# PREVALENCE AND FACTORS ASSOCIATED WITH MODERATE TO SEVERE ANAEMIA AMONG HIV INFECTED CHILDREN ADMITTED AT MULAGO HOSPITAL

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A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Masters of Medicine in Paediatrics and Child Health of Makerere University

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## Declaration

I here by declare that all the work presented in this dissertation is original unless otherwise acknowledged.

This work has not been presented to any university or higher institution for any academic award, publication or otherwise.

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# **DEDICATION**

This dissertation is dedicated to my beloved mother Olivia Munyagwa And

to the improved quality of life of all HIV infected children

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With this book, I would like to give the glory to the Almighty God for this far that He has brought me.

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## ABBREVIATIONS AND SYMBOLS

**ACU:** Acute Care Unit AIDS: Acquired immunodeficiency syndrome **AZT**: Zidovudine CMV: Cytomegalo virus **dl**: deci litre DNA: Deoxy-ribonucleic acid EDTA: Edetate calcium disodium **fl** : Femto litres **GIT:** Gastrointestinal tract GM CSF: Granulocyte Monocyte Colony Stimulating Factor **G** :gramme HIV: Human immunodeficiency virus HAART: Highly active anti retro viral therapy **IL**: Interleukin **IDI** : Infectious Disease Institute Hb: Haemoglobin Kg: Kilogram MAC: Mycobacterium Avium Complex MCV: Mean cell volume MCHC: Mean cell haemoglobin concentration mls : mills MNU: Mwanamugimu nutritional unit MUAC: Mid upper arm circumference **PIDC** : Paediatric Infectious Disease Clinic **PMTCT**: Prevention of mother to child transmission **RDW:** Red cell distribution width **RCT** : Routine Counseling and Testing. **RBC**: Red blood cell **SD**: Standard deviation

TIBC: Total iron binding capacity
TNF: Tumour necrosis factor
UNICEF: United Nations International Children's Emergency Fund
WBC: White blood cell
WHO: World Health Organization
< :less than
> : more than
≥ : more than or equal to

- $\leq$  : less than or equal to

## **OPERATIONAL DEFINITIONS**

Anaemia is defined as haemoglobin less than 11g/dl

Care giver is the person who provides the day to day care of the child.

**Height for age** expresses the height of a child in relation to age. It is an indicator of a child's long term nutritional status (chronic malnutrition)

**Hepatomegaly** is a palpable liver below the coastal margin (however in children less than 1 year in malaria endemic regions a palpable liver of less than 2 cm is not significant)

**Jaundice** is yellow discoloration of the skin, sclera and mucus membranes due to increased serum bilirubin.

Moderate to severe anaemia was defined as haemoglobin less than 9g/dl

Morphological type of anaemia was be defined as the erythrocyte shape, size and

haemoglobinisation as seen on microscopic examination of the blood film.

**MUAC** is a measure of the circumference of the upper arm at the point mid way between the acromion and the olecranon. It is a measure of muscle mass used in children of 12months to 59months.

Splenomegaly is a palpable spleen below the coastal margin in the longest axis.

**Under follow up care** refers to children who were earlier diagnosed sero positive and are regularly reviewed and managed in an HIV/AIDS care center.

Weight for height expresses the weight of a child in relation to height. It shows evidence of wasting and is an indicator of the child's present and intermediate nutritional status (acute malnutrition).

## ABSTRACT

#### Introduction/ background

Anaemia is a commonly encountered haematological complication of HIV infection that has a significant impact on quality of life and clinical outcome. It is estimated that up to 90% of children develop anaemia during HIV infection. Anaemia has been found to be a significant predictor of progression to AIDS and moderate to severe anaemia is associated with an increased risk of death. In Uganda no study has been done to determine the prevalence and identify the factors associated with moderate to severe anaemia among children aged 6 months to 12 years.

#### Objective

To determine the prevalence and describe the factors associated with moderate to severe anaemia in HIV infected children.

#### Study design

Cross sectional study

#### Setting

Paediatric wards of Mulago hospital

## **Participants**

Two hundred and fifteen HIV infected children aged 6 months to 12 years who were admitted on the paediatric wards from October 2006 to February 2007.

#### Measurements

The basic social demographic characteristics of the children and their care takers, the complaints on admission, nutrition, past medical and drug history were collected using a structured questionnaire and a detailed physical examination was then done. A blood sample was taken for full haemogram, blood film for malaria parasites and typing the anaemia, CD4 count and percentage. Stool was examined for ova/cysts and occult blood.

#### Statistical analysis

Data was processed using Epi Data 3.1 and analyzed using EPI Info version 6.04 and SPSS 13 with the help of a statistician. Bivariate analysis was done to test for association between moderate to severe anaemia and the different variables and for those which were significant, multivariate analysis was further done to test for independent association with moderate to severe anaemia.

## **Study Results**

The prevalence of moderate to severe anaemia was 50.7%. Moderate to severe anaemia was most prevalent among children age 6 to 24 months.

The factors independently associated with moderate to severe anaemia were age < 60 months (OR 4.51, 95% CI 1.77-11.47, p=0.002), not taking multivitamin supplementation (OR 4.67, 95% CI 1.97-11.06, p= 0.000), previous transfusion (OR 3.97 95% CI 1.47-10.68,p=0.006), Lymphadenopathy (OR 3.42 95% CI 1.26-9.27, p= 0.015), and malaria coinfection (OR 4.42, 95% CI 1.72-11.39, p=0.002) were independently associated with moderate to severe anaemia.

The types of anaemia included microcytic normochromic (48.4%), normocytic normochromic (34.9%), microcytic hypochromic (12%), and macrocytic anaemia (2.8%).

## Conclusions

Moderate to severe anaemia is highly prevalent among HIV infected children admitted on the paediatric wards of Mulago hospital. The factors independently associated with moderate to severe anaemia were age < 60 months, not taking multivitamin supplementation, previous blood transfusion, lymphadenopathy and malaria coinfection.

## Recommendations

Young HIV infected children should be routinely evaluated for presence of anaemia and appropriate preventive and treatment measures should be emphasized so as to reduce the frequency of anaemia in HIV infected children.

## **CHAPTER ONE**

## **1.0 BACKGROUND AND LITERATURE REVIEW**

#### **1.1 Introduction**

Anaemia is a common haematological complication of HIV infection that has a significant impact on the quality of life and clinical outcome.<sup>1</sup> It is estimated that up to 90% of adults and children develop anaemia during HIV infection.<sup>2</sup>

The pathogenesis of anaemia in HIV infection is multifactorial including chronic disease, opportunistic infections, drugs and nutritional deficiencies with several mechanisms occurring simultaneously in a single patient.<sup>2</sup>

Anaemia has been shown to be a significant predictor of progression to AIDS and several studies have shown that as haemoglobin levels decrease, the risk of HIV disease progression increases.<sup>3</sup> Moderate to severe anaemia is associated with an increased risk of death in both paediatric and adult patients.<sup>1,4-6</sup> Some of the identified risk factors for moderate to severe anaemia in a prospective cohort of HIV infected children in Uganda were hospitalization, suspected tuberculosis, malaria infection and height for age Z score < -2, however these were only children less than 3 years who were not on Highly Active Anti-retro viral Therapy (HAART).<sup>6</sup>

The epidemiology of anaemia in HIV infection appears to be changing since the introduction of Highly Active Anti-retro viral Therapy (HAART).<sup>3</sup> Studies have now shown that HAART is effective in the treatment of anaemia in HIV infection<sup>7</sup> and recovery from anaemia is associated with improved survival among HIV infected patients. In Uganda, many more children are now receiving HAART, however we do not know the prevalence and factors associated with moderate to severe anaemia among HIV positive children aged 6 months to 12 years.

#### **1.2 Literature review**

#### 1.2.1 Global burden of HIV

It is estimated that 39.5 million people were living with HIV/AIDS world wide by the end of 2006, of whom 2.3 million are children less than15 years.<sup>8</sup>

About 4.3 million people became newly infected in 2006 including 530,000 children and more than 60% of these were in Sub Saharan Africa.<sup>8</sup>

HIV/AIDS is a major cause of infant morbidity in Africa accounting for 7.7% of under 5 year mortality world wide. It accounts for a rise of more than 19% in infant mortality and a 36% rise in under five mortality.<sup>9</sup>

#### 1.2.2 Situation in Uganda

HIV/AIDS is a major public health problem in Uganda. The overall national HIV Seroprevalence is 6.4 % in adults according to the national sero survey<sup>10</sup> while that of children is 0.7%.<sup>10</sup> A study in Mulago hospital found a sero prevalence of 6.5% among children less than 5 years attending the out patient clinic.<sup>11</sup>

About 84,000 children aged 0-14 years were living with HIV in Uganda by the year 2003.<sup>12</sup>

#### 1.2.3 Definition of anaemia

The World Health Organisation defines anaemia as haemoglobin or haematocrit level below normal for the age, sex, altitude and physical state of an individual.<sup>13</sup>

In children aged 6 months to 5 years anaemia is defined as haemoglobin less than 11g/dl while those above 5 years to 14 years it is haemoglobin less than 12 g/dl.

## 1.2.4 Burden of anaemia

Anaemia is a wide spread public health problem. The World Health Organization estimates that over 2 billion people are anaemic world wide with more than 100million of these anaemic children living in Africa.<sup>14</sup>

Anaemia affects over 50% of pre-school children and pregnant women in developing countries and at least 30-40% in the developed countries.<sup>14</sup> In East Africa the prevalence of anaemia ranges from 15-93%.<sup>15</sup>

Anaemia is one of the 10 top commonest causes of out patient morbidity in Uganda contributing to 2.3% of the burden of disease.<sup>16</sup> According to the Uganda Demographic and Health survey of 2001, sixty four percent of children under the age of 5 years were anaemic.<sup>17</sup> A study by Karaire in 1989 found 62% of relatively healthy children attending the young child clinic in Mulago hospital were anaemic and Nakiboneka in 2003 found a prevalence of 21.4% of severe anaemia among children admitted in ACU at Mulago hospital.<sup>18,19</sup>

The causes of anaemia are often multifactorial including poor nutrition, micronutrient deficiency, Haemoglobinopathies and infections such as malaria, tuberculosis, HIV/AIDS, and helminth infestations.<sup>19,20</sup>

## 1.2.5 Epidemiology of anaemia in HIV infection

Anaemia is a frequent complication of HIV type-1 infection and is the most common haematological manifestation of HIV infection and AIDS.<sup>4,21,22</sup>

The prevalence of anemia in HIV disease varies considerably ranging from 1.3% - 95%.<sup>4</sup>

It depends on several factors such as the stage of HIV infection, sex, age, race, and concurrent illness as well as the definition of anaemia used. In general, as the HIV disease progresses, the prevalence and severity of anaemia increases.<sup>4</sup>

A study by Eley and others found anaemia in 73% of clinically stable HIV infected children in a children's hospital in Cape Town and was more prevalent in those with moderate to severe immunosuppression.<sup>23</sup>

In Uganda a study by Clark and others on a cohort of HIV infected children followed from 9- 36 months documented a baseline prevalence of anaemia (Hb <11g/dl) of 91.7% and the prevalence of moderate to severe anaemia (Hb < 9g/dl) was 35.1%.<sup>6</sup>

The high prevalence of anemia in HIV infected children in developing countries may be attributed to the fact that many of the children in these regions are also iron deficient which is compounded by poor social economic status.<sup>24,25</sup> However, Totin and others found no difference in the proportion of infants with iron deficiency and iron deficiency anaemia between HIV positive and HIV negative infants in Uganda.<sup>26</sup>

#### 1.2.6 Pathogenesis and risk factors for anaemia in HIV infection

The pathogenesis of anaemia in HIV infection is multifactorial with several mechanisms occurring simultaneously in a single patient. However the main mechanisms include decreased red blood cell production, increased red blood cell destruction, ineffective red blood cell production and blood loss.<sup>2,3,22,27</sup> These abnormalities may be attributable to the direct and indirect effects of HIV infection, opportunistic infection and toxicity of therapeutic agents.<sup>28</sup>

## 1.2.6.1 Ineffective red blood cell production

Anaemia may result from nutritional deficiencies, most commonly of iron, folate and vitamin B12.<sup>3</sup> Nutritional problems in HIV infected children may be due to several mechanisms working independently or synergistically.<sup>29</sup>

Insufficient consumption is one of the factors leading to nutrient deficiency and this may result from primary anorexia caused by infections or cancers.<sup>29,30</sup> Tumour necrosis factor and other cytokines cause delayed gastric emptying which increases the anorexia.<sup>29</sup>

Opportunistic infections of the oral cavity such as candidiasis<sup>31</sup>, CMV, herpes simplex, idiopathic apthathous ulcers cause anorexia and dysphagia thus reducing food intake.<sup>32,33</sup> The most common side effect of ART is nausea and vomiting which also reduces food intake.<sup>34</sup>

Gastrointestinal malabsorption may also contribute to altered nutrition. It may be due to HIV infection of the mucosal cells<sup>29</sup> or secondary enteric infections such as giardiasis, cryptosporidia, microsporidia, CMV, salmonella, shigella species and others.

