Addis Ababa University

College of Natural Sciences

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Prevalence and Determinants of Anemia and Iron Deficiency among HIV infected Children attending Antiretroviral Therapy Center at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Tesfanesh Abebe entitled:"Prevalence and determinants of anemia and iron deficiency among HIV infected children attending ART centers at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia"

A thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Food Science and Nutrition complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Prevalence and determinants of anemia and iron deficiency among HIV infected children attending ART center at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia.

Tesfanesh Abebe

Addis Ababa University, 2016

Background: Globally, HIV epidemic remains a serious challenge especially in children. Ongoing prenatal transmission impacts the incidence of pediatric HIV, adding to the large pool of HIV infected children in developing countries. Anemia and iron deficiency is common in HIV infected children.

Objectives: To assess prevalence and to identify determinants of anemia and iron deficiency among HIV infected children visiting ART center at Black Lion Hospital.

Methods: Institution-based cross sectional study was conducted among consecutively selected HIV infected children (n=108) visiting ART center from January to April 2016. The data was collected using pre-tested interview questionnaires. Dietary data was collected following a 24 hour recall. Blood sample was analyzed for hemoglobin, SGOT, SGPT, ALP, urea, crieatinin, CD4+count, serum ferritin and C-RP. In addition, anthropometrics measurement and 24hour dietary recall collected to assess their nutritional status.

Result: Majority of study children lived with their parents. However; substantial number 15 (13.9%) were orphans. Iron deficiency based on ferritin level was available from 108 children, of these study population were 12 (11.1%) had law ferritin levels<12mg/l indicating iron deficiency, and 19(17.6%) were anemic. Age, treatment regimen and serum SGOT level was significantly associated with anemia and iron deficiency.

Conclusion and Recommendation: Iron deficiency and anemia appear to be an important nutritional problem among HIV+ children at ART center. Therefore, effective actions aimed at the prevention and controls of this deficiency are strongly recommended in this context.

Declaration

I, the undersigned, declare that this research is my original work and that all source materials used for the research have been correctly acknowledged.

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Abbreviations and Acronyms

HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome
HAART	Highly active antiretroviral therapy
Hb	Hemoglobin
RUTF	Ready to use therapeutic food
EP	Erythrocyte protoprohperin
SF	Serum ferittin
CRPHS	C-reactive protein high sensitive
WHO	World health organization
BLH	Black Lion Hospital
OIs	Opportunistic infections
BUN	Bloody urea nitrogen
AGP	Alph-1-glicoprotein
URTI	Upper respiratory tract infection
ID	Iron deficiency
IDA	Iron deficiency anemia
TS	Transferin saturation
WHO	World health organization
UN	United Nations
SPSS	Statistical package for social science

1. Introduction

Globally, HIV epidemic remains a serious challenge especially in children. Ongoing pre-natal transmission substantially impacts the incidence of pediatric HIV, adding to the large pool of HIV infected children in developing countries. The two major co-morbidities include micronutrient deficiencies such as anemia and iron deficiency, whose detrimental effects are magnified in the context of HIV infection (WHO, 2001). Anemia is a common manifestation of pediatric HIV infection, and is a significant negative predictor of survival. Many etiological factors probably contribute to the development of low iron status in HIV-infected children, such as reduced dietary intake, the quality of dietary iron and altered iron absorption (Cook, *et al.,* 1994).

Iron is involved in many physiological functions in the body, and its deficiency can lead to iron deficiency and later to anemia and other health complications. Iron deficiency is the most common nutrient deficiency in the world. Symptoms of iron deficiency are: subtle, nonspecific and often only become apparent with severe anemia. The pallor of anemia was associated with weakness and tiredness long before its cause was known. Now, it is recognized that even without anemia, mild to moderate iron deficiency has adverse functional consequences on health (Oppenheimer, 2001).

Iron deficiency adversely affects the cognitive performance, behavior, physical growth of children and immune status of all age groups. The quantitatively dominating function of iron in the human body is as the oxygen-binding core of hemoglobin, the red pigment of blood and transportation of oxygen from the lungs to all tissues. During the progress of iron deficiency, hemoglobin synthesis in the bone marrow is restricted resulting anemia. Anemia caused by iron deficiency is called iron deficiency anemia, distinguishing this condition from other causes of anemia (Lutter & Rivera, 2003). Iron deficiency in children is caused by the combination of limited iron stores at birth, timing of umbilical cord clamping, timing and type of complementary food introduction and frequency of childhood infections.

1.1 Statement of the problem

Anemia is among the most common hematologic abnormalities in patients with HIV and has been associated with increasing disease progression to AIDS, high mortality and poor clinical outcomes. In HIV positive patients, the pathogenesis of anemia during human immunodeficiency virus, is multi-factorial and includes the effect of opportunistic infections, nutritional deficiencies, the anemia of chronic disease, impaired erythropoietin production, endogenous release of inhibitory cytokines, mycobacterium ovum complex infection, human parvovirus B19 infection ,HIV infection of hematopoietic progenitor cells, HIV infection of other bone marrow stromal cells and drug side effect(Semba, 2002).

Considerable emphasis is placed on reducing the prevalence of iron deficiency in children, for a large body of evidence indicates that poor iron status negatively affects cognitive, motor and social development during this period of rapid growth and development. Iron deficiency anemia has been shown to have significant adverse effects on children that include decreases in responsiveness and activity as well as increases in body tension and fatigue. There is also a well known association between IDA and delayed neurodevelopment in infants and children (Olivares, *et al.,* 1999).

The risk of iron deficiency is high during infancy and childhood, for only about 50% of the iron requirement of a normal six month old can be obtained from breast milk, by this age the stores received at birth are likely to have been used to support normal functions and growth even in children born at term of well nourished mothers. If the mother is anemic and/or the child is of low birth weight, the stores use up much earlier. Continued breastfeeding alone will supply only half of the infant's iron needs, where as the other half approximately 4 mg/day must come from complementary foods or an iron containing supplement in order to avoid iron deficiency. Where well fortified infant cereals are available and affordable, they provide the required iron, as does iron fortified infant formula (Domellöf, *et al.,* 2002).

Chronic iron deficency in infancy has a long term effect on late childhood ,for it is dependent on the socioeconomic charactersitics which has a long term consequence in

childhood. since the prevalence has been high, that needs special attention to over-come the problem by designinig program in prevention and treatment of iron deficiency. The current issue in prevention and treatment of iron deficiency would be increasing the use of iron rich foods, targeted fortification of foods for children, and the use of iron supplements (Zlotkin, 2002).

Pathogenesis of micronutrient deficiency is much more complex during human immune deficiency virus (HIV) infection. Insufficient dietary intake, mal-absorption, diarrhea, and impaired storage and altered metabolism of micronutrients can contribute to the development of micronutrient deficiency. Micronutrient deficiency may contribute to the pathogenesis of HIV/AIDS infection through increased oxidative stress and compromised immunity (Semba, 2001).

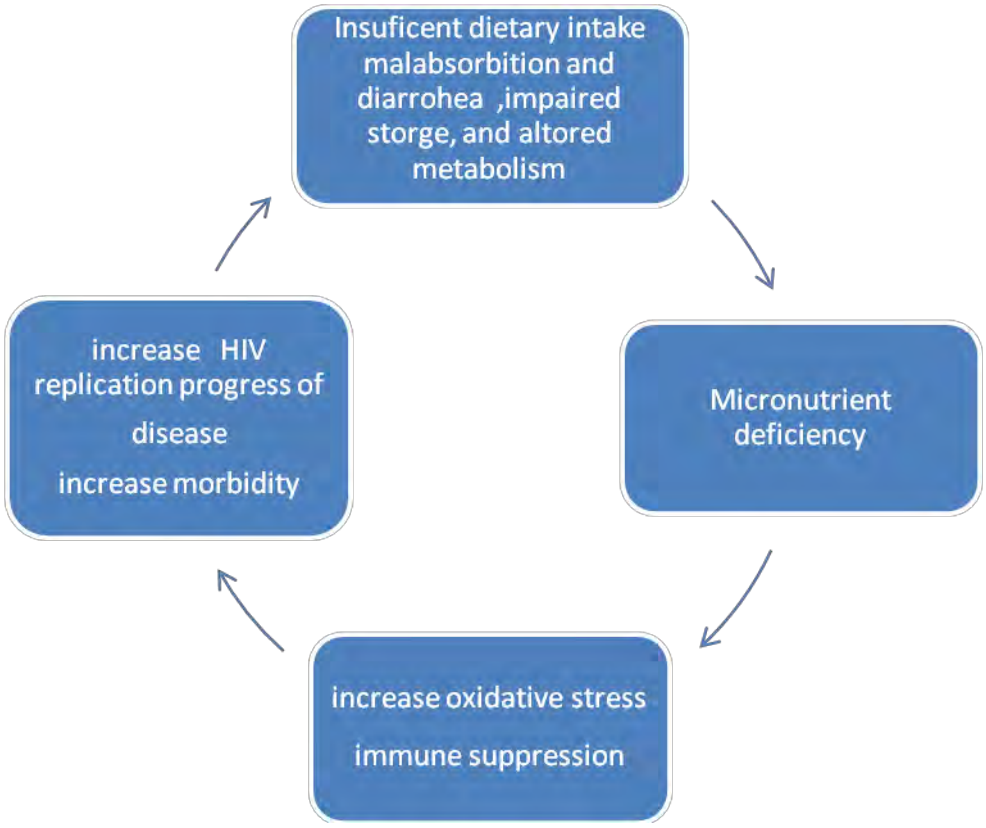


Figure1.Vicious cycle of micronutrient deficiency and HIV pathogenesis(Semba, 2001)

In analogy with other infections and on the basis of knowledge about the natural history of HIV infection, it is expected that HIV infection, through various mechanisms

operating at different levels, impairs micronutrient intake and status. Accordingly, available data suggest that HIV infection impairs the status of a range of micronutrients and that HIV positive individuals may have increased requirements; moreover, recommendations on micronutrient intake for people with HIV infection should address levels that prevent clinical and biochemical signs of deficiency. They should also be based on the effects of micronutrient status and intake, on HIV progression, morbidity and possibly transmission (Friis, 2005).

Other contributing factor for anemia in this patient is drug side effects.HIV treatment like highly active antiretroviral therapy (HAART) regimens, has been associated with hematological toxicity such as Zidovudin (ZDV) inhibits the proliferation of blood cell progenitor cells in a dose dependent manner and can cause anemia. Previous studies have demonstrated that patients with HIV infection and low hemoglobin (Hb) levels have a higher mortality risk than less anemic comparison groups, even after controlling for CD4 cell count and viral load. Low Hb levels have also been shown to be associated with disease progression to AIDS and with unfavorable outcomes of treatment, such as opportunistic infections and neurological deterioration. The risk of anemia and iron deficiency for ART treatment regimens containing ZDV was studied, but for the other treatment regimen, there are no available data and ART regimens not containing ZDV has not been rigorously established with that of ZDV contain in a previous study (Levine, 2001).

Nutrition is a significant component of comprehensive care for individuals living with HIV/AIDS, adequate and diversified nutrition is necessary to manage opportunistic infections, maintain the immune system, optimize response to medical treatment, and support optimal quality of life. Nutritional supplementation along with HAART can improve immune response, Body Mass Index (BMI), drug adherence and physical activity (Esna, *et al.,* 2012).

Prevalence of anemia and iron deficiency among children in Black Lion Hospital ART center, literature was not found. Doing research in that center will provide information about the predictor of anemia in association with their organ function test, and will help to obtain information which treatment regimens are risk for anemia and iron deficiency.

Use of Ready to Use Therapeutic Foods (RUTF) has a vital role in treatment for severe under-nutrition. However, there are little studies that document the magnitude of malnutrition, food variety and diversity among human immune deficiency virus infected children in clinical setting even the proportion of patient that they use supplementary foods needs study. Moreover, the prevalence of anemia and iron deficiency and its associated factors such as CD4+ count, renal and liver function test, and treatment regimens was not seen previously in Ethiopia. This research incorporates those factors to fill the gaps.

1.2. Significance of the Study

Iron deficiency anemia is one of the world's most widespread health problems especially among HIV infected children: approximately 44% of children in Ethiopia are anemic based on (EDHS, 2011).

Iron deficiency anemia leads to weakness, poor physical growth, and a compromised immune system decreasing the ability to fight infections and increasing the chance morbidity, doubling the problem for those HIV infected children, since they had been primarily immune compromised. It is also thought to impair cognitive performance and to delay psychomotor development. Iron deficiency is the most common form of micronutrient deficiency in childhood, affecting all socio-economic levels of society. It is a major public health concern in children and adolescent in the developing countries like Ethiopia.

Many studies have examined these at-risk group children; there is a scarcity of data on anemia in children living with HIV, but the response towards micronutrient deficiency including iron deficiency as well as iron deficiency anemia are increasing at an alarming rate and associating with the diseases prognosis and outcome. Putting this in to

consideration this study may have its own contribution in identifying iron deficiency, anemia, and determinant, and associated factors. In addition to this; this research will be help full in providing evidence to create screening test for iron deficiency for every child who come to HIV care and treatment center, and considering supplementation for those deficient patients and to decrease the consequence which come relating with it. Moreover, the researcher hopes that, this study may help for other researches as a spring board to work more on it and to conduct further studies.

