies [16,26,27] found significantly high prevalence of anaemia in female HIV infected patients than in males. Failure to see this difference in the present study can partly be explained by the fact that there was no statistical significant difference in employment status and level of income among males and females. Of the studied sociodemographic factors having no or low income was associated with high prevalences of anemia than it was seen in medium and high income patients. Having no income or low income may be associated with dietary insuficiences and thus anemia. As it was for income, anemia was more prevalent among the unemployed than it was for the employed, aproaching significant level. Again this could be explained by the fact that having no employment is associated with no income or low income.

Prevalence of anemia by clinical and laboratory characteristics among HIV Patients

Admitted patients had significantly higher anaemia prevalence 75.8% compared to 42.2% of patients who were recruited from the CTC, P value 0.001. This higher prevalence among admitted patients was also reported by Lyimo M et al on the study on

haematological manifestation of HIV[35] . This could be explained by the fact that admitted patients are likely to have low CD4 and advanced stage of HIV and these have been associated with anaemia [1,50].

Patients with history of malaria within three month before enrollment into the study were likely to be anaemic as 60% had anaemia. This could be explained by bone marrow suppression in the pathogenesis of anemia in both malaria and HIV [51] . Among patients with history of blood transfusion 75% were anaemic this could be due to effect of autoantibodies from previous transfusion or autoimmune effect caused by HIV itself[16,23, 26]. Cotrimoxazole use was associated with higher prevalence of anaemia 54.3% compared to 45.1% in patients not taking cotrimoxazole, this could be due to effect of cotrimoxazole on folate metabolism.

Of ARV naive patients 68.2% were anaemic compared to 49.7% of those on ARV. This could be due to effect of HIV on bone marrow suppression,having advanced stage of HIV and low CD4 count which are likely to occur in abscence of ARV[1,50].

Patients with silky hair were likely to be anaemic,this could be explained by the fact that anaemia is associated with advanced disease[1]. But in this study most patients were women (73%) and majority in child bearing age group who are likely to apply chemicals in their hair.

Anaemia was more prevalent in patients with black nails 62.9% compared to patients with normal coloured nails,other nail changes were not significant. This blackening of nails could be side effect of zidovudin use or due to fungal infection of the nail. Six patients (85.7%) among those with lymphadenopathy were anaemic.

Patients with tachypnoea were likely to be anaemic 91.7%, P value 0.01.this can be explained by the fact that anaemia when severe may present with tachypnea as a physiological compensatory mechanism even before the patient developes heart failure.

Mid upper arm circumference assessment revealed anaemia in all patients with severe acute malnutrition and 78.9% of patients with moderate acute malnutrition,P value was

0.01.These findings may offer an explanation to the association of micronutrient deficiency iron and folate, and anaemia that was observed in this study. Underweight as assessed by BMI and low waist circumference were also associated with anaemia.

On laboratory characteristics leucopenia,lymphopenia and thrombocytopenia were associated with anaemia, together with a low red blood cell, could indicate ineffective haematopoiesis. Similar findings were reported by Parinitha SS and Kulkarni MH [45],this could be due to direct effect of HIV on the haematopoietic system causing bone marrow suppression, secondary infections, neoplasms or side effects of therapy.

On the other hand,thrombocytosis in HIV could be reactive. Macrocytosis was prevalent in absence of anaemia as it was seen 60.6% of patients. Similar results have been reported. This observation could be due to side effect of medication such as Cotrimoxazole,and of ARV Zidovudine and Stavudine are implicated drugs [52, 53].

Association between anaemia and WHO clinical stage of HIV among HIV patients

Significantly high prevalences of anaemia were seen in patients in WHO stage II-IV, with the highest prevalence in stage III. Unexplained anemia of < 8g/dL is used to stage patients into WHO stage 3[39] and can partily explain the highest prevalence in Stage III. Todd S. Wills et al also reported higher prevalence of anemia in patients with advanced HIV disease [50].

Association between anaemia and CD4 count among HIV patients

Although anemia was seen in all levels of CD4 counts, anemia prevalence 89.5% was significantly high in patients with CD4 count < 200 cells/μL. A study in Mexico on risk factors and correlates for anemia in HIV treatment-naïve infected patients concluded that a CD4+ Cell Count <200 cells/mm^3 was associated with an increased risk of anemia after having observed that there is a positive correlation between hemoglobin and CD4+ cell count [1].

The study by Todd S et al on Anemia Prevalence and Associated Risk Factors in a Single-Center Ambulatory HIV Clinical Cohort which showed that patients with $CD4^+$

cell counts of less than 200/µL, 38.6% had an Hb level of less than 13 g/dL, compared with 12.2% of patients with a $CD4^+$ cell count of 500 or more/µL (P = .0001) [50]. These findings suggest that having low CD4+ cells is a risk for developing anaemia.

Association between Anaemia and type of ARV regimen among HIV patients

Not being on ARV treatment was significantly associated with high prevalence of anemia than being on ARV treatment, regardless of whether the regimen was AZT containing or not. Not being on ARV treatment would mean more opportunistic infections, low CD4 counts and advanced stage of HIV that are known to cause anaemia [1, 33]. These findings suggest that HIV infection by itself is a risk for anaemia as evidenced by results from the study.