Malabsorption may also be secondary to ARV drug induced diarrhea such as lamivudine.

Psychosocial factors which include unstable home environment with inadequate social and emotional support, poor social economic status as a result of illness in the biological parents or grand parents without an income, and loss of biological parents all predispose to inadequate food intake.<sup>29,35</sup>

#### Iron deficiency anaemia

Iron deficiency anaemia is defined as anaemia in the presence of deficient iron stores.<sup>2</sup>

Iron deficiency anaemia is a common haematological disease of infancy and childhood<sup>36</sup> and is the most common cause of nutritional anaemia in young children.

In 2003, Kizito found a prevalence of iron deficiency anaemia of 16% among children living in urban slums of Kampala<sup>37</sup>, while in 1994 Bakaki found a prevalence of iron deficiency anaemia among children aged 3 months to 13 years of 18% in rural areas of eastern Uganda.<sup>38</sup>

Elsewhere in Africa, the prevalence of iron deficiency anaemia among preschool children ranges from 7.4% in Kenya,<sup>39</sup>15.3% in Ghana<sup>40</sup> to 20% Nigeria.<sup>41</sup>

Iron deficiency is a common cause of HIV associated anaemia in women, infants and children worldwide.<sup>2</sup>

The prevalence of iron deficiency anaemia in HIV infected children has been documented as 18% by Eley and others in S. Africa<sup>23,42</sup> and 44.3% among ambulant children in Uganda by Totin and others.<sup>26</sup>

The main cause of iron deficiency is inadequate dietary intake and prolonged breastfeeding without complementing it in the first two years of life.<sup>42</sup> Iron is 2-3 times more efficiently absorbed from breast-milk than cows-milk<sup>36,43</sup> and is sufficient for the first 4-6 months in term infants. Therefore, infants of mothers who opt to give cow's milk from birth may be predisposed to iron deficiency during early infancy.

Malabsorption of dietary iron may also lead to iron deficiency.<sup>44</sup> This may be due to the hypoacidity as with AIDS.<sup>2</sup> Other causes of iron loss include secondary parasite infections such as hookworm <sup>45-47</sup> and occult neoplasms.<sup>2,48</sup>

Low social economic status and poverty have also been associated with iron deficiency.<sup>49</sup>

## Folate and vitamin B<sub>12</sub> deficiency

Folic acid is found in green leafy vegetables and is primarily absorbed in the jejunum.

HIV infected children with reduced food intake and pathology involving the jejunum are unable to absorb folic acid causing megaloblastic anaemia.<sup>2</sup>

The commonest cause of vitamin  $B_{12}$  deficiency in HIV disease is malabsorption secondary to HIV its self or opportunistic GIT infections.<sup>2</sup>

#### 1.2.6.2 Decreased red blood cell production

#### Infections of the bone marrow

The exact pathophysiology by which HIV causes anaemia is unclear.<sup>2</sup> However different mechanisms have been proposed. HIV directly infects the haematopoetic cells, progenitor cells and stromal cells causing reduced production of granulocyte colony stimulating factor (G.CSF) and Interleukin 3 (IL3) with resultant dysregulation of hematopoesis.<sup>2,50</sup> Infection of human macrophages with HIV stimulates the expression of proinflammatory cytokines such as TNF-alpha, IL-6 which inhibit expression of colony stimulating factor M,G and GM CSF.<sup>50,51</sup> Anaemic patients with HIV have been found to have a blunted response to erythropoietin.<sup>50</sup> Bone marrow infection with Mycobacterium Avium Complex (MAC) produces elevated concentrations of TNF-alpha that inhibits erythropoiesis. Characteristically in MAC infection anaemia occurs out of proportion of other cytopenias.<sup>52,53</sup>

Human Parvo virus B19 infection of the bone marrow also affects the erythroid progenitor cells causing chronic anaemia in HIV infection.<sup>53,54</sup>

Other infections of the bone marrow causing anaemia include tuberculosis, histoplasmosis, cryptococcal infection and Pneumocytis jiroveci.<sup>50</sup>

Bone marrow infiltration by HIV related malignancies such as Kaposi sarcoma, non –Hodgkin's lymphoma, and Hodgkin's disease cause marrow suppression with concomitant reduction in red blood cell production.<sup>55</sup>

#### Drug related anemia

Zidovudine (AZT) is the commonest cause of drug associated anaemia.<sup>53</sup> The mechanism through which it causes anaemia is not clearly known. However studies have shown that it causes a selective red cell aplasia or hypoplasia.<sup>55,56</sup> AZT related anaemia is less common in asymptomatic patients or those with less advanced disease and studies have shown that it improves with dose adjustment.<sup>57</sup> A study in India showed that 12% of children had AZT induced anaemia, of these 40% had the drug discontinued while 60% improved on dose adjustment.<sup>57</sup>

Other drugs associated with bone marrow suppression and causing anaemia include cotrimoxazole, phenytoin, carbamazepine, gancyclovir, amphotericin B, flucytosine, sulphonamides, methotraxate and doxorubicin.<sup>58</sup>

## Anaemia of chronic disease

Anaemia of chronic disease is a major cause of anaemia in HIV infection and is defined as anaemia occurring in association with inflammatory or infectious disease with hypoferremia in the absence of other known causes of anaemia.<sup>58,59</sup>

The pathogenesis of anemia of chronic disease involves shortened erythrocyte survival, impaired erythropoiesis, relative erythropoietin deficiency and decreased utilization of reticulo endothelial iron for hemoglobin synthesis.<sup>60,61</sup>

## 1.2.6.3 Increased red blood cell destruction (hemolytic anaemia)

Increased or premature RBC destruction in the spleen or circulatory system may occur in patients with HIV infection.<sup>3</sup> Hemolytic anaemia may result from RBC autoantibodies,<sup>62</sup> hemophagocytic syndrome,<sup>63</sup> disseminated intravascular coagulation,<sup>62</sup> thrombocytopenic papura<sup>64</sup> and glucose-6-phoshate dehydrogenase deficiency when exposed to drugs like quinine, sulphonamides and dapsone.<sup>36</sup>

#### 1.2.7 HIV and malaria

Malaria is an important and common cause of anaemia in endemic areas.<sup>65</sup>

Malaria associated anaemia is a major contributor to morbidity and mortality in children.<sup>65,66</sup> The prevalence of malaria increases with age in the first year of life. In the 2<sup>nd</sup> half of the first year most maternal antibodies and fetal haemoglobin have disappeared thus the greatest burden of malaria and malaria associated anaemia occurs in these infants.<sup>67,68</sup>

Malaria causes anaemia through haemolysis and increased splenic clearance of infected and uninfected red blood cells. Cytokine induced dyserythropoeisis also occurs.<sup>20</sup>

Severe malaria associated anaemia accounts for more than half of all childhood deaths in Africa.<sup>20</sup>

Some studies in malarial endemic areas have shown no effect of HIV infection on either the occurrence or severity of malaria.<sup>69,70</sup> However, in areas of unstable transmission of malaria and high immunodeficiency virus prevalence, HIV was found to be associated with severe complicated malaria.<sup>71-73</sup>

Infants with HIV and malaria are at particular risk of anaemia during the first year of life.<sup>20</sup>

A study in Western Kenya found that HIV infected infants after 16 weeks of age had significantly lower haemoglobin concentration when infected with malaria compared to those with out HIV or those with HIV infection but no malaria, suggesting that HIV infected infants are at risk of adverse consequences of malaria at this age.<sup>65</sup>

Othieno RO and others in Kisumu also found that both HIV 1 exposure and HIV 1 infection are associated with increased prevalence of severe malaria anaemia in infants during an acute P.falciparum infection, independent of parasite densities.<sup>74</sup>

#### 1.2.8 Evaluation of children with HIV related anaemia

A detailed clinical history, including nutritional assessment, drug history, and a physical exam should be done to evaluate for treatable causes or risk factors for anaemia.<sup>3</sup> Clinical and immunological staging is also indicated because severe anaemia has been associated with advancing HIV disease.<sup>5</sup>

#### Laboratory evaluation

#### **Reticulocyte count**

The reticulocyte count distinguishes patients with active bone marrow that is responding to anaemia ( counts above 2%) from those with suppressed bone marrow (counts below 2%).<sup>22</sup> The reticulocyte count in HIV related anaemia is usually low except in autoimmune hemolytic aneamia, acute blood loss, or response to replacement of iron, folate and B12.<sup>3</sup>

#### Full blood count

The mean cell volume is low (<80 ft) in iron deficiency, thalassaemia, and chronic lead poisoning and high in vitamin B12 deficiency, folate deficiency, drugs like AZT, gancyclovir and anti cancer drugs.<sup>22</sup> Anaemia of chronic disease normally presents with a normal MCV although it may some times be decreased (microcytosis)

Red cell distribution width (RDW) is elevated in iron deficiency.

## **Peripheral film**

The peripheral blood film may show microcytosis with hypochromia in iron deficiency, thalassaemia and lead poisoning. A macrocytic hypochromic picture is seen in vitamin B12, folate deficiency, liver disease and AZT related anaemia while normocytic normochromic is seen in hemolytic anaemia, bone marrow infiltration (infection or tumour), and in acute blood loss. Malaria parasites can also be demonstrated on both the thick and thin blood smears.

## Serum iron, ferritin and trasferrin receptor

Serum iron is a measure of the iron bound to transferrin and decreases in iron deficiency and liver failure.

Serum ferritin is the most useful single laboratory value for the diagnosis of iron deficiency, however both serum iron and ferritin are influenced by acute infection, inflammation, and malignant disease thus making their interpretation very difficult in HIV infection.<sup>75</sup>

## **Bone marrow examination**

This is the gold standard for evaluation of anaemia in HIV disease. However, it is an invasive procedure and unsuitable for routine evaluation.

Studies have also reported that absence of stainable iron in the bone marrow report can be inaccurate in >30% of cases and may thus miss iron deficiency.<sup>76</sup>

## Stool exam

Stool exam may reveal hook worm ova for children with hookworm infestations.

A positive occult test is indicative of GIT bleeding.