The aim of this work was to assess the prevalence of anemia, iron deficiency and its determinant, and risk factors among children visiting ART center. To fill this gap, the researcher conducted the study by using institution-based cross sectional study, by incorporating functional testes (renal and liver function test), and CD4+ count.

1.3. OBJECTIVES

1.3.1. General Objective

To assess prevalence and identify risk factors of anemia, iron deficiency and determinants of anemia and iron deficiency among HIV infected children attending care and treatment center at Black Lion Hospital, Addis Ababa.

1.3.2. Specific Objectives

To assess socio demographic factors of HIV infected children visiting ART center at Black Lion Hospital from January to April 2016.

To assess the nutritional /anthropometric status of these children visiting ART center at Black Lion Hospital from January to April 2016.

To identify the risk factors associated with anemia and iron deficiency in those children visiting ART center at Black Lion Hospital from January to April 2016.

To detect determinants of anemia and iron deficiency in those children visiting ART center at Black Lion Hospital from January to April 2016.

1. LITERATURE REVIEW

2.1. Epidemiological overview of HIV/AIDS, Anemia and Iron Deficiency.

HIV/AIDS epidemic is one of the most important challenges in global health today, with an estimated 33 million people worldwide are affected by the virus, in Sub-Saharan home to two-thirds of HIV-infected individuals are found. Sixty percent of those infected are women, this associated with mother to child transmission and the number of HIV infected children is increasing side by side (Joint United Nations Program on HIV/AIDS, 2008).

Despite progress in the prevention of mother-to-child-transmission of HIV and care for HIV exposed children, children born to HIV-infected women continue to experience increased risks of morbidity and mortality. It is well established that an infection may lead to micronutrient deficiencies, and on the reverse micronutrient deficiencies may affect the risk of morbidity from infectious diseases. In HIV infected individuals immunologic and hematologic abnormalities are common and they increase the risk of morbidity and mortality; however, little is known about the profile of immunologic and hematologic abnormalities in Ethiopia in those children who are on Highly Active Antiretroviral Therapy. Evaluating the hematological and immunological parameters in HIV/ AIDS patients on highly active antiretroviral therapy is important in order to monitor the body responses to the drugs. Therefore, assessment of hematological and immunological changes in HIV/ AIDS patients in highly active antiretroviral therapy is of a paramount importance. The effect of an infection is mediated via the acute phase response and localized lesions, leading to reduced intake and absorption, increased utilization and loss of micronutrients (Belperio, 2004).

Iron status depends not only on adequate intakes but also on the form of ingested iron (haem/non-haem), the presence of enhancers and inhibitors of dietary iron absorption and the extent of iron losses from the body. The vast majority (typically 90%) of dietary iron occurs in non-haem form (from vegetables and cereals), which is poorly absorbed than haem - iron (from meat and fish), and whose bioavailability is greatly affected by enhancers (e.g. meat, fish, vitamin C and A) or inhibitors (e.g. dietary fiber, tea, coffee,

calcium) that are present in the diet. Iron status may also be impaired through excessive loss of body iron due to infection, inflammation or parasitic infestation, all of which form a greater cause of iron deficiency and anemia in developing countries with poor sanitation and other factors related to poverty (Almeida, 2004).

A micronutrient deficiency may affect the risk of infection with a specific infectious agent as well as due to the severity of the infectious disease morbidity. These effects are mediated via the pathogen-city of the infectious agent, host risk behavior or the host defense and may be either synergistic or antagonistic. A synergistic relationship exists when a specific micronutrient deficiency increases infectious disease morbidity, in which case either improved micronutrient intake or treatment of the infection will break the vicious circle. An antagonistic relationship exists when a specific micronutrient deficiency reduces or increases intake increases infectious disease morbidity effects of HIV infection on micronutrient status (Friis, 2001).

The effect of an infection on nutritional status is determined by its natural history and actual course and is particularly detrimental if it is generalized, severe, long lasting or recurrent. Human immunodeficiency virus infection is associated with myriads of hematological abnormalities and complications, including anemia. There is epidemiologic evidence of HIV associated anemia, and also its effects on patient's survival and the need for specific diagnosis and treatment. Human immunodeficiency virus (HIV) is associated with numerous abnormalities of red blood cells production and lifespan. One of these consequences is anemia, the prevalence of these estimates varies widely from one population to another. However; anemia was consistently shown to be a predictor for increased disease progression and decreased survival of patient infected by HIV(Adias,2005).

Regular evaluation of patients infected by HIV is necessary to determine the specific causes of anemia in order to ensure the appropriate intervention of the institution.

2.2. Natural History of HIV Infection

HIV infection is characterized by an acute syndrome accompanying the primary infection, followed by a prolonged asymptomatic state eventually leading to advanced HIV disease (Harrison's, 2003).

Around 3–6 weeks after being infected with HIV, most individuals experience a febrile illness lasting a couple of weeks with anorexia, nausea and diarrhea followed by weight loss. During this acute HIV syndrome, the viral load peaks and is mirrored by a nadir in CD4+ counts which occasionally results in opportunistic infections. The CD4+ count then returns to almost normal values while the viral load stabilizes around an individual set point. A long asymptomatic period then follows during which viral load slowly increases and CD4+ count declines. This period is not a true latency period because viral replication continues (Kosalaraksa, *et al.,* 1993), resulting in a progressive decline in CD4+ counts of around 50 cells per year. After a number of years, opportunistic and other infections become increasingly frequent.

The length of the asymptomatic period and the type, timing and frequency of the subsequent opportunistic infections may vary depending on general health and exposure to pathogens. For example, in developing countries more than 90% of HIV-positive individuals get diarrhea compared to less than half in developed countries, and reactivation of latent tuberculosis is common. Although prevention or treatment of tuberculosis and other infections may delay HIV progression, the patient will eventually become wasted and die if antiretroviral (ARV) drugs are not started. Other baseline risk factors for overall mortality included severe anemia, severe immune suppression, history of tuberculosis, opportunistic infections, living in the poorest district, and advanced World Health Organization stage (Ruhinda, 2012).

2.3. Opportunistic Infections

Opportunistic infections (OIs) are infections that occur more frequently and are more severe in individuals with weakened immune systems, including people with HIV. OIs are less common now than they were in the early days of HIV and AIDS, because better treatments reduce the amount of HIV in a person's body and keep a person's immune system stronger. However, many people with HIV still develop OIs because they may not know of their HIV infection, they may not be on treatment or their treatment may not be keeping their HIV levels low enough for their immune system to fight off infections (WHO, 2013).

2.4. HIV and Dietary Intake, and Anthropometric Characteristics

Nutritional status and other baseline predictors of mortality among HIV infected children: were children with weight for age (WAZ) > -1, those with WAZ ≤ -2 to < -3 had a nearly double risk of death), and among those with WAZ ≤ -3, the risk more than tripled (Mwiru, *et al.,* 2013).

Energy requirements in children can vary according to the type and duration of HIV related infections, and whether there is weight loss along with acute infection. Although the finding of increased resting energy expenditure in asymptomatic disease has not been replicated in children, similar to asymptomatic HIV infected adults an average increment of 10% of energy intake is recommended to maintain growth. Based on clinical experience and existing guidelines to achieve catch-up growth in children irrespective of HIV status, energy intakes for HIV-infected children experiencing weight loss need to be increased by 50% to 100% over established requirements for otherwise healthy uninfected children (WHO, 2003).

2.5 Antiretroviral Therapy Treatment Regimens for HIV Virus Infection

Currently there are three classes of ARV drugs available for clinical use although the number and category of drugs are increasing from time to time. The drugs suppress the multiplication of the virus through interfering with the life cycle of the virus. The virus has enzymes that facilitate multiplication within the host cell. One of the vital enzymes for viral multiplication is reverse transcriptase, which copies the viral genome (RNA) to a complimentary provirus (DNA) using the DNA and cellular machineries and raw materials of the host cell. One big category of ARV drugs, called RTI (reverse transcriptase inhibitors), blocks this enzyme. These drugs are further divided into two: NRTI (nucleoside reverse transcriptase inhibitors) and NNRTI (non-nucleoside reverse transcriptase inhibitors). The virus has another essential enzyme called protease that is responsible for post-translational processing of viral proteins; this is blocked by a category of ARV drugs called protease inhibitors (PI). There are various combinations and formulations of these drugs. These drugs are given in different regimens; the most

effective combination currently is HAART (highly active antiretroviral therapy), which includes at least three drugs from one or all the three categories (DHHS panel, 2002).

According to WHO recommendation regular laboratory organ function tests are performed for those taking antiretroviral therapy, those are liver function test such as SGOT, SGPT and ALP are sensitive indicators of liver damage from different types of drug side effect. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. Under normal circumstances, these enzymes reside within the cells of the liver. But when the liver is injured, these enzymes are spilled into the blood stream, raising the enzyme levels in the blood and signaling liver damage. The second one is renal function test B.U.N (Blood Urea Nitrogen) is the end product of protein metabolism and its concentration is influenced by the rate of excretion. Increases can be caused by drug side effect, low fluid intake, intestinal bleeding, exercise or heart failure, and decreased levels may be due to a poor diet, mal- absorption, liver damage or low nitrogen intake. Creatinin is the waste product of muscle metabolism. Its level is a reflection of the bodies muscle mass. Low levels are sometimes seen in kidney damage, protein starvation, and liver disease. Elevated levels are sometimes seen in kidney disease due to the kidneys job of excreting creatinin, muscle degeneration, and drugs involved in impairment of kidney function this testes also included in this study (WHO, 2005).

2.6. Effects of Micronutrients on HIV Infection

Micronutrient deficiencies as well as interventions to increase micronutrient intake may be determinants of susceptibility to HIV infection, transmission and progression, including risk of opportunistic and other infections as well as of a range of non-HIV outcomes that will not be considered. Although, the micronutrient requirements are likely to be reduced when the HIV patient is on ARV, micronutrient deficiencies may persist and may affect absorption, pharmacokinetics and toxicity, and efficacy of the drugs (Raiten, 2005).

Given the routes of transmission of HIV infection, it is likely that micronutrient deficiencies may impair the epithelial integrity, the differentiation of target cells and other host defense mechanisms of an exposed sexual partner or offspring, thereby facilitating viral entry and replication. Micronutrient deficiencies may affect viral load of the HIV-positive individual, systemically or locally in genital secretions and breast milk, and thus affect both HIV progression and infectivity, these effects are mediated by oxidative stress and impaired immune functions. The oxidative stress may lead to activation of the nuclear transcription factor resulting in increased viral replication. The impaired immune functions resulting from lack of essential micronutrients have been called nutritionally acquired immune deficiency syndrome. Nutritionally acquired immune deficiency syndrome(NAIDS) may not only contributes to the depletion and dysfunction of CD4+ cells but also makes the host susceptible to other infections which may increase viral replication ,and hence quicken HIV progression (Whalen*, et al*., 2000).

Plasma viral load is a strong determinant of HIV progression but also of viral load in cervico-vaginal secretions, semen and breast milk although local infections may further boost local viral replication or shedding (Semba, *et al.,* 1999).

Anemia is a common complication of pediatric HIV infection and is associated with failure in growth. Prospective study reveals that, dietary iron intake was insufficient to protect against ID, pointing to a need for low-dose iron supplementation for iron-deficient HIV-infected children, and interventions to increase the consumption of animal protein (Kruger, 2013).

2.7. Iron and HIV Infection

Iron deserves special mention,for both the effect of HIV infection on status and the effect of status and intake on HIV infection seem to be different from those of other micronutrients. Iron stores decline in early asymptomatic HIV infection probably as a result of impaired absorption (Boelaert, *et al*., 1996). Iron may have adverse effects in HIV and other viral infections.

2.8. Causes of Iron Deficiency Anemia

Anemia is estimated to affect one-half of children in developing countries. There are two principal causes of IDA in young children. Anemia, defined as a low concentration of hemoglobin (Hb) in blood, can be caused by several factors. Besides diseases which lead to losses of blood or impairment of the production of Hb, nutrition plays the most important role. Increased iron losses through bleeding, and insufficient dietary iron intake (nutritional IDA). Sometimes, IDA can be caused by a combination of these two mechanisms. Hemorrhagic iron deficiency anemia due to blood loss is the most common cause of IDA (Mohamed,2015). The reason is almost always gastrointestinal bleeding, which is most often occult and can only be detected by a test for blood in stools. Common causes of chronic, occult gastrointestinal hemorrhage in early are intestinal infection (e.g. hookworm, Giardia) and food intolerance (e.g. cow's milk allergy) (Bunupuradah, *et al.,* 2013).