Among patients on ARV those on AZT containing regimen had higher prevalence of anaemia which is similar to observation from previous studies [33, 34]. Todd S et al also reported higher prevalence of anemia in patients who were currently being treated with HAART regimen containing zidovudine than in patients treated with non-zidovudine-containing HAART regimens; P< .0001 [50].

Association between anaemia and micronutrient levels (Vitamin B12, Folic acid and Iron)

Serum B12 levels

In this study only 2.5% of participants had vitamin B12 deficiency. The prevalence of vitamin B-12 deficiency was significantly lower in patients receiving HAART than in previously studied patients who did not receive HAART (8.7% compared with 27%) [54], which is higher compared to the current study. The lower prevalence of vitamin B12

deficiency in this study could be due to effect of HAART through down-regulation of an overactivated immune system in HIV patients, and antioxidant status improvement [54] as most patients were on ART or routine multivitamin supplementation in persons with HIV at our CTC. Prevalence of anaemia in patients with vitamin B12 deficiency in this study was 37.5%.Study by Mohsen Meidani et al reported anaemia in 23% of patients with vitamin B_{12} deficiency [38].

Serum Folate

Folate deficiency was seen in 40.5% of participants these findings are in tandem with Friis et al, in a study of HIV-positive asymptomatic subjects in Southern Brazil,which reported a plasma folate deficiency in 41% of the subjects studied[55]. Folate deficiency was highly associated with anaemia as it was cotrimoxazole chemoprophylaxis 56.2% and 54.3% respectively. This could be due to effect of cotrimoxazole on folate metabolism. Study done in Nigeria demonstrated anaemia in 52.8% of HIV-infected subjects with folate deficiency[44]. The observed findings could also be due to the fact that Folate is rapidly depleted in diseases, especially when there is prolonged anorexia, increased metabolic rate, and high cell turnover. The HIV virus is known to have a rapid turnover this could contribute to rapid depletion of folate stores [56].

Serum Iron

Analysis of data in current study revealed mean serum iron of 12.21 ± 7.06, this is markedly lower than findings by Banjoko et al[57] which showed mean iron in cohort of HIV patients to be 35.3 ± 0.8. Patients with Iron deficiency were likely to be anaemic as anaemia was seen in 85.4%. Mohsen Meidani et al reported anemia in 40% patients [38].The prevalence of iron deficiency in current study was high. This difference could be accounted by the fact that HIV -1 infection which is more common in our setting is known to affect virtually all the organs of the body causing different metabolic derangements and depression of the immune system [58]. These metabolic derangements include oxidative stress due to persistent immune activation associated with uncontrolled HIV-1 replication leading to excessive reactive oxygen species (ROS) generation [59].

Alterations in normal physiology as a result of immune response in HIV infections may also involve markers of iron metabolism [60, 61]. On assessing iron status only 46(14.6%) had adequate iron store, absolute Iron deficiency as indicated by serum ferritin <100 ng/ml and transferrin saturation (TSAT) <20% was seen in 26.9% patients and functional Iron deficience as indicated by serum ferritin ≥ 100ng/ml and < 20% TSAT was seen in 11.4% patients, iron overload was present in 0.6% of patients, these finding could be due to effect of HIV on Iron metabolism.

Predictors of anaemia among HIV patients Attending MNH Medical department

Factors with P-value ≤0.2 on univariate analysis were considered for multivariate analysis so as to control for confounder risk factors. Predictors of anaemia on multivariate logistic regression analysis were; History of blood transfusion since HIV diagnosis, thrombocytosis, low CD4 count, and low serum iron. Other factors could not maintain statistical significance on multivariate logistic regression. It was revealed that anaemia was more likely to occur 4.3 times in patients with history of blood transfusion since HIV diagnosis (aOR=4.27, 95%CI= 1.47-12.39, P value 0.01)

Patients who were found to have thrombocytosis were 15.8 likely to be anaemic compared to patients with normal platelates counts (aOR=15.81, 95%CI=1.72-145.5, P value 0.02).

Anemia was about 2 times more likely to occur in patients with CD4 counts 200-349 cells/µl when compared to patients with CD4 ≥ 349 cells/µl (aOR= 2.09, 95%CI=1.01-4.35, P value 0.05). Similar findings were reported by Mata-Marín.J et al, in his study CD4+ cells count <200 cells/mm^3 was associated with an increased risk of anemia in the multivariate analysis OR 8.8 (IC95% 5.3-15.8; p = 0.01) [1].

