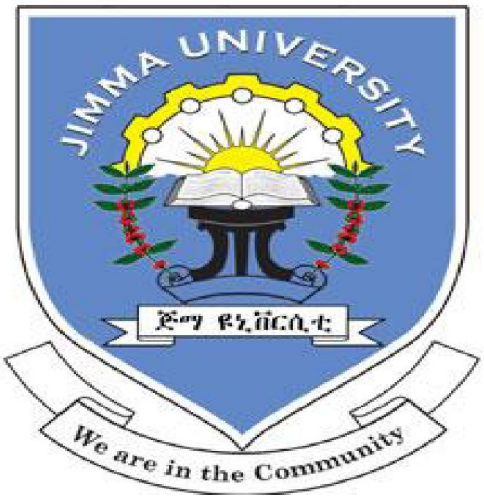
ANEMIA AND ASSOCIATEDFACTORS AMONGHIGHLY ACTIVE ANTI RETROVIRAL THERAPY EXPERIENCED HUMAN IMMUNO DEFICIENCYVIRUSPOSITIVE CHILDREN IN HAWASSA UNIVERSITY COMPREHENSIVE SPECIALIZED HOSPITAL, SOUTH ETHIOPIA



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Abstract

Background: Hematological complications have been documented to be one of the most common causes of morbidity and mortality in HIV positive children.Anemiais oneof the common hematological complications among children on Highly Active Antiretroviral Therapy (HAART) and the magnitude of anemia was associated with different factor such as opportunistic infection and the side effect of ART drug itself.Anemia has been identified as one of the predictors of early mortality in a cohort of HIV-infected children receiving HAART.

Objective: The aim of this study was to determine the prevalence of anemia and its associated factor amongHAART experienced HIV positive children.

Methods: Institutional-based crosses sectional study was conducted in Hawassa University comprehensive specialized hospital from February 15 to June, 15 2017.A total of 273HAART experienced children were included in the study. Socio-demographic characteristics and clinical data were collected using questionnaire based interview. After the interview, a detailed review of the medical records of each study participant was done using checklist. About 4 milliliters of venous blood sample was drawn from each study participant. The presence of Anemia was determined by hematology auto analyzer. Data was entered in to Epidata and analyzed by using SPSS version 20 software. Binary and multivariate logistic regression is used and Statistical significance was declared at P- value<0.05.

Result: The overall prevalence of anemia among study participants was 11.4%.Normocytic normochromic anemia (64.5%) took the higher proportion. In this study age<7(AOR=3, CI: 1.2-7.5, p=0.02), being rural dweller (AOR=2.6, CI=1.0-6.6, p=0.042) and having a viral load of >150 copies/ml (AOR=3.4, CI=1.36-8.3, p=0.009) were identified as determinants of anemia among study participants.

Conclusion and Recommendation: Thisstudy has shown that anemia is prevalent in HAART experienced children. Thereforeregular follow up management should be emphasized for those HAART experienced children who are anemic.Further study will be very important on the root causes and complications of anemia among anemic HAART experienced children.

Key Words: Anemia, Children, HIV, HAART, Hawassa, Ethiopia

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List of abbreviations and acronyms

AIDS Acquired Immune Deficiency Syndrome

ART Anti-Retroviral Therapy

CDCluster of Differentiation

CDC Center for Disease Control

EPO Erythropoeitin

HAART Highly Active Anti-Retroviral Therapy

HbHemoglobin

HCT Hematocrit

HIVHuman Immune Deficiency Virus

HUCSH Hawassa University comprehensive Specialized Hospital

IPI Intestinal Parasitic Infection

WHO World Health Organization

ZDV Zidovudine

CHAPTER ONE - INTRODUCTION

1.1 Background

The Human Immunodeficiency Virus (HIV) pandemic remains a serious challenge in the world. It continues to take its toll particularly on vulnerable populations such as children and has a profound impact on disease progression and mortality(1, 2). Hematological complications have been documented to be one of the most common causes of morbidity and mortality in HIV positive persons(1, 2).

Anemia is common among HIV-infected children worldwide including Africa (3, 4). In recent - years, several attempts have been undertaken to clarify the mechanisms leading to HIVassociated anemia and direct infection of erythroid progenitors has been discussed, but could not be proven(5-7). Furthermore, soluble factors like cytokines have been suggested to inhibit growth of hematopoietic cells in the bone marrow of HIV-infected patients(5).

Anemia refers to a condition in which the hemoglobin content of the blood is lower than normal for a person's age, gender, and environment, resulting in the oxygen carrying capacity of the blood being reduced(8).

In HIV-infected patients, anemia may be caused by nutrient deficiencies (iron, folic acid, and vitamin B12), sickle cell disease, HIV/AIDS itself, malaria, hookworm and other infections. Other mechanisms for HIV-associated anemia, although uncommon, include autoimmune destruction of erythrocytes(9). Direct infection of marrow precursor cells(10), has been hypothesized but not proven. Anemia has been reported as a very common complication of pediatric HIV infection, associated with a poor prognosis(11).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.

However, its multi factorial origin complicates its differential diagnosis and adequate treatment. Anemia has been shown to be a significant predictor of progression to Acquired Immune Deficiency Syndrome (AIDS) and several studies have shown that as hemoglobin levels decrease, the risk of HIV disease progression increases(12), and it is associated with an increased

risk of death in both children and adult patients. It is a wide spread public health problem. Opportunistic complications represent the underlying cause for anemia in a large number of HIV-infected patients(13).

HIV infected children demonstrated a robust increase in height and weight during Highly Active Anti-Retroviral Therapy (HAART) including those who failed to completely suppress virus. Older children initiating HAART with severe immune suppression were less likely to achieve a successful treatment outcome, emphasizing the importance of initiating HAART early to ensure adequate immune and growth responses(14). Unfortunately the drugs used in HAART regimens are often associated with adverse drug reactions(15, 16). The pathogenesis of anemia in HIV-infected children although multifactorial, relates primarily to a reduced production of erythrocytes. This reduction is influenced by several etiological factors including infection and neoplasm's drugs such as zidovudine (ZDV), a direct effect of HIV on erythropoiesis, a blunted response to erythropoietin and nutritional deficiencies(17).

1.2. Statement of the problem

Human Immuno deficiency Virus(HIV) infection is a worldwide public health problem. Globally, in 2012, there were an estimated 35.3 (32.2–38.8) million people living with HIV including 3.2 million children(18).Anemia, one of the commonest hematological complications with HIV infection, refers to a condition in which the hemoglobin content of the blood is lower than normal for a person's age, gender, and environment, resulting in the oxygen carrying capacity of the blood being reduced(19).Hematological complications have been documented to be the second most common cause of morbidity and mortality in HIV positive persons(1).It is a common manifestation of pediatric HIV infection and is a significant negative predictor of survival. It occurs in 50%–90% of children living in both resource-limited and resource-rich settings(6). It is also a significant public health problem especially in developing countries. In East Africa, approximately 75% of children under five years are suffering from anemia(17).The incidence of anemia ranges from 10% in people who have no HIV symptoms to 92% in individuals who have advanced AIDS(20).The prevalence of anemia in these children has a wide range(57% - 97%) as reported by number of researchers (11, 21-26).

Moreover, patients infected with intestinal parasites/helminthes, and HAART naive patients develop anemia(27).It is also associated with eating green leafy vegetables and being on cotrimoxazole treatment(28).Anemia has been reported as a very common complication of pediatric HIV infection, associated with a poor prognosis(11). It has been identified as one of the predictors of early mortality in a cohort of HIV-infected children receiving HAART(29).

Anemia is a significant negative predictor of survival among HIV infected children(30, 31).HIV related anemia decreases the quality of life and survival rate of HIV patients.Anemia may impair physical, socio-emotional and neurophysiological functioning of HIV infected children(11).It is a risk factor for delayed psychomotor development and cognitive function and also impairs cell-mediated immunity(32).Because of the long-term effects of iron deficiency anemia during infancy it is important to prevent and treat it appropriately as early in infancy as possible.

Increasing access to potent ART in resource-limited settings has transformed the prognosis of HIV infection, but adverse events may still occur after therapy initiation. Anemia is commonly

seen after ART initiation, whether due to pre-existing HIV related bone marrow suppression or as a side effect of ART(11).

On the other handMortality in ART children is associated with CD4 cell depletion, lower weight-for-age, younger age, and anemia (33).Anemia is also a common feature of HIV infection, occurring in approximately 35% of patients who initiate antiretroviral treatment (ART) in Europe and North America (34), which makes it to be recognized as an important clinical problem(35-37).During HAART, HIV infected children, including those who failed to completely suppress virus, displayed an increase in height and weight. Initiating HAART early is important in older HIV positive children with severe immune suppression as it will be less likely to achieve a successful treatment outcome to ensure adequate immune and growth responses(14).

HAART was reported to increase Hb levels in adults, despite the hematotoxic effects of some individual agents, and this may also apply to children.Several drugs given during the course of HIV disease could cause anemia in children including antiretroviral(38).