The use of ARVs boosts CD4+ and total lymphocyte counts. Prolonged use of antiretroviral drugs (ARVs) is associated with variable degrees of liver and pancreatic damage, hyper cholesteremia, and anemia in some patients. Since many of these side effects are multi-factorial, management of HIV patients should take into consideration such side effects in making treatment decisions based on periodic evaluation of these parameters *(*Mgogwe, *et al.,* 2011).

Anemia is one of the most common findings during human immunodeficiency virus (HIV) infection and is associated with fatigue, increased progression to acquired immune deficiency syndrome (AIDS) and higher mortality. Severe anemia increases the need for erythropoietin therapy and/or red blood cell transfusions. Up to 90% or more of adult's may develop anemia during HIV infection, especially individuals with advanced disease, tremendous therapeutic advances have been made in the management of HIV infection, and anemia remains a major problem (Mehta, *et al.*, 2011).

The etiology of anemia during HIV infection is multi factorial, and contributing factors include micronutrient deficiencies, the anemia of chronic disease, opportunistic infections, impaired erythropoietin production, infection of hematopoietic progenitor

cells or other bone marrow stromal cells by HIV, infiltrative opportunistic infections in bone marrow, antimicrobial therapy and antiretroviral therapy. HAART reduced the transfusion requirements for some HIV-infected patients with anemia of chronic disease (Shet, 2015).

A study among HIV-infected children in Baltimore, Maryland, showed that the prevalence of anemia after 1year was reduced among children receiving HAART compared to those who did not receive any antiretroviral therapy. (Richard, *et al,.* 2001).

Vitamins like "vitamin B12, folic acid and riboflavin" influence the formation of Hb but the most important nutritional factor is iron deficiency, the most frequently occurring micronutrient deficiency in both developed and less developed countries. Since iron deficiency usually responds to iron supplementation or fortification, the assessment of iron status has been crucial in the evaluation of nutritional anemia. There are some clinical indicators of iron deficiency, e.g. chronic fatigue being the most important, but also loss of appetite, pallor, general lassitude and apathy, increased susceptibility to infection, and delayed psychomotor and cognitive development. Despite iron therapy, deficits in the latter symptoms in early childhood may result in long lasting detriment but they are usually unspecific symptoms (Okechukwu, 2010).

2.9 .Classification of Anemia as a Public Health Problem

The prevalence of anemia, as a public health problem is categorized as follows: <5%, no public health problem; 5–19.9%, mild public health problem; 20–39.9%, moderate public health problem; 40%, severe public health problem (WHO, 2002).

2.10. Diagnosis of Iron Deficiency Anemia

The evaluation of the iron status in addition to dietary intake, mainly relies on biochemical indicators, especially for the early stages of iron deficiency, usually iron deficiency occurs in three sequentially developing stages: depleted iron stores, iron deficient erythropoiesis and iron deficiency anemia. These stages can be analyzed biochemically and there is now an agreement that the measurement of Hb, ferritin and

soluble transferrin receptor (sTfR), complemented with indicators of acute and chronic infections, is the best procedure for evaluating iron status. Unfortunately this is usually a difficult and costly procedure (WHO, 2011).

The most important indicator for the iron status is the measurement of ferritin. The serum or plasma content correlates well with the iron stores and in the first stage of iron deficiency the concentration of ferritin decreases, which makes it the most sensitive parameter. Low ferritin always indicates storage iron depletion. Since ferritin is increased by a number of factors, especially infection and inflammation, a high value is not inevitably a sign of a good iron status, to solve this problem it is helpful to measure parameters for acute and chronic infection, to discover subjects in which the ferritin concentration might be increased by infection or inflammation. Currently the most used parameter for acute infections is C-reactive protein (C-RP) and for chronic infections Alpha-1 glycoprotein (AGP). Another solution is to measure an indicator like sTfR, which is less influenced by infection (Zemerman, 2006).

Table1. Cut off values for hemoglobin to define anemia based on age (Zemerman 2006).

Age in year	**Hb (g/dl)**
<5	11
5-11	11.5
12-14	12

In addition, severe anemia was defined as hemoglobin concentration of less than 7 g/dl, (WHO, 2001) and the clear cut off values for ferritin range between 10 - 30 mg/l. A ferritin value below 12 mg/l certainly shows iron deficiency.

2.11 Prevalence of Anemia Globally and In different Countries

Anemia is one of the most common hematological disorders in the world; affecting two billion people worldwide (WHO, *et al.,* 2001). While there are many causes of anemia, iron deficiency is a major contributing factor, and women of reproductive age are among the most seriously affected due to blood loss during menstruation, increased demands during pregnancy to support fetal development, and insufficient dietary intake of iron and other nutrients. During pregnancy, anemia and iron deficiency are associated with increased maternal mortality, low birth weight, and preterm birth this problem persists to children. In the context of HIV infection, anemia and iron deficiency are highly prevalent (Friis, *et al.* 2001).

HIV associated anemia is always overseen and it could be a challenge for prognosis of patients who are taking ART. The prevalence of anemia due to HIV at the early stage of infection is more prevalent than in the late stage(Levine, *et al.,* 2001). Knowing the impact of HIV on the haematopotosis of HIV infected patients is very essential for the management and care of people living with HIV/AIDS. HIV related anemia decreases the quality of life and survival rate of HIV patients (Adias, 2005).

As anemia was frequently identified as an independent risk factor for disease progression and death, failure of erythropoiesis was the most important mechanism for anemia in HIV-infected children. Therapeutic options include highly active antiretroviral therapy and prevention or treatment of secondary infections and erythropoietin can improve anemia in children, but it has not been evaluated in developing countries. Micronutrient supplementation may be helpful in individual children. Childhood iron-deficiency anemia is associated with behavioral and cognitive delays. Given the detrimental long term effects and high prevalence of iron deficiency, its prevention in childhood is an important public health issue (Brotanek, *et al.,*2007).

Iron deficiency anemia (IDA) is a global public health problem, affecting an estimated 51% of children in developing countries and 12 % in developed countries. Owing to rapid body growth and a depletion of neonatal iron stores, iron requirements during late infancy

are higher than during any other period of life. Early weaning to cow's milk or formula unfortified with iron is known to increase the risk for IDA in this age group. Iron fortification of common child foods is recommended in many countries. Iron supplements are often recommended for infants who are breast fed for longer than4 – 6 months and who do not consumed equate amounts of iron fortified complementary foods (Dugdale, 2011).

As stated by the (WHO, 2001) about 2 billion people, over 30% of the world's population are anemic with about 1 billion suffering from iron deficiency anemia. In many developing countries more than one out of every three children is estimated to be anemic (Adish,1999). In countries where meat consumption is low, such as India and many in sub-Saharan Africa, up to 90% of women are or become anemic during pregnancy (Hamedani, 1991). WHO estimates that some 800,000 deaths worldwide are attributable to iron deficiency anemia and this disease remains among the 15 leading contributors to the global burden of disease (Alkhateeb,1990).

The rapid growth of infants, especially between 6–24 months, includes a major increase in overall red blood cell and tissue volume. Their need for iron during this period is proportionately nearly as great as that of pregnant women, and is difficult to meet through breast-feeding and common complementary feeding practices alone. If there is too little iron in the diet, if the iron consumed is poorly bio available or if the overall meal contents interact to curtail availability beyond the range of the body's ability to up regulate absorption to meet iron needs, stored iron will be used up and iron deficiency will occur(Skikne, 1990).

Results from nationwide probabilistic survey in Mexico, The highest prevalence of iron deficiency anemia was found in infants 12 to 24 months old (48.9%). Rural infants of this age tended to have a higher prevalence (52.9%) than their urban counterparts (46.8%), Anemia was more prevalent in rural than in urban children 5-6 years of age (Susan,2005).

Iron deficient children in Indonesia and India have been found to have poorer performance than those with normal iron status, and lower performance could be

substantially improved after 12 weeks of iron supplementation. A positive association between iron status and performance was also found in a large double blind study of 1,358 children 9–11 years (Ahmed, *et al.,*2010).

A research result from New Delhi among HIV+ children taking ART mild anemia was present in 15(31.3%) of total 36 anemic children, and 16(33.3%) were having moderate anemia. Severe anemia was present in 5(10.4%) of cases. The mean hemoglobin in study population was 9.8gms/dl with a range of minimum 2.9 gm/dl and maximum 9.8 gm/dl, ferritin was low in 7 (14.6%) out of the total 48 subjects. Ferritin deficiency was found to be causing anemia. Nain (25.0%) anemic patients (total anemic 36) were <5 years of age and 27(75.0%) were >5years age. Socioeconomic status was not found to be significantly associated with the development of anemia. Anemic patients had normal or low (but not in severe immune suppression) CD4 counts. In comparison 23(63.9%) of 36 anemic patients had low for age (severe immune suppression). Immunological stage was a risk factor for the development and severity of anemia. Hence, more the immune suppression higher the chances of anemia and of severe grade 8 (66.7%) of the 12 non-anemic were on ART and 12 (33.3%) of 36 anemic patients were on ART. Use of ART was found to be protective against development of anemia, but use of ART was not protective against the development of severe anemia.

Thus, ART which was protective for the development of anemia was not protective for the severity of anemia. Duration of ART, and the regimen used (Zidovudine and Stavudine) were not significantly associated with occurrence of anemia. 30 (83.3%) of total 36 anemic children were moderate to severe under-weight and 28(77.8%) were moderately to severely stunted. They were found as a significant risk factor for the development of anemia its severity. But wasting or low BMI as well as dietary intake were not statistically significant. It proves that malnutrition is definitely a major risk factor for the development of anemia and of severe grade in the children with HIV infection (Rajeshwari, *et al.,* 2015).

Another cross-sectional study conducted among 300 children from June 2011 to June 2012 visiting the pediatric outpatient clinics of Al-Fayoum University Hospital in Egypt,

it was found that 64% of studied children had IDA (20% mild, 41.7% moderate and 2.3% severe).The risk factors associated with it are found that children from rural areas, those from low social class and those of low maternal educational level had a higher risk for IDA than other children. Infants with IDA were found to consume foods with low iron content 50% below recommended daily allowance (Siti-Noor, 2006). Among children admitted to Erbil, Iraq Hospital pediatric emergency unit iron deficiency was noted in 51.9% and 48.1% according to serum iron and transferrin saturation respectively. Male have more risk to have lower TS<16% as compared with female , age and tea ingestion were significant independent predictor of iron deficiency, family income associated significantly with low serum iron.(Zlotkin, 2002).

Anemia is estimated to affect one-half of school-age children in developing countries. The school years are an opportune time to intervene, and interventions must be based on sound epidemiologic understanding of the problem in this age group.

A study from Pemba Is-land, Zanzibar a sample of 3595 schoolchildren, their iron status was assessed by hemoglobin, erythrocyte proto-porphyrin (EP), and serum ferritin concentrations from a venous blood sample. Overall, 62.3% of children were anemic and 82.7% of anemia was associated with iron deficiency. The overall prevalence of iron-deficient erythropoiesis was 48.5%, and the prevalence of exhausted iron stores was41.3%. In bivariate analyses, iron status was slightly better in girls than in boys, and was better in children aged 7-11y than in those older or younger. Hemoglobin but not EP or serum ferritin concentrations were lower in stunted children. Infection with malaria, Trichuris trichiura, Ascaris /lumbricoides, and Hookworms were all associated with worse iron status; the association with hookworms was strongest by far. In multivariate analyses, hookworm infection intensity was the strongest explanatory variable for hemoglobin,EP, and serum ferritin. Sex, malarial parasitemia, A/lumbricoides infection, and stunting were also retained in the multivariate model for hemoglobin. Twenty-five percent of all anemia, 35% of iron deficiency anemia, and 73% of severe anemia were attributable to hookworm infection; < 10% of anemia was attributable to A/lumbricoides, malaria infection, or stunting (Stoltzfus, *et al.*, 1997).

A cross-sectional survey, conducted in a Peri-urban health center in Nairobi, Kenya, determined the prevalence of iron deficiency anemia (IDA) and its risk factors among 403 children aged 6 months to 6 years. Findings revealed that the prevalence of IDA was 7.4% and was predominantly mild (93.6%). Age was found to be significantly associated with IDA, with a 14.6% prevalence rate in infants. (Iron deficiency anemia in children of a peri-urban health facility.).The prevalence of anemia was 16.2%. The mean hemoglobin concentration was 12.53 g/dl in boys and 12.52 g/dl in girls. The results suggest that iron deficiency is an important determinant of anemia in this population. There was a significant relationship between education of the mother and anemia in children but not with the family income. It is concluded that improving the economic status of the family, women education and health education about balanced animal and plant food consumption are recommended strategies to reduce the burden of iron deficiency anemia.(Achouri,2015).

A research result in Northwest Ethiopia Gonder town, The prevalence of stunting, underweight, wasting and intestinal parasitoses among school children was 23%, 21%, 11% and18%, respectively (Beminet, *et al.*2012).

