THE BURDEN AND DETERMINANTS OF ANAEMIA IN HIV INFECTED CHILDREN ATTENDING PUBLIC HOSPITALS IN DAR ES SALAAM

BY
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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences

2009

CERTIFICATION

The undersigned that they have read and hereby recommended for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled:: "THE BURDEN AND DETERMINANTS OF ANAEMIA IN HIV INFECTED CHILDREN ATTENDING PUBLIC HOSPITALS IN DAR ES SALAAM" in partial fulfillment of the requirements for the degree of Master of Science (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences

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ACKNOWLEDGEMENT

It is not possible for a single individual to cover all the aspects that made this dissertation into being, and I am therefore deeply grateful to all those who have, with such good grace, given their time and energy to supply valuable opinions, facts or even moral support.

Much of any merit this dissertation may have is due to the generosity of my supervisors Dr Pius Magesa and Prof Ferdinand Mugusi for their guidance and patience as they devoted their spare time so as to offer and contribute towards this dissertation. I am also thankful to Prof David Roberts (University of Oxford) for his close supervision to this dissertation

Special thanks go to the entire staff of the Departments of Haematology/Blood Transfusion and Paediatrics who contributed in one way or another to this dissertation. I also thank all children and their parents for accepting to participate in this study.

Much appreciation goes to Muhimbili University of Health and Allied Sciences (MUHAS) for the research grant to this dissertation.

Lastly I thank my sponsor-The German Academic Exchange Service (DAAD) for all material and financial support during my training.

DEDICATION

"To my Wife Domitila, parents and my sons Alphonce and Alvin for their love and encouragement"

ABSTRACT

Background: Though anaemia is still a major problem in patients infected with HIV, there is paucity of data describing its magnitude and determinants among HIV infected children in Tanzania. Most of the studies among anaemic Tanzanian children were conducted before the era of HIV and HAART.

Objectives: The study was aimed at determining the prevalence and contributing factors for anaemia among HIV-infected children attending public hospitals in Dar es Salaam.

Materials and Methods: This was a descriptive cross sectional study that was conducted at Muhimbili National Hospital and Mwananyamala Municipal Hospital. The target population for the study included 167 consecutive HIV infected children attending the HIV clinic or admitted in paediatric wards for the period of August to November 2008. The subjects were 6 months to 59 months of age to be recruited in the study
After written consent from the guardian of the child, information on social demographic and clinical characteristics was collected from the medical file and interview from the child's parents or guardians. Also, physical examination and laboratory tests on blood, stool, and urine were done for each study subject. The prevalence of anaemia was determined as a percentage among all children infected with HIV. Both univariate and multivariable logistic regression analyses were performed to identify possible risk factors associated with anemia in HIV-infected children.

Findings: In this study the overall prevalence of anaemia (Hb<11g/dl) among HIV infected children attending hospitals was 44%. Among 167 enrolled HIV infected children, 35 (21.1%) had mild anaemia, 14(8.4%) had moderate anaemia and 26(15.6%) had severe anaemia. In a univariate analysis, not being on HAART, advanced HIV disease, having a history of TB disease in the past 6 months at the study, a history of chronic diarrhoea (14 days or more), a history of malnutrition, a history of recurrent malarial attack (every month), being HIV positive for less than 2.5 yrs and hookworm infestation were all associated with anaemia. The use of anthelmintics and multivitamins were found to be protective against anaemia. The final model derived by multivariate logistic regression

demonstrated that not being on HAART (OR 3.4, 95%CI (1.20-9.60), advanced HIV disease, having a history of TB disease in the past six months at the study (OR 3.23, 95%CI (1.10-9.70) and having hookworm (OR 5.97, 95%CI (1.92-18.4) infestation were independent risk factors for anaemia among HIV infected children. Taking multivitamins (OR 0.07, 95%, CI (0.02-0.30) and antihelminthics (OR 0.27, 95%CI (0.10-0.74) were still the protective factors against anaemia.

Multivariate sub-analysis of factors associated with severe anaemia (HB<8g/dl) revealed that, having a history of TB disease in the past six months at the study, advanced HIV disease, being HIV positive for less than 2.5 yrs and having hookworm infestation were again independent risk factors for severe anaemia among HIV infected children. Taking multivitamins was also protective against severe anaemia.

Conclusions and recommendations: Despite the availability of HAART, the prevalence of anaemia was high among study patients and was multifactorial in nature. Efforts to correct anaemia in HIV infected children should include use of HAART, treatment of infections such as TB, malaria, hookworms and improving access to diverse diets rich in iron.

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LIST OF ABBREVIATIONS

AIDS-Acquired Immune Deficiency Syndrome

ANC-Absolute Neutrophil Count

ARV-AntiRetroVirals

CBC-Complete Blood Count

CD-Cluster Designation

CDC-Centre for Disease Control

CI- Confidence Interval

D4T-Stavudine

FBP-Full Blood Picture

G6PD-Gluose-6-phosphate Dehdrogenase enzyme Deficiency

Hb- Haemoglobin

HAART-Highly Active Anti Retroviral Therapy

HIV-Human Immunodeficiency Virus

IDA-Iron Deficiency Anaemia

LDH-Lactate Dehydrogenase

MAC-Mycobacterium Avium Complex

Mwana-Mwananyamala

MNH-Muhimbili National Hospital

OR-Odds Ratio

QOF-Quality of Life

SCID-hu- Severe Combined Immunodeficient -human

SSA-Sub Saharan Africa

TB-Tuberculosis

TNF-Tumour Necrotic Factor

WBC-White Blood Cell Count

WHO-World Health Organization

ZDV- Zidovudine

INTRODUCTION

In 2007, it was estimated that 2.5 million children were living with the Human Immunodeficiency Virus (HIV), and 420,000 children were newly infected [1]. Almost all infected children acquire HIV from their mothers in utero, during delivery or during breastfeeding. Nearly 90% of paediatric infections occur in Sub-Saharan Africa (SSA), where many countries report that more than one in five pregnant women visiting antenatal clinics are HIV-infected. In the absence of antiretroviral therapy, nearly one-third of vertically infected children living in the developed world rapidly progress to Acquired Immunodeficiency Syndrome (AIDS) or death in the first year of life [2].

Anaemia is a common complication of HIV infection and is independently associated with disease progression and mortality [3, 4]. The pathophysiology of HIV-related anaemia in childhood is not well understood and may be especially complicated amidst the dynamic changes associated with normal haematological development in early infancy.

Studies on the magnitude and determinants of HIV related anaemia among children in Tanzania are scarce. Most of the studies of anaemia in children were conducted before the era of HIV and were confined to anaemia associated with malaria and iron deficiency. A few studies available on HIV related anaemia have been focusing on pregnant women and marginalizing children. Thus this study was conducted to improve the understanding of HIV related anaemia among children attending hospitals in Dar es Salaam, Tanzania.

LITERATURE REVIEW

HUMAN IMMUNODEFICIENCY VIRUS AND AIDS IN CHILDREN

More than 1,000 children under 15 years of age are infected with HIV every day, most as a result of mother-to-child transmission of the virus[5]. Approximately 2.5 million children were living with HIV in 2007, up from 1.6 million in 2001. Children make up approximately 6 percent of the total number of people living with HIV. In 2007 there were about 1.8 million children living with HIV in sub-Saharan Africa. This represents nearly 90 percent of all HIV-positive children worldwide. As many other countries in Sub-Saharan Africa, Tanzania is struggling with a major HIV/AIDS disaster that wrecks havoc both in adult as well as child health. The first cases of AIDS in Tanzania were reported in 1983; by 1986 all regions of the country were reporting cases[6]. The prevalence of HIV in antenatal clinics has ranged from 4.2 percent in some districts to almost 32 percent in others. The Tanzania HIV/AIDS Indicator Survey (THIS, 2004) revealed an estimate of 7 percent in the 12-49 years of age cohort. In 2000, 11,673 cases of AIDS were reported to the National AIDS Control Program (NACP). Of the total, 433 cases (3.7 percent) were children under five. The cumulative number of AIDS cases in children under five between 1987 and 2000 is 4,357 (3.3 percent of the total). One study in rural Tanzania, revealed the overall prevalence of HIV antibodies in the children to be 16% (27/145) in 2002[7].
For 2003, the Ministry of Health and Social Welfare (MOHSW) estimated that 72,000 infants could be infected through mother (180,000 HIV-positive mothers) to child transmission of the HIV virus. Approximately 35 percent of the HIV positive infants (25,000) would contract the virus through breastfeeding. These populations (180,000 mothers and 25,000 infants) would place a significant stress of the health system, including staffing, laboratory services, logistics, etc. The MOHSW estimates that the few gains of the last 20 years in child mortality will be lost if the HIV/AIDS epidemic is not controlled[6].

ANAEMIA AS A PUBLIC HEALTH PROBLEM IN CHILDREN

Anaemia is a global public health problem affecting both poor and rich countries with major consequences for human health as well as social and economic development. It is the world's second leading cause of disability and one of the most serious global public health problems. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children. In 2002, iron deficiency anaemia (IDA) was considered to be among the most important contributing factors to the global burden of disease[8]

Anemia is a critical problem in Tanzania. According to some estimates, sixty two percent of children under five have anemia and up to 80 percent of pregnant women are anemic. Some experts estimate that in the hardest hit areas, up to 90 percent of children under five in Tanzania may have anemia[6]. The Tanzania Demographic and Health survey in 2004 approximated 70% of children aged six months to five years had anemia (<11g/di) and 4% had severe anemia. Children in rural areas were slightly more likely to be anemic than those in urban area. This varied across regions with 47% in Iringa and 88% in Lindi. In 2000, 88,933 cases of anemia in children under five were reported by health facilities placing anemia in fifth place as a cause for consultation(6). This number is most likely an underestimation as most cases of mild and moderate anemia are missed. It is estimated that 40 percent or more of all anemia in children under five is severe. It has been found that children living more than km from a facility are more likely to be anemic. Anemia was the third most common cause of hospitalization in children under five with 13.3 percent of the total (19,813 cases). However, another analysis of the data comprising more reports from facilities established that in 2003, there were 63,380 hospitalizations due to anemia. A case fatality rate of 5.7 percent has been established based on hospital reports.

It is likely that a substantial portion of this anemia is the result of malaria. Studies have shown that in malaria endemic areas, up to 60 percent of anemia in children could be prevented by anti-malarial chemoprophylaxis. When anemia hospitalizations are combined with malaria hospitalizations, the two are inextricably linked, a sobering 53.5 percent of all admissions of children under five are due to both anemia and malaria[6].