The multifactorial pathogenesis of anemia in HIV infection is also related to bleeding (gastrointestinal malignancy/severe infection), insufficient dietary intake (vitamins such as cobalamin and folate, iron, and general malnutrition), hemolytic anemia (i.e., malignancies, infections)(5). In HIV-infected patients, anemia may be caused by nutrient deficiencies (iron, folic acid, and vitamin B12), sickle cell disease, HIV/AIDS itself, malaria, hookworm, and other infections. Other mechanisms for HIV-associated anemia, although uncommon, include autoimmune destruction of erythrocytes (9). Direct infection of marrow precursor cells has been hypothesized but not proven.

Although prevention and elimination of pediatric HIV should remain high on the agenda, the antiretroviral needs of infected children should not be compromised. We need to draw attention to outcomes of children on pediatric ART and to ensure access to needed care for children with HIV globally. Hematological abnormalities are associated with increased risk of disease progression and death in individuals infected with HIV. Even though some studies have been carried out on prevalence of anemia giving information on the pattern of anemia in various geographical locations, age and social groups, and risk categories, the prevalence among children

and information regarding associated risk factor and hematological abnormalities is scanty from the southern region of the country particularly in the study area.

Hence, this study was conducted to determine the magnitude and associated risk factor of anemia in HIV infected children who are taking highly active antiretroviral therapy in Hawassa university comprehensive specialized hospital (HUCSH).

1.3. Significance of the study

This study was conducted to determine the prevalence and associated factor of anemia in HAART experiencedchildren in HUCSH.HIV associated anemia is always overseen and it could be a challenge for prognosis of patients who are taking ART.

Findings of this study will help us to set prevention programs for proper management, follow up and care for children on HAART. The study would be useful in managing children living with HIV in a more comprehensive manner by giving due attention to anemia and other co-morbidities. In addition, determining the prevalence of anemia at childhood has had paramount significance in reducing progression of disease. On the other hand, it can be a good indicator of general prevalence of anemia among ART children. Such data are fundamental for health planers and care givers for evidence-based intervention.

CHAPTER TWO - LITERATURE REVIEW

2.1. Global burden

A cross sectional study done on Hematologic abnormalities among children on HAART in Jimma University specialized hospital, south west of Ethiopia, showed that the prevalence of anemia among the study children was 21.9%(39). A retrospective study done in the period between on children attending the pediatric HIV comprehensive care clinic at Kenyatta National hospital showed that hematological abnormalities are common among HIV infected ARV naïve children, and anemia was the commonest hematological abnormality with almost half of the study population having microcytic hypochromic picture of anemia(40).

A follow up study undertaken in Indian Children and Dr. Ram ManoharLohia Hospital, showed that, after one year of ART anemia was seen in twenty (36.3%) children out of which eleven were moderately anemic and no one had severe anemia. This study concluded that there was a significant reduction in prevalence and severity of anemia in children following ART (p=0.00)(21). A retrospective study from 248 HIV-infected children aged 1–12 years attending three outpatient clinics in South India showed that the overall prevalence of anemia was 66% from whom 8% had severe anemia(41).

An observational cohort study among HIV-infected children in a rural Ugandan clinicMbarara, Uganda, showed that anemia was present in 148/257 (57.6%) of children, including (93/148) 62.2% with mild anemia, 47/148 (32.0%) moderate anemia, and 7/148 (4.8%) with severe anemia. The mean Hb was lower among children with more advanced HIV disease ($p<0.0001$). The proportion of children who had attained viral suppression (viral load <400 copies/ml) at 3 months was significantly lower among the anemic children, 31/58 (53.4%) compared to the non-anemic children 26/30 (86.7%) ($p=0.002$). However, the difference in clinical and immunological response between the anemic and non-anemic patients did not reach statistical significance(42).

A longitudinal cohort study nested within the Zimbabwe Vitamin A for Mothers and Babies Project showed that at one year of age, HIV-positive infants were 5.26 (AOR, $P < 0.001$) times more likely to be anemic compared to HIV-negative infants. Among, HIV-negative infants, EPO

was or tended to be inversely associated with Hb and was significantly positively associated with TfR throughout the first 6 months of life; TfR was significantly inversely associated with ferritin at 6 months; and EPO explained more of the variability in TfR than did ferritin. Among infected infants, the inverse association of EPO to hemoglobin was attenuated during early infancy, but significant at 6 month(43).

According to an observational prospective cohort study done in Nigeria, the mean haemoglobin level at baseline was 10.8±2.1g/dl for the ZDV group and 6.9±1.3g/dl for the d4T group (P = .001). At baseline, 232 (60.7%) of the patients had anemia while 26 (6.8%) had severe anemia. At the end of the 12 months evaluation period, 59 (16.8%) of the patients on ZDV had Hb<8g/dl and were switched to d4T with 16 (4.6%) of them requiring blood transfusion. The mean Hb level of ZDV group decreased from 10.8±2.1g/dl to 9.3±1.8g/dl while that of d4T group increased from 6.9±1.3g/dl to 11.2±1.5g/dl(15).

A cohort of children initiated on ART from 2005 to 2011 at Massey Street Children Hospital, Lagos, Nigeria, showed, After seven years of ART care, 64 % of the 660 study children were retained in care and on treatment, 16 % were lost to follow-up, 10 % were dead, and 9 % had discontinued HIV care at this facility for other reasons. World Health Organization disease stage, CD4 count, age, and year of ART initiation were highly predictive of mortality, while anemia at baseline was not statistically significantly associated(41).

Another cross sectional study in Nigeria on 68 HIV confirmed children showed Anemia (< 100 g/L) was present in 77.9%, severe (< 60 g/L) in 5.9%, moderate (60-70 g/L) in 32.3% and mild (80-99 g/L) in 39.7%. the mean Hb concentration decreased as disease progressed ($p < 0.05$); 6% had leucopenia, 17.5% had neutropenia and 2.5% (one case) had thrombocytopenia; also, the four (6%) subjects with leucopenia were in clinical stages B and C. Neutropenia, lymphocytopenia and thrombocytopenia were seen more in clinical stages B and C, though this relationship was not statistically significant(24).A systematic review from a global perspective on HIV-associated anemia in children showed the overall prevalence of mild or moderate anemia in HIV-infected children varied between 22–94 and 3– 82%, respectively(4).

A cross sectional research done in130 HIV infected Children (0-15 Years) Residing in Nkambe, North West Region, Cameroon aimed to assesseffect of ART on anemia showed that

there was a significant increase (P=0.00) in the mean CD4+, Hb and weight after ART intervention. The prevalence of non-anemia was higher 52(40.0%) after intervention compared to 30 (23.1%) at the beginning. After initiation, 30 (23.1%), 46(35.4%) and 11(8.5%) children experienced a decrease in their CD4, Hb and weight values, respectively. Of the 30 children with decreased CD4 count, 30(100.0%) were anemic and 11 (36.7%) were underweight. More females 47(78.3%) experienced increase in CD4+ count while the male children were non anemic 29(41.4%) and experienced an increase in weight 61(87.1%). Most children of the age group 6-10 years experienced an increase in CD4+ 26(89.7%), Hb 15(51.7%), and weight 25(86.2%) compared to the other age groups. But this is a cross sectional study with small sample size(44).

A study that compared the prevalence of pre-antiretroviral therapy hematological abnormalities among 1571 participants in a randomized trial of antiretroviral efficacy in Africa, Asia, South America, the Caribbean, and the USA showed that the frequencies of anemia (hemoglobin ≤ 10 g/dl) at initiation of antiretroviral therapy was 12% and varied by country ($p< 0.0001$ for each)(45).

Another cross sectional study conducted on 265 HIV infected children attending Gondar university Hospital ART clinic showed that Anemia was present in 16.2 % (43 /265) of children, 60.5 % of them had mild anemia, 37.2 % had moderate anemia and 2.3 % had severe anemia. The study concluded that the majority of HIV positive children in Northwest Ethiopia have a mild type of anemia and the increase in prevalence of anemia is due to being on cotrimoxazole and eating green leafy vegetables(28).Another cross sectional study in Ethiopia attending Zewditu Memorial Hospital (ZMH) ART Clinic in Addis Ababa, Ethiopia showed that the total prevalence of anemia was 22.2% from whom 21 (52.5%), 17 (42.5%), and 2 (5.0%) patients had mild, moderate, and severe anemia(46).A four-year retrospective study in HiwotFana Specialized University Hospital, Harrar, Ethiopia, on 108 children on ART indicated prevalence of anemia was 54.4%.However, there was a decline after initiation of ART (39.2%)(47).

2.2. Determinant factor of anemia

A study done on a total of 180 children patients attending Zewditu Memorial Hospital (ZMH) ART Clinic in Addis Ababa, Ethiopia showed that there was a significant increase in severity and prevalence of anemia in those with CD4+ T cell counts below 350 cells/μL ($P< 0.05$).