Low serum iron levels were associated with a 3.8 times increased likelihood of anemia (aOR=3.84, 95%CI=1.32-11.14, P value 0.01) than those with normal serum iron (Table 7).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study showed that risk factors for anaemia among HIV patients are multifactorial and they include low income, advanced WHO stage of HIV, CD4 count less than 200 cells/mm^3, being ARV naive, using AZT based regimen, defficiency of serum Folate and Iron. Anaemia is common problem among HIV patients and admitted patients had higher prevalence of severe anaemia compared to patients seen at clinic this would mean anaemia contributes to burden of HIV care as it is associated with increased morbidity among these patients. Unemployement and low income was shown to be associated with higher prevalence of anaemia. Regardless of ART regimen it was shown that ART naïve patients had higher prevalence of anaemia and ART treatment was associated with improvement of Haemoglobin. Furthermore being on AZT containing regimen was associated with higher prevalence of anaemia compared to non AZT regimen. Of the three studied micronutrients Iron deficiency was more prevalent among these patients and was associated with anaemia on multivariate analysis.

6.2 RECOMMENDATIONS

Findings from the study have shown that many patients who were ARV naive were anaemic as compared to their counterpart on ARV it is recomended that patients with HIV timely receive ARV as per guidline.

Early recognition of HIV associated anaemia may be achieved by HIV screening in adult patients presenting with unexplained anaemia and strictly measuring Haemoglobin level among HIV patients in care as it is done for CD4 monitoring in our setting.

As many patients were found to have iron deficiency it is highly recommended to do iron studies in these patients to establish types of iron deficiency as absolute iron deficiency will needs oral iron supplements while in other hand functional iron deficiency requires intravenous iron supplement.

This was a tertiary hospital based study, the results may not reflect true community picture, it is therefore recommended to do similar study in primary and secondary care facilities in order to capture other risk factors associated with anaemia among HIV patients.

6.3 STUDY STRENGTH

This study conducted among HIV patients included all stages of HIV disease as patients were recruited from both inpatients and outpatient. All patients were given feedback of their results and those with anaemia were treated as per guideline.

6.4 STUDY LIMITATIONS

One potential limitations is about generalisability of results as this study was conducted at Muhimbili National Hospital might not be a true representative of the general population but may be relevant to the population of HIV patients.

Other risk factors for anaemia including worm infestation and presence of opportunistic infections were not assessed. Iron status was evaluated by serum ferritin and transferrin saturation (TSAT) However, ferritin is an acute-phase reactant and can be elevated for reasons other than sufficient or excessive iron stores in infection and inflammation, similarly transferrin concentration are altered in states of hypoalbuminemia and chronic disease, resulting in false TSAT values. This might have either underestimated or overestimated iron deficiency in this HIV population.

Since many patients who were ARV naive were anaemic as compared to their counterpart on ARV it is recomended that patients with HIV timely receive ARV as per guidline.

REFERENCES

1. Mata-Marín J, Gaytan-Martinez J, Martínez-Martínez R, Arroyo-Anduiza C, Fuentes-Allen J and Casarrubias-Ramirez M. Risk factors and correlates for anemia in HIV treatment-naïve infected patients: a cross-sectional analytical study. BMC Res Notes, 2011. 3(230).

2. Beutler E and Waleen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood, 2006. 107(5): p. 1747–1750.

3. Buskin S and Sullivan P.Anemia and its treatment and outcomes in persons infected with human immunodeficiency virus. Transfusion, 2004. 44(6): p. 826–832.

4. Coyle.Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Med Clin North Am, 1997. 81(2): p. 449-70.

5. Huang S, Barbour J, Deeks S, Huang J, Grant R, Valerie L and McCune J., Reversal of human immunodeficiency virus type 1–associated hematosuppression by effective antiretroviral therapy. Clin Infect Dis, 2000. 30: p. 504–10.

6. Moore RD, Keruly Jc and Chaisson RE, Anemia and survival in HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 1998. 19: p. 29–33.

7. Hillman RS, Martin JB and Braunwald E, eds. Anemia. Harrison's principles of internal medicine, 1998(14th ed): p. 334–9.

8. WHO, Epidemic update and health sector Progress towards Universal Access, Progress Report GLOBAL HIV/AIDS RESPONSE, 2011.

9. (NACP), N.A.C.P, report, 2003 and 2006.

10. (NACP), N.A.C.P, report, 2012.

11. Hillman RS and Ault KA (Eds). Clinical approach to anemia. . In: Hematology in Clinical Practice , McGraw-Hill, 2001: p. 29.

12. Perera R,Isola L,Kaufmann H. Effect of recombinant erythropoietin on anemia and orthostatic hypotension in primary autonomic failure.Clin Auton Res, 1995. 5(4):p.211-3.

13. Gomes ME, Deinum J, Timmers HJ and Lenders JW. Anaemia and orthostatic hypotension. Lancet 2003. 362(9392): p. 1282.

14. Davenport , J. Macrocytic anemia. Am Fam Physician, 1996. 53(1): p.152-62.

15. Inelmen EM, D'Alessio M, Gatto MR, Baggio MB, Jimenez G, Bizzotto MG,Enzi G. Descriptive analysis of the prevalence of anemia in a randomly selected sample of elderly people living at home: some results of an Italian multicentric study.Aging(Milano), 1994. 6(2): p. 81-9.