THE IMPACT OF HIV INFECTION ON THE HAEMATOPOIETIC CELLS IN CHILDREN

HIV infection in paediatric patients is a multisystem chronic disease that manifests as a clinical spectrum from asymptomatic infection through symptomatic infection with opportunistic infections and malignancies[9]. Mononuclear phagocytic cells and CD4+ T lymphocytes represent the major targets for infection by HIV-1 in vivo [10]. The most severe pathogenic features associated with HIV-1 infection can be attributed to malfunction or premature death of these cells that are of haematopoietic origin. Patients with acquired immunodeficiency syndrome (AIDS), suffer from many haematological disorders, particularly those persons with long-term infection of HIV-1. These disorders include anaemia, lymphocytopenia, thrombocytopenia and neutropenia. The mechanisms that lead to the induction of these disorders are multi-factorial. However, sufficient evidence has accumulated which suggests that HIV-1 infection of cells within the microenvironment of the bone marrow can lead to the induction of haematopoietic deficits[10].

Most studies indicate that marrow-derived haematopoietic stem cells cannot be infected by HIV-1 until they undergo modest differentiation in order to express the appropriate receptors to enable virus entry and subsequent replication[10]. Some cells within the mixed environment of the marrow stroma appear to support HIV-1 replication however. These cells include marrow microvascular endothelial cells, sometimes referred to as blanket cells, stromal fibroblasts, as well as mononuclear phagocytes. Experiments suggest that the HIV-1 accessory protein, Vpr, plays some role in the activation of marrow-derived mononuclear phagocytes which appears to result in premature phagocytosis of non-adherent marrow cells present in the in vitro cultures. This phenomenon could account, in part, for the induction of cytopenias that are typical of individuals infected by HIV-1.These observations suggest that HIV-1 infection may affect processes important during early stages of haematopoiesis or stem cell differentiation[10].

Haematopoietic abnormalities may be also caused by altered stem cell differentiation possibly due to abnormal lineage specific expression of certain cellular genes such as cytokines relevant to haematopoiesis [11] . These cytokines could affect regulatory signals important in haematopoiesis. However, in HIV

infected individuals, it is not only the virus but also the highly active antiretroviral therapy (HAART) that both contribute to persistent haematopoietic suppression and ensuing cytopenias. Even if a lowering of HIV replication by HAART were to occur in infected individuals, prolonged HAART by itself and/or appearance of drug resistant mutants can contribute to haematopoietic suppression and resulting cytopenias. However, confounding factors such as opportunistic infections, immune mediated effects, or the consequences of prolonged physiological stress, which could contribute to decreased haematopoiesis in patients or other individuals, make the causative role of HIV in vivo, uncertain. The severe combined immunodeficient mouse transplanted with human fetal thymus and liver tissues (SCID-hu) is a small animal model which mimics HIV infection in humans, and is useful to determine the mechanisms of HIV-1 induced haematopoietic inhibition and development of drug therapies for interventions of stem cell differentiation. Further, SCID mouse serves as a useful small animal recipient of human progenitor cells and also allows us to study the differentiation of these cells in vivo.[11]

The haematopoietic system is involved early in the systemic manifestations of this disease. The haematological abnormalities seen are most probably a reflection of persistent viral infection, inflammation, and immune dysregulation, and may be complicated by secondary infections, chronic disease, drug toxicities, and nutritional deficiencies[12]. Anaemia and lymphopenia are commonly found in adult AIDS patients. Although both are also seen in pediatric patients, lymphopenia is much less common. Atypical lymphocytes with plasmacytoid characteristics have been identified in both adults and children. Pediatric bone marrow evaluation has shown an increase in plasma cells and plasmacytoid lymphocytes[9]. In a study by Ellaurie M et al, anaemia was present in 94% of HIV-infected infants and was a major predictor of disease progression. In 91% of their study patients had haematocrit of less than 25%. Leucopenia and thrombocytopenia occurred in 33% and 26% of HIV these children, respectively. Neutropenia was most severe in children with opportunistic infections[12].

A study on the haematological changes in Kenya by Mwanda OW showed that cytopaenias; leucopaenia, anaemia, thrombocytopaenia, were the salient features in

HIV infected individuals.Bone marrow hypoplasia, although occurs, was found in a minority of cases. Other changes included myelodysplasia, functionally defective cells, and enhanced bleeding tendency particularly in those with bleeding defects[13] One study described the haematological profile of pregnant women and compared them according to HIV serostatus in Abidjan, Cote d'Ivoire documented a prevalence significantly higher in HIV positive (81.7%, n = 161) than in HIV negative women (68.9%, n = 994) ($P < 0.001$). Severe anaemia (Hb < 7 g/dL) was present in 1.9% of the women (n = 31), 4.6% (n = 9) in HIV positive and 1.5% (n = 22) in HIV negative women ($P < 0.001$) [14]. Anaemia was highly prevalent in this population while severe anaemia was rare. HIV infection was a contributor to anaemia in pregnancy.

In a cohort of non-infected children of HIV positive mothers who were on Highly Active Antiretroviral Treatment (HAART), anaemia was observed in 188 (30.1%) children during follow-up and 161 (25.8%) had anaemia grade two or higher. Nadir haemoglobin values were reached by 6 weeks of life and anaemia was transient and disappeared by six months of age. Neutropenia was present in 41.9% (261 children) and 22.7% of the children had moderate-severe neutropenia. African infants had a higher percentage of neutropenia than the rest of the children (50% vs. 44%), although the differences were not significant[15].

HUMAN IMMUNODEFICIENCY VIRUS AND ANAEMIA

Anaemia is a common manifestation of paediatric HIV infection [16].The condition is often considered an inevitable complication of people living with HIV with prevalence ranging from 8%-95% in different settings[17, 18].The incidence of anaemia in the setting of HIV infection is dependent on the severity of HIV disease as well as the level of haemoglobin level . In one review [19], the prevalence of anaemia in HIV disease varied considerably, ranging from 1.3% to 95% depending on several factors, including the stage of HIV disease, sex, age, pregnancy status, and injection-drug use as well as the definition of anaemia used. In general, as HIV disease progresses, the prevalence and severity of anemia increase. Anaemia is also more prevalent in HIV-positive women, children, and injection-drug users than in HIV-negative women, children, and injection-drug users [19].

In Uganda, Clark TD et al found baseline prevalence and cumulative incidence of anaemia (haemoglobin < 110 g/L) of 91.7% and 100% and, for moderate anaemia (haemoglobin < 90 g/L), were 35.1% and 58.4%, respectively, among 225 HIV-infected children followed from 9 to 36 months of age. Hospitalization, suspected tuberculosis, malaria and height-for-age Z-score <-2 were significantly associated with moderate anaemia[20]. Nahlen et al in their study of 14,664 patients with documented haemoglobin results, 10% of them had haemoglobin < or = 10 gm/dl (anaemia) on enrolment into the study, 9% developed anaemia after enrolment, and 5% had received a blood transfusion and/or erythropoietin but did not have anaemia documented[21]. The incidence of anaemia increased with decreasing CD4+ count and patients with AIDS were more likely to develop anaemia than persons without AIDS (O.R. 4.4, 95% C.I. 3.5, 5.5). The 1-year incidence of anaemia was 36.9% for persons with one or more acquired immunodeficiency syndrome (AIDS)-defining opportunistic illnesses (clinical AIDS), 12.1% for patients with a CD4 count of less than 200 cells/μm or CD4 percentage of <14 but not clinical AIDS (immunologic AIDS), and 3.2% for persons without clinical or immunologic AIDS.

In assessing the importance of anemia in HIV-infected children in western and tropical settings, a systematic review with a descriptive component was conducted by Calis JC et al; thirty-six studies met the inclusion criteria[22]. Mild (hemoglobin <11 g/dl) and moderate (hemoglobin <9 g/dl) anemia were more prevalent with HIV infection (odds ratio 4.5; 95% confidence interval 2.5-8.3 and odds ratio 4.5; 95% confidence interval 2.0-10.3, respectively). Mean hemoglobin levels were lower (standardized mean difference; 0.79; 95% confidence interval 0.47-1.10). These differences were observed in both western and tropical settings. Anemia incidence ranged from 0.41 to 0.44 per person-year. There was limited data on more severe anemia (hemoglobin <7 or <5 g/dl)[22].

The high prevalence of anaemia and the increased morbidity and mortality associated with anaemia during AIDS has been well described yet there has been little information about anaemia and changes in haemoglobin levels during acute and early HIV-1 infection. A study done by Mlisana et al among patients with acute HIV infections, prevalence of anaemia was reported to range from 52.6% at 3

months post-infection, 61.1% at 6 months post-infection, and 51.4% at 12 months post-infection[23].

PATHOGENESIS OF ANAEMIA IN HIV INFECTED CHILDREN

Anemia occurs frequently among patients seropositive for human immunodeficiency virus (HIV), but its multifactorial origin complicates its differential diagnosis and adequate treatment[24]. In addition, the etiology of anemia in HIV infection often remains unclear. In recent years several attempts have been undertaken to elucidate the mechanisms leading to HIV-associated anemia.

The Direct effect of HIV infection on erythroid progenitors

Direct infection of erythroid progenitors has been discussed, but could not be proven[25]. Furthermore, soluble factors like HIV proteins and cytokines have been suggested to inhibit growth of haematopietic cells in the bone marrow of HIV-infected patients. Exactly how the virus mediates these effects remains uncertain, but both in vivo and in vitro studies have pointed up possible direct and indirect modes of haematopoietic suppression. Whether a significant fraction of CD34+ cells in vivo are infected with HIV remains controversial, but most studies using in situ polymerase chain reaction techniques would suggest not. Other more indirect modes of haematopoietic cell suppression such as production of autoantibodies, production of other humoral inhibitory factors, T-cell mediated suppression of haematopoiesis, or production of inhibitory or stimulatory cytokines may also be contributory. It is probable that several of these mechanisms may occur simultaneously, and an increased understanding of their role may lead to improved strategies to correct the cytopenias which often accompany HIV disease[25]. Inflammatory cytokines such as transforming growth factor-β, interleukin (IL)-1β, tumour necrosis factor (TNF)-α and interferon-γ also appear to play a role by inhibiting erythropoiesis in vitro. In HIV-infected patients with anaemia, TNF-α and IL-1β levels are elevated[26]. However, so far no statements can be made whether these factors are directly involved in myelosuppression or mediate their effect by inhibiting growth-factor synthesis.