Having intestinal parasitic infections (AOR = 2.7, 95% CI, 1.1–7.2), having lower CD4+ T cell count (AOR = 3.8, 95% CI, 1.6–9.4), and being HAART naive (AOR = 2.3, 95% CI, 1.6–9.4) were identified as significant predictors of anemia, concluding anemia was more prevalent and severe in patients with low CD4+ T cell counts(12, 46). Moreover patients infected with intestinal parasites/helminthes, and HAART naive patients also will develop anemia(27).

A retrospective study from 248 HIV-infected children aged 1–12 years attending three outpatient clinics in South India showed that the proportion of underweight and small children in the population was 55% and 46% respectively. Independent risk factors of anemia by multivariate analysis included the pre-school age group (age younger than 6 years), (OR: 2.87; $p < 0.01$), rural residence (OR: 12.04; $p < 0.01$), advanced HIV disease stage (OR: 6.95; $p < 0.01$) and presence of stunting (Height for- age Z score< -2) (OR: 3.24; 95% $p < 0.01$). Use of iron/multivitamin supplementation was protective against risk of anemia (OR: 0.44; 95%; $p = 0.03$). Pulmonary tuberculosis was an independent risk factor in multivariate analysis (OR: 3.36; 95% CI: 1.43, 7.89; $p < 0.01$) when correlated variables such as HIV disease stage and severe immunodeficiency, and nutritional supplement use were not included. Use of ART was associated with a reduced risk of anemia (OR: 0.29; 95% CI: 0.16, 0.53; $p < 0.01$). No significant association was found between anemia and gender, cotrimoxazole, or ART type(43).Anemia was independently associated with young age ($p <0.0001$), advanced HIV WHO disease stage ($p = 0.034$) and low CD4 percentage ($p = 0.048$)(22).

A cohort study done on Perinatally-infected children aged 2–12 years at three sites in southern India, which are followed for 1 year showed a high prevalence of anemia mediated by iron deficiency, vitamin A deficiency and chronic inflammation(27).

Several drugs given during the course of HIV disease could cause anemia in children including antiretroviral (35). HAART was reported to increase Hb levels in adults, despite the hematotoxic effects of some individual agents, and this may also apply to children. Cotrimoxazole is another drug associated with bone marrow suppression and causing anemia(11).

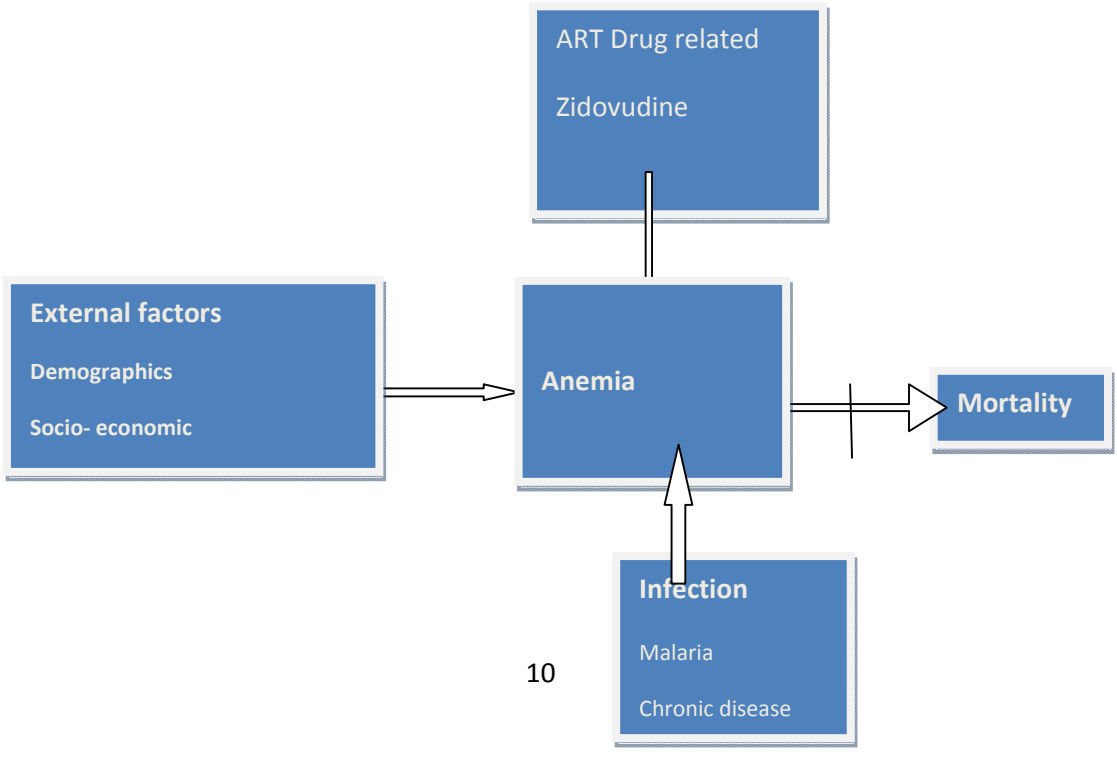
2.3. Types of anemia

Hematological abnormalities are common among HIV infected ARV naïve children, and anemia was the commonest hematological abnormality with almost half of the study population having microcytic hypochromic picture of anemia(40). An observational cohort study among HIV-infected children in a rural Ugandan clinicMbarara, Uganda, showed that microcytic-hypochromic anemia (44.9%) was the commonest type of anemia(42).

A cross-sectional study done on people on second line antiretroviral treatment in Lilongwe, Malawi showed that the prevalence of anemia was 33.2% − mild, moderate and severe anemia was 17.5%, 13.8%, and 1.9% respectively(48). Female participants had a higher prevalence than male participants. Another cross sectional study conducted on 265 HIV infected children attending Gondar university Hospital ART clinic showed that About 46.5 % of anemic children had normocytic-normochromic anemia followed by macrocytic-normochromic anemia (39.5 %)(28).

In general, anemia is high among ART users with different determinant factors with different types of anemia existing among ART users. However, the burden, the determinant factors and types of anemia among children on ART is not much known.

2.4 Conceptual Frame Work



CHAPTER THREE - OBJECTIVES

3.1. General Objective

To determine the prevalence of anemia and its associated factor among HIV positive children on HAART in HUCSH

3.2. Specific Objectives

- To determine the prevalence of anemia among HIV positive Children on HAART in HUCSH
- To assess factors associated with anemia among HIV positive children on HAART in HUCSH
- To describe the morphological types of anemia among HIV positive children on HAART in HUCSH

CHAPTER FOUR – MATERIALS AND METHODS

4.1. Study area and period

This study was conducted in HUCSH, Southern Nations Nationalities and Peoples Region (SNNPR) from February15 to June 15/ 2017. Hawassa is the capital city of the region located 275 Km from Addis Ababa. The altitude of the town is 1697meters above sea level with the mean annual temperature and rainfall of20.9OC and 997.6 mm respectively.

HUCSH has different clinical service providing units including HIV/AIDS free diagnosis, treatment and monitoring. The ART clinic diagnoses new cases and monitors those on therapy. Also, structured HIV/AIDS data were available at this Referral Hospital. There are 273 ART user children out of which134 of them are male and 139 of them are females.

4.2. Study design

Institutional based cross sectional study wasconducted.

4.3. Population

4.3.1. Source population

All children in HUCSH ART clinic under follow up

4.3.2. Study population

All children in HUCSH ART clinic under follow up who fulfill the inclusion criteria

4.4. Eligibility criteria

4.4.1. Inclusion criteria

Children aged 6 month to 14 years having followed up on ART clinic of HUCSH during the data collection period, who were voluntary to participate,were included in the study.The lower limit of 6 months is chosen because during infancy, physiological anemia occurs in the neonatal period and early infancy with the lowest level at about 2-3 months but by 6 months the hemoglobin has risen. The upper limit of 14 years is because this is the age limit for admission of children on the pediatric wards.

4.4.2. Exclusion criteria

Children on treatment for known anemia case and who had transfusion treatment within three months of data collection were excluded.

4.5. Sample size determination and sampling technique

All children on HAART(273) were includedas a study participant

4.6. Variables

4.6.1. Dependent variable

Anemia

4.6.2. Independent variables

- Sex
- Age
- Residence
- Family income
- Family size
- Guardians/parent Educational status
- Guardians/parents occupation
- Educational status of the children
- Malaria
- Opportunistic Infections
- CD4 count
- Viral load
- Type of drug/Duration
- Nutritional status

4.7. Data collection technique and instruments

4.7.1. Socio-demographic and clinical data collection

Socio-demographic and clinical data was collected by using structured questionnaire by trained data collector. It was prepared first in English version and translated to Amharic and again to English to confirm the correctness of the translation. A detailed review of the medical records was also done for each child to collect data for baseline Hb concentration, WHO clinical staging and types of drug by using check list. Laboratory results for hematological parameters, morphological examinations, CD4 count and parasitological examinations were collected by using laboratory format designed for this study.