16. Volberding,Levine A,Dieterich D,Mildvan D,Mitsuyasu R,Saag M and for anaemia in HIV working group. Anemia in HIV Infection: Clinical Impact and Evidence-Based Management Strategies. Oxford Journals Medicine Clinical Infectious Diseases 2004. 38(10): p. 1454-1463.

17. Bain.Pathogenesis and pathophysiology of anemia in HIV infection. Curr Opin Hematol. 1999 Mar;6(2):, 1999. 6(2): p. 89-93.

18. Kreuzer KA, Rockstroh J. Pathogenesis and pathophysiology of anemia in HIV infection. Ann Hematol.1997. 75(5-6): p. 179-87.

19. Zauli G, Carla Re M, Visani G, Furlini G, Mazza P, Vignoli M. Evidence for a human immunodeficiency virus type-l- mediated suppression of un infected hematopoietic (CD34+) cells in AIDS patients. J Infect Dis, 1992. 166: p. 707–10.

20. Horsburgh. Mycobacterium avium complex infection in the acquired immunodeficiency syndrome. N Engl J Med, 1991. 324: p. 1322–32.

21. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding, Laskin OL, Leedom JM, Groopman JE, Mildvan D and Hirsch MS.The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. New England Journal of Medicine,1987. 317(4): p. 192-197.

22. Sullivan P, Hanson D, Chu S, Jones J, Ward J and Adult/Adolescent Spectrum of disease group. Epidemiology of Anemia in Human Immunodeficiency Virus (HIV)-Infected Persons: Results from the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project. Blood, 1998. 91(1): p. 301-308.

23. Voth R, Rossol S, Graff E, Laubenstein HP, Schroder HC, Muller WE, Meyer zum Buschenfelde KH and Hess G. Natural killer cell activity as a prognostic parameter in the progression to AIDS. J Infect Dis, 1988. 157(4): p. 851–852.

24. Soriano V, Massimo P, Bergin C, Hatzakis A, Cacoub P, Katlama C, Cargnel A, Mauss S, Dieterich D, Moreno S, Ferrari C, Poynard T, Rockstroh J., Care of patients with chronic hepatitis C and HIV co-infection: recommendations from the HIV-HCV International Panel. AIDS, 2002. 16(6): p. 813-28.

25. Harriman GR, Smith PD, Home MK, Fox CH, Koenig S, Lack EE, Lane HC and Fauci AS. Vitamin B12 Malabsorption in Patients With Acquired Immunodeficiency Syndrome. Arch Intern Med,1989. 149(9): p. 2039-2041.

26. Levine AM, Berhane K, Masri-Lavine L, Sanchez M, Young M, Augenbraun M, Cohen M, Anastos K, Newman M, Gange SJ, Watts H. Prevalence and correlates of anemia in a large cohort of HIV-infected women: Women's Interagency HIV Study. J Acquir Immune Defic Syndr, 2001. 26(1): p. 28-35.

27. Semba RD, Shah N, Klein RS, Mayer KH, Schuman P,Vlahov D and HIV epidemiology research group. Prevalence and Cumulative Incidence of and Risk Factors for Anemia in a Multicenter Cohort Study of Human Immunodeficiency Virus Infected and Uninfected Women. Clinical Infectious Diseases,2002. 34(2): p. 260-266.

28. Mildvan D, Creagh T, Leitz G and anemia prevalence group. Prevalence of anemia and correlation with biomarkers and specific antiretroviral regimens in 9690 human-immunodeficiency-virus-infected patients. Curr Med Res Opin, 2007. 23(2): p. 343-55.

29. Semba RD. Iron-Deficiency Anemia and the Cycle of Poverty among Human Immunodeficiency Virus Infected Women in the Inner City. Clinical Infectious Diseases, 2003. 37(Supplement 2): p. S105-S111.

30. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, Pradier C, dArminio Monforte A, Ledergerber B, Lundgren JD. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS, 1999. 13(8): p. 943-50.

31. Abrams DI, Steinhart C and Frascino R. Epoetin alfa therapy for anaemia in HIV-infected patients: impact on quality of life. Int J STD AIDS, 2000. 11(10): p. 659-665

32. Moore RD, Forney D. Anemia in HIV-infected patients receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr, 2002. 29(1): p. 54-7.

33. Henry DH, Beall G, Benson C, Carey J, Cone L, Eron L, Fiala M, Fischl M, Gabin S, Gottlieb M, Galpin J, Groopman J, Hooton R, Miles D and Rinehart J. Recombinant Human Erythropoietin in the Treatment of Anemia Associated with Human Immunodeficiency Virus (HIV) Infection and Zidovudine Therapy. Annals of Internal Medicine,1992. 117(9): p. 739-748.

34. Martí-Carvajal AJ, Sola I, Peña-Martí GE, Comunián-Carrasco. Treatment for anemia in people with AIDS. Cochrane Database Syst Rev, 2011. 10.

35. Lyimo M. Haematological manifestation in HIV Muhimbili National Hospital. unpublished, 2003.

36. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance AFRICAN REGION WHO/HIV/2005.02

37. Makubi AN, Mugusi F, Magesa P and Roberts D. Risk factors for anaemia among HIV infected children attending care and treatment clinic at Muhimbili National Hospital in Dar es Salaam, Tanzania. Tanzania Journal of Health Research 2012.14(1).