The role of drugs for treatment of HIV and its complications

Many drugs for the treatment of HIV or its complications are myelosuppressive and account for a large number of anaemia in patients with HIV. Zidovudine is a common cause of drug-induced anemia in this patient population [26]. This type of anaemia is generally dose and time dependent and macrocytic in nature Inhibition of thymidine monophosphate by Zidovudine has been speculated to lead to intracellular thymidine triphosphate deficiency and subsequent suppression of haematopoietic colony formation, particularly erythroid colony formation [24, 26] It is of note, however, that modern dosages of < 4mg/kg zidovudine (ZDV) twice a day rarely cause anaemia. Instead, other drugs that can induce anemia itself or by enhancing ZDV plasma concentrations must be considered important contributing factors Other drugs that have been shown to cause anaemia in HIV are the following: amphotericin B, antineoplastic therapy, cidofovir, dapsone, flucytosine, foscarnet, ganciclovir, hydroxyurea, interferon-α, pentamidine, primaquine, pyrimethamine, ribavirin, sulfonamides, and trimethoprim. Many patients with HIV have a positive direct coombs test, indicating red blood cells coated with immunoglobulin. Surprisingly, the frequency of actual haemolysis is extremely low. In patients with glucose-6-phosphate dehydrogenase deficiency, oxidant drugs such as dapsone or sulfamethoxazole can precipitate hemolytic anaemia. Thrombotic thrombocytopenic purpura, characterized by hemolytic anemia and thrombocytopenia, also occurs with increased frequency in patients with HIV. Finally, haemolytic anaemia may occur secondary to hypersplenism, which largely is due to portal hypertension, a result of coinfection with hepatitis B or C [26].

Micronutrient deficiencies

Deficiency of vitamin B12, folate and iron are frequently reported in HIV patients[24]. However, specific investigations revealed appropriate storage amounts of these micronutrients. Supplementation may be beneficial in some patients, but often fails to reverse anemia in this population. In anaemic HIV patients reticulocytopenia is a consistent finding. Additionally, inadequately low endogenous erythropoietin concentrations have been repeatedly reported. Thus, it is speculated

that a blunted erythropoietin feedback mechanism contributes substantially to the pathogenesis of anemia in HIV patients[24].

Defects in iron metabolism are another common feature of anaemia of chronic disease. Iron studies reveal a low serum iron level, a low total iron-binding capacity, low transferrin saturation and normal or increased ferritin level. Normal or increased iron stores indicate that a functional block of iron release may disturb iron utilization in HIV infection [26]

Opportunistic complications

Opportunistic complications represent the underlying cause for anaemia in a large number of HIV-infected patients. Infections and malignancies can cause anaemia in this patient population. Atypical mycobacteria, particularly Mycobacterium avium complex, are the most common bacterial pathogens to infect bone marrow in patients with HIV [26, 27] .The mechanisms likely responsible for anaemia in these patients are elevated serum concentrations of TNF-α and direct invasion of the bone marrow by the organism [28]. Other pathogens that may contribute to the development of anaemia are cytomegalovirus, parvovirus B19, Cryptococcus neoformans, Toxoplasma gondii, Histoplasma sp, Pneumocystis jiroveci. and Leishmania sp [26]. Malignancies such as non-Hodgkin's lymphoma, Hodgkin's lymphoma, and Kaposi's sarcoma can infiltrate the bone marrow and subsequently cause anaemia. Patients with HIV may develop vitamin B12, folate, with resultant anaemia[26, 28]. Finally, blood loss, specifically gastrointestinal bleeding, may occur owing to complications of AIDS such as intestinal lymphoma, Kaposi's sarcoma, cytomegalovirus, and candidiasis[27, 28].

POSSIBLE FACTORS ASSOCIATED WITH ANAEMIA IN CHILDREN

Nutritional factors

A number of factors have been associated with anaemia. In a study conducted in Malawi among children, vitamin A deficiency and vitamin B_{12} deficiency were associated with severe anemia[29].However Iron deficiency was not prevalent in case patients. Another study done in South Africa indicated that iron depletion and iron deficiency anaemia was present in 73% of HIV-infected children in South Africa, something which is different from the developed countries[16].Significantly more children with moderate and severe disease, and severe immunosuppression had abnormal RBC morphology. 52% were iron-depleted, 20% had iron-deficient erythropoiesis and 18% iron deficiency anaemia (IDA). 16% (7/44) of anaemic children had microcytosis and hypochromia. Median soluble transferrin receptor concentration was significantly higher in those with microcytic hypochromic anaemia (42.0 nmol/L v 30.0 nmol/L, $p = 0.008$).

In their study of anaemia among HIV infected children, Balbaryski J et al[30] defined two groups of patients; one with microcytic anaemia (children under 24 months of age with mild disease) and the other group with normocytic or macrocytic anaemia (children above 24 months of age with advanced immunodeficiency) and thus concluded that the most frequent form of anaemia in children with mild HIV disease had features of Iron deficiency anaemia disease while anaemia in patients with advanced immunodeficiency had the characteristics of anaemia of chronic disease. Some literature has also reported macrocytic anaemia to be common among HIV infected individuals[30].

Zidovudine treatment

In a study done by Aurpibul L et al in Thailand, switching from stavudine(d4T) to zidovudine(ZDV) was associated with severe anaemia in 10% of the children receiving HAART[31]. Also there was a statistically significant decreases in haemoglobin level, white blood cell counts (WBC) count and absolute neutrophil count (ANC occurred following the substitution of d4T with ZDV, but the magnitudes of the decreases were small and not clinically significant .

Because antituberculosis agents and zidovudine are commonly used in HIV-infected patients , studies on their effect on the haematopoetic cells have also been performed. A group of 24 consecutive human immunodeficiency virus (HIV)-infected patients with tuberculosis who received concomitant antituberculosis therapy and zidovudine (tuberculosis group) were compared with 24 patients who received zidovudine but not antituberculosis medications (comparison group). Comparison patients were matched to tuberculosis patients by age, sex, ethnic group, month of starting zidovudine, and CD4 cell count. The frequency and severity of leukopenia and granulocytopenia were similar in tuberculosis and comparison patients, but marked anemia (hemoglobin less than 9.5 g/dl) developed in 50% of tuberculosis patients and 17% of comparison patients ($p = 0.03$). (32).

Role of infections

Anaemia is an important negative predictor for survival with disseminated Mycobacterium avium complex (MAC) infection in the acquired immunodeficiency syndrome (AIDS).The profound anaemia in MAC-infected AIDS patients is due to suppression of erythroid progenitors by a soluble factor(s) in the serum. The data suggest that the soluble factor(s) is probably elaborated by macrophages.[33].In another study, 100% of the 10 patients with disseminated TB were anaemic. A bone marrow aspirate and/or trephine biopsy performed in six of them revealed evidence of red cell hypoplasia[34]

Diarrhea has also been reported to be associated with anaemia in HIV. In one study Cryptosporidium associated diarrhea was detected in 21 (3.8%) individual stool samples collected from 553 pediatric patients hospitalized. Concomitant diseases observed were malnutrition, acute leukemia, bronchiolitis, HIV infection, anemia, celiac disease, myelofibrosis, vitelline sac tumor, neutropenia, osteosarcoma and dehydration[35].

Children with human immunodeficiency virus (HIV) infection have a higher prevalence of intestinal malabsorption[36]. Anemia is also a common feature in these children. In one study, iron deficiency was detected in 48% of patients, and it was significantly associated with intestinal iron malabsorption[36]. Low hemoglobin levels were detected in 66% of patients. The majority of children with iron

deficiency also had anemia. Preliminary data showed that oral iron administration was sufficient for raising hemoglobin in children with normal iron absorption, whereas parenteral administration was required in those with iron malabsorption. Therefore iron deficiency was a major feature of pediatric HIV infection was related to intestinal malabsorption contributed to anemia.

The relationship between HIV and intestinal helminths (Ascaris lumbricoides, Trichuris trichiura and hookworm) was assessed in a cross-sectional study conducted in 2002 among 907 adults in Tanga Region, Tanzania. Overall prevalences were, 1.2% for A. lumbricoides, 7.1% for T. trichiura and 75.7% for hookworm. When sex and age were controlled for, there was a statistically significant positive association between HIV and malaria. Infection with HIV and hookworms, was associated with a significant reduction in haemoglobin concentration [37].

HIV and malaria are among the leading causes of morbidity and mortality in sub-Saharan Africa, home to 10% of the world's population. An association between HIV and malaria is expected in theory, however, there is conflicting evidence regarding the impact of HIV infection on parasite loads[38]. HIV-associated immunosuppression contributes to more frequent and more severe malaria and reduced efficacy of antimalarials in pregnant women and adults Co-infection with malaria and HIV in pregnant women is associated with anemia, low birth weight, and increased risk of infant mortality to a greater extent than infection with either disease alone [38]. In a study done among 687 children 6-60 months of age who were admitted to hospital with pneumonia, HIV and malaria co-infected patients were found to have a high incidence of anemia [39].

Worsening HIV disease parameters

Low CD4 counts [(<25% for children less than 11 months, <20% for children 12-35months months, <15% for children 36 months and above)] and higher HIV-1 RNA levels in plasma have been associated independently associated with increased risk of anaemia in multivariate analyses[40].

MANAGEMENT OF ANAEMIA IN HIV INFECTED CHILDREN

Despite important advances in antiretroviral therapy, anaemia remains a problem in many HIV-infected patients. Although the incidence of anaemia in these patients has decreased, its prevalence appears to have stabilized or decreased only slightly. The management of anaemia typically includes correction of the underlying cause(s) .and blood transfusion or erythropoietin [41].

Prevention of anaemia

Prevention of anaemia should be the mainstay of management. As studies have identified that patients with CD4 level<25% and opportunistic disease are at increased risk, intervention with HAART along current treatment guidelines (i.e. before CD4 falls below 200/mm3) should reduce the risk of an individual developing anaemia[41].As randomized clinical trials in the HAART era have demonstrated that differences in anaemia risk exists between thymidine analogs it may be appropriate to consider regimens that do not contain AZT in these individuals, any person who presents with low haemoglobin and individuals requiring other myelosuppressive agents. Also HAART has been associated with an improvement in anaemia, and potential mechanisms that may be involved include a reduction in opportunistic infections and the anemia of chronic disease and an improvement in nutritional status[42]. A multivariate of the WIHS study found that HAART was significantly associated with correction; improvement was noted within six months and a greater resolution occurred after a longer duration of HAART[40]. Additionally, dietary advice to optimize nutrient intake, correction of nutritional deficiencies and to provide guidance in heart healthy eating should be initiated at presentation with HIV infection.