4.7.2 Blood and stool collection

After interview and detailed review of the medical record, the study participants were sent to laboratory where blood and stool was collected for determination of complete blood count, CD4 count and intestinal parasites, and the result was collected within six hours and filled on the data collection format.About Four milliliter of venous blood was collected from each study participant using ethylene diamine tetra acetic acid (EDTA) anticoagulated test tube by Vacutainer technique.

One gram of stool sample was collected from each study participant and assessed for intestinal parasitic infection using wet mount preparation.A single stool specimen was collected from each patient using clean, dry, leak proof, and wide-mouthed caps; a drop of saline was mixed by an applicator stick on the slide.

4.7.3 Blood and Stool sample analysis

Hematological parameters;Hb concentration, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell(RBC) count and red cell distribution width (RDW) was determined using hematological auto-analyzer (ruby Cell-Dyne USA) within 30 minute after collection. CD4 count was assayed using the Becton Dickenson (BD) FACS count(BD, USA).Low, normal and high control reagent was used for

both hematology and CD4 analyzer to check the accuracy and precision of the results.Analysis will only continue when three of the control reagent passed. Thin and tick blood film was done for morphological identification and for malaria parasite and analyzed by senior laboratory technologist. The result of the test was recorded on the laboratory format designed for this research. (Detailed information regarding blood collection and analysis was described under ANNEX III and IV)

The collected stool specimen was examined for intestinal parasites by experienced laboratory technologist within 10–15 minutes of collection (ANNEX IV).

4.7.4 Anthropometric data collection

The weight of the children was measured using automatic weighing scale which was calibrated twice daily. It was recorded to the nearest 0.1kg. Height of the child was also measured by height scale(batch number-M-37/123/5097, INDIA). For those less than two years of age, length measurement was conducted using a length measuring board in a recumbent position on a hard and flat surface. For those who were two years old and above, height was measured on a standing position by prestige weight scale(manufacturer-hospital equipment manufacturing company(HENC). Height (length) of the child was recorded to the nearest 0.1cm. HAZ and WAZ score was analyzed using the WHO Anthroplus software and was expressed as standard deviation (mean ±SD) designed(49).

4.8. Data processing and statistical analysis

Data was cleaned, edited, checked for completeness and entered in to Epidata version 3.4, and transferred toSPSS version 20. Descriptive statistics was used to analyze frequency of anemia and other descriptive variables.Bivariate analysis was done to see the relationship between the independent and dependent variables while multivariate logistic regression was used to identify independent predictors of anemia.Those variables which have significant statistical association in the bivariate analysis (p-value <0.2)were considered as a candidate variable for multivariate analysis. In all cases,p-value less than 0.05were considered as statistically significant.

4.9. Data quality assurance

Training was given to sample collectors and supervisor for one day on how to approach study subject. To ensure quality of data, sample was handled and checked depending on the standard operating procedure in place(ANNEX III and IV), and proper handling of quality control materials and reagents was checked daily. Calibration of analyzers was also checked and quality control testing for BD FACS count and CBC analyzer was performed before running tests. The quality of the Geimsa stains also maintained daily. The questioner was also checked daily for the completeness of necessary information required and appropriate measure was taken on time for completeness before data entry and running of the test. Data clean up and cross checking was done before analysis.

4.10. Ethical consideration

Ethical clearance was obtained fromJimma University, School of Health Sciences Ethical Review Committee. This ethical clearance was taken to HUCSH clinical director office and a permission letter to conduct the study was given from the clinical director office. Next, the permission letter was given to the HUCSH ART clinic and HUCSH laboratory office beforethe study wasconducted. Then, the aim, purpose, benefits and method of the study was clearly explained to the participant. Informed written consent was taken from the parents/guardians and in addition assent was obtained from children above 7 years before enrollment in the study. Participation in the study was voluntary and refusal was possible at anytime. All the data was kept confidential. To ensure confidentiality of data, study participants was identified using codes and unauthorized persons was not be able to access the collected data. The study participants' result was reported to the physician for proper management.

4.11. Plan for dissemination and utilization of results

The findings will be presented to Jimma University and the copy of the result will be submitted to HUCSH; it will also be published on reputable peer reviewed journals.

4.12. Operational definition

ART: a treatmentregimen containing at least three different anti-retroviral drugs.

ANEMIA: According to the Federal Democratic Republic of Ethiopia Ministry of Health National Guidelines for HIV/AIDS and Nutrition Revised September 2008 Anemia: is a hemoglobin (Hb) concentration of < 11 g/dL for children <5 years old, <11.5 g/dl for children 5–11.9 years old and <12 g/dl for children 12–14.9 years old after altitude adjustment(50).

Mild anemia: hemoglobin level between 10 and 10.9 g/dL for under 5 and between 11 and 11.9 g/dL for under 18 years of age children;

Moderate anemia: hemoglobin level between 7.0 and 9.9 g/dL for under 5 and between 8.0 and 10.9 g/dL for under 18 years of age children.

Severe anemia: hemoglobin level <7.0 g/dL for under 5 and <8.0 g/dL for under 18 years of age children

Anemia by RBC indices: Normocytosis-MCV level of between 76-96fl; Microcytosis- MCV less than 76fl; MacrocytosisMCV level of greater than 96fl; Normochromic-MCHC level of between 31-35g/dl and hypochromic MCHC level of less than 31 g/dl

HAART Experienced: people who have already taken one or more forms of HIV medication.

HAART Naïve: HIV positive patients who have never taken any antiretroviral therapy for their infection.

Preschool children: Are those children whose age is less than seven

School children: Are those children whose age is greater than seven

CHAPTER FIVE –RESULT

5.1. Socio-demographiccharacteristics of the participants

A total of 273 children participated in this study, from whom: 50.9% were female, 80.6% were preschool children, and 82.1% were urban dwellers. Most of the participants (80.6%) were above >7 years oldwith mean age of10.2 years (±3.2), with a rangedfrom1.0–14.0 years (Table 1).

Table 1: Socio-demographic characteristics of the study participants at HUCSH, southern Ethiopia, from February 15 to June 15/ 2017

Variable	Category	N(%)
Age	≤7	53(19.4)
	>7	220(80.6)
Sex	Male	134(49.1)
	Female	139(50.9)
Residence	Urban	224(82.1)
	Rural	49(17.9)
Educational status	Did not begin	38(13.9)
	Primary	219(80.2)
	Secondary	16(5.9)
Family size	≤3	46(16.8)
	4-7	192(70.3)
	>7	35(12.8)

5.2. Clinical and immunologic characteristics of the study participants

Most of the participants (n=269) were in WHO clinical stage of either 1 or 2. From the total study participants,4.4% hada temperature of >37 degree centigrade (C^o). None of the participants has diarrhea. The prevalence of intestinal parasitosis was 0.4%. From the total study participants,89.4% were on AZT/3TC/EFV, 83.9% did not take other drug and 11.7 % had CD4 cell count of less than or equal to350 (Table 2).

Table 2: Clinical and immunologic characteristics of the study participants at HUCSH, south Ethiopia, from February 15 to June 15/2017

VARIABLES	CATEGORY	N=273(%)
WHO stage	1 and 2	269(98.5)
	3 and 4	4(1.5)
Diarrhealdisease	Yes	0
	No	273(100)
Intestinal parasites	Yes	1(0.4)
	No	272(99.6)
Malaria	Yes	1(0.4)
	No	272(99.6)
Temperature	≤37	261(95.6)
	>37	12(4.4)
Other medication	No	229(83.9)
	Cotrimoxaxol	31(11.4)
	Amoxacillin	5(1.8)
	Ougmentin	2(0.7)
	Nutritional supplement	6(2.2)
ART Drugs	AZT/3TC/EFV	244(89.4)
	ABC/3TC/CALETR	20(7.3)
	TDF/3TC/CALETRA	9(3.3)
CD4 cell	≤350	32(11.7)
	>350	241(88.3)
viral load	≤150	57(20.1)
	>150	216(79.1%)

WHO,world health organization; ART, antiretroviral therapy

5.3. Prevalence of anemia

The prevalence of anemia among participant was 11.4% (n=31). Out of the total anemic children females had a higher prevalence, 11.5% (n=16),of anemia, though the difference was not significant. In addition, rural dwellers had a higher prevalence (20.4%) than the urban dwellers (9.4%)(Table3).