## **CHAPTER TWO**

## 2.0 PROBLEM STATEMENT, JUSTIFICATION AND OBJECTIVES

#### 2.1 Statement of the problem

HIV/AIDS is a major public health problem in Uganda with an overall national sero-prevalence of 6.4% in adults and  $0.7\%^{10}$  in children and accounting for a rise of > 19% in infant mortality and a 36% rise in under five mortality.<sup>9</sup>

Anaemia is a significant public health problem especially in developing countries.<sup>24</sup>

It is one of the ten top commonest causes of out patient morbidity in Uganda and is responsible for 2.3% of the burden of disease.<sup>16</sup> According to the Uganda Demographic and Health Survey, 64% of children under 5 years were found to be anaemic.<sup>17</sup> A recent study done in the Acute Care Unit (ACU) found that 21.4% of children attending ACU, Mulago hospital were severely anaemic.<sup>19</sup>

HIV infection has greatly increased the prevalence of anaemia in children. A previous study in Uganda found 90.9% of HIV infected infants anaemic compared to 76.9% HIV negative infants seen in an out patient clinic.<sup>26</sup>

The consequences of anaemia include lowered resistance to disease, increased susceptibility to infection, poor cognitive development, impaired physical development, poor school performance, reduced work capacity with impaired social and economic development. All these are made worse by the chronic ill health associated with HIV infection.

Anaemia has been shown to be a significant negative predictor of progression from HIV infection to AIDS and progression to moderate to severe anaemia is associated with reduced survival in HIV infected children.<sup>5,6</sup> The magnitude, types and associated factors of moderate to severe anaemia among these children in Uganda is not known.

#### 2.2 Justification of the Study

Anaemia is an independent predictor of clinical prognosis in HIV and moderate to severe anemia is associated with increased morbidity and mortality in children infected with HIV.

The causative factors of anaemia are multifactorial and the most commonly identified risk factors include nutritional deficiency, hook worm infestations, anaemia of chronic disease, malaria and opportunistic infections.

The factors associated with anaemia in HIV infected children have not been well characterized in sub Saharan Africa. The previous few studies in Uganda only looked at HIV infected children less than 3 years none of whom was receiving antiretroviral therapy.

The management of HIV infected children has improved over the years with more children now receiving ART and management of opportunistic infections. However there is still a gap in knowledge of the proportion of HIV infected children with moderate to severe anaemia, the associated factors and the types of anaemia in HIV infected children aged between 6 months to 12 years, hence the need for this study.

The results of this study will provide information on the factors associated with moderate to severe anaemia and the types of anaemia in HIV infected children in Uganda which can be used to effectively address anaemia in HIV infected children.

## 2.3 Research questions

- 1. What is the prevalence of moderate to severe anemia among HIV infected children admitted at Mulago hospital?
- 2. What factors are associated with moderate to severe anaemia among HIV infected children admitted at Mulago hospital?
- 3. What are the types of anaemia among HIV infected children admitted at Mulago hospital?

## **2.4 Objectives**

## 2.4.1 General objectives

To determine the prevalence and describe the factors associated with moderate to severe anaemia among HIV infected children aged 6months to 12 years admitted at Mulago hospital.

## 2.4.2 Specific objectives

- 1. To determine the prevalence of moderate to severe anemia among HIV infected children aged 6 months to 12 years admitted at Mulago hospital.
- 2. To describe factors associated with moderate to severe anaemia among HIV infected children aged 6 months to 12 years admitted at Mulago hospital.
- 3. To describe the morphological types of anaemia among HIV infected children aged 6 months to 12 years admitted at Mulago hospital.

# **CHAPTER THREE**

## **3.0 METHODS**

## 3.1 Study design

Cross sectional study

## 3.2 Study setting

The study was carried out on the general paediatric wards of Mulago hospital which is a national referral and teaching hospital for Makerere University. It is situated in Kawempe, one of Uganda's administrative divisions in Kampala district.

Mulago receives patients from health units in and around Kampala and also from district and regional referral hospitals. Mulago hospital has 4 main paediatric wards and a nutritional unit and each ward has a bed capacity of about 50 although commonly there are more than 50 children admitted on each ward at any one time. The children admitted to the paediatric wards routinely go through counseling and testing for HIV using a rapid antibody test and those below 18 months who test positive, further DNA PCR is done to confirm their status. All the children confirmed to have HIV are then referred to the Paediatric Infectious Disease Clinic (PIDC) for follow up management.

## **3.3 Population**

## 3.3.1 Target population

Children aged between 6 months to 12 years. The lower limit of 6 months was chosen because during infancy, physiological anaemia occurs in the neonatal period and early infancy with the lowest level at about 2-3 months but by 6 months the haemoglobin has risen to normal values. The upper limit of 12 years was because this was the age limit for admission of children on the paediatric wards.

## 3.3.2 Accessible population

Children aged 6 months to 12 years admitted on the paediatric wards of Mulago hospital.

## **3.3.3 Study population**

Children aged 6 months to 12 years admitted on the paediatric wards of Mulago hospital during the study period.

## 3.3.4 Study unit

HIV infected child aged 6 months to 12 years admitted on the paediatric wards of Mulago hospital who fulfilled the inclusion criteria.

## 3.4.1 Sample size estimation for prevalence

The number of children studied was calculated using a formula by Kish and Leslie using the available population.

$$N = \underline{n}$$

$$1+ n$$

$$popn$$
where n = 
$$\underline{Z^2P(1-P)}$$

$$D^2$$

Z is the value corresponding to 95% confidence interval = 1.96

P is the assumed prevalence of moderate anaemia in HIV infected children of 50%. This is because no study has been done to find out the prevalence of anaemia in HIV infected children aged 6 months to 12 years.

D is the absolute error = 0.05.

n = 384.

According to the RCT register, 10% of the children admitted on the paediatric wards and tested were found to be HIV positive. From a pilot retrospective survey, it was assumed that 80 children admitted on the paediatric wards per month were found to be HIV infected. Therefore 480 HIV infected children would be available in a period of 6 months, the maximum time frame in which the study could be carried out.

Therefore using the available finite population according to this formula,

$$N = \underline{384}$$
$$\underline{1 + \underline{384}}$$
$$\underline{480}$$

N = 213

#### 3.4.2 Sample size calculation for associated factors

Using a formula for unmatched cohort and cross- sectional studies<sup>77</sup> (exposed and unexposed) with the expected frequency of moderate to severe anaemia in severely malnourished children (exposed) of 57% and that in those without severe malnutrition (unexposed) 30%, using a confidence interval of 95%, a power of 80%, taking the disease in the exposed group to be 57%, risk ratio of 1.9 and the ratio of controls to cases as 5:1 the sample size in the exposed group would be 34 while that in the unexposed group would be 170 giving a total sample size for associated factors of 204.

$$N = \frac{2(Z.\alpha + Z.\beta)^2 \cdot P.Q}{(P_1 - P_2)^2}$$

 $Z.\alpha = 1.96$ 

 $Z.\beta$  = standard normal percentage corresponding to power of 80% (0.842)

 $P_1$  = expected out come in the exposed group of 57%

 $P_0$  = expected out come in the unexposed group of 30%

$$\mathbf{P} = (\underline{\mathbf{P}_1 \mathbf{x} \mathbf{R} \mathbf{R}}) + (\underline{\mathbf{P}_0 \mathbf{x} \mathbf{C}})$$
$$1 + \mathbf{C}$$

Q = 100-P

C = 5 the ratio of controls to cases

Therefore n<sub>1</sub> for exposed group =  $2(1.96 + 0.842)^2$  x P.Q = 34

 $(57-30)^2$ 

 $n_2$  for unexposed = 5 x 34 = 170

 $N = n_1 + n_2$ 

$$N = 34 + 170 = 204$$

The large sample size of 213 was used to determine the prevalence and also identify the associated factors.

## 3.5 Selection criteria

## 3.5.1 Inclusion criteria

- i. Children aged 6 months to 12 years admitted on the paediatric wards of Mulago hospital.
- ii. Confirmed HIV infection.
- iii. Consent from the care takers and additional assent from older children above 8 years.

## 3.5.2 Exclusion criteria

- 1. Children whose care taker could not give a detailed history.
- 2. Children whose care taker is less than 18 years.

#### 3.6 Data collection

#### 3.6.1 Sampling procedure

Children admitted on the paediatric wards who had gone through routine HIV counseling and testing (RCT) and turned out positive on a rapid antibody test or those who were already diagnosed HIV positive were identified through a counselor who then told them about the study during post test counseling. Those who agreed to participate in the study were approached by the Principal Investigator or Research Assistant who confirmed their acceptance to participate in the study. The study was further explained to the care takers and the older children, after which a written consent from the care takers and additional assent from older children to take part in the study was obtained. Upon obtaining a signed consent form and additional assent from children aged 8 years and above, the children who fulfilled the inclusion criteria were consecutively enrolled into the study until the sample size of 215 was achieved.

#### 3.6.2 Study instrument

A pre-tested and standardized questionnaire written in English was used, the non clinical part of the questionnaire such as social demographics and social economic history was translated into the local language (Luganda) to the guardians who could not understand English but the appropriate clinical terms remained in English.

## **3.6.3 Measurement variables 3.6.3.1 Clinical history**

A detailed history including the social demographic characteristics, presenting symptoms, presence of symptoms related to anaemia, nutritional history, past medical history, previous and current drug history were obtained from the care taker. A previous history of blood transfusion irrespective of when the patient was transfused was considered to be an indicator of moderate to severe anaemia. Caretaker characteristics including education level and occupation were obtained. The caretaker/ family's level of income was assessed by asking the caretaker how much they earned per month including donation or handout from other persons or organizations.

## 3.6.3.2 Physical examination

Each child had a detailed physical exam including temperature, presence of pallor, jaundice, angular stomatitis, koilonychia, pedal oedema and signs of opportunistic infections. The weight was measured in kg to the nearest 100g using a 25kg Salter scale and round dial flat, weighing scale for those above 25kg. The length for children below 2 years was measured using a stadiometer and height for those above 2 years was measured while standing against a wall. Anthropometric measurements for wasting and stunting were calculated from the recorded weight and height using a nutritional statistical package.

Any other diagnoses made including concurrent opportunistic infections were recorded. The WHO clinical staging of HIV disease was also done for each child.

## 3.6.3.3 Laboratory investigations

The skin around the cubital fossa, dorsum of the hand or inguinal area was cleaned with 70% alcohol before drawing blood from the patient.

A 5ml syringe and needle was used to draw 5mls of blood and this was done once from each child then aliquoted as follows

- -0.5mls for blood film, malaria parasite and reticulocyte count.
- -2mls for complete blood count
- -2.5 mls for CD4 absolute counts and percentages

## **Blood film for malaria**

A drop of blood was put on a slide to make a thick film which was then stained with Giemsa stain to look for malaria parasites.

For those who had a positive blood slide for malaria, quantification by counting the parasites against 200 WBCs on a thick film and parasite density calculated as follows.

<u>No of parasites/200WBC</u> x WBC counts/ $\mu$ l (corrected to 8000cells) = parasitaemia / $\mu$ l 200

#### Blood film for typing the anaemia

At least 2 thin films were made and the best was stained with Giemsa stain and examined for erythrocyte morphology. The anaemia was typed by a competent laboratory technician in the Mulago-Mbarara Joint AIDS Programme in Mulago hospital. A sickling test was done for those with morphology suggestive of sickle cell disease.

#### **Reticulocyte count**

Two drops of the anticoagulated venous blood were added to an equal amount of methylene blue solution in a test tube. The mixture was gently shaken and left to incubate for 15 minutes after which it was gently shaken again. A drop of this mixture was then placed on a slide and a thin smear made. Using the x 100 oil-immersion objective, the number of reticulocytes seen in observing 500 erythrocytes was recorded and the percentage of reticulocytes was then calculated to determine whether the bone marrow was appropriately responding to anaemia. This was indicated by an increase in the reticulocyte count above 2% and reticulocyte index of greater than1.