A results from ART Clinic of Minilik II Hospital Addis Ababa, Ethiopia: Of the 230 study subjects 121 (52.6%) were anemic before ART. However, the prevalence of anemia after ART (37.4%) was significantly decreased. The prevalence of anemia was higher in females than in males at base line (70.25% vs. 29.75%), and after ART treatment (69.23% vs. 30.77%). Mean CD4 cell count of study subjects was112cells/microl ± 67/microl at baseline. The mean CD4+ T cell count is significantly increased after ART and found to be211cells/microl ±120/microl. Significant association was observed between Hb and CD4+ T cell count after ART .There was a decline in the prevalence of anemia and increment of mean CD4+ T cell count among HIV infected patients after ART(Adane, 2012).

A retrospective cohort study among HIV infected children who received HAART at Zewditu Memorial Hospital in Addis Ababa, Ethiopia ART clinic. The mean level of hemoglobin, thrombocyte count and CD4 count showed statistically significant increment from the baseline.

After six months of HAART, the prevalence of anemia, thrombocytopenia and neutropenia among the study children was 21%, 8.3% and 13.3%, respectively. The result indicates that the mean hemoglobin, thrombocyte count and CD4+ count increased significantly in children who received HAART, but anemia, neutropenia and thrombocytopenia were common before and after treatment among the study subjects (Abebe, 2014).

A retrospective study in Harar in Hiwot Fana specialized hospital. The prevalence of anemia among HIV infected children was 39.2% 1 year after initiation of ART. At baseline, 51.6% of the study subjects were underweight (weight-for-age Z score less than -2 standard deviation, 49.1% were stunted (height-for-age Z score less than -2 SD); and 31.5% were wasted (body mass index less than -2 SD), which, after a year on ART declined to 8.9%, 15.9%, and 9.8%, respectively(Teklemariam, *et al.,* 2015).

Malnutrition is a common condition in HIV-infected children, A retrospective cohort study at Zewditu memorial hospital, Addis Ababa, Ethiopia evidenced that from a total of 475 HIV infected children starting antiretroviral treatment. Of whom 42 (8.8%) died during a median study follow up of 12 months. The average survival time for the entire cohort was 27.9 months. Independent baseline predictors of mortality were severe wasting (Hazard ratio = 4.99) absolute CD4 below the threshold for severe immunodeficiency (HR = 3.02) and low hemoglobin value (HR = 2.92) for those hemoglobin value < 7.0 gm/dl. Severe wasting (WHZ < -3) appear to be strong independent predictor of survival in HIV infected children receiving ART (Habteselassie, 2014).

Another study in northern Ethiopia Anemia was highly prevalent (42%) and constituted an important nutritional problem in the region. In a sub-sample of 230 anemic children, 56% had a low red blood cell (RBC) count, and 43% had a serum ferritin of less than 12 mg/l indicating that the anemia was largely due to iron deficiency. Dietary factors associated with iron deficiency anemia included frequent consumption of inhibitors, such as fenugreek and coffee, and poor health in the child such as diarrhea and stunting. Among school age children in southwest Ethiopia the overall, prevalence of anemia was 43.7%, and that of IDA was 37.4%. Not-consuming protein source foods, not consuming

dairy products, not-consuming discretionary calories, low family income and intestinal parasitic infections were predictors of IDA (Desalegn,2014).

3. METHOD AND MATERIALS

3.1. Study Area

The study was conducted in Black Lion Hospital located in Addis Ababa, the capital city of Ethiopia. It is situated in central Ethiopia at an elevation of about 2440 m (about 8000 ft) above sea level on a plateau. According to Central Statistics Agency (CSA, 2013) report, Addis Ababa which has an estimated population of 3,103,700, of them 92,000 - 120,000 children aged 0 to 14 living with HIV in the city (UNAIDS,2014). The city has an estimated area of 2,723,000 square kilometer. There is only one tertiary hospital in the town; Black Lion Hospital (BLH) one of the largest hospitals in Ethiopia. It has 200 doctors, 379 nurses and 115 other health professionals dedicated to providing health care service. The hospital treats 640 number of HIV cases of patient per year.

3.2. Study Design

Institution based cross – sectional study was employed among consecutively selected HIV infected children, who were visiting care and treatment center at Black Lion Hospital until the desired sample size attained, from January to April 2016.

3.3. Source Population

All registered children visiting Black Lion Hospital for antiretroviral treatment.

3.4. Study Copulation

The study populations were consecutively selected HIV infected children attending care and treatment center for follow-up were included.

3.5. Inclusion Criteria: all children visiting Black Lion Hospital for antiretroviral treatment.

3.6. Exclusion Criteria: children who take iron supplementation and hematologic patients.

3.7. Sample size Determination

The sample size required for this study was determined using a single population proportion sample size calculation formula by considering the following assumptions, prevalence of iron deficiency from previous study (39.4%), level of confidence (95%) and margin of error (5%). Then the formula for calculating the sample size was.

$$N = \frac{(Za/2)^2 P(1-p)}{D^2}$$

$$= \frac{(1.96)^2 (0.39)(0.61)}{(0.05)^2}$$

= **365.56 ≈ 366 +**5% non response rate

By using correction formula, because the total number of children who visit the ART clinic were less than 10, 0000.

$$S = 1 + \frac{n}{n/N}$$

$$S = 1 + \frac{366}{366/153} \approx 108$$

3.8 Sampling Procedure

A consecutive sampling procedure was employed until the determined sample size is completed.

3.9. Data Collection Tools and Procedures

3.9.1 Data Collection

Data was collected from January to April 2016. A structured questioner was used to collect information about socio- demographic characteristics, health, educational status, employment status and marital status of the care giver, family monthly income and number of children in the family. In addition, morbidity two weeks prior to the interview was collected. Presence of diarrhea defined as at least three loose stools in 1 day.

Health status during the interview was assessed through clinical evaluation and history taking for the presence of opportunistic infections based on the signs and symptoms ,and check the individual patient ART monitoring and record book to know the treatment regimens that the child get, and the presence of diagnosed current tuberculosis infection.

The questionnaire was developed in English, translated into the local language (Amharic) and back translated in to English for consistency. The questioner was pre-tested in 5 % of the total samples of study participants in non targeted hospitals before application. Any error and comments during pre-test was corrected in to the final version of the questionnaire. Interviewers were trained on interviewing techniques and were supervised by the investigator to maintain consistency in information gathering.

3.9.2. Dietary Assessment

For all children food items consumed in the 24hours preceding the interview was reported using a 24 dietary recall questioner. Food items were categorized as (1) starchy staples (foods made from grain, roots, or tubers); 2) legumes(soya beans ,nuts, peas, beans, chickpea); 3) dairy (milk, cheese, or yogurt); 4) meat, poultry, fish, or eggs; 5) vitamin A-rich fruits and vegetables (red or yellow yams ; carrots or red sweet potatoes; green leafy vegetables; fruits such as mango, papaya); 6) other fruits and vegetables (or fruit juices); and 7)foods made with oil, margarine, or butter according to (Arimond, *et al.,* 2010) were used to assess dietary diversity and food variety.

Participants were asked to report the frequency of consumption of each food using the last 24 hours. Using fixed cutoff points to define these 3 categories based on (Kennedy, *et al.,* 2011). A low diversity (having consumed only 1-2 food groups in the last 24 hours) whereas, medium diversity group (having consumed 3and 4 food groups in the last 24 hours) and high diversity that was ≥5food groups per 24hour.

3.9.3 Anthropometric Measurement

Weight and height were measured and recorded by the experienced nurses, working in the ART center. The children's age was obtained from the mothers/care givers.

Weight of the participants was measured in kilograms using standardized beam balance (Secagmbh and co.kg, SN.57552201, Germany) to the nearest 0.1kg while the children wore light clothes and bare foot. The scale was checked at zero before and after each measurement.

Measurement of height was conducted using the standard measuring height scale (Stadiometer, CE 0123, Germany) with sliding head bar and recorded to the nearest 0.1cm. The participants were asked to take off their shoes, stand erect, and look straight in vertical plain.

Nutritional status of the children was classified according to (WHO, BMI chart for determining nutritional status of HIV+ children and adolescents age 5-18years to assess the nutritional status of the study participant(WHO,2007). Since weight-for-age (WAZ) is inadequate indicator for monitoring child growth beyond pre-school years due to its inability to distinguish between relative height and body mass, therefore, BMI-for-age is recommended by the WHO and USCDC to assess thinness/wasting in school-aged children and adolescents, those lower than -3SD were classified as severe and between

-2SD taken as moderate, and -1SD considered as normal/mild malnutrition.

3.9.4 Clinical Data

All 108 children were subjected to detailed history with special(WHO,2006) references to symptoms; co-morbidities and the presence of opportunistic infections, at list one symptom for each illness were considered as having the disease and in addition to the symptoms inspection were done in order to detect the signs. Moreover, medical checkup was also done by the clinic Doctors.

Specifically wasting syndrome was detected in comparison of the current weight measurement with the previous 1 month measurement reviewed from their chart and considered as having wasting syndrome, if it was beyond 10% of the total body weight.

3.9.5. Laboratory Methods

The following tests were analyzed for each study subject, CD4+ count, renal function test, liver function test and hemoglobin tests, blood was collected from the study subjects using sterile vacutainers with EDTA anticoagulant and anticoagulant free tube for serum. To avoid any possible transmission of blood borne diseases, all lancets and vacutainers to draw blood were disposable; for the safety of the study participants antiseptic solution (alcohol with cotton) was used before the vein puncture in order to prevent infections.

A total of 5mls of venous blood was collected from each child from the cephalic/anti cubital vein (depending on the accessibility to the vein) by the laboratory technicians.

The 2ml blood sample in EDTA ant coagulated vacutainer was utilized for hemoglobin count, it was analyzed using (Sysmex hematology analyzer, SN, kx-5014,Japan) and CD4+count using (FACS Caliber count-BD flow cytometer) machine at Black Lion Hospital half to one hour after collection.

The rest 3ml of blood sample in anticoagulant free vacutainer was allowed to clot at ambient temperature and centrifuged at 3000rpm by (Humax 14k, centrifuge, SN, 042587, China) for 5 minute until the serum and cell fragment fully separated to measure serum ferritin (SF) and serum C-reactive protein high sensitive (CRPHS) in 108 study participant, the serum separated in to 1.8cc Nunc tube/serum container and stored at -20^{o}c until transported to Ethiopian Public Health Institute (EPHI) for analysis.

Finally, the serum was transported to EPHI with sample rack in ice box. Samples of serum were analyzed for serum ferritin using automated clinical analyzer (electro chem. ilumenescense immuno assay cobas 601 E SN; 14L3-03, Germany) and CRPHS analyzed

with clinical chemistry analyzer (immune turbid metric cobas, C 501, SN; 14L3-03, Germany) method and hemoglobin by (Sysmex. Hematology Analyzer, SN: kx-5014.Japan).

Data analyses, the following cutoffs were used for analyses. Anemia was defined as hemoglobin (Hb), <11.5 g/l for children age 5-11 year and 12g/l for age 12-14year, according to the WHO-recommended cutoff elevated C-RP as 5 mg/l (WHO, 2011). Hemoglobin value was adjusted for altitude according to WHO hemoglobin adjustment formula

$$Hb = -0.32 \times (\text{altitude in meters} \times .0033) + 0.22 \times (\text{altitude in meters} \times .0033)2$$

$$Hb = -0.32 \times (2440 \times .0033) + 0.22 \times (2440 \times .0033)2 = 11.86 g/l$$

3.10. Study Variables

3.10.1. Independent Variable

Socio-demographic characteristics: age, sex, educational status of parents/care givers, marital status of care giver, residence, employment status, number of children in the family, family income. In addition; CD4+count, illness history, and food frequency are independent variables.

Treatment regimen: There are various combinations and formulations of ART drugs. These drugs are given in different regimens; the most effective combination currently is HAART (highly active antiretroviral therapy), which includes at least three drugs from one or all the three categories, those are Lamivudine(3TC), Abacavir(ABC), Stavudine(d4T), Zidovudine(ZDV), Nevirapine(NVP), Efavirenz(EFZ).

3.10.1. Dependent Variable: Iron deficiency (iron depletion), nutritional status, and anemia

3.11 Operational Definition

Iron deficiency: The state of having depleted iron store, a serum ferritin < 12 ng/dl or <30 ng/dl in the presence of acute infection.

Iron deficiency anemia: combination of iron deficiency and anemia (WHO, 2001).

Nutritional status: were defined, sever acute malnutrition (Z score < -3), moderate acute malnutrition (Z score -2) and normal/mild acute malnutrition (Z score -1) based on BMI, (WHO, 2007).

Wasting syndrome: a total weight loss >10% within 1month.

3.12 Data Quality Control

To ensure data quality, since the target group had been children living with HIV special care and training were given for the data collectors for one day. Before collecting the data, the questionnaire was pretested in similar groups with the study population in non-selected hospitals (St Paulo's and Zewditu Memorial hospital) to familiarize the data collectors with the data collection tools, specimen collection and sample handling. Any error found during the process of pre-testing was corrected in the final version of the questionnaire. The principal investigator was made frequent checks on the data collection process to ensure the completeness and consistency of the information gathered.