Table 3: prevalence of anemia among HAART experienced children at HUCSH, from February 15 to June 15/2017

Variables	CATEGORY	ANEMIA		
		YES(31)	NO(242)	Total (%)
Sex	Male	15(11.2)	119(88.8)	134(49.1)
	Female	16(11.5)	123(88.5)	139(50.9)
Age in year	≤7	11(20.7)	42(79.3)	53(19.4)
	>7	20(9.1)	200(90.9)	220(80.6)
Residence	Rural	10(20.4)	39(79.6)	49(17.9)
	Urban	21(9.4)	203(90.6)	224(82.1)
Educational status	Not formal educated	6(15.9)	32(84.2)	38(13.9)
	Primary	22(10.1)	197(89.9)	219(80.2)
	Secondary	3(18.8)	13(81.2)	16(5.9)
Family size	≤3	8(17.4)	38(82.6)	46(16.8)
	4-7	21(10.9)	171(89.1)	192(70.3)
	>7	2(5.7)	33(94.3)	35(12.8)
WHO stage	1 and 2	29(10.8)	240(89.2)	269(88.6)
	3 and 4	2(50)	2(50)	4(11.4)
ART drug	AZT/3TC/EFV	28(11.5)	216(88.5)	244(90.3)
	ABC/3TC/CALETRA	1(5)	19(95)	20(3.2)
	TDF/3TC/CALETRA	2(22.2)	7(77.8)	9(6.5)
Other medication	No	23(10.0)	206(90.0)	229(83.9)
And supplement	Cotrimoxazol	5(16.1)	26(83.9)	31(11.4)
	Amoxicillin	2(40)	3(60)	5(1.8)
	Augmentin		2(0.8)	2(0.7)
	Plamplet	1(3.2)	5(2.1)	6(2.2)
CD4 Count	≤350	6(18.7)	26(81.3)	32(11.7)
	>350	25(10.4)	216(89.6)	241(88.3)
HAZ	≤-2	11(10.5)	94(89.5)	105(38.5)
	>-2	20(11.9)	148(88.1)	168(61.5)
WAZ	≤-2	5(18.5)	22(81.5)	27(9.9)
	>-2	26(10.6)	220(89.4)	246(90.1)

EDU,educational;WHO, world health organization; HAZ, standard deviation; WAZ, weight to age Z score

5.4. Associated factors of Anemia

Variables whose P value less than 0.2 in binary logistic regression model were selected as a candidate for multivariate analysis model to identify independent predictorsof anemia. Accordingly, the age, residence, temperature, being on WHO stage 1&2 and viral load >150 were selected for multivariate analysis. In multivariate analysis, age≤ 7(AOR=3, CI: 1.2-7.5,

p=0.02), being ruraldweller (AOR=2.6, CI: 1.0-6.6, p=0.042) and having a viral load of >150 copies/ml (AOR=3.4, CI; 1.36-8.3, p=0.009) are associated with being anemic the study participants (Table 4).

Table 4: Associated factors of anemia among HAART experienced children at HUCSH,south Ethiopia, from February 15 to June 15/2017

Variable	Category	COR(95%CI)	P-Value	AOR(95%CI)	P-Value
Age	≤7	2.28(1.17-5.87)	0.019*	3(1.2-7.5)	0.02**
	>7	1		1	
Sex	Male	1			
	Female	1.03(0.49-2.18)	0.93		
Residence	Urban	1			
	Rural	2.18(1.08-5.67)	0.032*	2.6(1.0-6.6)	0.04**
EDU.status	No	1.23(0.27-5.68)	0.79		
	Primary	2.07(0.55-7.82)	0.29		
	Secondary	1			
Family size	≤3	1			
	4-7	1.7(0.71-4.2)	0.23		
	>7	3.5(0.69-17.5)	0.25		
Caretaker EDU	No	1			
	primary	1.6(0.58-4.6)	0.35		
	Secondary	2.1(0.69-6.4)	0.21		
	College	0.76(0.24-2.43)	0.66		
Prim caretaker	Mother or father	1.02(0.42-2.48)	0.97		
	other	1			
Temperature	≤37	4.33(1.22-15.35)	0.023*	2.6(0.6-11.1)	0.2
	>37	1			
ART Drug	AZT/3TC/EFV	1			
	ABC/3TC/CALETR	2.46(0.32-19.1)	0.4		
	TDF/3TC/CALETRA	0.45(0.09-2.29)	0.3		
Other drugs	no	1		1	
	Cotrimoxazol	0.58(0.20-1.65)	0.31	1.1(0.3-4.4)	0.8
	OTHER	0.37(0.09-1.45)	0.15*	0.3(0.07-1.57)	0.28
WHO stage	1&2	15.3(3.46-67.8)	0.00*	9.9(1.7-57.4)	0.01**
	3&4	1		1	
CD4 count	≤350	0.50(0.19-1.34)	0.2		
	>350	1			
Viral load	≤150	1		1	

	>150	3.8(1.75-8.32)	0.001*	3.4(1.36-8.3)	0.009**
WAZ	<-2	1			
	≥-2	0.3(0.06-1.46)	0.21		
HAZ	<-2	0.87(0.40-1.89	0.72		
	≥-2	1			

EDU, educational; WHO, world health organization; HAZ, height to age ratio; WAZ, weight to age z score; COR, crude odds ratio: AOR, adjusted odds ratio

5.5. Type and severity of anemia among participants

Typing of anemia was done by morphological examination of the peripheral blood film (Figure 1) and RBC indices (figure 2).

Three different morphological types of anemia were observed; microcytichypochromic, normocytic normochromic and macrocytic normochromic (Figure 1).

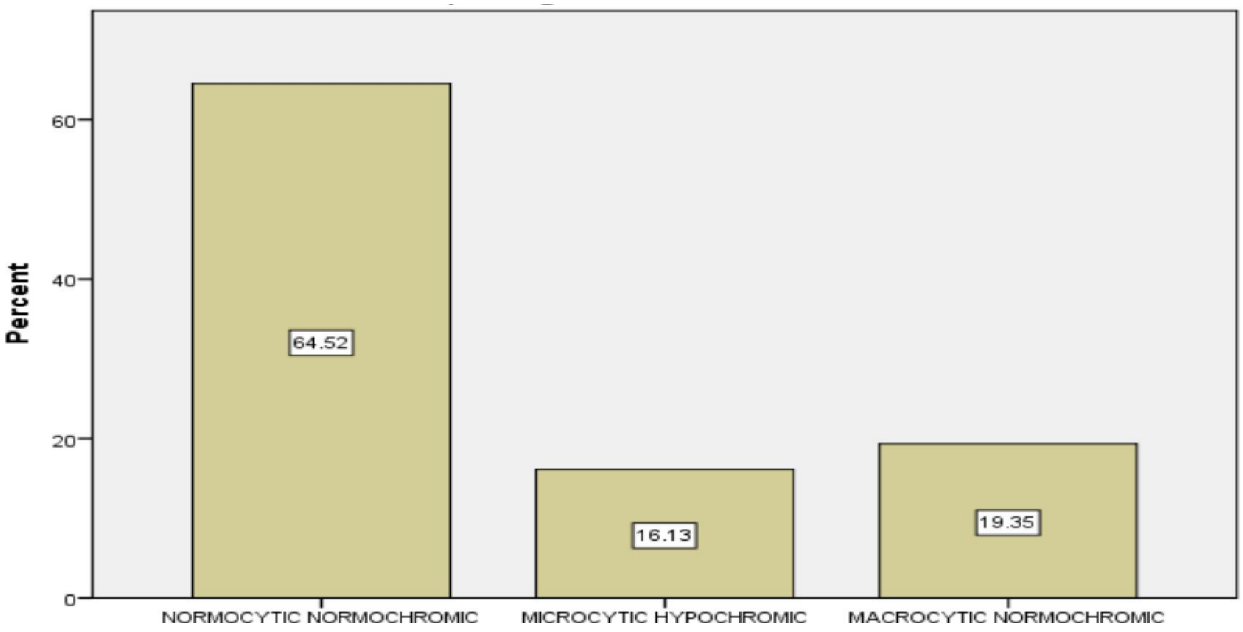


Figure 1: Morphological distribution of anemia among HAART experienced children at HUCSH, southern Ethiopia, from February 15 to June 15/2017

Four different types of anemia were observed by using RBC indices (table 5). Macrocytic anemia was the most prevalent (54.8%).

Table 5: Types of anemia classified by RBC indices among HAART experienced children at HUCSH, from February 15 to June 15/2017

Types of anemia	N	Percentage
Normocytic Normochromic	1	3.2
Microcytic Hypochromic	2	6.5
Macrocytic	17	54.8
Normocytic Hypochromic	11	35.5

The severity of anemia among study participants were studied by classifying it as mild, moderate and severs (figure 2).