Elimination of causation

Once anaemia develops intervention is appropriate as it may both improve quality of life and reduce morbidity and mortality. The short-term risks of anaemia may be greatest in individuals with other health problems most notably cardiac and renal disease therefore these individuals may require early intervention. The investigation of anaemia in HIV infection follows along the same lines as in general medicine and

should routinely include drug history, enquiry about blood loss particularly in stools, urine, evaluation of vitamin B12, folate and iron stores, as well as investigation for parvovirus B19 and if the CD4 level is <25% for other infections such as Mycobacterium avium or Cryptococcus neoformans. Assessment of erythropoietin levels may be appropriate if intervention with this agent is being considered and may be useful in assessing whether anaemia is related to AZT (where levels are generally normal or elevated) or chronic disease (where levels may be low). Interventions for anaemia are limited. Drug switching, to a regimen which does not contain AZT should be considered, but has not been specifically investigated in the HAART era. In the absence of clear evidence of iron eficiency, iron supplementation should be avoided, as this may be associated with accelerated disease progression. Evidence for the risk of iron overload comes from several sources. Simultaneous administration of low doses of oral iron with dapsone for the prophylaxis of Pneumocystis pneumonia in HIV-positive patients was associated with excess mortality relative to those receiving aerosolised pentamadine. A retrospective cohort study in thallasemia major patients found the rate of disease progression was significantly faster in patients receiving lower doses of iron-chelating desferrioxamine and higher serum ferritin concentrations. A study of haptoglobin polymorphisms in HIV positive subjects indicated that the haptoglobin 2-2 polymorphism was associated with both higher iron stores and shortened survival as compared with the haptoglobin 1-1 or 2-1 phenotypes and a retrospective study of bone marrow macrophage iron in HIVpositive patients suggested that survival is shorter with high iron stores[43, 44]. These studies were not conducted in the HAART era and we all performed in the developed world. Investigations in other settings are required to confirm these concerns are real and generally applicable. Supplementation with B12 and folate has not been suggested to be harmful but is not effective in the setting of AZT related anaemia[45].

Use of recombinant human erythropoietin

Recombinant human erythropoietin (rHuEPO) is the mainstay of treatment for anaemia. The evidence for effectiveness of this agent, at least at a dose of 100 U/kg thrice weekly by intravenous bolus, is limited to persons with endogenous

erythropoietin levels ≤500 IU/L6[46, 47]. Above this level benefit has not been demonstrated. As individuals with AZT related anaemia may have erythropoietin levels ranging from 9-3,390 mU/ml10 it is clear that rHuEPO will not be suitable for all patients. Therefore, measurement of endogenous erythropoietin levels should be performed prior to initiation of rHuEPO treatment. Erythropoietin, administered as 100-300 units/kg subcutaneously 3 times a week, has been demonstrated to increase haemoglobin levels by 2.5 g/dl over a 4 month period in 221 patients with HIV-infection and Hb <11 g/dl[34]. A second study of 251 anaemic patients (defined as hematocrit <30%) with AIDS and serum erythropoietin level ≤500 IU/L used an initial rHuEPO dosage of 4,000 units subcutaneously for 6 days each week. Based on the patient's response to therapy, the dosage was increased sequentially to 8,000 units subcutaneously for 6 days per week. Changes in mean hematocrit level from a baseline of 27.9% to 33.6% were observed at week 12 ($p < .0001$) and to 34.5% at week 24 ($p < .0001$)35. Quality of life improvements were demonstrated in both these studies most notably in terms of energy and physical functioning[34,35]. Additionally, rHuEPO reduces transfusion requirements in HIV-infected patients who have endogenous erythropoietin levels of ≤500 U/L[46]. Therapy with rHuEPO is generally well tolerated with adverse events rates similar to placebo injections6. Current recommendations for rHuEPO use in persons with endogenous erythropoietin <500 U/ml are to initiate with 100-300 U/kg subcutaneously thrice weekly with the aim to increase Hb by 1 g/dl by week 4. Once weekly dosing of rHuEPO is under consideration. Higher doses, up to 60,000 U/week, of rHuEPO can be considered if a satifactory response is not observed. Once Hb has reached 13 g/dl rHuEPO can be discontinued and resumed once Hb falls below 12 g/dl3. These expert opinion based guidelines encourage more aggressive treatment of anaemia than is currently routine in clinical practice and are likely to, in part, reflect the authors' appreciation from clinical trials experience of the quality of life benefits that treatment of anaemia brings. The main obstacle to the prescription of rHuEPO is cost. Cost effectiveness studies in a range of disease areas including HIV-infected children (but not

adults) suggest the drug costs may readily be offset against transfusion costs[48]. As rHuEPO treatment improves quality of life in HIV and treatment of anaemia in HIV

is associated with improved survival analysis of cost per quality adjusted life years (QALYs) saved would be feasible in HIV. However such an analysis has not been reported to date. In cancer-associated anaemia, where rHuEPO improves quality of life but generally not survival, rHuEPO is cost effective. Estimates from a range of case based scenarios with varying assumptions are that the effectiveness resulting from $US 1 spent on standard care can be achieved with only $US 0.81 of rHuEPO care[49]. That is to say, all costs considered rHuEPO may actually save the healthcare budget money

Role of Blood Transfusion

Transfusions should be used when rapid recovery is required, and the underlying conditions causing anemia should be treated, if possible. Recent research has shown that anemia and transfusion are associated with accelerated mortality in patients with human immunodeficiency virus type 1 (HIV-1) infection. It appears that blood transfusions may directly accelerate HIV-1 disease progression through activation of HIV-1 expression and/or transfusion related immunosuppression. In vitro and in vivo evidence suggests that immunization with common recall antigens (eg, tetanus toxoid) can significantly increase plasma HIV viremia, as well as the in vitro susceptibility of peripheral blood mononuclear cells to acute infection. Allogeneic leukocytes also present an antigenic challenge to HIV-infected mononuclear cells, which may cause them to proliferate and increase viral production. In vitro incubation of HIV-infected mononuclear cells with allogeneic blood components (mainly leukocytes) results in increased viral replication. Whether the effects of transfusion on HIV viral load are due to a reduced immune response and/or activation of the virus itself remains to be determined. Preliminary data suggest that leukocyte depletion prior to RBC transfusion does not alter viral replication compared with standard packed RBC transfusions[50].

PROGNOSTIC IMPLICATIONS OF ANAEMIA IN HIV

Anaemia is a prognostic marker of future disease progression or death, independent of CD4 and viral load. Recovery from anaemia reduces the risk of disease progression to approximately the same level as seen among patients who have never had anaemia. Additionally, anaemia impacts a range of dimensions of quality of life, most commonly through fatigue[41].

Impact on survival

The prospective, multicenter cohort study EuroSIDA has previously reported on predictors and outcomes of anemia in patients infected with human immunodeficiency virus. In a Cox proportional-hazards model with serial measures of CD4+ cell count, plasma viral load, and degrees of anemia fitted as time-dependent variables, the relative hazard of death increased markedly for patients with anemia versus no anemia[51]. A clinical scoring system was developed and validated for patients receiving highly active antiretroviral therapy using the most recent laboratory measures. Mild and severe anaemia were independently (P<.01) associated with clinical disease progression, with a relative hazard of disease progression of 2.2 (95% confidence interval [CI], 1.6-2.9) and 7.1 (95% CI, 2.5-20.1), respectively, compared with patients with no anemia[51].

Studies have consistently found anemia to be associated with reduced survival, even when potentially confounding factors were controlled for[52]. Although HIV infection is a strong predictor of death among children , preventable conditions including anaemia contribute significantly to infant and child mortality independent of HIV infection[39].

Impact on the quality of life

The relationship between changes in hemoglobin level and energy and physical functioning in the anemic and "normal" ranges of hemoglobin among individuals with AIDS has also been studied, a higher hemoglobin level was associated with a higher energy score and a higher physical functioning score (P < .001 for both), after adjusting for CD4 lymphocyte count, sex, age, education, and HIV risk factor. In longitudinal analyses, increases in hemoglobin were associated with increases in

energy and physical functioning scores ($P < .001$ for both), after adjusting for CD4 lymphocyte count, sex, age, education, and HIV risk factor. Higher levels of hemoglobin are associated with better quality of life among individuals with AIDS. Changes in hemoglobin level within the conventional normal range of hemoglobin are also significantly associated with changes in quality of life[53].

The negative impact of anaemia on patient quality of life (QOL), functional status, and treatment outcomes underscores the need for its correction in these patients. It has also been found that the gains in overall QOL have been significantly and directly related to increases in Hb, with maximum QOL gains in the range of Hb levels of 11-13 g/dL, supporting the need to achieve and maintain Hb levels > or =12 g/dL in an effort to preserve and maximize QOL benefits[54]. Anaemia in HIV-infected individuals, still a common haematological complication in the highly active antiretroviral therapy era, is associated with shortened survival, increases in the rate of disease progression, and reduction in quality of life[55].

STATEMENT OF THE PROBLEM

Anaemia is a major cause of sickness and death among children in sub-Saharan Africa [56, 57]. In various settings 12-20% of HIV hospitalized children are severely anaemic [56, 57]Certainly children admitted to hospitals with severe anemia (Haemoglobin <8g/dl) are more likely to die than those children admitted with mild or without anemia. Even when blood transfusion services are available, there is a significant case fatality rate of 6-18 %[56].