3.13. Statistical Analyses

Data was cleaned for inconsistencies and missing values, described and summarized using univariate, descriptive statistics mean, standard deviation, median, quartiles, range, minimum and maximum values, percentage, and frequencies was done to determine the proportion of socio-demographic and other independent variables of the study participant. A bivariate and multivariate logistic regression (crud and adjusted odds ratio) analysis was performed to identify associated factors related to anemia and iron deficiency. Statistical significant in this study was defined as P value 0.05, analyses were performed using SPSS software version 20.

3.14. Ethical Consideration

A formal letter for ethical approval was obtained from College of Natural Science Institutional Review Board of Addis Ababa University Center for Food Science and Nutrition to secure permission.

Before proceeding with the interview and sample collection, parents of participants were informed about the general purpose of the study and issues of confidentiality, and informed consent was obtained to participate in the study. Additionally, participants were informed that their participation in this study would not involve any risks, and also they have a full right to refuse or discontinue participating in the study at any time.

Effort was done to overcome ethical concerns of the participants due to the sensitivity of taking blood sample; clear explanation about the purpose and usefulness of the study was given. Confidentiality of information was assured by excluding names and other identifying numbers from the questionnaires.

4. RESULT

4.1. Socio-Demographic Characteristics of the Study Participants

A total of 108 children participated in the study. A Socio demographic characteristic of the study participants is indicated in table 2. The age range of the study participants were from 6-18, majority of study children live with their parents. However, substantial number of them 15 (13.9%) were orphan.

Majority 94(92.6%) of their fathers and 47(43.5) mothers were secondary school completed. Out of 108 the total study populations 57 (52.8%) were male 51 (47.2%) were female that was 1.11ratio, 94(87%) of them live in Addis Ababa the rest 14 (13 %) were live regions outside Addis Ababa (9.3% were from Oromia, 1.9% Amhara, 0.9% Tigray and 0.9% Harar). More than fifty percent 67(62%) of the study participants' family were earned<1000 ETB per month, 41(38%) >1000ETB with a median of 600ETB. Significant number of the study participants' mothers were house wives 38(35.2%) and 41(38%) fathers were daily labourer. Majority 92 (85.2%) the family of the participants have <3 children, 10(9.3) 4-6 children very small amount of study participant were live with>6 children in their family. Marital status of the parents/care givers of the study participants, 53(49.1%) of them were married, 17(15.7%) were single, 29(26.9%) widowed and the rest 9(8.3%) divorced at the time of the study (Table 2).

Table2. Socio- demographic characteristics of the study participants (n=108) among HIV$^+$ children visiting ART center at Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Variables	Frequency	ercent	ean ±SD
Child age			
6-12	30	27.8	7.9±2.49
13-18	78	72.2	15.2±1.66
Child residence			
Addis Ababa	94	87	
Regions outside Addis Ababa	14	13	
Respondents relation with child			
Mother	53	49.1	
Father	21	19.4	
Orphan	15	13.9	
Other	19	17.5	
Maternal education			
Unable to read and write	28	25.9	
Primary education	22	20.4	2.38±0.98
Secondary education	47	43.5	
Higher education	11	10.2	
Maternal/care giver Occupation			
House wife	38	35.2	
Civil servant	25	23.1	
Merchant	13	12	
Daily laborer	9	8.3	
Other	29	18.5	
Marital status			
Single	17	15.7	
Married	53	49.1	
Widowed	29	26.9	
Divorced	9	8.3	

Table2. Socio- demographic characteristics of the study participants (n=108) among HIV$^+$ children visiting ART center at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia 2016.

Variables	Frequency	percent	Mean ±SD
Unable to read and write	2	1.9	
Primary education	6	5.6	2.91±0.35
Secondary education	94	92.6	
Higher education	6	5.6	
Paternal Occupation			
Civil servant	20	18.5	
Farmer	11	10.2	
Merchant	10	9.3	
Daily laborer	41	38	
Other	26	24	
Number of children in the family			
≤3	92	85.2	2.19±1.64
4-6	10	9.3	
≥6	6	5.6	
Family monthly income			
<1000	67	62	
>1000	41	38	

4.2. Opportunistic Infections and Other Illness of the Study Population

According to the care givers response, history, and clinical examination, 15(13.9%), 12(11.1%) of the study populations had upper respiratory tract infection in the week prior to the interview and within the last 14 days. Thirteen (12%) of the children had diarrhea in 14 days, 6(5.6%) in 7 days preceding the study. none of the study participants were diagnosed for malaria neither 7days nor 14 days (Table 3).

Table3. Morbidity among HIV$^+$ children (n=108) two weeks preceding the data

	Last 7day		Last 14 day	
Morbidities	**Frequency**	**Percent**	**Frequency**	**Percent**
Diarrhea	6	5.6	13	12
Malaria	0	0	0	0
URTI	15	13.9	12	11.1

* upper respiratory tract infections

From a total of (n=108) children 10(9.3%) were diagnose and on treatment for tuberculosis during the study period, 11((10.2%) of the children had wasting syndrome, 12(11.1%) oral ulcer, 11(10.2%) acute/chronic diarrhea, 11(10.2%) pneumocystis carrini (Table4).

Table 4. Opportunistic infections among HIV$^+$ children (n=108) at Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Opportunistic infections	**Frequency**	**Percentage**
Acute/chronic diarrhea	11	10.2
Tuberculosis	10	9.3
Oral thrush	6	5.4
Oral ulcer	12	11.1
Pneumocystis carrini	11	10.2
Wasting syndrome	11	10.2

4.3. Laboratory and Treatment Regimen Results

A total of 108 children were tested for CD4+ cells count. The median CD4+ cell counts was 672cells/µl, the range were between 132cells/µl-2335cells/µl.

Substantial number of children 42 (38.9%) were on 4C (AZT, 3TC, NVP) ART treatment combination, while 20(18.5%) on 4D (AZT, 3TC, EFZ), 22(20.4%) 1E (TDF+3TC+NVP) and 4.9% 1G (ABC, 3TC, EFZ).

Iron deficiencies based on serum ferritin levels were available from 108 children of these study population 12(11.11%) low ferritin level <12mg/l, 96(88.89%) had a ferritin level of 12 mg/l and above, the overall median ferritin was 53.73mg/l, above 75% percent of the study population had ferritin level of 100.25, below 25% percent of the participants were 23.14mg/dl ferritin level. The overall anemia among the study population 19 (17.6%) were anemic based on their hemoglobin value but severe anemia was 0(0%). A total of 20(18.5%) patients had acute infection based on C-RP level >5mg/l (Table5).

Table5. Biochemical, hematological, treatment regimens, clinical and nutritional profiles among HIV^+ children (n=108) at Black Lion Hospital Addis Ababa, Ethiopia 2016.

Variables	Frequency	Percent
$CD4^+$ count		
<500cells/µl	67	62
Treatment regimen		
4C(AZT,3TC,NVP)	42	38.9
1E(TDF+3TC+NVP)	22	20.4
4D(AZT,3TC,EFZ)	20	18.5
1G(ABC,3TC,EFZ)	5	4.9
Other	19	17.5
Iron deficiency status		
Iron deficient	12	11.1
Non iron deficient	96	88.9
Anemia status		
Anemic	19	17.6
Non-anemic	89	82.4
C-reactive result		
≤5mg/l	88	81.5
>5mg/l	20	18.5
Nutritional supplementation (RUFT)	15	14.2
Yes	15	14.2
No	93	85.8

*Lamivudine(3TC),Abacavir(ABC),Stavudine(d4T),Zidovudine(ZDV/AZT),Nevirapine(NVP),Efavirenz(EFZ)

Majority of the study population had normal renal function test result only10 (9.25%) with a mean value of (0.8mg/dl±0.23mg/dl, SD) were found with abnormal creatinine result.

From the liver function test result the alkaline phosphate result were elevated among 86(76.62%, Mean=558.09U/L±280.86u/l, SD) with minimum value of 43u/l and a maximum of 1495u/l. 14(12.96%) had elevated SGOT minimum 0.6u/l,77u/l maximum which was double of the normal maximum reference rang and the SGPT was 11(10.18%) (Table6).

Table6. Organ function test result among HIV^+ children (n=108) at Black Lion Hospital Addis Ababa, Ethiopia 2016.

Functional testes	Minimum	Maximum	Mean	Std. Deviation	Proportion with elevated value	Normal range
Renal fun. test						
Urea mg/dl	0.60	36.00	16.67	7.13	108(0.0)	10-50
Creatinine mg/dl	0.30	1.80	0.80	0.23	10(9.25)	0.6-1.1
Liver fun. test						
SGOT u/l	0.60	77.00	26.37	12.93	14(12.96)	≤37
SGPT u/l	8.00	75.00	21.54	11.50	11(10.18)	≤37
ALP u/l	43.00	1495.00	558.09	280.87	86(76.62)	64-306

* ALP; Alkaline phosphatase, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase.

4.4 Nutritional Status and Dietary Diversity Indicators

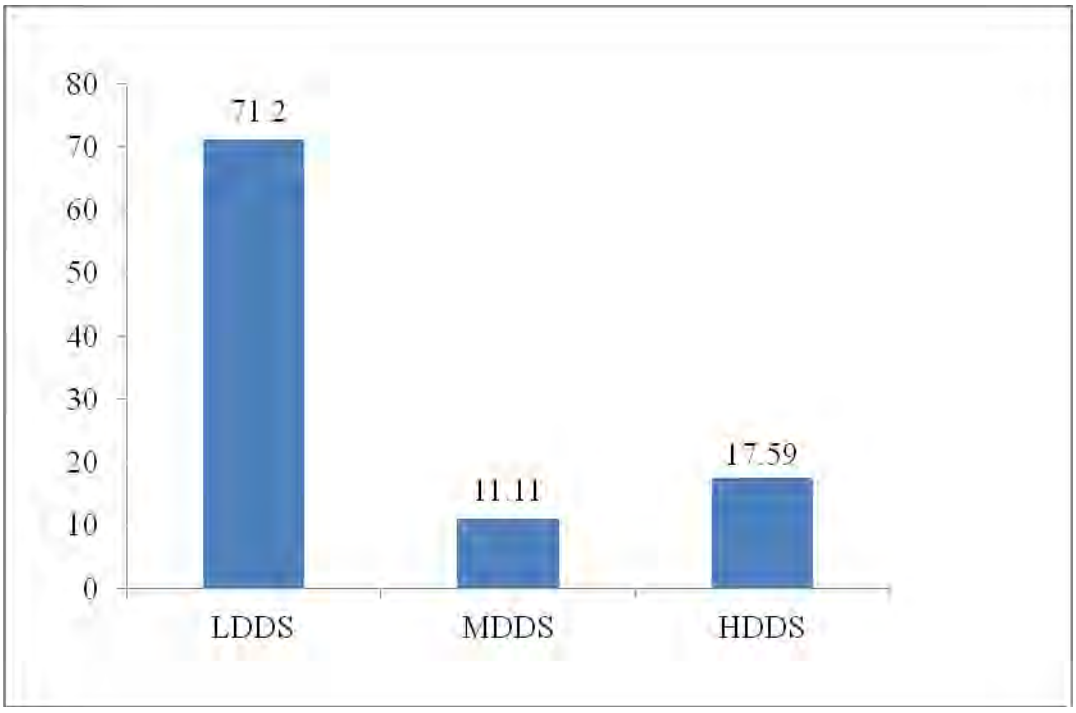
The prevalence of sever acute malnutrition (SAM) among children 23(21.29%) had (BMI less than -3 SD) and 28 (25.92%) moderately wasted (BMI less than -2 SD), 49(45.37%) were normal or had mild malnutrition (BMI less than -1 SD). About 15 (13.9 %) participants were supplemented with plump nut (RUTF).

4.5 Food Varity Score and Dietary Diversity Score

The children had a mean FVS of (3.43± 2.38SD) with a minimum variety of 1and 11, maximum variety while the theoretical maximum was15. The mean DDS of the study population was 2.96(SD ±1.71) the range of DDS was 1-6. When considering the dietary diversity and consumption pattern of the sample population, all the children had used the same kind of cereals. Furthermore, a higher proportion had starchy staples were more than 90 percent.

The food items with the highest frequency of consumption were from the starchy staple (foods made from grain, roots, or tubers), Teff were consumed by 99(91.7%) of all children. Followed by the legumes and food made with fat, oil, margarine/ butter 56(51.9%), vitamin-A-rich by 27(25%) off those food group. Yam, were consumed infrequently by only 1(0.9%) of the children were consumed. Other fruit 25(23.1%), dairy group were consumed by 22(20.4%), meat group by 17(15.7%), Fish were consumed infrequently only by 1(0.9%) of the children. Moreover, 77(71.29%) participants were with inadequate/low dietary diversity12 (11.11%), 19(17.59%) were taking moderate to high variety per day (Figure2).

Figure2: Bar graph of LDDS, MDDS, HDDS



*HDDS: high dietary diversity score, MDDS: moderate dietary diversity score, LDDS low dietary diversity score.