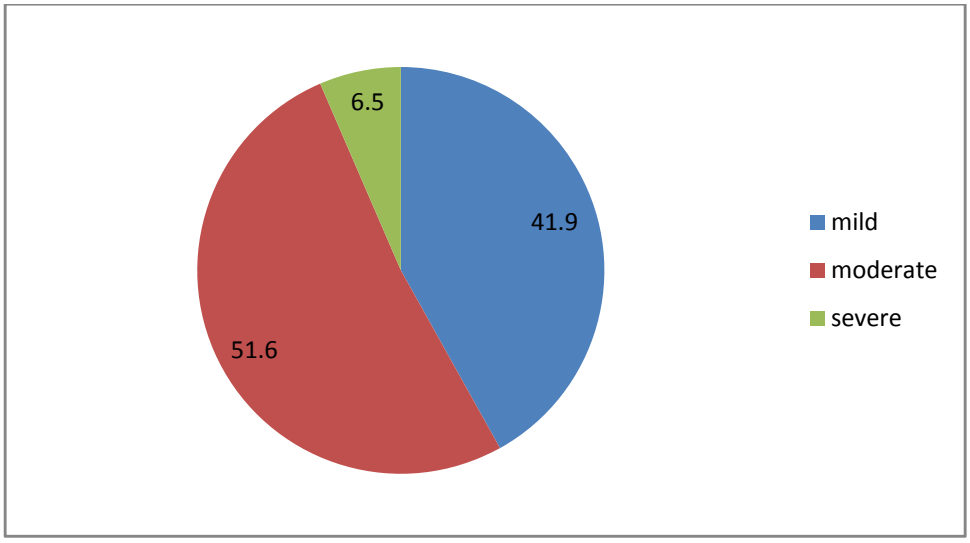


Figure 2: Distribution of severity of anemia among HAART experienced children at HUCSH, southern Ethiopia, from February 15 to June 15/2017

CHAPTER SIX - DISCUSSION

Anemia is a condition in which the hemoglobin concentration of a RBC is lower than the reference interval for a person age, gender and environment(8). It isa major public health problem and very common feature in HAART experienced pediatric patients. Itscause is multifactorial which may complicate the treatment and prognosis(12).Therefore the current study tried to address the prevalence, morphological features of anemia and its associated factors among HAART experienced children at HUCSH.

The overall prevalence of anemia among study participants was 11.4%.This is relatively comparable with the study conducted in Gondar,(16.2%)(28). A comparison study done on children on HAART selected from Asia, Africa and America obtained anemia prevalence of 11.9%(45). Which is comparable with our study.In the other hand the findings of the current study was lower than the studies done in Zewditu memorial hospital, Addis Ababa(22.2%)(51),Jimma(21.9%)(39), Eastern Ethiopia(39.2%)(47), Nigeria(54.2%)(52), north eastern Nigeria(24.3%)(53), western Uganda(57.6%)(42)andIndia(47.1%)(27).The difference in prevalence between the present study and the study done in Nigeria, western Uganda and India could be explained by the difference in ethnicityand geographicallocation. Theage differences in the study participants may also be another explanation for the difference between the current study and other studies because some of the studies included between the age of 3month and 18 year(42) and different sample size used. In addition, another explanation to this low prevalence of anemia could be low prevalence of malaria and intestinal helminthes which are the main predisposing factors of anemia among the study group(54, 55).Aprospective cohort study in Tanzania showed that malaria parasiticinfection in children increases the risk of anemia(55).Different studies also supports the effect of intestinal parasitosis on increasing anemia in children(46).

From the present study, Anemia was slightly higher among female (11.5%) than male (11.2%) but not statistically significant. This is in line with the study done in Addis Ababa(46), Uganda(42) and Malawi(48), and in contrast with the study done in eastern Ethiopia(47).In addition prevalence of anemia was also higher among preschool children(20.7%) than school children which is comparable with the study done in eastern Ethiopia(47).Moreover, anemia is

more prevalent in rural dwellers than the urban dwellers. The same result was found from other part of Ethiopia(46).

In the current study microscopic examination of blood film for morphological classification indicated majority of them were classified as normocytic normochromic anemia (64.5%) followed by macrocytic normochromic (19.4%) and microcytic hypochromic (16.1%). Our result was concordant with study done in Gondar which showed that About 46.5 % of anemic children had normocytic normochromic anemia followed by macrocytic-normochromic anemia (39.5 %)(28).The study done in Ugandashowed that however, microcytic-hypochromic(44.9%) anemiafollowed by normocytic-hypochromic (26.5%) and (19.0%) patients had normocytic normochromic anemia and (8.2%) patients(56).

The severity of anemia in this study was 6.5% severe, 51.6% moderate and 41.9%mild anemiarespectively. 6.8% and 6.7% of severe anemia was reported from Nigeria(57) and India(27)respectively which is comparable with the current study. Different report fromthe current study was reported from northwest Ethiopia with 60.5%, 37.2% and 2.3% mild, moderate and severe anemia, Addis Ababa which reported 52.5% mild and 42.5% moderate anemia (46) and Uganda with 62.2%, 32% and 4.8% mild, moderate and severe anemia respectively (56).

In multivariate analysis, there was a significant association between the age of ≤7(AOR=3, CI:1.2-7.5) at P =0.02 which was comparable with the study reported from eastern Ethiopia(AOR=4.24, CI:1.85-9.73, P<0.001)(47) and Uganda(p <0.001)which found anemia to be independently associated with younger age(56).This might be due to the increased growth requirements of youngerchildren, as described by other study(22).Anemia is caused by different ethological agents, which include nutrient deficiency,immunosuppression of erythropoiesis, drug side effects, opportunistic infections, HIV-associated malignancies, and other factors(4, 13).

Form the current study, having a viral load of >150copies/ml is another risk factor for anemia, which is relatively comparable with the prospective cohort study done insouthern India which stated that having a detectable viral load (AOR 2.4, 95%CI:1.1-5.4) could be a risk factor for anemia(27).

The use of any form of combination of ART drug is not associated with the prevalence of anemia in our study which is supported by other different studies(53). In contrary a study done in harar, Ethiopia(58)and Asia(16)showed that the odds of anemia in zidovudine based HAART experienced children are higher than the other.

From the current study, anemia was also associated with being rural dweller.Such findings reflect that lack ofbetter socio-demographic conditions could mean lack of access to better feeding, health care and consequently higher prevalence of anemia.

In this study, there was no significant association between nutrition status (wasting) and anemia.Same finding was obtained by other study.In this study only 1child (0.4%) had intestinal helminthiasis and 1 child (0.4%) had malaria parasite infection. Due to a smallnumber of children with intestinal helminthiasis and malaria infection, the association between intestinalhelminthiasis and anemia, the association between malaria infection and anemia could not be established. Routine use of antihelminthiasis may have reduced the prevalence of intestinal helminthiasis in this group of children.

Limitation of the study

Duration of ART drug and hepatitis B was not included as a predictor of anemia which has an effect on prevalence of anemia. Diagnostic studies for iron deficiency: serum iron, ferritin and TIBC were not done because of budget constraints and the results of this study may not be generalizable because it is institution based cross sectional study.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

From the present study we can conclude that Anemia was prevalent among HAART experienced HIV positive children. Children on ART are at risk of becoming severely anemic and should be monitored. Anemia was also associated with being younger age, urban dweller and with viral >150copies/ml. Normocytic normochromic anemia was the highly prevalent anemia.

7.2. Recommendation

This study has shown that anemia is prevalent among HAART experienced children. Therefore the government should state follow up guidelines for anemia for HAART experienced children and the clinician regularly follow hose HAART experienced children and emphasis should be given for those children who are anemic and severely anemic. Guardians of the children also should follow closely the symptom of anemia on their children.Further study will be very important on the root causes and complications of anemia among anemic HAART experienced children.

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ANNEXES

Annex I questionnaires in English

A questionnaire prepared to assess prevalence of anemia and associated factors among HIV-infected children in antiretroviral therapy clinicat HUCSH.

Date……. **Study number**.....

PART I: Socio-demographic characteristics

Instruction: for the following question please circle one from the given chooses/write in words.

1. Sex 1.Mele 2.Femel
2. Age _____year
3. Residence 1. Urban 2. Rural
4. Educational status?1. No formal education 2. Primary 3. Secondary 4. College

Family and social history

5. Who is the primary care taker to the child? *(circle)* 1. Father 2.Mother 3.Grandmother 4. Older sibling 5. Aunt 6. Uncle 7.Other specify
6. What is the occupation of the care taker 1. Farmer 2. Merchant 3. Government employ 4. Daily labor 5. Other specify
7. Care takers Annual Income in ETB …….
8. What is the highest level of education of the primary care taker (tick against the level of education*)*1. No formal education 2. Primary 3. Secondary 4. College

PART II: Nutritional Habit

9. Breast feeding history 1.yes 2. No
10. Is the child still breast feeding 1.yes 2. No
11. When were complimentary feeds started (age in months)_____
12. How many times does the child feed in a day_____

Past medical/ drug history

13. Previous transfusion 1. Yes 2. No

When was the last transfusion done? (Weeks) _____

14. is the child on ART 1.Yes 2.No If yes specify_____
15. Other drugs (specify) 1._____ 2._____

PART III: Menstrual history (for girls aged 8 years and above)

16. Have you started menstrual periods? 1.Yes 2.No

17. If yes, what is the duration of the period? _____ (Days)

Measuredvalues

19. Anthropometry

a) Length / Height …… (cm)

b) Weight …….. (kg)

c) MUAC …….. (cm)

d)Weight for height Z score ……

e) Height for age Z score …….