38. Statistics TNc. 2012.

39. Mohsen Meidani, Razael F, Mohammad Reza Maracy, Majid Avijgan, Katayoun Tayeri. Prevalence, severity and related factors of anemia in HIV/AIDS patients.Journal of Research in Medical Sciences, 2012. 17(2).

40. Lewis M, Bain B and Bates I. Practical Haematology: Tenth Edition Churchill Livingstone. 2010: p. 146-147.

41. World Health Organization.Iron deficiency anemia: assessment,prevention and control;a guide for programmer manager.www.who.int/entity/nutrition/publications/GFF References en.pdf..2011.

42. SYSTEM,A.A, Folate assay laboratory procedure manual.http://www.inlexmedical.com/files/ .PDF/Folate_AXS.pdf., 2011(REF 7K46, 49- 3690/R3, B7K460).

43. AxSYM, A. Active-B12 (Holotranscobalamin) .Accessed on 12th.August.at. http://www.inlexmedical.com/files/ PDF/AXS-ActiveB12Holt.pdf,2011(manual IVD REF 1P43-20 ABOL039/R1).

44. Akanmu A, Vincent O, Adediran A,Titilope A,Onogu E,Ralph A and Hab C. Plasma folate studies in HIV-positive patients at the Lagos university teaching hospital, Nigeria. Indian J Sex Transm, 2010. 31(2): p. 99-103

45. Parinitha SS and Kulkarni MH. Haematological changes in HIV infection with correlation to CD4 cell count Australas Med J, 2012. 5(3): p. 157–162.

46. Mukaya JE,Ddungu H,Ssall F,Oshea T and Crowther MA. Prevalence and morphological types of anaemia and hookworm infestation in the medical emergency ward, Mulago Hospital, Uganda. SAMJ, S. Afr. med. j. , 2009. 99(12).

47. Ragnarsson A, Ekstrom A, Carter J, Ilako F, Lukhwaro A, Marrone G and Thorson A.Sexual risk taking among patients on antiretroviral therapy in an urban informal settlement in Kenya: a cross sectional survey. J Int AIDS Soc, 2011((1186/1758-265).

48. ASAP. The HIV epidemic in Tanzania Mainland:Where have we come from,where is it going, and how are we responding? 2008.

49. Dube NM,Summers R,Tint KS and Mayayaise G. pharmacovigilance study of adults on highly active antiretroviral therapy, South Africa: 2007 – 2011.Pan Afr Med J, 2012.

50. Wills TS, Nadler JP,Somboonwit C,Vincent A,Leitz G,Marino K,Nalk E,Powers S,Khan N and Laartz B. Anemia Prevalence and Associated Risk Factors in a Single-Center Ambulatory HIV Clinical Cohort AIDS Read, 2004. 14(6).

51. Sarah Hochman and Kami Kim. The Impact of HIV and Malaria Coinfection: What Is Known and Suggested Venues for Further Stud, 2009.

52. Geene D, Sudre P, Anwar D, Goerhring C, Saaidia A,Hirschel B. Causes of macrocytosis in HIV-infected patients not treated with zidovudine. Swiss HIV cohort study. J.Infectious ,2000,40(2):160-3

53. Oliveira OC,Oliveira RA,Souza Ldo R. Impact of antiretroviral therapy on occurrences of macrocytosis in patients with HIV/AIDS in Maringá, State of Paraná. Med Trop. 2011 Jan-Feb;44(1):35-9.

54. Rechama AF,Cadafalch J,Sarda P,Barcelo M and Fuster M. Vitamin B-12 metabolism in HIV-infected patients in the age of highly active antiretroviral

therapy: role of homocysteine in assessing vitamin B-12 status. Am J Clin Nutr 2003. 77(2): p. 420-424.

55. Fuchs D, Jaeger M, Widner B, Wirleitner B, Artner-Dworzak E and Leblhuber F. Is hyperhomocysteinemia due to the oxidative depletion of folate rather than to insufficient dietary intake? . Clin Chem Lab Med, 2001. 39: p. 691-4.

56. Friis H, Gomo F, Koestel N,Nyameza N,Krarup H and Michaelsen KF. HIV and other predictors of serum folate, serum ferritin, and hemoglobin in pregnancy: A cross-sectional study in Zimbabwe. Am J Clin Nutr., 2001. 73: p. 1066–73.

57. Banjoko O, Oseni F, Togun R, Onayemi O, Emma-Okon B and Fakunle J. Iron status in HIV-1 infection: implications in disease pathology, BMC Clinical Pathology, 2011. 12.

58. Martinez E and Gattel JM. Metabolic abnormalities and body fat redistribution in HIV-1 infected patients: lipodystrophy syndrome.Curr Opinium Infect Dis, 1999. 12(1): p.13-20.

59. Salmen S and Berrueta L. Immune modulators of HIV infection: the role of reactive oxygen species. 3:121. J Clin Cell Immunol 2012. 3(121).