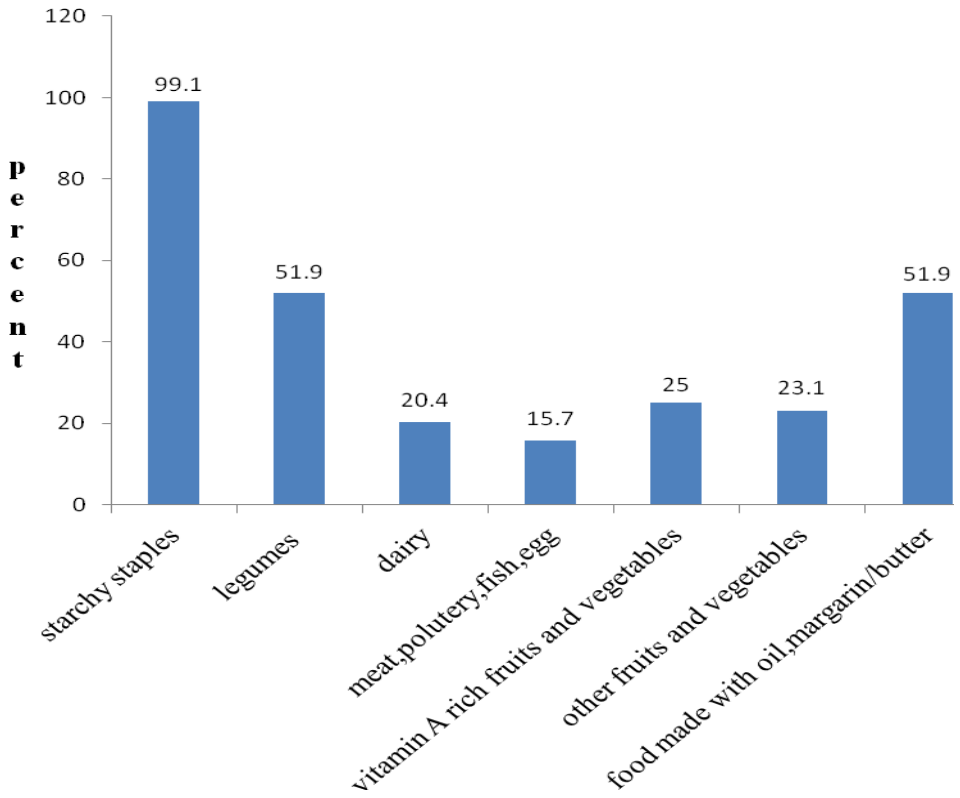
Table7.Percentage, distribution and intake of different food items in the 24hr preceding the study among the study population (n=108), at Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Food item	Frequency	Percent
Starchy staples		
Teff	99	91.7
Sorghum	13	12
Millet	2	1.9
Corn(Maize)	5	4.6
Wheat	82	75.9
Barely	17	15.7
Rice	13	12
Oats	15	13.9
Legumes		
Beans	43	39.8
Pea	43	39.8
Soya bean	7	6.5
Nut	17	15.7
Dairy		
Milk	18	16.7
Cheese	9	8.3
Yogurt	7	6.5
Meat, poultry, fish, or eggs		
Egg	9	8.3
Chicken	8	7.4
Fish	1	.9
Meat	11	10.2
vitamin A-rich fruits and vegetables		
Orange	15	13.9
Mango	12	11.1

Food item	Frequency	Percent
Yam	1	.9
Carrot	18	16.7
Sweet potato	12	11.1
Green leafy vegetables	8	7.4
Tomato	55	50.9
Other fruits and vegetables		
Apple	3	2.8
Banana	14	13
Strawberry	4	3.7
Lemon	11	10.2
Pineapple	2	1.9
foods made with oil, margarine, or butter		
Margarine	43	39.8
Butter	22	20.4

Percentage, distribution and intake of different food items in the 24hr preceding the study among the study population (n=108), at Black Lion Hospital Addis Ababa, Ethiopia 2016.

Figure3. Consumption of different food groups by the study population 24hour preceding the study.



4.6 Factors Associated with Anemia and Iron Deficiency

Bivariate logistic regression analysis was done to assess association of socio demographic variables and other factors with child anemia and iron deficiency. Accordingly, from the child variables only age shows statistically significant association with anemia, but not with iron deficiency. Children age from 13-18years had significantly greater unadjusted odds of anemia (COR: 5.06; 95% CI: 1.78–14.37) which mean prevalence of anemia increased with age in this study in comparison with children age from 6=12 years (Table 8).But it did not show statistically significant association in multivariate logistic regression. The remaining maternal and paternal factors did not show statistical significant association with child anemia and iron deficiency.

Table8. Socio-demographic characteristics of the study participants associated with anemia and iron deficiency (n=108) among HIV^+ children visiting ART center at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia 2016.

Variables	Anemia		Iron deficiency	
	COR	AOR	COR	AOR
Child age				
6-12year	1	1	1	
13-18year	5.06(1.78,14.37)*	3.49(0.76,16.07	1.35(0.37,4.85)	-
Child sex				
Male	1	1	1	
Female	0.46(0.16,1.26)	0.23(0.04,1.24)	1.66(0.49,5.58)	-
Child residence				
Addis Ababa	1	1	1	
Regions outside Addis Ababa	0.47(0.13,1.71)	3.77(0.33,43.30)	2.58(0.60,10.98	-
Maternal education				
Unable to read&write	1	1	1	
Primary education	0.03(0.21,0.05)	0.81(0.06,1.06)	1.30(0.11,15.98	-
Secondary education	0.82(0.19,3.58)	0.69(0.78,6.28)	0.34(0.04,3.34)	-
Higher education	0.54(0.08,3.77)	0.39(0.02,6.20)	1.08(0.11,10.69	-
Mother Occupation				
House wife	1		1	
Civil servant	4.11(0.82,20.56)	-	0.61(0.06,6.32)	
Merchant	1.96(0.37,10.44)	-	0.39(0.04,4.03)	
Daily laborer	2.86(0.32,25.80)	-	0.29(0.02,3.57)	
Other	2.02(0.49,8.41)	-	0.11(0.01,1.21)	
Marital status				
Single	1	1	1	
Married	0.82(0.21,3.36)	0.58(0.02,14.61)	-	-
Widowed	1.34(0.26,6.86)	0.19(0.00,8.40)	3.50(0.54,22.78	
Divorced	1.71(0.15,19.36)	-	1.09(0.78,6.60	
Paternal education				
Unable to read & write	1			
Primary education	1.05(0.19,5.89)			
Secondary education	0.78(0.21,2.84)	-	-	-
Higher education	0.41(0.11,1.46)			

¶

Variables	Anemia		Iron deficiency	
	COR	AOR	COR	AOR
Paternal occupation	1.2(0.22,6.52)	0.58(0.35,9.56)	0.61(0.06,6.32)	
Farmer				
Merchant	0.8(0.13,5.09	3.81(0.02,5.93	0.39(0.04,4.03)	
Daily laborer	1.2(0.16,9.01)	1.16(0.16,8.33)	0.29(0.02,3.57)	
Other	2.16(0.44,10.63)	1.99(0.12,32.74	0.11(0.01,1.21)	
Number of children in the family				
≤3	1	1	1	
4-6	0.45(0.10,1.96)	0.09(0.00,6.34)	1.22(0.14,10.59	-
≥6	0.97(0.11,8.94)	-	-	
Family monthly income				
<1000 ETB	1	1	1	-
>1000 ETB	0.62(0.21,1.85)	0.44(0.09,2.15)	1.51(0.34,6.74)	

*Significant:

4.7. Opportunistic and Other Illness Associated with Anemia and Iron Deficiency

There was no significant association in the prevalence of anemia and iron deficiency between children with or without opportunistic infections and other illness in the previous week/14 days preceding the study (Table9).

Table9. Opportunistic and other illness variables associated with anemia and iron deficiency state among HIV^{+} children in Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Opportunistic and other illness	Anemia		Iron deficiency	
	COR	AOR	COR	AOR
(URTI)	2.70(0.72,10.11)	1.07(0.35,3.24)	0.40(0.12,1.39)	0.37(0.09,1.35)
Acute/chronic diarrhea	1.96(0.65,5.89)	0.69(0.19,2.44)	0.75(0.22,2.51)	1.77(0.32,9.85)
Tuberculosis	5.37(0.99,29.04)	1.27(0.38,4.17)	1.36(0.40,4.63	1.71(0.34,8.58)
Oral thrush	1.18(0.12,11.19)	1.19(0.47,3.05)	1.79(0.54,5.99)	1.12(0.20,6.21)
Oral ulcer	1.05(0.21,5.28)	0.79(0.26,2.36)	0.92(0.27,3.10	0.94(0.22,3.99)
PCP	3.37(0.52,21.74)	-	2.97(0.53,16.71)	1.90(0.25,4.77)
Wasting syndrome	5.11(0.67,38.88)	0.87(0.21,3.64)	0.40(0.12,1.59)	1.69(0.28,10.11

4.8. Functional Tests, Treatment Regimens, Clinical and Nutritional Profiles Associated with Anemia and Iron Deficiency

Treatment regimen was a significant variable highly associated with anemia at (AOR=0.09; 95% CI: 0.01, 0.91). Participants who take treatment regimen 1E (TDF+3TC+NVP) combination were less likely to develop anemia relative to other treatment regimens which prevents anemia by 9%.

The other relevant determinant was serum ferritin (AOR=22.85; 95% CI: 3.67, 142.23), strongly associated with anemia. Participants, whose serum ferritin <12mg/l were 22.85 times more likely to be anemic as compared to those serum ferritin >12mg/l,this number gives insight the anemia among HIV infected children was because of iron deficiency. Serum glutamic oxaloacetic transaminase (SGOT) (AOR=0.48; 95% CI: 0.00, 0.88) significantly associated with serum ferittin which were at lower risk to develop iron deficiency. It prevents iron deficiency by 48% relatively (Table10).

Table10. Functional tests, treatment regimens, clinical and nutritional profiles associated with anemia and iron deficiency state among HIV$^+$ children visiting ART center at Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Variables	**Anemia**		**Iron deficiency**	
Functional testes	**COR**	**AOR**	**COR**	**AOR**
Renal function test				
BUN	-	0.94(0.03,29.69)	0.23(0.02,2.79)	-
Creatinin	0.75(0.14,3.89)	0.30(0.04,2.40)	1.27(0.15,11.01)	1.44(0.13,16.18
Liver function test				
SGOT	0.58(0.11,3.03)	0.52(0.05,4.98)	1.67(0.49,6.84)	0.48(0.00,0.88)*
SGPT	0.49(0.06,4.15)	-	0.88(0.10,7.61)	2.89(0.19,43.26)
ALP	0.43(0.09,2.01)	-	2.93(0.36,24.09)	0.47(0.05,4.85)
CD4$^+$ count				
<500	1		-	1
>500	1.07(0.37,3.13)	-	1.07(0.29,3.80)	1.11(0.26,4.64)
Treatment regimen				
4C(AZT,3TC,NVP)	1	1	1	1
1E(TDF+3TC+NVP)	0.44(0.10,1.96)	0.09(0.01,0.91)*	2.80(0.31,25.5)	3.16(0.29,34.92)
4D(AZT,3TC,EFV)	1.13(0.19,6.54)	0.24(0.02,2.83)	3.00(0.33,27.23)	3.42(0.35,33.76)
1G(ABC,3TC,EFV)	0.40(0.10,2.68)	0.79(0.48,12.930)	1.20(0.12,11.86)	1.47(0.12,18.06)
Other	0.43(0.11,1.63)	0.15(0.02,1.33)	1.27(0.28,5.68)	1.20(0.23,6.36)
Nutritional status				
SAM	0.99(0.25,3.87)	-	1.95(0.47,8.11)	0.66(0.43,10.06
MAM	0.16(0.39,1.05)		1.29(0.33,5.15)	1.17(0.08,16.78)
Normal/mild malnutrition	0.40(0.14,1.16)		1.49(0.44,5.03)	1.99(0.16,24.53)
Iron deficiency				
S. ferittin>12mg/l	1			
S. ferittin<12mg/l	9.8(2.68,35.89)	22.85(3.67,142.23)*		

*Significant

4.9 Dietary Variables Associated with Anemia and Iron Deficiency State

None of the 24hour recalls DDS or FVS were associated with neither anemia nor iron deficiency (Table11).

Table11. Dietary variables associated with anemia and iron deficiency state among HIV^+ children visiting ART center at Black Lion Hospital, Addis Ababa, Ethiopia 2016.