f) Temperature ……$_{O}^{C}$

PART IV: CLINICAL DATA

1. WHO clinical staging_____
2. Current CD4 count_____
3. Current CD4 percentage_____
4. Malaria parasites1.yes 2. No
5. If yes What species _____
6. How often
7. What is the parasite density_____

PART V: Laboratory investigations for CBC

RBC total _____x106/ul

Hb_____ g/dl

HCT _____%

MCV __ fl

MCH _____ pg

MCHC _____ g/dl

RDW _____ %

ANNEXE II : QUESTIONNAIRE IN AMHARIC VERSION

መጠይቅክፍል ፣ ፡- የቤተሰብናማህበረዊ ጽታ

መመሪያ: ለሚከተሉትጥያቄዎችከአማራጮቱአንዱንበመክበብ / በጽሁፍ/በመሙላትመመለስ።።

1. ጾታ 1.ወንድ 2.ሴት
2. እድሜ? _____ዓመት
3. የሚኖሩትየትነው? 1. ከተማ 2. ገጠር
4. የት/ትሁኔታ? 1. ያልተማረ 2. አንደኛደርጃ 3. ሁለተኛደርጃ 4. ሶስተኛደርጃ
5. አሳዳጊማነው? (የምትኖረው/ሪውከማንጋነው) 1. ከአባት 2. ከእናት 3. ከአያት 4. ከታላቅእህት/ወንድም 5.ከአክስት 6. ከአጎት
6. የአሳዳጊስራ? ……
7. የቤተሰብአመታዊገቢ? ……
8. ያሳዳጊየት/ትደረጃ 1. ያለተማረ 2. አንደኛደረጃ 3. ሁለተኛደረጃ 4. ኮሌጅ

መጠይቅክፍል 2; የአመጋገብልምድ

9. ጡትጠብተሃል/ሻል? 1.አዎ 2.አይ
10. እስካሁንእየጠባ/ችነው? 1.አዎ 2.አይ
11. ተጨማሪምግብመችጀመለ/ች? …….
12. በቀንስንቴይመገባል/ትመገባለች/? ……..

የቀድሞየጤናሁኔታ

13. ደምተሰጥቶህ/ሽያወቃለ? 1.አዎ 2.አይ
14. አዎከሆነመልስዎደምየወሰዱበትየመጨለሻቀን?
15. (ART) መድሀኒትይጠቀማል/ትጠቀማለች? 1.አዎ 2.አይ

    አዎካሉየትኛወን? …….

መጠይቅክፍል 3 የወርአበባሁኔታ(ከ 8 አመትበላይ)

16. የወርአበባማየትጀምረሻለነው? 1.አዎ2.አይ

    አዎካሉምንያህልቀንይቆያለ? ……..

መጠይቅክፍል 4.አንትሮፖሜትሪክልኬቶች

17. ቁመት
18. ክብደት
19. የደምግፊት

መጠይቅክፍል5፡የደምምርመራዉጠየቶች

RBC total _____x106/ul

Hb_____ g/dl

HCT _____%

MCV __ fl

MCH _____ pg

MCHC _____ g/dl

RDW _____ %

Cd4 ……..

አመሰግናለሁ!!! ጥያቄየንጨርሻለሁ!!

Annex III Procedure for Venous Blood Collection

The blood samples will be obtained from the antecubital vein for analysis.First, the subject will be told that he is going to give the blood sample and will be asked for his permission.Thena sterile syringe of the capacity required will be Selectedand apply a soft tubing tourniquet or fastening arm band to the upper arm of the subject then cleanse the puncture site with 70% ethanol and allow drying. Do not re-touch the cleansed area!

When sufficient blood has been collected, the tourniquet will be releaseed and instruct the subject to open his or her fist then Remove the needle and immediately press on the puncture site with a piece of dry cotton wool and Remove the tourniquet completely. Instruct the subject to continue pressing on the puncture site until the bleeding has stopped.

The collected blood will be filled in to EDTA tube quality of sample will be handled and checked depending on the standard operating procedure in place and used for the required analysis.

Annex IV SOPs

A. ForStool Examination

- Label a clean slide with patient's number and initials.
- Perform macroscopic examination of the stool.
- Prepare 2 smears on the slide.
- Place a drop of normal saline using a Pasteur pipette.
- Transfer a portion of the sample emulsifying using an applicator stick.
- Cover smear with cover slip ready for examination.
- Microscopic examination to identify and report parasite seen by species, if no abnormality seen will be negative.

B. How to collect thick malaria smear

Thick film examination is about 20 times more sensitive than thin film examination for parasite detection.

C. Prepare thick film in the following way:

- Hold the third finger of the left hand of the patient between your left thumb and finger at the first phalangeal joint.
- Wipe fingertip with swab dipped in antiseptic solution.
- Allow the fingertip to dry.
- Hold pricking needle in right hand and prick the finger and allow blood drop tooozeout.
- Take a clean, dust free, grease free slide and take 3 drops of the blood 1 cm from the edge of the glass slide.
- Make thick smear by joining the 3 drops of blood and spreading it in an area of10 mm diameter.
- Allow it to air dry.

D. Giemsa staining thick blood smear for hemoparasites

- Dilute Giemsa stock solution to 1:10 using buffer ph 6.8.
- Place blood smear on a staining rack facing upward.
- Flood smear with freshly dilute giemsa stain for 15 minutes.
- Wash with clean water using a wash bottle.
- Put stained smear on a standing rack and leave to air dry.
- Stained blood smear is now ready for microscopic examination.

E. SOP FOR CBC

Purpose

It helps to instruct to measure red blood cell using Cell dyne ruby CBC analyzer.

Principle

The CELL-DYN Ruby uses flow cytometric techniques to analyze the RBC/PLT, WBC and NOC populations. It is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light. A sensor or sensors measure, by the loss or scattering of light, the physical or chemical characteristics of the cells or particles. Flow cytometry enables the rapid screening of large numbers of cells and provides quantitative cell analysis at the single-cell level and uses the scatter plots to differentiate the WBC into five subpopulations.

Procedure for start up of CELL-DYN ruby CBC analyzer

1. Power on to the display
2. perform daily maintenance
3. Let the laser warm up for 15 min
4. Prime the analyzer
5. verify back ground count
6. perform QC and verify QC result, if pass
7. prepare and run specimens

Procedure for running the specimen

For Open mode analysis

1. After specimen is collected, place it in a rack near CELL- DYN ruby
2. Since the Open mode does not use rack and tube position processing
3. Enter Specimen ID in the Open Tube Entry region
4. Mix Specimen Tube
5. Open the sample tube and place it under the Open Mode Probe Raise the tube until the end of the probe is deeply immersed in the sample.
6. Press the Touch Plate to activate aspiration Remove the tube when the beep sounds and Replace the cap.

7. When the cycle is finished, the results post to the Data log and are displayed in the Run View

For Closed Mode Analysis

1. After specimen is collected, place it in a rack near CELL- DYN ruby
2. Verify the Analyzer Status indicates **Ready** state and is in the **closed** mode.
3. Mix the specimens and place them in the Sample Loader racks
4. Place the racks in the Sample Loader to the right of the Processor Cover with the rack bar code labels facing the Operator.
5. **Start** Loader.
6. The results are posted to the **Data log** and are displayed in the **Run View**.
7. Review Results

Reference rang

For RBC

Male =4.1 – 5.6 X 10^6/ul

Female = 3.5 – 5.1X 10^6/ul

For HCT

Children at birth . . . . . 0.44–0.54(44-54%)

Children 2–5 y . . . . . 0.34–0.40(34-40 %)

Children 6–12 y . . . . . 0.35–0.45(35-45%)

Adult men. . . . . 0.40–0.54(40-54%)

Adult women 0.36–0.46 (36-46%)

For HGB

Female: 11.5-15.0g/dl

Male: 12.5-17.0g/dl

Newborn infants 14 -18 g/dl

Child 6 months–4 years 11.0 -14g/dl

For RBC indices

MVC 80 – 100 fl

MCH 27 – 34 pg

MCHC 32 – 36g/dl

Result interpretation

High RBC count indicates polycytemia

Low RBC count indicates anemia

High HCT value indicates Polycythemia

Low HCT value indicates Anemia

High HGB value indicates Polycythemia

Low HGB value indicates Anemia

High Red Cell Indices value could be megaloblastic anemia

Low Red Cell Indices value indicates Anemia

Quality control

The quality control programs on the CELL-DYN Ruby help assess precision and accuracy, identify shifts and trends, and

Determine the nature and cause of errors.

QC PROCEDURE TO PERFORM IN OPEN MODE

1. From the Open Tube Entry (NOTE) region using the mouse, click on the QCID icon to display the QCID Look up list of QCID files
2. Then select the QCID Specimen ID you would like to run.
3. The QCID Specimen ID selected will automatically fill the NOTE region fields with the QCID, Specimen Type, and Test Selection.
4. Remove the cap from a well-mixed control specimen tube and place the open tube under the Open Mode Probe.
5. Raise the tube so that the end of the probe is deeply immersed in the specimen.
6. Press the Touch Plate to activate aspiration.