60. Doherty CT. Host pathogen interaction: the role of iron. J Nut, 2007.137(5):p.1341-1344.

61. Drakesmith H and Prentice A. Viral infection and iron metabolism. Nat Rev Microbiol, 2008. 6(7): p. 541-542.

APPENDICES

Appendix i: REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR ADULTS AND ADOLESCENTS

Primary HIV infection

Asymptomatic

Acute retroviral syndrome

Clinical stage 1

Asymptomatic

Persistent generalized lymphadenopathy (PGL)

Clinical stage 2

Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media, pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulcerations

Papular pruritic eruptions

Seborrhoeic dermatitis

Fungal nail infections of fingers

Clinical stage 3

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

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

Unexplained chronic diarrhoea for longer than one month

Unexplained persistent fever (intermittent or constant for longer than one month)

Oral candidiasis

Oral hairy leukoplakia

Pulmonary tuberculosis (TB) diagnosed in last two years

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

Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary

Unexplained anaemia (< 8 g/dl), and or neutropenia (<500/mm3) and or thrombocytopenia (<50 000/ mm3) for more than one month

Clinical stage 4

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

HIV wasting syndrome

Pneumocystis pneumonia

Recurrent severe or radiological bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)

Oesophageal candidiasis

Extrapulmonary TB

Kaposi's sarcoma

Central nervous system (CNS) toxoplasmosis

HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary:

Extrapulmonary cryptococcosis including meningitis

Disseminated non-tuberculous mycobacteria infection

Progressive multifocal leukoencephalopathy (PML)

Candida of trachea, bronchi or lungs

Cryptosporidiosis

Isosporiasis

Visceral herpes simplex infection

Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)

Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)

Recurrent non-typhoidal salmonella septicaemia

Lymphoma (cerebral or B cell non-Hodgkin)

Invasive cervical carcinoma

Visceral leishmaniasis

Appendix ii: QUESTIONNAIRE (ENGLISH VERSION)

A: S0CIAL DERMOGRAPHIC INFORMATION

1. Identification Number----------
2. Patient hospital No---------- phone number----------(optional)
3. Age(years)----------
4. Sex 1. Male 2. Female
5. Residence ----------
6. Level of education

   1. No formal education 2. Primary education 3. Secondary education

   4. Diploma/Advanced diploma 5. University education
7. Occupation

   1. Unemployed 2. Self employed 3. Private employed 4. Government employed
8. Approximate income per month

   1. No constant income 2. Less than 200,000/=

   3. 200,000/= to 500,000/= 4. More than 500,000/=
9. Marital status

   1. Single 2. Married 3. Divorced/Separated 4. Widowed/widower

B: PAST MEDICAL HISTORY

10. (i) Have you suffered from malaria within past three month? 1. Yes 2. No

    (ii) Is your stool blood stained? 1. Yes 2. No

    (iii)When were you diagnosed to have HIV……year

    (iv) History of Blood transfusion since HIV diagnosis 1.Yes 2.No
11. Concomitant drug use

    (i) Cotrimoxazole prophylaxis? 1. Yes 2. No

    (ii) Herbal preparation 1. Yes 2. No

    (iii) Alcohol 1. Yes 2. No

(iv) Aspirin/ Non steroidal anti-inflammatory drugs 1. Yes 2. No

(v) Haematenics 1. Yes 2. No

(For questions 13 to 16 look into patient file and CTC card number 2)

12. Are you on ARV? 1. Yes 2. No

13. If yes, for how long ……. .(Month)

14. Types ……..

15. Since initiation of ARV were they changed at some point in time? 1. Yes 2. No

16. If Yes; types(from record) Duration of use reasons for changing

....

17. Clinical stage of the disease……..

C: EXAMINATION FINDINGS

18. (i)Pallor 1.Yes 2.No

(ii) Jaundice 1. Present 2.Absent

(iii) Cyanosis 1.Present 2. Absent

(iv) Mouth ulceration 1.Present 2.Absent

(v) Hair changes 1.Present 2.Absent

(vi) Gum bleeding 1.present 2.Absent

(vii)Nail changes 1.present 2.absent

(viii) Lymphadenopathy 1.present 2.Absent

(ix) Skin changes 1.present 2.absent

(x) Body Temperature……degree centigrade

(xi) Respiratory rate----------b/min,

(xii) Pulse rate----------b/min,

(xiii) Blood pressure……..mmHg

(xiv)Mid Upper Arm Circumference (MUAC) ---------cm

(xv) Weight ---------kg Height----------cm

(xvi) BMI……wt (kg)/height (m2)

a.Underweight = <18.5 b.Normal weight = 18.5–24.9

c.Overweight = 25–29.9 d.Obesity = BMI of 30 or greater

(xvii) Waist circumference ……..cm 1.female a.88 b.<88 c.>88

(xviii)Spleen size BLCM----------cm

(xix) Liver span BRCM----------cm

D: LABORATORY FINDINGS

21. CD4 count ……. 1.>500 2.349-499 3.200-349 4. <200
22. FBP

RBC……. WBC…… PLT……

HGB…… MCV…… MCH……..