#### **Complete blood count and CD4%**

Two and a half mls of blood were put in an EDTA vacutainer and an automated coulter counter was used to determine haemoglobin concentration, total red blood cell count, mean cell volume, mean cell haemoglobin concentration, mean cell haemoglobin, packed cell volume, red cell distribution width, WBC count (total and differential), and platelet count. A portion of this blood sample was used to determine the absolute CD4 count and CD4% by flow cytometry using the

BD FACScalibur method. These were then used to determine the immunological stage of each child using the guidelines in appendix v

#### Stool exam

A stool container with a scoop was given to the care taker to collect a sample of approximately 2-5gm of stool using the scoop. Macroscopic exam for consistency and colour was done. A sample of stool was then emulsified with two drops of normal saline and examined for ova/cyst, white and red blood cells using a microscope under x 10 power.

Occult blood test was done using haematoccult test method. This was done by adding an activator then a developer to a sample of emulsified stool, which was then applied on a test strip and colour change observed for 30 seconds. A positive reaction produced a blue colour while absence of blue colour on the test strip indicated a negative reaction.

Occult blood screen was based on the haemoglobin –catalysed oxidation of phenolic compounds present in the guaiac to blue coloured quinones.

When a feacal sample containing occult blood is applied to the test strip, contact is made between haemoglobin and the guaiac, a pseudoperoxidase reaction occurs upon addition of a developer forming a blue chromatogen.

## 3.7 Data management and analysis

The data collected was cleaned and edited by the principle investigator on the same day of collection.

Data obtained was coded then entered into the computer using Epi data 3.1 and analysed using EPI Info version 6.04 and SSPS 13 soft ware package with the help of a statistician.

Categorical variables were summarized as proportions, while means, median and standard deviations were used for continuous variables.

In the bivariate analysis odd's ratios, 95% confidence interval, and chi-square test were used to test for association between moderate to severe anaemia and categorical variables, while the student's t test was used for continuous variables.

Multivariate analysis using logistic regression was done to determine the factors that were independently associated with moderate to severe anaemia.

## 3.8 Quality control

The questionnaire was pre tested and standardized before commencement of data collection. The research assistant was trained on how to collect the blood samples from the patients. The principal investigator took the history, carried out the physical examination and filled the questionnaires which were then crosschecked by the principal investigator for completeness before leaving the study site. The specimens were examined by competent laboratory technicians in the Mulago-Mbarara Joint AIDS programme laboratory in Mulago hospital which undergoes regular quality control.

## 3.9 Ethical considerations

Institutional consent was obtained from department of Paediatrics and Child Health, Makerere University, Faculty of Medicine Research and Ethics Committee, and the National Council for Science and Technology.

Informed written consent was sought from the caretakers and in addition assent was obtained from older children above 8 years who were able to sign consent before enrollment in the study.

Laboratory results were availed to the attending doctors for appropriate management of the patients.

Participation in the study was voluntary and refusal to do so did not affect the hospital or ward management of the child.

HIV infected children were referred to PIDC and their parents to the Infectious Disease Institute (IDI) for further follow up and management.

# **CHAPTER FOUR**

## 4.0 RESULTS

## 4.1 Description of study participants

A total of 225 HIV positive children admitted on the Paediatric wards of Mulago hospital between October 2006 and February 2007 were eligible for the study. Three children were excluded because the care takers did not consent to taking a blood sample, 1 child had a care taker who was less than 18 years, and 6 were later excluded following a negative DNA PCR. Therefore results are reported for 215 HIV infected children. One hundred and twelve (52.1%) were males and 103 (47.9%) were females, M:F 1.09: 1.

#### Figure1. Study profile



## 4.1.1 Age distribution

Children aged between 6 months and 144 months were studied. The mean age was  $45.18 \pm 2.878$  months. The age distribution of the study population was as shown in figure 2





- The biggest percentage of children [101/215 (47%)] were aged 6-24 months.
### 4.1.2 Residence and tribe distribution

The majority of children 126/215 (59%) were resident in Kampala district where the study site is located, and a significant proportion of the subjects were also resident in the neighboring districts of Wakiso 54/215 (25%), Mukono 15/215 (7%), and Luwero 6/215 (3%) as shown in figure 3. Consequently most of the study population (71%) comprised of the ethnic group of Baganda which is the predominant resident group in the districts mentioned above.

# Figure 3. Districts of residents of the study children



# 4.1.3 Characteristics of the study population

One hundred and fifty one children (70.2%) were below 60 months. Ninety children (41.9%) were receiving cotrimoxazole prophylaxis, and 47 (21.9%) were on antiretroviral therapy. One hundred eighty nine (87.9%) had a biological parent/s as the primary care taker.

The caretaker's level of income was ranging from those who did not earn anything to a maximum of sh.300,000. A cut off of < sh.60,000 was used to categorize those who were living below the poverty line of less than a dollar per day converted to Ugandan shillings.

The other baseline social demographic characteristics of the study population are shown in table1

	Number	Percentage
	N= 215	
Child characteristics		
Age		
< 60months	151	70.2
$\geq$ 60months	64	29.8
Sex		
Male	112	52.1
Female	103	47.9
Child under follow up care	90	41.9
In an HIV clinic		
Cotrimoxazole prophylaxis	90	41.9
Multivitamin supplementation	70	32.6
ART	47	21.9
Care taker characteristics		
Primary care taker		
Biological parent	189	87.9
Other care taker	26	12.1
Mothers education level		
$\leq$ primary	125	69.8
> primary	54	30.2
Father's educational level		
≤ primary	49	45.4
> primary	59	54.6
Caretaker's level of income per		
month(in Uganda shillings)		
< 60,000	163	75.8
$\geq$ 60,000	52	24.2

Table 1. Baseline and social demographic characteristics

#### 4.2 Clinical presentation of the study children

One hundred and sixty three children (75.8%) presented with a fever, 164/215 (76.3%) had cough and 114/215 (53%) had diarrhoea. Thirteen children (6%) presented with bloody stools. One hundred sixteen (54%) of the children had history of previous admission and 46/215 (21.4%) had received a blood transfusion of whom 16 had been transfused in the last one week. On physical examination, majority of children 166/215 (77.2%) had pallor of mucus membranes and palms and only 8 children (3.8%) had jaundice. Ninety two children (42.8%) were severely wasted (weight/ height -2SD) and 94/215 (43.7%) were stunted (height/age -2SD). The other findings on history and physical examination are shown in table 2.

	Number	Percentage
Findings on history		
Fever	163	75.8
Cough	164	76.3
Loss of appetite	88	40.9
Mouth sores	67	31.2
Diarrhoea	114	53.0
Blood in stools	13	6
Bleeding from other site	4	1.9
Weight loss	138	64.2
Previous admission	116	54
Previous transfusion	46	21.4
Transfusion in the past 7 days	16	7.4
Findings on Physical signs		
Signs related to anaemia		
Pallor	166	77.2
Jaundice	8	3.7
Smooth tongue	40	18.6
Koilonychia	29	13.5
Pedal oedema	5	2.3
Gallop rhythm	9	4.2
Hepatomegaly	69	32.1
Splenomegaly	31	14.4
Independent signs		
Oral thrush	65	30.2
Lymphadenopathy	48	22.3
Nutritional status		
Wasting (-2 SD)	92	42.8
Stunting (-2 SD)	94	43.7

# Table 2. Clinical presentation of the study children

SD = standard deviation

#### 4.3 Laboratory findings of the study children

The haemoglobin ranged from 2.9 to 15.6g/dl with mean haemoglobin of 8.8205 ( $\pm$  2.315).

The White blood cell counts (WBC) ranged from 0.4 x  $10^3/\mu$ l to 39.0 x  $10^3/\mu$ l and the mean WBC was 10.668 x  $10^3/\mu$ l ( 6.7461). Leucopenia (WBC < 4,000 x  $10^3/\mu$ l) was detected in 23/215 (10.7 %) of the children. The Platelet count ranged from 4.0 x  $10^3/\mu$ l to 1091x  $10^3/\mu$ l with a mean of 294.477 x  $10^3/\mu$ l. Thrombocytopenia (platelets < 150,000 x  $10^3/\mu$ l) was found in 52/215 (24.5%) of the study children as shown in table 3 below. The reticulocyte count ranged from 0 to 17 % and 137/215 (63.7%) had a reticulocyte index < 1 showing that the bone marrow was not responding adequately to their degree of anaemia.

Malaria parasites were detected in 41/215 (19%) of the study children and the mean parasite density was 1301.46 (1694.369). The laboratory findings are shown in table 3.

Test	Minimum	Maximum	Mean (SD)
Haemoglobin	2.9	15.60	8.8205 (2.315)
WBC ( 10 <sup>3</sup> /ul)	0.4	39.0	10.668 ( 6.7461)
RBC ( 10 <sup>3</sup> /ul)	1.1	5.9	3.749 ( 0.9457)
MCV (fl)	47.0	107.0	73.267 (11.2966)
MCHC (g/dl)	26.2	42.6	32.925 ( 1.7600)
RDW	9	27.1	15.595 ( 3.1347)
Platelets ( 10 <sup>3</sup> /ul)	4.0	1091.0	323.927 (208.5248)
Reticulocyte count (%)	0	17.0	1.619 ( 2.0495)
Malaria parasite density**	40	4200	1301.46 ( 1694.369)

Table 3. Laboratory investigations of the study children

SD = Standard deviation

WBC = White blood cell count

RBC = Red blood cell count

MCHC = Mean cell haemoglobin concentration

MCV = Mean cell volume RDW = Red cell distribution width

\*\* Results apply to those who had a positive malaria smear.

#### 4.4 Prevalence of anaemia

The prevalence of moderate to severe anaemia (Hb < 9g/dl) among the study population was 50.7% (109 / 205). However overall anaemia (Hb < 11g/dl) occurred in 183/ 215 (85%) children of whom 74 (34.4%) had mild anaemia, 96 (44.7%) had moderate anaemia and 13(6%) had severe anaemia.

The age distribution of children with moderate to severe anaemia is shown in figure 4 below.



Figure 4. Age distribution of children with moderate to severe anaemia

Moderate to severe anaemia was commonest in the age group 6- 24 months [61/109 (56%)] as shown in the figure above.

# 4.5 Factors associated with moderate to severe

#### 4.5.1 Baseline and social demographic characteristics

On bivariate analysis, the baseline characteristics associated with moderate to severe anaemia are

shown in table 4 below.

characteristic	Mod-Severe anaemia Hb < 9g/dl n(%)	Mild-No anaemia Hb≥9g/dl n(%) N = 106	OR	95% CI	p- value
A	N = 109				
Age	(50, 7)	(2)		1 54 5 60	0.0014
< 60 months	88 (58.3)	63 (41.7)		1.54 - 5.28	0.001*
$\geq$ 60 months	21 (32.8)	43 (67.2)	2.86		
Sex					
Female	53 (51.5)	50 (48.5)	1.06	0.62-1.81	0.831
Male	56 (50)	56 (50)			
Child under Follow up					
care in an HIV clinic					
No	77 (61.6)	48 (38.4)	2.90	1.65-5.10	0.000*
Yes	32 (35.6)	58 (64.4)			
Cotrimoxazole					
prophylaxis					
No	77 (61.6)	48 (38.4)	2.90	1.65-5.10	0.000*
Yes	32 (35.6)	58 (64 4)			0.000
Multivitamin	52 (55.6)	56 (61.1)			
supplementation					
No	87 (60 0)	58 (40.0)	3 27	1 78-5 98	0 000*
Ves	22(31.4)	<u> 18 (68 6)</u>	5.41	1.70-3.70	0.000
ADV thereasy	22 (31.4)	40 (00.0)			
AK v therapy	02(54.9)	76(452)	0 1 2	1 00 4 16	0.024*
INO Ver	92 (54.8)	70 (45.2)	2.13	1.09-4.10	0.024*
Yes	17 (30.2)	30 (63.8)			
AZI					0.000
Yes	8 (30.8)	18 (69.2)	0.38	0.16-0.93	0.030*
No	101 (53.4)	88 (46.6)			
n = number of children	OR = Odds ratio	* = statistical	ly signi	ficant p < 0.05	5
Hb = haemoglobin	CI = Confidence	ce Interval			

Age < 60 months, not being under care, not taking cotrimoxazole prophylaxis, multivitamin supplementation or ARV therapy were significantly associated with moderate to severe anaemia. The children on AZT were less likely to have moderate to severe anaemia. The duration of AZT therapy ranged from 2 months to 24 months with a mean duration of 7 months.