According to the available literature, data on the magnitude of anaemia among African HIV children are scarce [29]. There is also a knowledge gap about the clear pathophysiology of anaemia among African HIV children. Earlier it was reported that nomocytic nomochromic anaemia was common among HIV infected individuals, however a few published studies indicated that hypochromic microcytic anaemia to be the commonest type of anaemia particularly in Sub Saharan Africa. Without a better understanding of its pathogenesis in our local setting, it will be difficult to make rational improvement in management of anaemia among HIV infected children

STUDY RATIONALE

Though anaemia is still a major problem in patients infected with HIV/AIDS despite the introduction of HAART, there is paucity of data describing the magnitude and determinants among children in Tanzania. Most studies in Africa were conducted before the era of HIV and HAART. These studies were mainly confined to the anaemia associated with malaria, iron deficiency or with other individual factors [29, 57]. As a result, the treatment guidelines advocated by the World Health Organization (WHO) deal specifically with malaria, folate deficiency, and iron deficiency, which are widely held to be the most common causes of anaemia in African children[58]. To improve the understanding of anaemia in HIV era, a cross-sectional study among HIV children was conducted to determine its magnitude and the factors involved. Thus it was important to identify the various risks factors that may be implicated in anaemia, thus prevent or retard its occurrence or ameliorate its severity. Identification of these factors would also assist in development of effective strategies for controlling anaemia.

RESEARCH QUESTIONS

1. To what extent was anaemia prevalent among HIV infected children in attending public hospital in Dar es Salaam?

2. What were the possible factors that could be associated with anaemia among HIV infected children attending public hospitals in Dar es Salaam

3. What was the spectrum of blood cells' morphology on blood film in anaemic HIV infected children?

STUDY OBJECTIVES

Broad objective

To determine the prevalence and factors contributing to anemia in HIV infected children attending public hospitals in Dar es Salaam.

Specific Objectives

1. To determine the prevalence of anaemia in HIV infected children
3. To determine the factors contributing to anaemia in HIV infected children
2. To describe the spectrum of blood cells' morphology on blood films in anaemic HIV infected children

METHODOLOGY

Study setting

The study was conducted in the HIV paediatric care and treatment clinics and wards at the Muhimbili National Hospital (MNH) and Mwanayamala Municipal Hospital in the city of Dar es Salaam, Tanzania. The Dar es Salaam city has four referral government hospitals being Muhimbili National Hospital (MNH) and three municipal hospitals. The MNH is a national referral hospital for both inpatient and outpatient from municipal and other referral hospitals across the country. The MNH runs a paediatric HIV clinic from Monday to Friday with an average of 15-20 children attending (at least once) per day and 2-5 patients /week with HIV admitted in a paediatric ward. Mwananyamala Hospital is one of the three municipal hospitals in Dar es Salaam serving as a secondary referral hospital. It has also a paediatric HIV clinic which runs from Monday to Saturday with 10-15 children attended per day with an average of 2-3 children with HIV being admitted in the paediatric ward.

Study design

This was a descriptive cross sectional study. Standard structured questionnaires (as data instruments) were used to collect information from the children's parents/guardians and from the patients' file and laboratory results.

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Study subjects

The target population for the study included all consecutive HIV infected children attending the HIV clinic or admitted in paediatric wards for the period of August to November 2008. The subjects were of 6 months to 59 months of age. Those who were declined to participate in the study were excluded from the study

Sample size and Sample calculations

A minimum sample size for the study was obtained using the sample size estimation formula of $N=Z^2 P (1-P)/d^2$ whereby

N=Minimum sample size

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

P= Prevalence of anaemia among HIV children. As per literature review [19] this is 12%

d=Margin of error (taken as 5% in this study)

Putting P=12%, d=5%, N=162

Data collection

All children's parents/guardians were interviewed by the research physician using a structured questionnaire No 1 assigned an identity number. The following information at the time of study was collected: The name of child, his/her file number, age, sex, residence, ethnicity, level of education of the guardian/parent, and occupation. The history also included the HAART and cotrimoxazole (TMP-SMX) status, having a history of TB disease in the past six months at the study and whether the child was known to have sickle cell disease. Other information included history of chronic diarrhea (in two weeks or more), history of being malnourished as documented in the file, history of recurrent fevers (one episode every month), history of recurrent malarial attack (every month) and being treated for helminthiasis in the past three months. The questionnaire was translated into Swahili language for ease understanding.

Physical examination

Each study subject had physical examination on anthropometric measurements, clinical WHO stage, and presence of pallor, jaundice, lymphadenopathy (>2cm length), hepatomegaly and splenomegaly. This was done by the attending research physician. The information was captured in the structured questionnaire No 1

Collection of Specimens

The following tests were requested for each study subject: full blood picture, peripheral smear, reticulocyte count, LDH, bilirubin, Coombs test, CD4 level, stool for ova or cyst, occult blood, urine for rbc/ova, blood smear for malaria, and sickling test.The results were entered in a questionnaire No 2. The specimens were transported to the laboratory and processed within 4 hours. All specimens were processed at Muhimbili National Hospital Central Laboratory. A laboratory technician was available to assist in storing, transporting and processing these specimens.

Laboratory Methods

Haematology tests

Blood for haematological tests was collected from the study subjects using sterile vacutainers with EDTA anticoagulants. A total of 6mls of venous blood was collected from each child from the cephalic/medial vein or femoral vein (depending on the accessibility to the vein) by the research physician. The blood sample was utilized for full blood picture, smear, sickling solubility test Coombs test and reticulocyte count. Full blood picture was done using a CBC Coulter Counter (Coulter Corp, Miami, FL). The blood films stained with leishman stain were examined by the principal investigator with support from experienced haematologists who identified the blood cell morphology and presence of malaria parasites. Reticulocyte count was determined using New Methylene blue staining.

Biochemical tests and Immunological tests

Measurement of bilirubin and lactate dehydrogenase (LDH) was done in biochemistry laboratory.

The CD4 count was analyzed using FACS Calibur-BD machine.

Parasitological tests

Kato technique and filter method was used to examine stool for ova/cyst and urine for schistosoma ova, respectively. N-multistix with ten parameters was used to

examine red blood cells in urine. Specimens were collected using special containers for stool and bottles for urine.

Statistical Methods

The data were entered into a computer and cleaned using the SPSS package software Version 15.0.The prevalence of anemia was determined as a percentage among all HIV infected children whose haemoglobin level was measured during the study. To describe the spectrum of red blood cell (RBC) morphology on blood films a pairwise comparison was conducted using Chi squire test or Fisher's exact test. Both univariate and multivariate logistic regression analyses were performed to identify possible risk factors associated with anaemia (Hb<11g/dl) in HIV-infected children. Subnalysis of factors associated with severe anaemia (Hb<8g/dl) was also done. Adjusted and unadjusted odds ratios (OR) were calculated to find out the strength of association. The final models for factors strongly associated with either anaemia or severe anaemia was derived by forward likelihood ratio method. All analyses were conducted with SPSS v 15.0. Test of significance was 2-sided with a probability cut-off value of 0.05.

Recruitment of research assistants

One clinician and one laboratory technician were recruited in the process of data collection at the clinics, ward and laboratory. The training was conducted for one day and emphasis was on understanding the objective of the study and the contents of the questions to be asked to the study subjects.

Definition of Terms

Anaemia was defined as hemoglobin level of less than 11g/dl. It was graded as follows; (basing on WHO classification)[59]

Grade 1 anaemia (mild); Haemoglobin level (g/dl) of 9.5-10.9

Grade 2 anaemia (moderate); Haemoglobin level (g/dl) of 8.0-9.4

Grade 3 anaemia (severe); Haemoglobin level (g/dl) of 6.5-7.9

Grade 4 anaemia (life-threatening); Haemoglobin level (g/dl) of less than 6.5g/dl

RESULTS

Social demographic and clinical characteristics of study subjects

A total of 167 HIV infected children were recruited for the study. The mean age of study subjects was 3.7 yrs, the minimum being 0.6 yr and maximum being 4.9yrs. The majority of the study subjects were males (53.9%) as seen in Table 1. There was almost an equal number of study subjects who were recruited at Muhimbili National Hospital (49.7%) and those who were recruited at Mwananyamala Hospital (50.3%). Likewise, 49.7% of the children were seen at the outpatient clinic and 50.3% were seen in the inpatient unit. The majority of the children had mothers/guardian who were unemployed (64.7%) but had some formal education (86.8%).

The study also indicated that about two thirds of the study population were on HAART at the time of recruitment. Similarly the majority of study subjects were in WHO clinical stage 3 or 4 and had CD4% level of <25% at the time of the study. Lymphadenopathy appeared to be a common clinical manifestation while few patients had jaundice, splenomegaly or hepatomegaly (Table 1).

It was also found that HIV infected children recruited at the outpatient clinic did not differ from those recruited in the admitting wards with respect to age, their maternal occupation, maternal level of education, ARV status, WHO stage , % CD4 level status and presentation with hepatomegaly (see appendix on table 1a). Jaundice and splenomegaly were more common in admitted patients (13% and 10%, respectively) than in outpatients (4% and 3.6%, respectively) though the differences were not statistically significant (P=0.06 and 0.07, respectively). Lymphadenopathy was common in outpatients.

Table 1: Characteristics of HIV infected children by sex

Variable	Male n %	Female n %	Total n %
All	90(53.9)	77(46.1)	167(100)
Mean age ±SD*	3.8±1.2	3.5±1.3	3.7±1.3
Hospital of Recruitment			
Muhimbili	45(50.0)	38(49.4)	83(49.7)
Mwananyamala	45(50.0)	39(50.8)	84(50.3)
Source of recruitment			
Inpatient	52(57.8)	31(40.3)	83(49.7)
Outpatient	38(42.2)	46(59.7)	84(50.3)
Maternal occupation			
Unemployed	56(62.2)	52(67.5)	108(64.7)
Employed	22(24.4)	19(13.0)	32(19.2)
Business	12(13.3)	15(19.5)	27(16.2)
Maternal education			
% with no formal education	08(08.9)	14(18.2)	22(13.2)
HAART$^{\epsilon}$ status			
% on HAART	60(66.7)	48(62.3)	108(64.7)
WHO stage			
2	02(02.2)	05(07.8)	08(4.8)
3	76(84.4)	59(76.6)	135(80.8)
4	12(13.3)	12(15.6)	24(14.4)
CD4 %			
>30	41(45.6)	39(50.6)	80(47;9)
25-30	10(11.1)	07(09.1)	17(10.2)
<25	39(43.3)	31(40.3)	70(41.9)
% Jaundice	06(6.7)	09 (11.7)	15(09.0)
% Lymphadenopathy	23(25.8)	16(20.8)	39(23.4)
% Hepatomegaly	07(07.80	07(09.1)	14(08.4)
% Splenomegaly	07(7.8)	05(06.5)	12(7.2)

Note. .*indicate numbers and not %. $^{\epsilon}$HAART- Highly Active Anti Retroviral Therapy. SD*-Standard deviation

Prevalence of anaemia in HIV infected children

In this study the overall prevalence of anaemia (Hb<11g/dl) among HIV infected children attending public hospitals was 44% (Table 2). Among 167 enrolled HIV infected children, 35 (21.1% had mild anaemia, 14(8.4%) had moderate anaemia and 26(15.6%) had severe anaemia. No difference was found in the prevalence of anaemia between HIV infected children recruited at the outpatient clinic and those recruited in the admitting wards. However, on grading of anaemia the prevalence of severe

anaemia was significantly higher (P=0.002) among children who were recruited in the admitting wards (19 %) than those who were recruited at the outpatient clinic (12%). Microcytic anaemia was found to be the commonest (73%) type of anaemia followed by normocytic normochromic anaemia.