Food group		Anemia		Iron deficiency	
	Frequency (%)	COR	AOR	COR	AOR
Starchy stables					
No	1	-	-	-	-
yes	107(99.1%)				
Legumes					
No	52(48.1%)	1	1	1	1
Yes	56(51.9%)	0.96(0.36,2.59)	1.10(0.38,3.23)	0.74(0.22,2.51)	0.57(0.15,2.08)
Dairy					
No	86(79.6%)	1	1	1	
yes	22(20.4)	0.66(0.21,2.09)	0.91(0.23,3.60)	-	-
Meat poultry fish egg					
No	91(84.3%)	1	1	1	1
Yes	17(15.7%)	0.64(0.18,2.24)	0.59(1.25,2.79)	2.20(0.26,18.26)	0.97(0.08,11.22)
Vitamin A rich foods and vegetables					
No	81(75%)	1	1	1	1
yes	27(25%)	0.67(0.23,1.98)	0.84(0.19,3.58)	1.00(0.25,3.99)	0.44(0.08,2.51)
Other fruits and vegetables					
No	83(76.9%)	1	1	1	
Yes	25(23.1%)	0.81(0.26,2.52)	1.42(0.29,6.95	3.67(0.45,29.90)	3.41(0.29,40.03)
Food made with oil, margarine ,butter					
No	52(48.1%)	1	1	1	
Yes	56(51.9%)	0.74(0.27,2.02)	0.89(0.29,2.64)	2.36(0.67,8.38)	1.69(0.42,6.77)

5. DISCUSSION

A total of 108 children included in the study. The age range of the study participants was 6-18years old children. Majority of the children were living with their parents, but substantial number of children were orphan and of their parents had completed secondary school, housewife mothers, and daily laborer fathers.

All children were on different ART treatment combinations with the majority taking treatment 4C (AZT, 3TC, NVP) combination. The median CD4+ cell counts was indicating that most of them were within the normal range, but the maximum value was out of the normal range which based on WHO guideline this might be as a result of unidentified hematological problems.

Small number of the study population had abnormal renal function test result based on creatinine. Unlike to the renal function test their liver function test result was significantly elevated above the normal value especially, the alkaline phosphate result were highly elevated, in fact this enzyme was expected to rise in children in a normal physiological condition, but also shows some abnormality with their liver. More than 75% of the children had elevated SGOT value, which was double of the normal maximum reference rang while the SGPT not prevalent but still it was significant, this might be due to long term use of antiretroviral therapy which has hepatotoxcicity effect or as a result of other pathological disorders and undiagnosed infections.

None of the participants had malaria infection based on history, but they acquired upper respiratory infections, diarrhea and some of them were suffered from opportunistic infections.

When considering the dietary diversity and consumption pattern of the sample population, all the children had used the same kind of cereals. Furthermore, a higher proportion had used starchy staples. The food items with the highest frequency of consumption were from the starchy staple (foods made from grain, roots, or tubers), Teff were consumed by all children,followed by the legumes and food made with fat, oil, margarine/ butter and Fish, were consumed infrequently, based on the mean individual dietary diversity score in the study subjects, near to similar patterns were observed when examining the percentages of children with low, moderate, or high dietary diversity, more than 50% children were acutely wasted based on BMI. Anemia was prevalent among HIV+ children, which is explained by iron deficiency. Opportunistic infections, socio-demographic data, nutritional status and dietary diversity were not predictor of anemia and iron deficiency. The determinants of anemia and iron deficiency in this study were age, iron deficiency, SGOT, and treatment regimen.

Literatures from different countries have found anemia as the most common hematological abnormality in HIV infected children. In this study 19(17.6%), were having anemia, these findings were similar to the prevalence of severity of the anemia observed in other studies. Socio demographic variables, wasting or low BMI as well as dietary intake were not statistically significant, which was consistent with study finding of (Rajeshwari, *et al.* 2015).

prevalence of anemia from this study lower than the study from pediatric outpatient clinics of Al-Fayoum university hospital in Egypt.This might be resulted from basic socio demographic, cultural, health and dietary habit difference, it was found that 64% of studied children had IDA (Mohammed, *et al.* 2015).

Among children admitted to Erbil, Iraq hospital pediatric emergency unit iron deficiency was noted in 51.9% and 48.1% according to serum iron and transferrin saturation respectively, but there was similarity in the predictors that was age. Found that the prevalence increases with age it might be associated with HIV disease progress (Zlotkin, 2002). This discripancy might resulted from the parameters and labratoy method used to determine anemia and iron deficency, a clinical history of the study population, and treatment regimn.

The prevalence of anemia was found to be relatively higher than that of research from Per-iurban health center in Nairobi, Kenya findings revealed that the prevalence of IDA was 7.4%, which was less than this study result. It might be due to basic socio demographical, health and cultural difference in feeding practice but similar in the determinate of anemia among HIV+ children. The percentage of anemia in HIV infected children were similar to what was found in the other studies of similar kind, which was consistent with the prevalence of Kenitra, Northwest of Morocco, the prevalence of anemia was 16.2%. The mean hemoglobin concentration was 12.53 g/dl in boys and 12.52 g/dl in girls, the results suggest that iron deficiency is an important determinant of anemia in this population. There was a significant relationship between education of the mother and anemia in children which was different from the current study but not with the family income which was consistent with this study (Achouri, 2015).

A result from Pemba Is-land, Zanzibar, iron status was assessed by hemoglobin, erythro-cyte protoporphyrin (EP), and serum ferritin concentrations from a venous blood sample, overall, 62.3% of children were anemic (hemoglobin <110g/l), and 82.7% of anemia was associated with iron deficiency which were triple of this study but the predictors were the same. The overall prevalence of iron-deficient erythropoiesis was 48.5%, and the prevalence of exhausted iron stores

(serum ferritin <12g/L) was 41.3%. There were a difference in the parameters drawn to detect anemia ,the previous method was to detect iron deficiency and anemia at the two levels this increase the prevalence, but the current study parameter that I used only gave the number of iron deficiency and anemia at the first stage (iron depilation stage) this reduced the prevalence relatively. It was consistent in bivariate analyses iron status was slightly better in children age 7-11 year than in those older.

A retrospective study in Harar Hiwot Fana specialized hospital, the prevalence of anemia among HIV infected children was 39.2%, 1 year after initiation of ART. This difference may arise from the difference between treatment regimens and duration of intake, from multivariate analysis of the current study shows treatment regimen 1E (TDF, 3TC, NVP) had preventive effect than the other medications and also resulted from long term use of anti retroviral therapy.

At baseline, 51.6% of the study subjects were underweight (weight-for-age Z score less than -2 SD); 49.1% were stunted (height-for-age Z score less than -2 SD); and 31.5% were wasted (body mass index less than -2 SD), which, after a year on ART, declined to 8.9%, 15.9%, and 9.8%, respectively.(Teklemariam, *et al.* 2015). The prevalence of sever acute malnutrition (SAM) among children were 23(21.29%) (BMI less than -3 SD) and 28 (25.92%) moderately wasted (BMI less than -2 SD) the rest 49(45.37%) were normal or had mild malnutrition (BMI less than -1 SD). About 13.9 %(15) different from the previous study it might be due to the difference in parameter taken to classify this was based on specific WHO BMI chart for determining nutritional status of HIV^+ children and adolescents, but the previous study was based on weight for age and weight for height and it also explained by the use of plumpy nut supplementation about 15(14.2%) of the study population was supplemented.

This significant number of under nutrition was not considered totally as protein energy malnutrition, it might be as a result of HIV wasting syndrome, because from the total study population 11(10.2) of them had been wasting syndrome, since, BMI defines acute wasting.

The finding of this study was not consistence with a study in northern Ethiopia; anemia was highly prevalent (42%) and constituted an important nutritional problem in the region. In a sub-sample of 230 anemic children, 56% had a low red blood cell (RBC) count, and 43% had a serum ferritin of less than 12 mg/l indicating that the anemia was as a result of iron deficiency. This difference was associated with a long term time lapse between the studies (Addish, 1999).

Similar study among school age children in southwest Ethiopia,the Overall, prevalence of anemia was 43.7% and IDA was 37.4% (Desalegn,2014), which was two times double with the current study ,because the current study population had elevated serum SGOT which was statically significant in preventing iron deficiency anemia relatively. This functional test was not included as parameter, so the difference might arise from this parameter and periodic examination of complete blood count was done for those HIV infected children based on the protocol for care and treatment of HIV infection.

6. Conclusion and Recommendation

To explore anemia and iron deficiency among children living with HIV, cross-sectional study was conducted to examine the prevalence, determinant and risk factors of anemia in a group of HIV infected children in ART clinic. Even though, the prevalence of anemia in the clinic was high but it is not severe anemia.

Treatment with ART prevents anemia among HIV-infected children specifically treatment regimen1E (TDF, 3TC, EVZ) has a protective effect relative to other combinations. The independent predictor of anemia was iron deficiency and age. The socio demographic variables were not determinants of anemia and iron deficiency.

This research hypothesized that, nutritional factors including dietary intake, had no etiological role in childhood anemia in the context of HIV infection. Significant number of study participant was their serum liver enzyme elevated above normal, which shows liver abnormality, but those patients with elevated serum specifically the SGOT were less likely to develop iron deficiency which also implies directly or indirectly to iron deficiency anemia. Malnutrition (SAM, MAM) were highly prevalent among HIV+ children.

The limitation of the current study could be the study design as the cross-sectional study design by its nature limits information about cause and effect relationship in the majority of predictors, dietary diversity and variety was based on 24 hour recall method, respectively they may create a possibility of recall bias.

Iron deficiency and anemia appear to be an important nutritional problem among HIV+ children at ART center. Therefore, effective actions aimed at the prevention and controls of this deficiency are strongly recommended in this context.

Reference

Abebe H, (2014). Study of the immunologic and hematologic profile of children on HAART: a retrospective cohort study at Zewditu Memorial Hospital (doctoral dissertation, AAU).

Achouri A, Sbaibi R, Ahami A and El Hioui M (2015). prevalence of iron deficiency anaemia among school children in Kenitra, Northwest of Morocco. *Pakistan Journal of Biological Sciences,* ***18***: 191-195.

Adane A, Desta K, Bezabih A, Gashaye A & Kassa D (2012). HIV associated anemia before and after initiation of antiretroviral therapy at ART Centre of Minilik II Hospital, Addis Ababa, Ethiopia. *Ethiopian medical journal*, ***50***: 13-21.

Adish A, Esrey S, Gyorkos T, & Johns T (1999). Risk factors for iron deficiency anaemia in preschool children in northern Ethiopia. *Public health nutrition,* ***2*****:** 243-252.

Adias T, Uko E & Erhabor O (2005). Anemia in human immunodeficiency virus infection: a review. *Nigerian journal of medicine: journal of the National Association of Resident Doctors of Nigeria*, ***15***: 203-206.

Ahmed A, Al-Rabaty A & AlNakshabandi A(2010). Relationship between hemoglobin level and feeding pattern in apparently healthy children below two years. *Zanco Journal of Medical Sciences,* ***14*****:** 9-15.

Alkhateeb N, Risk Factors Associated with Iron Deficiency Among Children Admitted to Paediatric Emergency Unit in Erbil, Iraq. *Hemoglobin,* ***11***: 0.001.

Arimond M, Wiesmann D, Becquey E, Carriquiry A, Daniels M, Deitchler M & *et al*. (2010). Simple food group diversity indicators predict micronutrient adequacy of women's diets in 5 diverse, resource-poor settings. *The Journal of Nutrition*, ***140***, 2059S-2069S.

Bunupuradah T, Kariminia A, Chan K, Ramautarsing R, Huy B, Han N & et al (2013). Incidence and predictors of severe anemia in Asian HIV-infected children using first-line antiretroviral therapy. *International Journal of Infectious Diseases*, ***17*****:** 806-810.

Bemnet A, Beyene M, Bereket F, Ketema T, Desalegn W, Gizachew Y, & et al (2012).Micronutrient levels and nutritional status of school children living in Northwest Ethiopia. *Nutrition Journal* ***10****:1475-2891*

Belperio P*,* Rhew D (2004).Prevalence and outcomes of anemia in individuals with human immunodeficiency virus*: a systematic review of the literature. Am J Med;* ***116****: 27S–43S.*

Cook J, Skikne B, Baynes R. (1994).Iron Deficiency: The Global Perspective. Progress in iron research. New York: PlenumPress: 219–28

Desalegn A, Mossie A, & Gedefaw L (2014). Nutritional iron deficiency anemia: magnitude and its predictors among school age children, southwest ethiopia: a community based cross-sectional study. *Ethiopian medical journal*, ***50***: 13-21.

Domellöf M & Hernell O (2002). Iron-deficiency anaemia during the first two years of life. *Food & Nutrition Research,* ***46***: 20-30.

Dugdale A (2011). Diagnosis and management of iron deficiency anaemia: a clinical update. *The Medical journal of Australia,* ***194***: 429.

Demographic Health Survey (EDHS) (2011)Preliminary Report. *Addis Ababa: Federal Ministry of Health*.

Esan M, Jonker F, van Hensbroek M, Calis J, & Phiri K (2012). Iron deficiency in children with HIV-associated anaemia: a systematic review and meta-analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, ***106***: 579-587.

Friis H (2005). *Micronutrients and HIV infection: a review of current evidence* (p. 159181).

Hamedani P, Raza R, Bachand R, Manji M, & Hashml K(1991). Laboratory diagnosis of iron deficiency in a developing country, Pakistan. *Journal of international medical research,* ***19***: 19-23.