#### **4.5.2 Presenting symptoms**

The mean duration of fever was  $17.30 \pm 2.233$  days, while that of cough was  $23.12 \pm 2.207$  days and diarrhea  $14.11 \pm 2.111$  days. Forty six children had received a transfusion at one point in time. A history of previous blood transfusion irrespective of when transfusion was done was statistically and significantly associated with moderate to severe anaemia (OR 2.73, 95% CI 1.36-5.48, p=0.004) as shown in table 5.

symptom	Mod-Severe naemia	Mild-No naemia	OR	95% CI	p- value	
	Hb < 9g/dl n(%)	$Hb \ge 9g/dl n(\%)$				
Fever $\geq$ 14 days	43 (70.5)	18 (29.5)	2.68	1.37-5.27	0.004*	
$Cough \ge 30 \text{ days}$	35 (68.6)	16 (31.4)	3.07	1.52-6.18	0.001*	
Difficult breathing	37 (56.1)	29 (43.9)	1.36	0.76-2.44	0.295	
Mouth sores	38 (56.7)	29 (43.3)	1.42	0.79-2.54	0.235	
Loss of appetite	53 (60.2)	35 (39.8)	1.92	1.10-3.33	0.020*	
Diarrhoea ≥14 days	25 (67.6)	12 (32.4)	2.37	1.04-5.39	0.037*	
Blood in stools	9 (69.2)	4 (30.8)	2.29	0.68-7.69	$0.137^{\Psi}$	
Weight loss	81 (58.7)	57 (41.3)	2.48	1.40-4.41	0.002*	
Previous admission	64 (55.2)	52 (44.8)	1.47	0.86-2.53	0.155	
Previous blood	32 (69.6)	14 (30.4)	2.73	1.36-5.48	0.004*	
transfusion						
n = number of children	* = statistically sig	nificant p < 0.05 $\Psi$	= Fishe	rs exact test		

Table 5. Symptoms associated with moderate to severe anaemia

Chronic illness (fever lasting more than 14 days, cough for more than 30 days, diarrhoea for more than 14 days, loss of appetite), weight loss and previous transfusion were significantly associated with moderate to severe anaemia.

# 4.5.3 Feeding practices and care taker characteristics

All the children below 60 months were breastfed. One hundred and seventeen (77.5%) of these children had complimentary feeds started by 6 months of age. There was no statistical significance between the children who started complimentary feeding after 6 months versus those who started before 6 months (OR 0.648, p 0.266) as shown in table 6.

	Mod-Severe	Mild-No	OR	95% CI	p- value
	anaemia n (%)	Anaemia n (%)			
Feeding practices					
Complimentary feeds started					
(child<60 months)					
> 6 months	17 (50)	17 (50)	0.64	0.39-1.39	0.266
$\leq$ 6 months	71 (60.7)	46 (39.3)			
No of feeds /day					
<4 feeds	43 (53.8)	37 (46.3)	1.21	0.69-2.11	0.475
$\geq$ 4 feeds	66 (48.9)	69 (51.1)			
Caretaker characteristics					
Mother's education					
≤ primary	69 (55.2)	56 (44.8)	1.14	0.60-2.16	0.680
> primary	28 (51.9)	26 (48.1)			
Father's education					
$\leq$ primary	21 (42.9)	28 (57.1)	0.51	0.23-1.10	0.088
> primary	35 (59.3)	24 (40.7)			
Level of income per month					
< 60,000	84 (51.8)	79 (48.5)	1.14	0.61-2.14	0.664
$\geq$ 60,000	25 (48.1)	27 (51.9)			

#### Table 6. Feeding practices and caretaker characteristics

OR = Odd's ratio

CI = Confidence interval

Caretaker characteristics and feeding practices were not associated with moderate to severe anaemia.

# 4.5.4 Physical signs

The physical signs associated with moderate to severe anaemia are shown in the table below.

Sign	Mod-Severe anaemia	Mild-No anaemia	OR	95% CI	p- value
	Hb < 9g/dl n(%)	$Hb \ge 9g/dl n(\%)$			
	N = 109	N = 106			
Temp≥ 37.5	40 (58.0)	29 (42.0)	1.53	0.86-2.74	0.143
Wasted (-2SD)	54 (58.7)	38 (41.3)	1.75	1.01-3.03	0.042*
Stunted (-2SD)	55 (58.5)	39 (41.5)	1.75	1.01-3.01	0.043*
Jaundice	7 (87.5)	1 (12.5)	7.00	0.87- 59.9	0.036 <sup>Ψ</sup> *
Oral thrush	34 (52.3)	31 (47.7)	1.09	0.61-1.96	0.758
Smooth tongue	27 (67.6)	13 (32.5)	2.35	1.14- 4.86	0.018*
Koilonychia	18 (62.1)	11 (37.9)	1.70	0.76-3.81	0.188
Peripheral					
Lymphadenopathy	31 (64.6)	17 (35.4)	2.08	1.07-4.04	0.029*
Pedal oedema	4 (80.0)	1 (20.0)	4.00	0.44-36.38	$0.193^{\Psi}$
Gallop rhythm	9 (100.0)	0	2.06	1.79-2.37	$0.002^{\Psi^*}$
Chest in drawing	15 (68.2)	7 (31.8)	2.25	0.88-5.78	0.143
Hepatomegaly	40 (58.0)	29 (42.0)	1.53	0.86-2.74	0.143
Splenomegaly	18 (58.1)	13 (41.9)	1.41	0.65-3.05	0.375

Table 7. Physical signs associated with moderate to severe anaemia

n = number of children \* = statistically significant p < 0.05  $\Psi$  = Fishers exact test

The physical signs statistically and significantly associated with moderate to severe anaemia were wasting, stunting, jaundice, smooth tongue, peripheral lymphadenopathy and gallop rhythm.

#### 4.5.5 Coinfection with other common illnesses and HIV staging of the study children

Most of the children had more than one diagnosis as shown in table 8. The commonest coinfections included pneumonia (45.1%), tuberculosis (7%), malaria (20.5%), persistent diarrhoea (12%) and oral candidiasis (19.5%). The mean malaria parasite density was 1456.25 (1787.81) among the children with moderate to severe anaemia compared to 751.11 (1257.86) in those with mild to no anaemia and this difference was statistically significant (p=0.003).

Four children (1.9%) were classified as WHO clinical stage I, 46 (21.4%) stage II, 86 (40%) stage III and 79 (36.7%) stage IV.

The associations between HIV comorbidity, WHO clinical staging and immunological stage are as shown in table 8 below.

	Mod-Severe anaemia Hb < 9g/dl n(%)	Mild-No anaemia Hb≥9g/dl n(%)	OR	95% CI	p- value
Diagnosis					
Pneumonia	41 (42.3)	56 (57.7)	0.53	0.31-0.92	0.025
Tuberculosis	9 (56.3)	7 (43.8)	1.27	0.45-3.55	0.644
Malaria	33 (75.0)	11 (25.0)	3.75	1.77-7.90	0.000*
Oral candidiasis	25 (59.5)	17 (40.5)	1.55	0.78-3.82	0.202
K.S	3 ( 75.0)	1 (25.0)	2.97	0.30-29.03	$0.321^{\Psi}$
Sickle cell anaemia	3 (100)	0	2.00	1.74-2.28	$0.247^{\Psi}$
WHO staging					
III & IV	84 (50.9)	81 ( 49.1)	1.10	0.55-1.95	0.910
Immunological stage					
CD4 < 15% or	74 ( 60.2)	49 (39.8)	2.45	1.41-4.28	0.001*
<200 (>5yrs)					
* statistically signification	nt value < 0.05	$\Psi$ fisher's exact test	significa	antly	

Table 8. HI	V comorbidity	y and staging	of the 215	study children
				•/

Only coinfection with malaria and CD4% <15 or counts <200 were statistically and significantly associated with moderate to severe anaemia.

# 4.6 Logistic regression for factors associated with moderate to severe anaemia

Since there might be an interaction between the factors associated with moderate to severe anaemia, all the factors that had a p value of  $\leq 0.2$  were entered into the logistic model to establish those that were independently associated with moderate to severe anaemia as shown in table 10.

	<b>Bivariate analysis</b>		<u>Multivariate analysis</u>	
Variable	Unadjusted OR		Adjusted OR	
	(95% CI)	p-value	(95% CI)	p-value
Age < 60months	2.86 (1.54-5.28)	0.001	4.51 (1.77-11.47)	0.002*
Not on multivitamins	3.27 (1.78-5.98)	0.000	4.67 (1.97-11.06)	0.000*
Duration of fever	2.68 (1.37-5.27)	0.004	2.18 (0.97-4.86)	0.056
Blood in stools	2.29 (0.68-7.69)	0.137	9.52 (0.93-97.16)	0.057
Previous transfusion	2.08 (1.36-5.48)	0.004	3.97 (1.47-10.68)	0.006*
Lymphadenopathy	2.08 (1.07-4.04)	0.029	3.42 (1.26-9.27)	0.015*
Malaria	3.75 (1.77-7.90)	0.000	4.42 (1.72-11.39)	0.002*
CD4 < 15% or	2.45 (1.41-4.28)	0.001	1.67 (0.74-3.76)	0.211
<200 (> 5yrs)				

Table 9. Logistic regression for factors associated with moderate to severe ana	emia.
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After logistic regression, only age < 60 months, not taking multivitamin supplementation, previous transfusion, lymphadenopathy and malaria remained independently associated with moderate to severe anaemia.

#### 4.7 Predictors of severe anaemia

A sub analysis was done for the children with severe anemia to determine the factors predicting severe anaemia among the study children as shown in table 11 below.

Variable	Hb < 5g/dl	$Hb \ge 5g/dl$	OR (95% CI)	P value
	n (%)	n (%)		
	N=13	N=202		
Not on multivitamins	11 (7.6)	134 (92.8)	2.79 (0.60-12.94)	0.173
Fever $\geq$ 14 days	10 (16.4)	51 (83.6)	6.47 (1.70-24.55)	0.002*
Diarrhoea $\geq$ 14 days	4 (10.8)	33 (89.2)	4.54 (0.79-26.05)	$0.086^{\Psi}$
Loss of appetite	11 (12.5)	77 (87.5)	8.929 (1.92-41.36)	0.001*
Blood in stools	4 (30.8)	9 (69.2)	9.53 (2.46-36.92)	$0.004^{\Psi_{*}}$
Previous admission	12 (10.3)	104 (89.7)	11.30 (1.44-88.59)	0.004*
Previous transfusion	10 (21.7)	36 (78.3)	15.37 (4.02-58.6)	0.000*
Wasting	9 (9.8)	83 (90.2)	3.22 (0.96-0.82)	0.047*
Jaundice	2 (25)	6 (75)	5.93 (1.07-32.89)	$0.022^{\Psi_{*}}$
lymphadenopathy	8 (16.7)	40 (83.3)	6.48 (2.01-20.87)	0.000*
Gallop rhythm	7 (77.8)	2 (22.2)	116.6 (19.89-684)	$0.000^{\Psi_{*}}$
Hepatomegaly	9 (13)	60 (87)	5.32 (1.57-17.96)	0.003*
Malaria	6 (3.6)	38 (86.4)	3.69 (1.17-11.63)	0.018*

Table 10. Predictors of severe anaemia

On bivariate analysis, the factors that were statistically and significantly associated with severe anaemia were fever  $\geq 14$  days, loss of appetite, blood in stools, previous admission, previous transfusion, wasting, jaundice, lymphadenopathy, gallop rhythm, Hepatomegaly and malaria.