23. B12 levels……..
24. Folate levels……..
25. Serum iron levels…….
26. Ferritin levels……
27. Transferrin concentration……..

Transferrin saturation %= Serum iron x 100 / TIBC

TIBC =Transferrin level x 25 ……..

8.2.2. QUESTIONNAIRE (SWAHILI VERSION)

A: UTAMBULISHO WA MSHIRIKI

1. Namba ya utambulisho..... Namba ya simu.....(hiari)
2. Namba ya jalada.....
3. Umri (miaka).....
4. Jinsia 1. Mwanaume 2.Mwanamke
5. Mahali unakoishi.....
6. Kiwango cha elimu

   1. Sijasoma 2.Elimu ya msingi 3.Elimu ya sekondari

   4. Stashahada/Shashahada ya juu 5.Digrii
7. Ajira

   1. Sina ajira 2.Nimejiajiri 3.nimeajiriwa sekta binafsi 4.Mwajiriwa wa serikali

8. Kipato kwa mwezi

   1. Sina kipato 2.<200,000/- 3. 200,000/- mpaka 500,000/= 4.> 500,000/=
9. Hali ya ndoa

   1. Sijaoa/Sijaolewa 2. Nimeoa/Nimeolewa 3. Nimeachika 4. Mjane/mgane

B: HISTORIA YA HALI YA AFYA

10. (i)Umeugua ugonjwa wa malaria kipindi cha miezi 3 iliyopita ? 1. Ndio 2. Hapana

    (ii)Unapata kinyesi chenye damu? 1. Ndio 2. Hapana

    (iii)Uligundulika lini kuwa VVU? ..... (mwaka)

    (iv)Umewahi kuongezewa damu tangu ugundulike kuwa na VVU? 1.Ndio 2.Hapana

11. Dawa nyingine unazotumia

    (i) Septrin 1. Ndio 2. Hapana

    (ii) Dawa za miti shamba 1. Ndio 2. Hapana

(iii) Pombe 1. Ndio 2. Hapana

(iv) Aspirin/Non steroidal anti-inflammatory 1. Ndio 2. Hapana

(v)Dawa za kuongeza damu 1. Ndio 2. Hapana

C:HISTORIA YA UGONJWA WA UKIMWI

(Swali 13-16 angalia kwenye faili na kadi namba 2)

12.Je unatumia dawa za kupunguza makali ya ugonjwa wa ukimwi. 1. Ndio 2. Hapana

13. Kama ndio kwa muda gani sasa.....

14. Aina 1.....2.....3.....

15. Je tangu kuanza dawa umewahi kubadilishiwa dawa 1. Ndio..... 2. Hapana

16. Kama ndio; Aina za dawa muda aliotumia sababu kubadilisha

…..... ....

17. Hatua ya ugonjwa.....

D:VIPIMO VYA MWILI

18. (i)Dalili ya upungufu wa damu 1. Ndio 2.Hapana

(ii) Manjano 1. Ndio 2.Hapana

(iii) Hali ya rangi ya samawati 1. Ndio 2.Hapana

(iv) Mouth ulceration 1. Ndio 2.Hapana

(v) Mabadiliko ya nywele 1. Ndio 2.Hapana

(vi) kutoka damu kwenye fizi 1. Ndio 2.Hapana

(vii)Nail changes 1. Ndio 2.Hapana

(viii) Uwepo wa matezi 1. Ndio 2.Hapana

(ix) mabadiliko ya ngozi 1. Ndio 2.Hapana

(x) Joto la mwili……

(xi) Kasi ya upumuaji----------/dakika

(xii) Kasi ya mapigo ya moyo-----/dakika

(xiii) Mgandamizo wa damu……..mmHg

(xiv)Ukubwa wa mzunguko wa katikati ya mkono(MUAC) ---------sm

(xv) Uzito ---------kg Urefu----------sm

(xvi) Uwiano wa uzito na kimo……uzito (kg)/urefu (m2)

a.Uzito pungufu = <18.5 b.Uzito unaotakiwa = 18.5–24.9

c.Uzito zaidi = 25–29.9 d.Kiriba tumbo = BMI of 30 or greater

(xvii) Ukubwa wa mzunguko wa kiuno……..sm 1.female a.<88 b.>88

(xviii)Ukubwa wa Bandama chini ya mbavu upande wa kushoto ----------sm

(xix) Ukubwa wa Ini chini ya mbavu upande wa kulia ---------- sm

E: VIPIMO VYA MAABARA

21. Kiasi cha chembe hai kinga..... 1. >500 2. 349-499 3.200-349 4. <200

22. Picha ya damu WBC…… PLT……

HGB…… MCV…… MCH…….