The findings also indicated that the mean ages were slightly lower for HIV infected children with moderate and severe anaemia (Table 3).The prevalence of anaemia was higher among males (48.9%) compared to females (40.3%). The severity of anaemia did not differ much with respect to the hospital from which study subjects were recruited though moderate anaemia was slightly more prevalence among children who were recruited at Muhimbili National Hospital (10.8%) compared to those who were recruited at Mwananyamala Hospital (6.0%).

Table 2: Prevalence of anaemia (Hb<11g/dl) in HIV infected children

Variable	All N=167 n (%)
Anaemia status	
Normal	92(55.1)
Anaemia	75(44.9)
Degree of anaemia	
Normal (Hb≥11g/dl)	92(55.1)
Mild (Hb=9.5-10.9g/dl)	35(21.1)
Moderate(Hb=8.0-9.4g/dl)	14(08.4)
Severe(Hb <8.0g/dl)	26(15.6)
Type of anaemia	
Normocytic	19/75(25.3)
Macrocytic	03/75(04.0)
Microcytic	53/75(70.7)

Children whose mothers were unemployed had a higher prevalence of severe anaemia (19%) than those whose mothers were employed (12.5%) or had business (3.7%). The prevalence of anaemia was high in children with advanced WHO

clinical stage 4 (75%) and severe immunosuppression (51.2%). Children with hepatomegaly and splenomegaly appeared to have a high prevalence of anaemia (50% and 66% respectively). Likewise, the majority of HIV infected children with hepatomegaly splenomegaly and jaundice appeared to have a higher prevalence of severe anaemia.

Table 3: Characteristics of HIV infected children by prevalence and severity of anaemia

Characteristic	Total 167	Normal ≥11g/dl %	Mild anemia 10.9-9.5g/dl %	Moderate anemia 9.4-8 g/dl %	Severe anemia[¥] <8g/dl %
Mean age N(in yrs)*	167/ (3.6)	92 (4.2)	35(3.5)	14(1.8)	26(2.9)
Range (in yrs)+	0.6-4.9	1.6-4.9	1.0-4.9	0.6-4.8	0.9-4.9
Sex					
Male	90	51.1	22.2	7.8	18.9
Female	77	59.7	19.5	9.1	11.7
Hospital					
MNH®	83	54.2	20.5	10.8	14.5
MWANA©	84	56.0	21.4	6.0	16.7
Source of recruitment					
OPD	83	55.4	30.1	2.4	12.0
IPD	84	54.8	11.9	14.3	19.0
Maternal occupation					
Unemployed	102	54.6	16.7	9.3	19.4
Employed	32	56.3	25.0	6.3	12.5
Business	27	55.6	33.3	7.4	3.7
Maternal level of education					
No formal education	22	63.6	18.2	0.0	18.2
Formal education	145	53.8	21.4	9.4	15.2
HAART status					
On HAART	108	69.4	20.4	1.9	8.3
Not on HAART	59	28.8	22.0	20.3	28.8
WHO stage					
2	8	87.5	12.5	00.0	0.0
3	135	58.5	20.7	08.1	12.6
4	24	25.0	25.0	12.5	37.5
CD4 % level					
>30	80	60.0	21.3	10.0	08.8
25-30	17	58.8	23.5	05.9	11.8
<25	70	48.6	20.0	07.1	24.3
Jaundice	15	46.7	13.3	06.7	33.4
Lymphadenopathy	39	53.8	17.9	07.7	20.5
Hepatomegaly	14	35.7	07.1	07.1	50. 0
Splenomegaly	12	25.0	08.3	0 .0	66.7

Note. .*[and +]indicate numbers and not %. ® Muhimbili National Hospital,© Mwananyamala, ¥ severe anaemia included both severe to life threatening anaemia as per WHO definition, IPD-inpatient ,OPD- outpatient

Risk factors for anaemia in HIV infected children

In a univariate analysis (Table 4), not being on HAART, advanced WHO stage, CD% <25%, having a history of TB disease in the past six months at the study, history of chronic diarrhoea (14 days or more), history of malnutrition or history of recurrent malarial attacks (every month), and having hookworm infestation were all associated with anaemia. Study subjects who were > 2.7 years of age had a lower risk of developing anaemia than those who were ≤ 2.7 years. Likewise, being HIV positive for ≥ 2.5 years resulted into a low risk of anaemia compared to being HIV positive for <2.5 years. The of use of anthelmintics in three months prior the study and taking multivitamins were found to be protective against anaemia in HIV children

Table 4: Univariate analysis of factors associated with anaemia (<11g/dl) in HIV infected children

Variable	Univariate	
	Anaemic children n/N (%)	OR(95%CI)
Age group(yrs)		
≤2.7	32(97.0)	1
>2.7	43/134(32.1)	0.1(0.02-0.8)*
Sex		
Male	44/90(48.9)	1
Female	31/77(40.3)	0.7(0.3-1.3)
Maternal occupation		
Unemployed	49/108(45.4)	1
Employed	14/32(43.8)	0.9(0.4-2.0)
Business	12/27(44.4)	0.9(0.4-2.3)
Maternal educations		
No formal education	8/22(36.4)	1
Formal education	67/145(33.3)	0.6 (0.1-1.9)
Duration of HIV positivity(yrs)		
<2.5	55/80(68.8)	1
≥2.5	20/87(23.0)	0.1(0.6-0.3)*
HAART status		
On HAART	33/108(30.8)	1
Not on HAART	42/59(71.2)	5.6 (2.7-11.2)*
HAART regimen		
ZDV based regimen	50/108(46.2)	1
Non ZDV based regimen	25/59(42.3)	0.8 (0.1-3.2)
WHO stage+		
3	56/135(41.5)	1
4	18/24(75.0)	4.2(1.6-11.3)*
CD4 %		
>30	32/80(40.0)	1
25-30	07/17(41.2)	1.0(0.3-3.0)
<25	36/70(51.4)	1.5(1.1-4.3)*
History of TB disease		
No	52/135(38.5)	1
Yes	23/32(71.9)	4.0(1.7-9.4)*
History of chronic diarrhoea		
No	48/128(37.5)	1
Yes	27/39(69.2)	3.7(1.7-8.0)*
History of recurrent fever		
No	26/65(40.0)	1
Yes	49/102(48.0)	1.3(0.7-2.6)*
History of recurrent malaria		
No	53/137(38.7)	1
Yes	22/30(73.3)	4.3(1.8-10.5)*

* P value <0.05, +8 subjects in WHO stage 2 excluded for analysis

Cont.. Table 4: Univariate analysis of factors associated with anaemia (<11g/dl) in HIV infected children

Variable	Anaemic children n/N (%)	Univariate OR(95%CI)
History of recurrent malaria		
No	53/137(38.7)	1
Yes	22/30(73.3)	4.3(1.8-10.5)*
History of malnutrition		
No	52/135(38.50	1
Yes	23/32(71.9)	4.0(1.7-9.4)*
Use of anthelmintics		
No	63/115(54.8)	1
Yes	12/52(23.1)	0.2(0.1-0.5)*
Use of multivitamins		
No	36/124(29.0)	1
Yes	39/43(90.7)	0.04(0.01-0.12)*
Cotrimoxazole(TMP-SMX) use		
No	16/31(51.6)	1
Yes	59/136(43.4)	0.7(0.3-1.5)
Hookworm infestation		
No	39/124(31.5)	1
Yes	36/43(63.7)	11(4.5-27.4)*
Microscopic haematuria		
No	57/132(43.2)	1
Yes	18/35(51.4)	1.3(0.7-2.9)

* P value <0.05,

The final model derived by forward step multivariate logistic regression (Table 5) demonstrated that not being on HAART (OR 3.4, 95%CI (1.20-9.60), having history of TB disease in the past six months at the study (OR 3.23, 95%CI (1.10-9.70), CD4<25%(OR 2.3, 95%CI (1.2-36.1) and having hookworm infestation (OR 5.97, 95%CI (1.92-18.4) were independent risk factors for anaemia among HIV infected children. Also, being HIV positive for ≥ 2.5 years resulted into a low risk of anaemia (OR 0.12, 95%CI (0.04-0.36) compared to being HIV positive for <2.5 years .Taking multivitamins (OR 0.07, 95%, CI (0.02-0.30) and anthelmintics (OR 0.27, 95%CI (0.10-0.74) were the protective factors against anaemia.

Table 5: Multivariate analysis of risk factors for anaemia in HIV infected children

Variable	Adjusted OR	95% CI	P-*value*
Not being on HAART	3.4	1.20-9.6	0.026
Having a history of TB disease in the past six months at the study	3.23	1.10-9.7	0.037
Having hookworm infestation	5.97	1.92-18.4	0.002
Being HIV positive for ≥2.5 years	0.12	0.04-0.36	<0.001
CD4% <25%	2.3	1.2-36.4	0.021
Taking anthelmintics	0.27	0.10-0.74	0.011
Taking multivitamins	0.07	0.02-0.3	<0.001

To further evaluate the factors in anaemia, sub analysis of risk factors for severe anaemia in HIV infected children was carried out (Table 6). In a univariate ,not being on HAART, advanced WHO stage, CD4<25%, having a history of TB disease in the past six months at the study, history of chronic diarrhoea (14 days or more), history of malnutrition or history of recurrent malarial attacks (every month),having hookworm infestation and microscopic haematuria were associated with severe anaemia. The children who were > 2.7 years of age had a lower risk of developing severe anaemia than those who were ≤ 2.7 years. The analyses also showed that being HIV positive for ≥ 2.5 years resulted into a low risk of severe anaemia compared to being HIV positive for <2.5 years. Use of antihelmithics and multivitamins appeared to be protective against severe anaemia.