# 4.8 Logistic regression for predictors of severe anaemia

All the factors that had a p value of  $\leq 0.2$  on bivariate analysis were entered into the logistic model to determine the independent predictors of severe anaemia as shown in table 12.

Variable	<u>Bivariate analysis</u>		Multivariate analysis		
	Unadjusted OR		Adjusted OR		
	(95% CI)	p-value	(95% CI)	p-value	
Loss of appetite	8.92 (1.92-41.36)	0.001	9.54 (1.00-90.8)	0.050*	
Previous transfusion	15.37 (4.02-58.6)	0.000	21.38 (3.58-127.6)	0.001*	
Fever $\geq$ 14 days	6.47 (1.70-24.55)	0.002	7.17 (1.15-44.57)	0.035*	

Table 11. Logistic regression for predictors of severe anaemia

From the above table, the factors predicting severe anaemia were loss of appetite, fever for more than 14 days and a previous transfusion irrespective of when the child was last transfused.

#### 4.9 Types of anaemia among the study children

Microcytosis was found in 130/215 (60.5%) of the children and 72/215 (33.5%) had both microcytosis and RDW > 16. One hundred and four (48.4%) had a microcytic normochromic picture, 75/215 (34.9%) had a normocytic normochromic picture, 26/215 (12.0%) had a microcytic hypochromic picture. Only 6/215 (2.8%) children had macrocytosis and 2 of these were on AZT therapy and 4/215 (1.9%) had a normocytic hypochromic picture.

Coulter counter values were used to determine the above types according to the following values.

Normocytosis	MCV = 76-96 fl
Microcytosis	$MCV < 76 \ fl$
Macrocytosis	MCV > 96 fl
Normochromic	MCHC = 31- 35 g/dl
Hypochromic	MCHC $< 31 \text{ g/dl}$

Table 12.Type	s of anaemia
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Туре	Number	Percentage
Microcytic normochromic	104	48.4
Normocytic normochromic	75	34.9
Microcytic hypochromic	26	12.0
Macrocytic	6	2.8
Normocytic hypochromic	4	1.9

### 4.8.1 Type of anaemia and its relation to degree of anaemia

Normocytic normochromic picture was more common in the group with mild to no anaemia and this difference was statistically significant [44/75(58.7%) vs 31/75 (41.3%) p= 0.044]. Children with microcytic hypochromic picture were thrice more likely to have moderate to severe anaemia compared to those with mild to no anaemia [20/26 (76.9%) vs 6/26 (23.1%) p= 0.004] as shown in table 14.

Туре	Mod-Severe anaemia	Mild-No anaemi	a OR	95% CI	p- value
	Hb < 9g/dl n(%)	$Hb \ge 9g/dl  n(\%$	)		
Normocytic					
Normochromic					
Yes	31 ( 41.3)	44 (58.7)	0.56	0.31-0.98	0.044*
No	78 (55.7)	62 (44.3)			
Microcytic					
Hypochromic					
Yes	20 (76.9)	6 (23.1)	3.74	1.44-9.74	0.004*
No	89 (47.1)	100 (52.9)			
Microcytic					
Normochromic					
Yes	55 (52.9)	49 (47.1)	1.18	0.69-2.02	0.535
No	54 (48.6)	57 (51.4)			
Macrocytic					
Yes	0	6 (100)	2.09	1.81-2.40	$0.013^{\Psi_{*}}$
No	109 (52.2)	100 (47.8)			
n = number of children $OR = Odds ratio$		atio $*=$	statistica	lly significan	t p < 0.05

Table 13. Type of anaemia and its relation to degree of anaemia

#### 4.10 Stool analysis

Stool samples were received from 164 out of 215 children. There were no ova or cysts detected among the stool samples. Yeast cells were seen in 9.7% of the stool samples.

The occult blood test was positive in 23% (38/164) of the children. Of the children with positive occult test, 76.3% (29/38) also had microcytosis. There was no statistically significant association between positive occult blood test and moderate to severe anaemia (OR 1.634, 95% CI 0.78-3.41, p= 0.190).

# **CHAPTER FIVE**

#### **5.0 DISCUSSION**

This study was designed to determine the prevalence of moderate to severe anaemia, factors associated with moderate to severe anaemia and the type of anaemia among HIV infected children aged 6 months to 12 years who were admitted on the paediatric wards of Mulago hospital.

#### 5.1 Prevalence of anaemia

The prevalence of moderate to severe anaemia (Hb< 9g/dl) was 50.7%. A previous study done in Uganda among children (aged 9 months to 36 months) found that  $35.1\%^6$  had moderate to severe anaemia. The higher prevalence could be explained by the fact that the present study looked at hospitalized children whose comorbidities could have increased the occurrence of anaemia while the previous study measured baseline haemoglobin of out patient children.

Secondly the present study looked at children between the age of 6 months and 12 years and it was found that moderate to severe anaemia was highest among 6 to 24 months, its prevalence went down reaching lowest values between 72-120 months then went up again between 121-144 months. The increasing occurrence of anaemia during adolescence also raised the overall prevalence of moderate to severe anaemia in this study.

Severe anaemia (Hb < 5g/dl) was found in 6% of the study children. This is however lower than 21.4%<sup>19</sup> found among children (3 months to 60 months) presenting to the Acute Care Unit of Mulago hospital regardless of their HIV status. This can be explained by the fact that the major causes of severe anaemia such as malaria and sickle cell disease were less prevalent in this study compared to the previous study in ACU. Malaria occurred in 20% of the study children and only 1.4% had sickle cell anemia compared to 58.7% and 13% respectively in the previous study.

Secondly the lower prevalence of severe anaemia in this study could also be explained by the fact that some of the children on the general wards were recruited after they had received an emergency blood transfusion prior to determination of their HIV status (16 children had received a blood transfusion in the previous 7 days prior to enrollment).

#### 5.2 Factors associated with moderate to severe anaemia

#### 5.2.1 Age

The younger age group (< 60 months) was independently associated with moderate to severe anaemia (OR 4.51, 95% CI 1.77-11.47, p 0.002). This could be explained by the fact malaria which is a major cause of anaemia occurred in  $[31/44 \ (70.5\%)]$  of children less than 60 months and similarly 67/92 (72.8%) of the wasted children in this study were also less than 60 months. The increased body demands secondary to rapid physical growth in children less than 60 months, chronic infection associated with HIV coupled with inadequate intake of nutrients also predisposes these children to anaemia especially that secondary to nutritional deficiency.

Studies in Zaire<sup>78</sup> and Tanzania<sup>79</sup> have also found anaemia to be highly prevalent in children less than 60 months and this could be due to the fact that both Tanzania and Zaire are within the malaria endemic region.

#### **5.2.2 Caretaker characteristics**

In this study none of the caretaker characteristics were statistically and significantly associated with moderate to severe anaemia. Most of the caretaker's level of education was less than or only upto primary. Mothers who had studied up to primary level or less were more likely to have children with moderate to severe anaemia although this was not statistically significant (OR 1.14, 95% CI 0.60-2.11, p 0.68). Similarly a low level of family income was not statistically and significantly associated with moderate to severe anaemia (OR 1.14, 95% CI 0.61-2.14, p 0.664). Studies in Uganda<sup>37</sup> and Kenya<sup>39</sup> have shown similar findings however in Tanzania<sup>80</sup>, parental education level was associated with anaemia although this did not remain significant after multivariate analysis. Other studies have found that poor social economic status and unstable home environment especially loss of a biological parent was associated with inadequate food intake and nutritional anaemia.<sup>29,35</sup> In the present study the majority of children had a biological parent which could possibly explain the difference.

#### 5.2.3 Follow up management in an HIV/AIDS care center

In this study, children who were newly diagnosed and not yet in the Paediatric Infectious disease clinic or other HIV/AIDS care centers were significantly more likely to have moderate to severe

anaemia on bivariate analysis [OR 2.908(1.657-5.102), p= 0.000]. This is because the children not under an HIV/AIDS care programme were not on cotrimoxazole prophylaxis, multivitamin supplementation or HAART and were more likely to have severe immune suppression, with an increased risk of opportunistic infections which were not diagnosed and treated promptly thus increasing the risk of anaemia.

#### 5.2.4 Clinical presentation

On clinical history, symptoms of chronic illness (persistent fever, chronic cough, persistent diarrhoea, loss of appetite and weight loss) were significantly associated with moderate to severe anaemia. Persistent or chronic diarrhoea causes intestinal malabsorption thus contributing to anaemia of chronic disease. Similarly, chronic infections associated with HIV infection cause anorexia leading to inadequate food intake and this, coupled with the increased energy demands associated with infection contribute to nutritional anaemia. HIV infection is also associated with persistent fever which results from release of proinflammatory cytokines such as TNF alpha and IL-6 which further inhibit erythropoiesis ultimately causing anaemia.

In this study both severe stunting and wasting were associated with moderate to severe anaemia. Similarly a previous study done in Uganda found that anaemia was highly prevalent among severely malnourished children irrespective of their HIV status.<sup>81</sup> In Tanzania, malnutrition was found to be independently associated with anaemia although the HIV status of these children was not reported.<sup>80</sup> Another study in HIV infected Ugandan children found that hospitalization, chronic diarrhoea, suspected tuberculosis, and stunting were significantly associated with moderate anaemia.<sup>6</sup> These findings therefore suggest that the cause of anaemia in HIV infection is multifactorial, including chronic diseases and nutritional deficiencies.

A gallop rhythm was statistically and significantly associated with moderate to severe anaemia on bivariate analysis and was predictive of severe anaemia. This is because severe anaemia is a common cause of hyperdynamic state of heart failure. Lymphadenopathy was statistically and significantly associated with moderate to severe anaemia. This is because the children with lymphadenopathy also had chronic diseases like tuberculosis, and Kaposi sarcoma which are causes of anaemia. A previous transfusion at any point in time was also independently associated with moderate to severe anaemia in this study (OR 3.97, 95% CI 1.47-10.68, p=0.006). This could be because these children were severely anaemic prior to the transfusion and the blood given did not sufficiently raise their haemoglobin level. Secondly if the cause of anaemia is not addressed, then the child will have recurrent anaemia even after the previous transfusion. In this study, among the children who had a history of blood transfusion, 29/46 (63%) were severely immune suppressed (CD4% <15) showing that if the immunity is not improved, blood transfusion would only be a temporary treatment of anaemia.

Other studies in Uganda and Tanzania also found that a history of blood transfusion was independently associated with anaemia although in these two studies the HIV status of the children were not identified.<sup>19,80</sup>

# 5.2.5 Co-infection with other common illnesses Malaria

Among the common illnesses, only malaria was independently associated with moderate to severe anaemia (OR 4.42, 95% CI 1.72-11.39, p=0.002). Malaria is a major cause of anaemia in endemic areas. P. falciparum causes anaemia through direct destruction of both parasitized and unparasitized red blood cells with increased splenic clearance of these cells and also by direct suppression of the bone marrow through cytokine induced dyserythropoiesis.

Earlier studies showed no effect of HIV infection on the occurrence or severity of malaria<sup>69,70</sup> however more recent studies have shown that HIV is associated with severe complicated malaria<sup>72,73</sup> and that especially infants are at greater risk of malaria anaemia during an acute P. falciparum infection.<sup>20,74</sup> Another study by Clark found that malaria was significantly associated with moderate anaemia among HIV infected children in Uganda.<sup>6</sup>

#### 5.3 Advancing HIV disease

There was no association between advanced WHO clinical stage (III and IV) with moderate to severe anaemia. However, on Immunological staging severe immune suppression (CD4% < 15 or CD4 counts < 200 for children above 5years) was significantly associated with moderate to severe anaemia. This is because children with severe immune suppression are prone to multiple

opportunistic infections such as giardiasis, isosporiasis and chronic chest infections all of which predispose HIV infected children to anaemia. Systemic review of literature has shown that worsening HIV disease and higher viral loads are independently associated with increasing risk of anaemia.<sup>3,4</sup>

#### 5.4 Drugs

#### **5.4.1 HAART**

Forty seven children (21.8%) were on Highly Active Antiretroviral Therapy. The drugs taken included stavudine, zidovudine, lamivudine, didanosine, abacavir, niverapine, efavirenz, kaletra, and nelfinavir. Not receiving HAART was significantly associated with moderate to severe anaemia (p=0.024) on bivariate analysis.