23. Kiasi cha Vitamini B12 mwilini …….

24. Kiasi cha Folate mwilini.....

25. Kiasi cha madini chuma .....

26. Kiasi cha Ferritin mwilini.....

27.Kiasi cha Transferrin concentration

Transferrin saturation %= Serum iron x 100 / TIBC

TIBC =Transferrin level X 25 ……

Appendix iii: CONSENT FORM

8.3.1 CONSENT FORM (ENGLISH VERSION)
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES DIRECTORATE OF RESEARCH AND PUBLICATIONS, MUHAS CONSENT FORM

CODE NO_____

CONSENT TO PARTICIPATE IN THE STUDY TITLED ANAEMIA AND ITS ASSOCIATED RISK FACTORS AMONG PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS ATTENDING MUHIMBILI NATIONAL HOSPITAL - MEDICAL DEPARTMENT

Greetings, I am Dr Avelina Mgasa, a resident in the department of Internal Medicine. I would like to conduct the study above as a necessary requirement for fulfillment of my postgraduate studies .

The purpose of the study is to Assessment of risk factors for development of anemia among admitted patients with human immunodeficiency virus Muhimbili National Hospital Medical department.

This study requires you to participate so that important information can be obtained from you regarding your health.

If you agree to participate in the study, you will be interviewed, and a detailed clinical history will be requested. A blood sample will also be requested from you.

Confidentiality:
All information collected on questionnaires will be entered into computer with identification number. The questionnaires will be handled with greater secrecy in order to maintain confidentiality.
There is no risk associated with this study

Taking part in this study is completely voluntary. If you choose not to participate in the study, you will continue to receive all services that are normally provided in the ward.

If you agree to take part in this study, you will be evaluated for Anaemia risk factors, and in case of any appropriate measure will be taken including appropriate advice or prescription of medication if required.

If you have any question about the study, you can contact Dr Avelina Mgasa 0786 570933 and Dr Magdalena Lyimo, Department of Hematology and Pro. F.mugusi 0784613354 of department of Internal Medicine, MUHAS. If you have questions about your rights as a participant, you may contact Prof M.M.Aboud, Chairman of MUHAS Research and Publications Committee. P.O.BOX 65001 Dar es Salaam.

Do you agree?

Participant agrees…….. ……..Participant does NOT agree……

I, _____have read/been told of the contents of this form and understood its meaning; hence, I do agree to participate in this study.

Signature_____ (Participant), Date_____

Signature_____ (Researcher), Date_____

Appendix iii: CONSENT FORM (SWAHILI VERSION)
KUKUBALI KUSHIRIKI KWENYE UTAFITI

NAMBA YA SIRI_____

KUKUBALI KUSHIRIKI KATIKA UTAFITI KUHUSU VIASHIRIA HATARISHI VYA KUPATA UPUNGUFU WA DAMU MIONGONI MWA WAGONJWA WANAOISHI NA VIRUSI VYA UKIMWI KATIKA HOSPITALI YA TAIFA MUHIMBILI IDARA YA TIBA.

Salaaam!
Mimi naitwa Dr.Avelina Mgasa ni mwanafunzi wa udhamili Chuo Kikuu cha Sayansi za Afya. Nafanya utafiti kuhusu viashiria hatarishi vya kupata upungufu wa damu miongoni mwa wagonjwa wanaoishi na virusi vya ukimwi katika hospitali ya taifa Muhimbili.

Jinsi ya kushiriki
Kama utakubali kushiriki katika utafiti huu ,nita kuhoji maswali machache kuhusu ugonjwa wako na nitakuchukua damu kwa ajili ya vipimo.

Madhara/usiri
Hakuna madhara yoyote yanayotegemewa kutokana na utafiti huu.Taarifa za ugonjwa wako zitatunzwa kwa kutumia herufi maalum ili kuwa na usiri.

Uhuru wa kushiriki ;
Kushiriki kwenye utafiti ni hiari yako. Unaweza kujitoa wakati wowote. Kama utachagua kutoshiriki, utaendelea kupata huduma kama kawaida hapa hospitalini.

Faida ya utafiti
Ukishiriki kwenye utafiti huu , utachunguzwa kama unakiashiriria chochote cha kupata upungufu wa damu utatibiwa au kupewa ushauri.

Taarifa

Kuna kamati ya kusimamia udhibiti wa Utafiti huu.

Endapo unahitaji kupata maelezo kuhusu haki zako au taarifa ,wasiliana na Dr Avelina Mgasa 0786570933 au Dr Magdalena Lyimo 0659 120100 au Prof. F.Mugusi 0784 613354 wa chuo Kikuu cha Afya na tiba Muhimbili. Kama unaswali lolote kuhusu haki yako kama mshiriki wasiliana na Prof M.M.Aboud, ambaye ni mwenyekiti wa bodi ya utafiti chuo kikuu cha Afya na Tiba Muhimbili, kwa S.L.P **65001 Dar es Salaam**

Baada ya maelezo hayo , Je unakubali kushiriki kwenye utafiti? (weka alama) ya vema,

Ndiyo..... Hapana.....

Mimi ....., nimeelezwa na nimesoma maelezo haya. Maswali yangu yamejibiwa.

Nimekubali kushiriki katika utafiti huu.

Sahihi ya mshiriki..... Tarehe…….

Sahihi ya Mtafiti ..... . Tarehe……