Table 6. Univariate analysis of factors associated with severe anaemia (<8g/dl) in HIV infected children

Variable	Univariate	
	Severely anaemic children n/N (%)	OR(95%CI)
Age group(yrs)		
≤2.7	12/33(36.4)	1
>2.7	14/134(10.4)	0.2(0.08-0.8)*
Sex		
Male	17/90(18.9)	1
Female	09/77(11.7)	0.6(0.2-1.3)
Maternal occupation		
Unemployed	21/108(19.4)	1
Employed	04/32(12.5)	0.6(0.1-1.8)
Business	01/27(03.7)	0.1(0.02-1.2)
Maternal education		
No formal education	04/22(18.2)	1
Formal education	22/145(15.2)	0.8(0.2-2.6)
Duration of HIV positivity(yrs)		
<2.5	21/80(26.3)	1
≥2.5	05/87(05.7)	0.1(0.6-0.5)*
HAART status		
On HAART	09/108(08.3)	1
Not on HAART	17/59(28.8)	4.4 (1.8-10.7)*
HAART regimen		
ZDV based regimen	18/108(16.6)	1
Non ZDV based regimen	08/59(13.3)	0.7 (0.1-4.9)
WHO stage		
3	17/135(12.6)	1
4	09/24(37.5)	4.2(1.5-10.9)*
CD4 %		
>30	05/90(05.6)	1
25-30	02/17(11.8)	2.2(0.4-12.7)
<25	19/60(31.7)	7.8(2.7-22.5)*
History of TB diseases		
No	13/135(09.6)	1
Yes	13/32(40.6)	6.4(2.6-15.9)*
History of chronic diarrhoea		
No	11/128(08.6)	1
Yes	15/39(38.5))	6.6(2.7-16.2)*
History of recurrent fever		
No	06/65(09.2)	1
Yes	20/102(19.6)	2.3(0.9-6.3)
History of recurrent malaria		
No	15/137(10.9)	1
Yes	11/30(36.7)	4.7(1.8-11.7)*

Cont... Table 6 Univariate analysis of factors associated with severe anaemia (<8g/dl) in HIV infected children

Variable	UNIVARIATE	
	Severely anaemic n/N (%)	OR(95%CI)
History of malnutrition		1
No	14/135(10.5)	2.0(2.0-12.8)*
Yes	12/32(37.5)	
Use of ant helminthes		
No	23/115(20.0)	1
Yes	03/52(05.8)	0.2(0.07-0.9)*
Use of multivitamins		
No	17/43(39.5)	1
Yes	09/124(07.3)	0.1(0.04-0.3)*
Cotrimoxazole(TMP-SMX) use		
No	06/31(19.4)	1
Yes	20/136(14.7)	0.7(0.2-1.9)
Hookworm infestation		
No	09/124(07.3)	1
Yes	17/43(39.5)	8.4(3.3-20.8)*
Microscopic haematuria		
No	16/132(12.1)	1
Yes	10/35(28.6)	2.9(1.1-7.1))*

* P value <0.05, +8 subjects in WHO stage 2 excluded for analysis

Multivariate analysis (Table 7) revealed, having a history of TB disease in the past six months at the study, CD4 <25%, having hookworm infestation were independent risk factors for severe anaemia among HIV infected children.The analyses also showed that being HIV positive for ≥ 2.5 years resulted into a low risk of severe anaemia compared to being HIV positive for <2.5 years. Taking multivitamins was consistently a protective factor against severe anaemia.

The results from analyses indicated lack of association (with statistical significance) between gender, maternal occupation and education, type of HAART regimen, history of recurrent fevers and use of cotrimoxazole with mild anaemia and severe anaemia in HIV infected children.

Table 7: Multivariate analysis of risk factors for severe anaemia in HIV infected children

Variable	Adjusted OR	95% CI	P-*value*
Having a history of TB disease in the past six months at the study	5.7	1.30-25.′	0.021
CD4%<25%	3.6	6.1-21.9	<0.001
Having hookworm infestation	12.2	2.28-65.9	0.003
Being HIV positive for ≥2.5 years	0.12	0.02-0.6	0.01
Taking multivitamins	0.18	0.04-0.7	<0.021

Description of the spectrum of blood cells' morphology on blood films

Tables 8 shows that abnormal red blood cell shape (P=0.02), microcytosis (P<0.001), hypochromia (P<0.001), and abnormal platelet morphology (P<0.001), were common in HIV infected children compared with anaemia compared to those who had no anaemia

Table 8: Blood cells' morphological patterns in HIV infected children by anaemia status (Hb<11/dl)

Variable	Anaemic n/N	%	Non anaemic n/N	%	*P-value*
RBC morphology"					
Normal	03	04.0	14	15.2	0.02
Abnormal	72	96.0	78	84.2	
RBC Size					
Normal	25	33.3	69	75.0	
Microcytic	46	61.3	07	07.6	<0.001
Macrocytic	20	05.3	16	17.4	
Chromic state.					
Normochromia	37	49.3	74	80.4	<0.001
Hypochromia	32	42.7	05	05.4	
Nucleated red blood cells	12	16.0	14	15.2	0.89
Yes	63	84.0	78	84.8	
No					
Polychromasia	03	04.0	02	02.2	0.66
Yes	72	96.0	90	97.8	
No					
Target cells	06	08.0	00	0.0	
Yes	69	92.0	92	100.0	0.49
No					
Neutrophil morphology					
Normal	48	64.0	45	48.9	
Abnormal[∂]	27	36.0	47	51.1	0.05
Lymphocyte morphology					
Normal	41	54.7	43	46.7	
Abnormal[+]	34	45.3	49	53.3	0.30
Platelet morphology					
Normal	43	57.3	82	89.1	
Abnormal[ℓ]	32	42.7	10	10.9	<0.001

"Abnormal RBC morphology-anisocytosis , poikiolocytosis, spherocytes, and fragmentation

[∂]Abnormal neutrophil means dysplasia, hypersegmentation, hyposegmentation

[+]Abnormal lymphocyte includes atypical lymphocytes

[ℓ]Abnormal platelets includes giant platelets, hypogranular platelets

DISCUSSION

Social-demographic and clinical characteristics of the study population

This study included HIV infected children seen at Muhimbili National and Mwananyamala Municipal Hospitals in Dar es Salaam. This was representing a study population of Tanzanian children who could access service at secondary and tertiary levels of health care delivery basing on severity or complication of the diseases and the availability of HIV service. In Tanzania, HIV care and treatment centers are mainly available at these levels of health care system, thus the population studied was representative of a hospital setting at secondary and tertiary levels of health care delivery.

The study subjects were aged 0.5 yr to 4.9 years with overall mean age of 3.8 years. This created a homogenous study population in which there were no marked differences in age to influence the interpretation of the haematogical findings considering the age related physiological variation on blood cell counts and red blood cell indices. Normally at the age of six months to six years, these haematological parameters tend to remain constant [60]

In this study despite the fact that about two thirds of the study population were already on highly active antiretroviral therapy, the majority of them were in WHO clinical stage 3 and 4 and had CD4% level of <25% at the time of study. This meant that most of the patients were in intermediate to advanced stage of immunosuppression despite being on ARV which could be expected to boost the immunity and the clinical stage. The majority of the study subjects looked clinically better than their CD4 % level. This discrepancy might be partly explained by a possible short duration of HAART for many of the study subjects to allow significant increment in clinical status and CD4 level by the time of this study. The other possible explanation for this lack of correlation between proportion of patients who were on HAART and their clinical stage and immunity status might be a delay to seek or access HIV service thus presenting at the hospital with severe immunosuppression which could take longer time of improvement by HAART.

Prevalence of anaemia in HIV infected children

Anaemia is one of the leading reasons for paediatric admission in many African hospitals and a major contributor of death [56, 57].The prevalence of anaemia among HIV infected children in African setting has not been comprehensively investigated. This study has shown that anaemia (44%) in HIV infected children attending hospitals is still common. The finding suggests that anaemia is common among HIV children even in the era of HAART. This finding is similar to reports from other studies which have indicated that the prevalence of anaemia ranged from 1.3 to 95% [17-19]. The findings from this study however differed from the study done by Clack T.D et al who reported a prevalence of 91.7% among Ugandan HIV infected children [20]. This finding also contradict those reported by Brian S E et al in Sout[1] Africa in which the prevalence was 73%[16]. As it has been documented in other studies prevalence of anaemia depends on several factors such as stage of HIV diseases, age, sex, as well as the definition of anaemia used[3, 19]. In this study 94% of the study subjects were in stage 3 to 4 of WHO clinical staging indicating moderate to severe forms of HIV disease, thus contributing to the high prevalence of anaemia found. The use of highly active antiretroviral therapy (HAART) has been associated with a significant increase in haemoglobin concentration and a decrease in the prevalence [19].Despite the fact that about 65% of the HIV infected children were on ARV during this study, the prevalence of anaemia remained high. Possibly most of the study subjects had taken HAART for a short duration before this study to induce significant increment in haemoglobin level.

Although the overall prevalence of children recruited at the outpatient clinic did not differ much from those recruited in the admitting wards, the prevalence of severe anaemia was higher among inpatients than outpatients. This can be partly explained by the fact that severely sick patients are more generally admitted than being attended in the out patient clinics. The overall prevalence of severe anaemia of 15% in this study also appeared to be high. The findings correspond to reports from other studies [3, 59] This high prevalence is possibly due to most of the patient being moderately to severely immunosuppresed. This confirms the previous findings that as HIV progresses, the prevalence of HIV and severity of anaemia increase[3].

A hypochromic microcytic type of anaemia was found to be common among HIV infected children with anaemia. This indicated that possibly iron deficiency was mainly responsible for anaemia. This finding did not differ to the general population in Tanzania where iron deficiency is the commonest cause of anaemia in children. In Romania, a similar finding were reported by Strauss I in which microcytic anaemia accounted for 80% of all anaemia in HIV infected children [61]. It had also been reported that deficiencies in the nutrients such iron contribute to the development of anaemia among HIV/AIDS patients in less developed countries[62]. A similar finding was also reported in South Africa in which more HIV infected children with moderate and severe disease and severe immunosuppression had abnormal RBC morphology to suggest iron deficiency. The finding from this study, however differed from the study of Balbarysk J et al who documented that the most frequent form of anaemia in children with mild HIV disease had features of iron deficiency anaemia(hypochromic microcytic picture) while anaemia in patients with advanced immunodeficiency has the characteristics of anaemia of chronic disease (normochromic normocytic picture)[30]. In the current study, most of the children had advanced immunodeficiency and yet hypochromic microcytic anaemia was the more common than normocyitic normochromic anaemia. The variation could be partly attributed to the study population in which Balbarysk et all defined two groups of patients; one with microcytic anaemia (children under 24 months of age with mild disease) and the other group with normocytic or macrocytic anaemia (children above 24 months of age with advanced immunodeficiency). In this study all children aged 6-59 months were analysed together.