HAART has been found effective in reducing the risk of anaemia.<sup>4</sup> HAART improves the immune system and quality of life of HIV infected children, reducing the risk of opportunistic infections, suppressing the HIV virus and ultimately reducing the risk of anaemia

A study in Blatimore USA found that on multivariate analysis, use of HAART was strongly associated with not having anaemia after adjusting for gender, race, baseline CD4, HIV-1-RNA levels and treatment of anaemia.<sup>7</sup> On the contrary, in the present study HAART was not independently associated with moderate to severe anaemia showing that there may be interplay between HAART and other factors in the prevention of anaemia in HIV infection. This could also be because in this study only a small proportion of children (21.9%) were on HAART. Furthermore only a smaller proportion (13.9%) of the children < 60 months who were most affected by moderate to severe anaemia were on HAART.

A study in Combodia found that while HAART was associated with improvement in CD4% it was not associated with improvement in the haemoglobin level and nutritional status of severely malnourished children.<sup>82</sup> This suggests that nutrition has to be effectively addressed in order to improve the haemoglobin of HIV infected children.

Among the antiretroviral drugs, AZT is known to cause anaemia. However in this study, children on AZT were less likely to have moderate to severe anaemia (OR 0.387, 95% CI 0.16-0.93, p = 0.030). The reason for this could be that the overall benefit of using the combination therapy in

the AZT regimen exceeds the myelosupressive property of AZT especially when given in optimal doses.

A cohort analysis from John Hopkins university USA also suggested that use of AZT was not a significant risk factor for anaemia in HAART treated patients.<sup>7</sup> While on the contrary another study in India found that AZT induced anaemia was the second most common adverse effect and occurred at a mean interval of 2 years after starting therapy.<sup>83</sup> Therefore it is possible that the difference with the present study could be because the majority of children on AZT had taken it for a shorter duration. The duration on AZT therapy in the present study ranged from 2 months to 24 months with a mean duration of 7 months.

#### 5.4.2 Cotrimoxazole

Not taking cotrimoxazole prophylaxis in this study was associated with moderate to severe anaemia (p = 0.000). This could be due to the fact that cotrimoxazole prophylaxis prevented most of the chronic infections and malaria which would otherwise cause anaemia. In this study, most of the children with malaria had not been on cotrimoxazole prophylaxis [31/44 (70.5%) vs 13/44 (29.5%)]. Similarly, among the children who presented with persistent diarrhoea 42/61 (68.9%) were not on cotrimoxazole prophylaxis compared to 19/61(27.9%) on cotrimoxazole prophylaxis. Among those with a chronic cough, 38/51 (74.5%) had not been on cotrimoxazole prophylaxis compared to 13/51 (25.5%) on cotrimoxazole.

Cotrimoxazole has been found to reduce morbidity and mortality among HIV infected children in Africa through prevention of respiratory infections,<sup>84,85</sup> gastrointestinal infections<sup>86</sup> (Isospora and enteric nontyphi salmonella infections) and malaria.<sup>87</sup> A study in Mali also found that cotrimoxazole prophylaxis had a 99.5% protective efficacy against episodes of clinical malaria.<sup>86</sup> On the contrary, in Zambia cotrimoxazole prophylaxis did not prevent malaria. However it was also noted that the prevalence of malaria was very low in Zambia.<sup>84</sup>

#### 5.4.3 Multivitamin supplementation

In this study not taking multivitamin supplementation was independently associated with moderate to severe anaemia (OR 4.67, 95% CI 1.97-11.06, p = 0.000). Among the children who presented with persistent diarrhoea, 31/37 (83.8%) were not taking multivitamin supplementation compared to 6/37 (16.2%) who were taking multivitamins. Similarly among the children with chronic cough, 44/51 (86.3%) were not taking multivitamins compared to 7/51 (13.7%) on multivitamin supplementation. Forty seven (77%) of the children with fever were not on multivitamins versus 14/61 (23%) who were taking multivitamins. Sixty nine (75%) of children with severe wasting were not on multivitamins compared to 23/92 (25%) who were taking multivitamins. This shows that children who were not on multivitamin supplementation were more likely to have chronic ill health and thus, increased risk of anaemia.

The oxidative stress associated with HIV infection leads to increased viral replication, quickening the progression of HIV disease and increasing the risk of HIV associated comorbidities. This effect worsens the anaemia due to HIV its self and also that associated with inflammation and chronic disease.

Multivitamins including the vitamin B group, C, and E improve the immune system because of their antioxidant properties and in so doing, multivitamins prevent progression of HIV infection thus reducing the risk of HIV comorbidities like anaemia.<sup>88</sup> Secondly the antioxidant vitamin C increases absorption of non heme iron thus reducing the risk of iron deficiency which is otherwise a major cause of anaemia in children.

#### 5.5 Laboratory findings and type of anaemia

One hundred and thirty children (60.5%) presented with microcytosis (MCV <76). This is similar to 57.1% (MCV<70) found among HIV infected infants in Uganda<sup>6</sup>. A microcytic hypochromic picture was found in 26 (12%) of the study children and 20 of these (76.9%) had moderate to severe anaemia (p= 0.004). In another study, Totin also found that a microcytic hypochromic picture was associated with an Hb< 9g/dl.<sup>26</sup>

A microcytic normochromic picture was seen in 104/215 (48.4%). This was more in the children with moderate to severe anaemia although the difference was not statistically significant

(p=0.535). This large percentage could be due to iron deficiency (the early stage) and anaemia of chronic disease.

A recent blood transfusion may modify the morphological picture of anaemia. In this study 27 children had received a blood transfusion with in the previous 30 days and therefore this could have modified their picture of anaemia.

The most likely cause of microcytosis in our setting is iron deficiency and other less likely causes include thalassaemia, and lead poisoning. Anaemia of chronic disease may also present with microcytosis in the late stage.

The RDW was used to differentiate between iron deficiency and thalassaemia in this study. Seventy two out of two hundred and fifteen children (33.5%) had both microcytosis and RDW > 16 suggesting iron deficiency. The prevalence of iron deficiency (33.5%) in the present study was much less than that found by Totin (47.1%) among HIV infected children.<sup>26</sup> This difference could be explained by the fact that the previous study used serum ferritin which is a more specific measure of iron deficiency than that used in the present study. It is therefore possible that this study could have missed some children with iron deficiency.

A normocytic normochromic picture was seen in 75/215 (34.9%) of the children. This picture was less common among the patients with moderate to severe anaemia (OR 0.56, 95% CI 0.31-0.98, p= 0.044). The commonest cause of a normocytic normochromic picture is anaemia of chronic disease or aplastic anaemia associated with HIV virus.

Macrocytosis (MCV>96) was found in 6 (2.8%) of the study children. AZT is a common cause of macrocytosis. However, in this study only 2 children with macrocytosis were on AZT. The possible causes of macrocytosis in the other 4 children could have been vitamin B12 and folate deficiency which were not tested for in this study.

## 5.6 Study limitations

- Diagnostic studies for iron deficiency i.e serum iron, ferritin and TIBC were not done because of budget constraints. Similarly other contributing factors to anaemia in HIV like vitamin B12 and folic acid deficiency, Parvo virus B19 infection, mycobacterium avium complex infection and autoimmune disease were not investigated due to the same reasons.
- The prevalence of severe anaemia could have been underestimated because some children had received a blood transfusion prior to enrollment and drawing a blood sample for analysis. A previous transfusion could also have modified the morphological type of anaemia
- 3. The results of this study may not be generalisable because it is a hospital based study and may not represent the out patient and rural population of HIV infected children.

# **CHAPTER SIX**

#### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### **6.1 CONCLUSIONS**

- The prevalence of moderate to severe anaemia among HIV infected children admitted on the Paediatric wards in Mulago hospital was 50.7%
- Moderate to severe anaemia was more prevalent between the ages of 6 to 24 months (60.4%)
- The factors independently associated with moderate to severe anaemia were age < 60 months, not taking multivitamin supplementation, previous blood transfusion, lymphadenopathy and coinfection with malaria.</li>
- 4. Almost half of the children presented with a microcytic normochromic picture (48.4%) and the other types included normocytic normochromic (34.9%) microcytic hypochromic (12%), macrocytosis (2.8%) and normocytic hypochromic (1.9%).

#### **6.2 RECOMMENDATIONS**

- 1. HIV infected children should routinely be evaluated for the presence of anaemia.
- Prevention of malaria should be emphasized among HIV infected children so as to prevent anaemia due to malaria.
- 3. Early diagnosis and referral of HIV infected children to HIV/AIDS care centers for regular follow up management should be emphasized.
- In addition to cotrimoxazole prophylaxis, HIV infected children should receive multivitamin supplementation so as to reduce the occurrence of moderate to severe anaemia.
- 5. Another study should be done to identify the risk factors for anaemia in HIV positive children compared to those who are HIV negative.

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# Appendix (i)

# **CONSENT FORM**

# Title of study

Prevalence and factors associated with moderate to severe anaemia in HIV infected children receiving treatment in Mulago hospital.

# Introduction

I am Dr Mary Munyagwa from department of Paediatrics and Child Health Mulago hospital.

I am going to carry out a study on the risk factors for anaemia in HIV infected children.

This study will help us understand the factors that are likely to contribute to anaemia in HIV infected children so that they can be addressed in order to improve the quality of life of these children.

I am therefore requesting you to participate in this study by allowing your child to be enrolled in the study.

# **Study procedure**

During the study, the following will be done

- 1) You will be asked questions about your child's current and past medical and nutritional history.
- 2) Your child will be examined.
- 3) We shall collect samples of blood, stool and urine from your child which will include blood and stool. During sample collection, strict aseptic measures will be used to make sure that your child does not get exposed to infection.

# **Risks and discomforts**

During the process of removing blood, your child will feel some pain however this will be mild and short lived. We shall draw about a teaspoon of blood from your child to do the blood tests. This amount of blood will be too little to cause harm to your child's health.

# Benefits

Your child will receive a complete medical exam, the investigations done during the study will be free of charge and availed to the doctors taking care of the child for proper management of your child.

Accepting your child to be enrolled in the study is completely voluntary and refusal to participate will not affect the management of your child.

# Confidentiality

Your child's records will be kept confidential and only the people working on the study will have access to them. A study number will be used instead of the child's name.

# Questions

You are free to ask any questions now or later.

In case you have any questions related to the study later, you can contact Dr Munyagwa on 0772629873 at the department of Paediatrics and Child Health Mulago hospital.

# Statement of consent.

The purpose and nature of this study has been explained to me and I understand that the participation of my child in the study is voluntary and that no consequences will result if I refuse my child to participate.

I have the right to know the results of the laboratory tests for my child.

Name of parent/ guardian

Signature/ finger print

Date

Name of investigator

Signature

Date.

# Appendix ii

### ASSENT FORM

### **Purpose of the study**

A research is a way of finding out new information about something.

We are doing a research to find out some factors that can cause a child to have little blood in their body. We are asking children who are receiving treatment from Mulago hospital to be part of the study.

When you accept to be part of the study, we shall request you to respond to some questions concerning your health and family. A doctor will then examine and also remove some blood from you (about a tea spoon) using a needle so that we can test it. During the process of removing blood, you will feel some pain as the needle enters your body but this will not last long and after the needle is out you will not feel pain again. We shall also give you two containers so that you put a small part of your stool in one and a little amount of urine in the other.