Habteselassie A. (2014). Hematological outcomes of children with highly active anti-retroviral therapy at Zewditu memorial hospital. *Research*.

HHS panel: Guidelines for the use of antiretroviral agents in ely-infected adults and adolescents. Feb. 4, 2002,

Harrison, J. (2003). *Off to the side: a memoir*. Grove Press.

Joint United Nations Program me on HIV/AIDS (UNAIDS). (2014). The gap report. *Geneva: UNAIDS*.

Kosalaraksa P, Bunupuradah T, Vonthanak S, Wiangnon S, Hansudewechakul R,Vibol U, & et al. (2012). Prevalence of anemia and underlying iron status in naive antiretroviral therapy HIV-infected children with moderate immune suppression. *AIDS research and human retroviruses*, ***28***: 1679-1686.

Kruger H, Balk L, Viljoen M & Meyers T. (2013). Positive association between dietary iron intake and iron status in HIV-infected children in Johannesburg, South Africa. *Nutrition Research*, ***33***, 50-58.

Kennedy G, Ballard T, & Dop M. (2011). *Guidelines for measuring household and individual dietary diversity*. Food and Agriculture Organization of the United Nations.

Lutter K & Rivera J (2003). Nutritional status of infants and young children and characteristics of their diets. *The Journal of Nutrition, **133**:* 2941S-2949S.

Levine A, Berhane K, Masri-Lavine L, & *et al* **(**2001). Prevalence and correlates of anemia in a large cohort of HIV-infected women*:* women's interagency HIV study. *J Acquired Immune Deficiency Syndrome;* **26**: 28–35.

Mehta S, Mugusi F, Spiegelman D, Villamor E, Finkelstein L, Hertzmark, E& *et al* Fawzi, W (2011). Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. *AIDS patient care and STDs*, ***25***: 579-585.

Mgogwe J, Semvua H, Msangi R, Mataro C, Kajeguka D & Chilongola J (2012). The evolution of haematological and biochemical indices in HIV patients during a six-month treatment period. *African health sciences*, ***12***: 2-7

Mohamed M, Al Ghwass E, Samar M, Dalia A (2015). Iron deficiency anemia in an Egyptian pediatric population: A*nnuals of African Medicien, **14**:* 25-31.

Mwiru R, Spiegelman D, Duggan C, Seage G, Semu H, Chalamilla, G & et al (2013). Nutritional status and other baseline predictors of mortality among HIV-infected children initiating antiretroviral therapy in Tanzania. *Journal of the International Association of Providers of AIDS Care*, 23: 259-57.

Olivares M, Walter T, Hertrampf E, & Pizarro F(1999). Anaemia and iron deficiency disease in children. *British medical bulletin, **55***: 534-543.

Okechukwu A, Gambo D & Okechukwu I (2010). Prevalence of anemia in HIV- infected children at the university of teaching hospital, Gwagwalada. *Nigerian Journal of Medicine*, ***19***.

Oppenheimer S (2001). Iron and its relation to immunity and infectious disease. *The Journal of Nutrition, **131***: 616S-635S.

Rajeshwari K, Kumar K, Singh T & Anuradh A. Spectrum and risk factors of anemia in HIV infected Indian children presenting to a tertiary level teaching hospital at New Delhi.

Ruhinda E, Bajunirwe F & Kiwanuka J (2012). Anaemia in HIV-infected children:severity, types and effect on response to HAART. *BMC pediatrics*, ***12***: 1.

Raiten D, Grinspoon S & Arpadi S (2005). Nutritional considerations in the use of ART in resource-limited settings. *Geneva: World Health Organization Department of Nutrition for Health and Development*.

Stoltzfus R, Chwaya H , Tielsch J, Schulze K, Albonico M, & Savioli L. (1997). Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *The American journal of clinical nutrition*, *65*(1), 153-159.

Shet A, Bhavani P, Kumarasamy N, Arumugam K, Poongulali et al (2015). Anemia, diet and therapeutic iron among children living with HIV: a prospective cohort study. *BMC pediatrics*, ***15***: 1

Siti-Noor A, Wan-Maziah W, Narazah M & Quah B, (2006). Prevalence and risk factors for iron deficiency in Kelantanese pre-school children. *Singapore medical journal,* ***47***: 935-939.

Skikne B, Flowers C & Cook J (1990). Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood,* ***75***, 1870-1876.

Semba R, Shah N, Klein R S, Mayer K, Schuman P, Vlahov D & *et al* (2002). Prevalence and cumulative incidence of and risk factors for anemia in a multicenter cohort study of human immunodeficiency virus–infected and–uninfected women. *Clinical infectious diseases*, ***34*****,** 260-266.

Susan G & Benjamin J (2005). The incidence, treatment and follow up of Iron deficiency in a tertiary care Pediatric unit. *Clinic Ped,* ***333***: 7.

Teklemariam Z, Mitiku H & Mesfin F (2015). Prevalence of anemia and nutritional status among HIV-positive children receiving antiretroviral therapy in Harar, Eastern Ethiopa. *HIV/AIDS (Auckland, NZ)*, *7*, 191.

W. H.O (2001). Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers.

W. H.O (2007). BMI chart for HIV+children and adolecent age(5-18)

W. H. O (2011) Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Geneva: Vitamin and Mineral Nutrition Information System.

W. H.O (2013). Global update on HIV treatment 2013: results, impact and opportunities.

Zlotkin S (2002). Current issues for the prevention and treatment of iron deficiency anemia. *Indian pediatrics,* ***39***: 125-129.

Zimmermann M & Hurrell R (2007). Nutritional iron deficiency. *The Lancet*, ***370***, 511-520.

Annex

To be answered by participant

Dear parents!

In the case of promotion of children health to understand the existing prevalence of anemia, iron deficiency and risk factors associated with iron deficiency among HIV infected children to be evidence based. You are chosen to participate in this study. The choice is made consecutively. The questions include various private & personal lives and small amount of blood will be taken from your child for analysis.

In order to attain the goal effectively, I request your will full cooperation. Confidentiality is strictly protected. It is your right to participate or to refuse in the study, if you do not won't to participate in the study, you can discontinue at any time. But your honest participation will have contribution to generate valid information that can be used for investigation design. So please be cooperative to answer these questions. If there is anything that require clarification please ask the facilitator.

Do you wish to participate in the study?

Yes I want to participate ( )

No I do not want to participate ( )

Thank you!

Consent form

Title: Prevalence and determinates of anemia and iron deficiency among HIVinfected children attending antiretroviral therapy center at Black Lion Tertiary Hospital, Addis Ababa, Ethiopia.

Principal investigator: Tesfanesh Abebe

Institution: Addis Ababa University; center for food science and nutrition

Introduction: Globally, the HIV epidemic remains a serious challenge especially in children. Ongoing prenatal transmission substantially impacts the incidence of pediatric HIV, adding to the large pool of HIV-infected children in developing countries like Ethiopia and background co-morbidities compound the problem. Two such major co-morbidities include anemia and poor nutrition, whose detrimental effects are magnified in the context of HIV infection.

Iron deficiency is the single most common nutritional disorder world-wide and the main cause of anemia in infancy, childhood and pregnancy. It is prevalent in most of the developing world and it is probably the only nutritional deficiency of consideration in industrialized countries. in the developing world the prevalence of iron deficiency is high, and is due mainly to a low intake of bio available iron. However, in the other hand, iron deficiency often co-exists with other conditions such as, malnutrition, vitamin A deficiency, folate deficiency and infection. In tropical regions, parasitic infestation and haemoglobinopathies are also a common cause of anemia.

Procedures

If you agree to participate, I will collect the 5ml blood and small amount of stool of your child with for analysis.

Risks

Nothing harmful will come from your child participation.

Benefits

There are no direct benefits to you or your child. However the results will possibly help others. Based on the finding I will inform the authorized person to work on it

Cost

There is no cost to you for participating

Compensation

There will be no compensation to you for participating

Participant's right

If I have said things that are not clear to you, you may ask me without any fear, and I will give you answer and explanation .You may feel free and ask questions. Your child participation in the study is entirely volunteer and up to you to decide. There is no penalty if you don't agree to participate. You can say no without worry. The hospital and the health care provider will continue to give care for your child as usual.

Confidentiality

By excluding names and other identifying numbers from the questionnaire confidentiality of information will be assure. Test results and any information about your child will be kept privet.

Persons to contact

If you have any question, you can ask at any time, if you have additional questions about the study you may contact Tesfanesh Abebe (tesfanesh.abebe@yahoo.com)

Do you wish to participate in the study?

Yes I want to participate ( ) Name _____Signature_____Date

No I do not want to participate ( )

Thank you for your cooperation!

ለአዲስ አበባ ዩኒቨርስቲ ስነ የምግብ ሳይንስና ኒውትሪሽን ማዕከል

የሦስተኛ ዓመት የድህረ ምረቃ ተማሪ የሚያደርጉት ጥናት

የጥናት ፈቃድ ፎርም

በጥቁር አንበሳ ስፔሻላይዝ ሆስፒታል የሜሞግሎቢን መጠን እና የአይረን ማነስ እና ሊያስከትሉ የሚችሉ ተያያዥ ምክንያቶችን በኢችአይቪ ህመምተኞች ህፃናት ላይ የሚካሄድ ጥናት፡፡

ዋና ተመራማሪ፡- ተስፋነሽ አበበ, ከአዲስ አበባ ዩኒቨርስቲ ስነ የምግብ ሳይንስና ኒውትሪሽን ማዕከል

ማብራሪያ፡- አይረን የሚባለው ንጥረ ነገር ለሰውነታችን በጣም አስፈላጊ በመሆኑ በተለይም ደግሞ ለህጻናት እድገትና የነርቭ ስርዓት እናም የኦክስጅን ዝውውር ሰውነታችን በሚፈልገው መጠን እንዲከናወን ያደርጋል፡፡ በመሆኑም ህጻናት በይበልጥ ለችግሩ ተጋላጭ በመሆናቸው ምክንያት ጥናቱ በእነሱ ላይ እንዲሆን አድርጎታል፡፡ እንዲሁም በአይረን እጥረት የሚጠቁ ህጻናት ቁጥር በከፍተኛ ደረጃ ቁጥራቸው እየጨመረ በምጣቱ ችግሩን አውቆ መፍትሄ ለመስጠት ያስችል ዘንደ የሚደረግ ጥናት ነው፡

መመሪያዎች፡-ጥናቱ ላይ ለመሳተፍ ከተስማማሽ/ህ 5 ሲሲ ደም እና ትንሽ ሰገራ ከልጅሽ/ህ ላይ ለምርምር ይወሳል

ሰጋት፡- ያንቺ /ተ መሳተፍ ምንም አይነት ጉዳት በልጅሽ /ህ ላይ አያደርስም

ጥቅም፡- ምንም አይነት ቀጥተኛ ጥቅም ላንቺም/ተም ለልጅህ/ሽ አይኖርም፡፡ ግን በተዘዋዋሪ በሌሎች ልጆችም ሆነ ላንቺ/ተ ልጅ የጥናቱ ውጤት ላይ ተመርኩዞ ለውጥ ሊያመጡ የሚችሉ ሰዎች በማሳወቅ እንዲሰሩበት ያስችላል፡፡

ዋጋ፡- በማሳተፍሽ/ህ ምንም አይነት ዋጋ አያስከፍልም

ማካካሻ፡- በማሳተፍሽ/ህ ምንም አይነት ማካካሻ አይኖረውም፡፡

<u>የተሳታፊ መብቶች፡-</u> ምንም አይነት ግልጽ ያልሆነ ነገር ካለ የፈለግሽውን/ከውን ጥያቄ ካለምንም ፍርሀትና ጭንቀት መጠየቅ ትችያለሽ/ላለህ፡፡ የልጅሽ /ህ ተሳታፊ በሙሉ ፍቃደኝነት ላይ የተመሰረተ ነው፡፡

ባለመሳተፍሽ የሚደርስብሽ/ህ ምንም አይነት ቅጣት አይኖረውም፡፡ ሆስፒታሉም ሆነ የጤና ባለሞያዎቹ የሚያስፈልገውን አገልግሎት ልክ እንደበፊቱ ይሰጣሉ፡፡

ሚስጥራዊነቱ ማንኛውም ገላጭ የሆኑ ነገሮች ማለትም ስም ቁጥር የመሳሰሉትን በማስወገድ ማንኛውን መረጃ በሚስጥር ይጠበቃል፡፡

ጉዳዩ፡- የሚመለከታቸው ሰዎች አድራሻ

ምንም አይነት ጥያቄ ወይም አስተያየት ቢኖራችሁ (Tesfansh.abebe@yahoo.com) ስልክ ቁጥር ፡ 0910102685 ተስፋነሽ አበበ ብለው ይጻፉልን/ይደወሉልን፡፡

ጥናቱ ላይ ለመሳተፍ ፍቃደኛ ኖት

ስም ---------- ፊርማ ---------- ቀን ----------

የጥናቱ አስተባባሪ ፊርማ ----------

ለትብብርዎት እናመሰግናለን፡፡