Risk factors for anaemia in HIV infected children

Potential causes of anaemia in the context of HIV disease include HIV infection of the haematopoietic stem cell/erythroid progenitor, immune mediated haemolysis, aplastic anaemia, malignancies, blood loss, bone marrow infections, and deficiency of erythropoietin [62]. The results of this study show the multifactorial nature of anaemia causation in African children setting. The study revealed that infections particularly tuberculosis, hookworm and malaria contribute significant to anaemia in children.

This study found that having a history of tuberculosis in the past six months at the study was a strong independent risk factor for both mild and severe anaemia in HIV infected children. Strong association of tuberculosis and anaemia was also reported by Ramnath S when studying anaemia in HIV infected persons in Southern India[63]. This finding is also consistent with results of another study done in northern part of Tanzania where anaemia was found to be common among HIV-TB co-infected with prevalence of anaemia of 15% [64]. The etiology of anemia in TB is likely multifactorial, deriving partially from anemia of chronic disease (associated with increased IL-6) and partly from deficiencies of nutrients such as iron, vitamin A, and selenium[65].Also, in some cases, severe anaemia may be the only clue to diagnosing occult tuberculosis infection of the bone marrow[34]. A study done by Sei Won Lee M et al revealed treating tuberculosis in some patients with HIV leads to complete resolution of anaemia(66). The study in Malawi also showed that occult mycobacterium disease was highly associated with anaemia in HIV infected children [29]

In this study, hookworm infestation was found to be strongly associated with both mild and severe anaemia in HIV infected children. This indicated hookworm was common in anaemic children with HIV.A similar finding was reported by Viroj W et al in Thai who found a high prevalence (50%) of intestinal parasites among the HIV- infected patients.

Hookworm and Ascaris lumbricoides appeared to have the highest prevalence (63.33 % and 13.33 % , followed by Opisthorchis viverrini (10 %), Isospora belli (5

%), strongyloides stercolaris (3.33 %), Cryptosporidium (3.33 %), and Microsporidium (1.67 %) infestation[67]. This finding also corresponds to the study done in Tanga Region, Tanzania in which the prevalence of hookworm in HIV patients was 75.7%. It was further found that infestations with hookworms was associated with a significant reduction in haemoglobin concentration[37]. However these results differ on the population studied; whereas the current and Thai studies included children, the study in Tanga included adults only.

The association of malaria and anaemia has been well documented in various studies[37, 38]This study adds important information that recurrent malaria is also a potential risk factor for anaemia in HIV infected children. It is possible that the effect of malaria and HIV infection seem to be synergistic in causation of anaemia. The association between malaria and anaemia in HIV infected children was also similarly reported by Villamor et al in Tanzania who established that HIV and malaria co-infected patients ad a high incidence of anaemia[39].

The role of HAART in anaemia of HIV infection was also established in this study. It was demonstrated that not being on HAART was an independent risk factors for anaemia. A similar finding was reported by Feyler A et al in which the use of HAART led to the reduction in the prevalence of anaemia from 13% in 1995 to 4% in 1999 [68]. They pointed out that the improvement of immunological and clinical status associated with the increasing efficacy of antiretroviral therapy possibly explained a large part of the reduction of the risk of anaemia. However it has to be remembered that Zidovudine (ZDV) based regimen may cause anaemia in HIV patients due to the myelosuppressive effect and can worsen the existing enaemia. A randomized clinical trial by Graeme Moyle in the HAART era have demonstrated that differences in anaemia risk exists between thymidine analogs and it may be appropriate to consider regimens that do not contain AZT in these individuals, any person who presents with low haemoglobin and individuals requiring other myelosuppressive agents [41] Addition of 3TC may marginally increase the anaemia risk with AZT. Precipitous drops in haemoglobin after the introduction of 3TC to ongoing AZT therapy has been reported. However in this study there was a tendency

to have anaemia in subjects who were on ZDV based regimen compared those who were non ZDV based regimen but the association was not statistically significant.

Low CD4 counts (<25%)and higher HIV-1 RNA levels in plasma have been associated independently associated with increased risk of anaemia[40]. This finding is also consistent with the results from this study in which subjects who had CD4 levels of <25% had a higher risk of developing severe anaemia than those with CD4 levels of <25%. How ever did not correlate with advanced WHO clinical stage whose association was not statistically significant in multivariate analysis. This may be due to the finding that though most of the patients had CD4 levels of <25%, the majority were in WHO clinical stage 3 and not stage 4 which normally corresponds with CD4 levels of <25%. It was interesting to find that children who had long duration of HIV positivity were less likely to be anaemic, both in univariate and multivariate analysis. The finding is different from a number of studies which have demonstrated strong association between anaemia and long duration of HIV[3, 19]. In this study the explanation for this unexpected finding could be the possibility that most of the children with a long duration of HIV, have been on HAART for a long duration which could partly protect them from anaemia and opportunistic infections. It could be also due to longer regular check on their anaemia status at the clinics allowing immediate correction compared to those children who were diagnosed HIV positive for a short duration.

Multivitamins appeared to be protective against mild anaemia and severe anaemia in HIV infected children. This is consistent with the study done in Tanzania which showed that multivitamin supplementation could improve haematological status in HIV-infected women and their children in Tanzania[69]. Micronutrient supplements have also been reported to delay HIV disease progression and reduce mortality in HIV-positive persons not receiving highly active antiretroviral therapy[70].The use of anthelmintics was also found to be protective against mild and severe anaemia among HIV infected children. This was expected since anthelmintics are recommended for community prophylaxis of children against infestation with hookworms. It has also been suggested that treatment for helminth infestation may

also decrease both the rate of viral replication in those infected with both types of organism[71].

In this study malnutrition was associated with anaemia only on multivariate analysis. However malnutrition contributes significantly to anaemia due to the deficiency of iron, folate and B12 as well as increased infections. In one study it was shown that nutrient supplement can correct anaemia and weight loss in HIV-infected children, though this is more quickly in HIV-negative undernourished children than those who are HIV positive[72].

The findings from analysis demonstrated lack of association between taking a prophylactic dose of cotrimaxazole(TMP-SMX) and anaemia in HIV infected children. A similar finding was documented by Sullivan PS et al in the US[3]. Although administration of TMP-SMX can cause drug associated aplastic anaemia or immune mediated destruction of specific population of blood cells, the effect did no appear in this study. This is possibly due to the effect of TMP-SMX on prevention of some opportunistic infections which are more predictable causes of anaemia, or other infections that could promote the development of the anaemia associated with chronic disease or with inflammation.

There was lack of statistical association between chronic fever, recurrent malaria, chronic diarrhea and WHO clinical stage 4 in multivariate analysis though in univariate analysis they were all associated with anaemia. The lack of association is may be due to the small sample size. Therefore, the absence of statistical significance of these associations has to be taken cautiously.

Morphology of blood cells on peripheral smear

This study showed that shows that microcytosis and hypochromia and low MCV were common in HIV infected children with anaemia compared to those who had no anaemia. This is similar to the finding reported by Megan et al in which hypochromasia and microcytosis were identified in the blood smears of 45% of the women with anaemia, and 86% had mean corpuscular volume values less 80 fl, suggesting that iron deficiency was an important contributing factor to the high rates

of anaemia we observed .These results are also in accordance with other findings from Sub-Saharan Africa[62, 69].

Study limitations

The findings of this study were also limited by other factors. The lack of association in multivariate analysis of anaemia with factors such as WHO clinical stage and malaria may be attributed by the small sample size in this study. Therefore, the absence of the statistical significance in some of the associations has to be taken cautiously.

Studies on serum iron, ferritin, and total iron binding capacity, folate, cobalamin level and bone marrow could give more information on the findings of microcytic anaemia and micronutrient deficiencies in HIV infected children. However, limited funding and time for data collection resulted in failure to carry out some of these tests.

Some parameters for blood counts and indices (such as MCV) were not paediatically adjusted .This is due to the fact that there are no locally reported levels for children in Tanzania. This will be addressed in future studies when we have paediatrically adjusted references

Even with these limitations in mind, the observed findings may still be a good reflection of true situation and this study serves as a reference for further recommendations to improve care of HIV infected children and a step for further studies on the pathophysiology of HIV related anaemia in African children.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- The prevalence of anaemia is common among HIV infected children despite the availability of HAART.
- Tuberculosis, hookworm infestation and not being on HAART were potential risk factors for both mild and severe anaemia.
- Multivitamin supplementation and use of anthelmintics appeared protective against anaemia in HIV infected children.
- Abnormal red blood cell morphology, microcytosis and hypochromia were more common in HIV infected children with anaemia than those who had no anaemia.

Recommendations

- All HIV positive children should be regularly screened for anaemia.
- Continue encouraging the use of multivitamins in all HIV infected children.
- The three monthly anthelmintics should be should given to all HIV infected children.
- HAART should be made available to all eligible HIV infected children.
- Efforts to correct anaemia in HIV infected children should also include treatment of infections such as TB, malaria and hookworms.
- A case controlled study can be considered in future to highlight whether some factors are stronger risks for anaemia in HIV infected children.

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APPENDICES

Questionnaire 1 (English Version)

Questionnaire 1 on a Research Study about the Burden and Determinants of Anaemia among HIV infected Children in Dar es Salaam

A INTRODUCTION

First name_____, **Family name**_____S/N____

File_____ **Residence**_____**Mobile no**_____

Site of study 1. MNH 2. Mwananyamla **Source** 1. OPD 2. Ward

B. Social demographic characteristics and medical history

Qn	Question	Response	Instructions
1	How old is the child?	Years_____ Months_____	
2	Sex of the child	1. Male 2. Female	
3	Ethinicity	1.African 2.Arab 3.Indian 4.Mixed 5.others (mention)_____	
4	Parents/guardian's (mothers, grandmother, ant or the like) Occupation	1. Peasant 2. Civil servant 3.Business 4. Student/pupil 5.Self employed 6.Unembployed	
5	Parents/guardian's level of education	1. No formal education 2. Less than primary	