You are free to decline to participate in this study or withdraw from the study at any time and this will not affect your management in any way.

You are free to ask any questions now or if you get any questions later, you can call or ask your parent to call Dr Mary Munyagwa at 0772629873.

# Statement of consent

I have fully understood the purpose and nature of this study and also understand that my participation is voluntary and no consequences will result if I refuse to participate.

Name	of	child/	thumb	print
1 (unit	<b>U</b> 1	CIIII(4/	tilaino	prim

Witness

Date

If the child is not able to read this consent form and verbal consent is obtained using the content in this form, the person obtaining assent should sign below.

Witness

Date
### Appendix iii

# PREVALENCE AND FACTORS ASSOCIATED WITH MODERATE TO SEVERE ANAEMIA IN HIV INFECTED CHILD ADMITTED AT MULAGO HOSPITAL

### QUESTIONNAIRE

Date		Study number
Social demogr	aphics	
IP No/clinic No	- )	
Name		
Date of birth	/ /	
Age (months / )	years)	
Sex: female =	=1, male =2	
Tribe		
Village	Sub county	County District
Presenting co	mplaints.	
	<b>r</b>	
Presence of syr	nptoms related to anaemia	If a symptom is present check the box for yes and vice
versa		
a) Fever		
1. $\Box$ Yes	2. □ No	Duration(days)
b) Cough		
1. $\Box$ Yes	2. □ No	Duration
c) Fast / difficu	lt breathing	
$1. \Box$ Yes	$2. \square No$	Duration
d) Easy fatigab	ility	
1. $\Box$ Yes	2. □No	Duration
e) Loss of appe	tite/ poor feeding	
I. ⊔ Yes	$2. \sqcup No$	Duration
f) Mouth sores		
$I. \sqcup Yes$	2. ⊔ No	Duration
g) Yellow eyes, $1 \Box V_{ex}$	$\sqrt{SK1n}$	Devertien
$1. \sqcup Y es$	$2. \square INO$	Duration
n) vomiting $1 \Box Vac$	$1 \square \mathbf{N}_{2}$	Duration
$1. \sqcup 1 \text{ es}$	$2. \Box INO$	Duration
1) Iffiliability $1 \Box V_{22}$	$2 \Box N_{c}$	Duration
i) Diarrhood	$2. \square INO$	
$\int Diamoea$	$2 \square N_0$	Duration
$1. \Box 1 \nabla S$	2. LINU	
$\Gamma$ I $\Box$ Vec	$2 \square N_0$	Duration
1. 105	$2.$ $\Box$ INU	

1) Blood in stool	/ black stools		
1. $\Box$ Yes	2. □ No	Duration	
If yes specify	y		
m) Bleeding from	n other site		
$I. \sqcup Yes$	2. ⊔ No	Duration	
n) Joint pains			
$I. \sqcup Yes$	2. ⊔ No	Duration	
o)Weight loss			
$I. \sqcup Yes$	$2. \sqcup No$	Duration	
Past medical/ dr	ng history		
a). Previous admi	ssion		
$1. \square$ Yes	$2. \square$ No If ves. number	of admissions	
When was the las	st admission (in weeks)?		
What was the cau	use of the last admission?	(Look	at previous notes
<i>if available</i> )		(	
5			
b).Previous trans	fusion		
1. $\Box$ Yes	2. $\Box$ No If yes, number	of transfusions	
When was the las	st transfusion done? (Weeks)		
c).Which drugs h	as the child taken over the pas	st 1 month (Look <i>at previous note</i> )	s)
i) Iron	$1. \Box \text{ Yes } 2. \Box \text{ No}$	v) Folate $1.\Box$	Yes $2.\square$ No
ii) Co-trimox	azole 1. $\Box$ Yes 2. $\Box$ No	vi) Anti- TB drugs $1.\Box$	Yes $2.\square$ No
iii) Chorampl	henical 1. $\Box$ Yes 2. $\Box$ No		
iv) Anticance	er drugs 1. $\Box$ Yes 2. $\Box$ No		
Speci	fy		
vii) Is the chi	$ld on ART 1. \Box Yes 2.$	□ No	
If yes sp	pecify		
viii) Other dr	ugs (specify)		
l			
2			
3			
d) Is the immuni	$\mathbf{x} = \mathbf{x} + $	$2 \square \mathbf{N}_{2}$	
a) is the minutes	sation up to date? $1.\Box$ res	2. 🗆 INO	
Nutritional histo	Drv		
a) Breast feeding	history		
i) Was the child	d ever breast fed $1. \Box$ Yes	2. 🗆 No	
ii) Is the child s	still breast feeding $1. \Box$ Yes	2. 🗆 No	
	6		
b).When were co	mplimentary feeds started (ag	e in months)	
c).Which complia	mentary foods were/are given		
1\ TT			
d).How many tin	hes does the child feed in a day	У	•

# Menstrual history (for girls aged 8 years and above)

Have you started	l menstrual	periods?	$1. \square$	Yes	2.⊔ No	
If yes, what is	s the duration	on of the	period			(Days)

#### Family and social history

a). Who is the primary care taker to the child? (circle)

- 1. Father 4. Older sibling
- 2. Mother 5. Aunt
- 3. Grandmother 6. Uncle

b).What is the occupation of the care taker .....

c) What is the care takers level of income.....

d).What is the highest level of education of the primary care taker (tick against the level of education)

	father	mother	other care taker
No formal education			
Primary level			
O level			
A level			
Tertiary education			
	No formal education Primary level O level A level Tertiary education	fatherNo formal educationPrimary levelO levelA levelTertiary education	fathermotherNo formal educationPrimary levelO levelA levelTertiary education

#### **Physical examination**

a). Anthropometry				
i) Length / Heig	,ht	(cm)		
ii) Weight		(kg)		
iii) MUAC		(cm)		
iv) Weight for he	ight Z scor	re		
v) Height for age	e Z score	e		
<ul> <li>b) Temperature</li> <li>c) Pallor ( <i>circle</i>)</li> <li>i). Mild</li> <li>ii). Moderate</li> <li>iii). Severe</li> </ul>		<sup>0</sup> C		
d) Jaundice	1.□ Yes	2.□ No	e) Oral thrush	1. Ves
f) Angular stomatitis	1.□ Yes	2.□ No	g) Smooth Tongue	1.□ Yes
h) Angular cheilitis	1.□ Yes	2.□ No	i) Lymphadenopathy	1.□ Yes
<ul><li>j) Skin and nails</li><li>i). Petechiae</li><li>iii).Echymoses</li></ul>	1.□ Yes 1.□ Yes	2.□ No 2.□ No	ii).Koilonychia	1.□ Yes

2.□ No

2.□ No

2.□ No

2. 🗆 No

m) Kaposi sarcoma lesions 1. $\Box$ Yes 2. $\Box$ No	o n) Pe	dal oedema	1. 🗆 Yes	2. 🗆 No
Cardiovascular system 1. Pulse rate(beats/min) 2. JVP raised 1.□ Yes 2.□ No 3. Heart sounds (circle) i). Normal ii). Gallop iii) Others e.g murmurs (specify)	Blood press	suremm	ηHg	
Respiratory system         1. Rate	nin)			
Abdominal exami) Hepatomegaly.1.□ Yes2.□ No				
ii) Splenomegaly $1. \Box$ Yes $2. \Box$ No				
Central nervous system.1. Is the child conscious1.□ YIf No, i) are there meningeal signs1.□ Yii) Are there localising sins1.□ Y	Yes $2.\square$ Yes $2.\square$ Yes $2.\square$	No No No		
Clinical diagnosis 1 2 3				
HIV WHO Clinical staging				
Current treatment given           1           2           3           4				

k) Parotid enlargement  $1.\Box$  Yes  $2.\Box$  No l) Bone tenderness  $1.\Box$  Yes  $2.\Box$  No

# Laboratory investigations

 g/dl
 x10 <sup>6</sup> /ul
 x 10 <sup>3</sup> /ul
 %
 fl
 pg
 g/dl
 %
 x 10 <sup>3</sup> /ul
 %
······································

#### **Blood film report** (*circle against the result*)

a) Erythrocyte hemoglobinisation	b) Erythrocyte size
1. Normochromic	1. Normocytosis
2. Hypochromic	2. Microcytosis
3. Polychromasia	3. Anisocytosis
	4. Macrocytosi

#### c) Red blood cell shape (circle)

- 1. Normal
- 6. Schistocytes
   7. Elliptocytes
- Sickle
   Target
   Tear drop cells
- 4. Acanthocytes 9. Helmet cells
- 5. Poikilocytes 10.Other (specify).....

### Malaria parasites 1.□ Yes 2.□ No

if	yes
	What species
	What is the parasite density

. . . . . . .

. . . . . . .

. . . . . . .

#### Stool analysis

- a) Microscopy (*circle*)
  - 1. RBC
  - 2. Pus cells
  - 3. Ova/cyst
  - 4. Type of ova/cyst (specify)
- b) Occult blood test ( *circle*)
  - 1. Positive
  - 2. Negative

## Appendix iv INTERIM REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR

**INFANTS AND CHILDREN** (For persons aged under 15 years with confirmed laboratory evidence of HIV infection: HIV antibody if aged 18 months and above; virologicalal or p24 antigen testing if aged under 18 months)

### **Clinical Stage 1**

Asymptomatic

PGL

#### **Clinical Stage 2**

Hepatosplenomegaly

Papular pruritic eruptions

Extensive wart virus infection

Extensive molluscum contagiosum

Fungal nail infections

Recurrent oral ulcerations

Lineal gingival erythema (LGE)

Angular cheilitis

Parotid enlargement

Herpes zoster

Recurrent or chronic RTIs (otitis media, otorrhoea, sinusitis)

#### **Clinical Stage 3**

Moderate unexplained malnutrition not adequately responding to standard therapy

Unexplained persistent diarrhoea (14 days or more )

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

Oral candidiasis (outside neonatal period)

Oral hairy leukoplakia

Acute necrotizing ulcerative gingivitis/periodontitis

Pulmonary TB

Severe recurrent presumed bacterial pneumonia

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

Chronic HIV-associated lung disease including brochiectasis

Symptomatic Lymphoid interstitial pneumonitis (LIP)

#### **Clinical Stage 4**

Unexplained severe wasting or severe malnutrition not adequately responding to standard therapy

Pneumocystis carini pneumonia

Recurrent severe presumed bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)

Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration, visceral of any duration)

Extrapulmonary TB

Kaposi's sarcoma

Oesophageal candidiasis

CNS toxoplasmosis (outside the first 6weeks of life)

HIV encephalopathy

CMV infection (CMV retinitis or infection of organs other than liver, spleen or lymph nodes; onset at the age one month or more)

Extrapulmonary cryptococcosis including meningitis

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

Cryptosporidiosis

Isosporiasis

Disseminated non-tuberculous mycobacteria infection

Candida of trachea, bronchi or lungs

Acquired HIV associated rectal fistula

Cerebral or B cell non-Hodgkin lymphoma

Progressive multifocal leukoencephalopathy (PML)

HIV-associated cardiomyopathy or HIV-associated nephropathy

## Appendix v Immunological staging

Immunological staging of HIV disease will be done using CD4 counts according to the criteria below.

	< 11 mo	12-35 mo	36-59 mo	>5 years
	(%)	(%)	(%)	(%)
No suppression	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Moderate	25-30	20-25	15-20	200-349
Severe	<25	<20	<15	<200 or <15%

## Appendix vi

Blood Indices (coulter counter normal ranges)

WBC count	$4.0 - 11(103/\mu l)$
RBC	4.5- 6.5 (106/µl)
НСТ	40 - 54 (%)
MCV	76-96 (fl)
MCHC	27-32 (g/dl)
RDW	11-16(%)
Platelet count	150- 400 (103/µl)