7. After the cycle is completed the result displayed on the run screen

8. Verify that all controls are within limits

9. If any values are out of limit repeat control, if controls are within limit you can run patient Specimens

QC For Closed Mode Analysis

1. Put the control specimen tube near CELL-DYN ruby

2. Verify the Analyzer Status indicates **Ready** state and is in the **closed** mode.

3. Place a well-mixed control specimen tubein the rack and place it in the Sample Loader to the right of the Processor Cover with the rack bar code labels facing the Operator.

4. Select **F12 – Start** Loader.

5. The results are posted to the **Data log** and are displayed in the **Run View**.

6. Verify that all controls are within limits

7. If any values are out of limit repeat control, if controls are within limit you can run patient Specimens

G. CD4 count

Purpose To enumerate the absolute and percentage of lymphocytes that are CD4 + T-lymphocyte in unlysed whole blood using FACS Count analyzer

Principle When whole blood is added to the reagent tubes, the fluorochrome labeled antibodies, bind specifically to antigens on the surface of lymphocytes. It detects two fluorescent colors and measures relative cell size. CD3 cell fluoresce red and CD4+ cell fluoresce yellow. In the analysis the software identifies the T-.lymphocyte populations and calculates the absolute counts

Materials **Reagents**

Name	Manufacturer	Catalog No
BD FACSCount reagent kit	BD	340167
BD FACSCount control kit,	BD	340166

FACS flow,	BD	342003

Sample type	Amount required	Transport and Storage	Stability
K_3 EDTA blood	4-5ml ***N.B:-*** *Not less than 1/3 ml of the standard collection tube.*	Room Temperature (20^{O}c - 25^{O}c).	48 hours before preparing & 48 hours after preparing

Procedure **Preparing Controls**

Step **Action**

1. Label reagent CD4-Zero, CD4-Low and CD4-Medium, CD4-High
2. Vortex the reagent upside for 5 seconds and upright for 5 seconds.
3. Open the reagent tubes using the coring station
4. Mix the normal whole blood by inverting the tube five times
5. Reverse pipette 50µl of normal whole blood in to each control run tubes
6. Cap the tubes and vortex upright for 5 seconds
7. Incubate for 30 to 60 minutes at room temperature in the dark
8. Un cap the tube and pipette 50µl of fixative solution into tube
9. Recap the tube and vortex upright for 5 seconds
10 Stained samples can be stored in the workstation up to 24 hours before adding the control beads

11 Place the Zero/Low control bead pair and Medium/High control bead pair in the Control area of the workstation

12 Uncap the reagent tubes

13 Vortex the Zero/Low control bead pair and reverse pipette 50µl of Zero control Beads into the CD4 reagent tube labeled Zero

14 Vortex the medium/High control bead pair and reverse pipette 50µl of Medium Control beads into the CD4 reagent tube labeled Medium

15 Run on the FACSCount instrument within 1 hours of adding the control Bead

Running patients sample

Step	Action
1	Vortex the reagent upright for 5 seconds.
2	Uncap the CD4 tube and place the reagent in the sample holder so the CD4 tube in the run position
3	Press [RUN]. .
4	Remove the reagent and recap the CD4 tube
5	Discard the reagent pair in an appropriate biohazard container

Annex V INFORMATION SHEET IN ENGLISH VERSION

Information sheet

Title of studyprevalence of anemia and associated factors among HIV- infected children in antiretroviral therapy clinicatHawassa University Comprehensive Specialized Hospital.

Principal Investigator: Metsihet Mohammed, postgraduate student from Jimma University. **Purpose of the study**: To know the burden of anemia and its associated factors. HIV positive children are at high risk of developing anemia.

Introduction I am Metsihet Mohammed from department of medical laboratory science HUCSH. I am going to carry out a study on the risk factors for anemia in HIV infected children. This study will help us understand the factors that are likely to contribute to anemia in HIV infected children so that they can be addressed in order to improve the quality of life of these children. I am therefore requesting you to participate in this study by allowing your child to be enrolled in the study.

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

BenefitYour child will receive a complete medical exam, the investigations done during the study will be free of charge. Findings from this study will help us in setting prevention programmes and developing treatment protocols. For those who will be anemic will get proper treatment and counseling.

Risks and discomforts During the process of drowning blood, your child will feel some pain however this will be mild. We shall draw about a teaspoon of blood from your child to do the blood tests. This amount of blood will be too little to cause harm to your child's health. All information obtained in this study will be considered confidential

and used only for research purpose and patient care. Patient identity will be kept confidential.\

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

Who to contact This research has been reviewed and approved by the ethics committee. If you have any questions, you may ask those now or later. If you wish to ask questions later, you may contact me at: Metsihet Mohammed Tel: +251911562769 I have been invited to allow my child to participate in this research.

Statement of Consent The purpose and nature of this study has been explained to me and I understand that the participation of my child in the study is voluntary and that no consequences will result if I refuse my child to participate. I have the right to know the results of the laboratory tests for my child. By signing this form, I agree to participate in this study:

Parent /guardian signature…….. Date……

Patient ID……Patient initials……

PI signature……Date……..

ANNEX-VI: INFORMATION SHEET IN AMHARIC VERSION

ለጥናቱመረጃናመገለጫቅጽ

የጥናቱአላማ

የዚህጥናትአላማመሰረትያደረገውየደምማነስችግርከኤችአይቪመድሀኒትጋርያለውንቁርኝትበኤችአይቪታማሚልጆችላይለማጥናትነው፡፡እርሶበዚህጥናትመሳተፍዋበኤችአይቪታማሚልጆችየደምማነስችግርየሚያስከትለዉንህመምለመቆጣጠርይረዳል፡፡

በጥናቱስለመሳተፍ

በዚህጥናትመሳተፍበሙሉፈቃደኝነትላይየተመሠረተነው፡፡ስለሆነምበመጀመሪያበጥናቱእንዲሣተፉፈቃደኝነተዎንበትህትናእንጠይቃለን፡፡በዚህጥናትለመሳተፍከፈቀዱለአመስትደቂቃያህልለጥያቄዎችምላሽይሰጡናል፡፡በተጨማሪምለተለያዩምርመራዎች የደምናሙናለመስጠትፈቃደኛእንዲሆኑእንጠይቆታለን፡፡

በጥናቱበመሳተፍየሚገኝጥቅም

የደመዎናሙናበላብራቶሪሲመረመርጠቃሚዉጠየትካለከዶክመንቶጋርዕንዲያያዝእናየህኪምክትትልናአስፈላጊውንምክርእንዲሰጦይደረጋል፡፡

ምስጢርንስለመጠበቅ

በጥናቱውስጥየተሰበሰቡማናቸውምግላዊመረጃዎችሚስጥራዊነታቸውየተጠበቀይሆናል፡፡ከማንነትዎጋርበቀጥታተያያዥነትያላቸውመረጃዎችበሙሉበዋናተመራማሪውሚስጥራዊበሆነየመረጃጥንቅርዘዴከተቀየሩበኋላብቻለምርምርሂደቱየሚውሉይሆናሉ ::

ከጥናቱስለመውጣትናስለማቋረጥ

ይህጥናትበፈቃደኝነትላይየተመሰረተእንደመሆኑመጠንጥናቱዉስጥአለመሳተፍናበማናቸውምወቅትበፈቃደዎከጥናቱመውጣት ይችላሉ፡፡ከጥናቱበመዉጣቶበህክምናዎላይምንምአይነትችግርአያመጣም፡፡

ከጥናቱጋርበተያያዘማናቸውምጥያቄቢኖርዎበሚከተለውአድራሻጥያቄዎንማቅረብይችላሉ፡፡

ዋናተመራማሪ፡- መጽሄትመሀመድ

አድራሻ፡ሀዋሳዩኒቨርስቲሜዲካልላቦራቶሪሳይንስትምህርትክፍል

ሀዋሳ፣ኢትዮጵያ
ስልክ: 09-11-56-27-69፣ኢ-ሜል: m.metsihet2006@gmail.com

ANNEX VII: CONSENT FORM ENGLISH VERSION

Code No_____

I consent acceptance, has been explained to me in a language I understand on anemia and its association factors among HIV positive HAART experienced children in our country. I understand that anemia and its association with HAART in HIV patients is not well known. For this reason, doing research on this title will be paramount important to decrease the complication.

Therefore, I am informed about giving blood sample in no harm method and I will be interviewed for five minutes. More over all the data obtained will be kept strictly confidential. Anonymous testing will be undertaken, that is sample will be coded and result will not be identified by names in this paper and in the other reports; decline to answer the questions; the right not to participate or withdraw and decide not to participate has no influence on any services that I seek to get. Even I have been assured that I am benefited from cost free laboratory examination and based on the test result I will get the usual professional support from the assigned physician.

Therefore, by understanding the objective of the consent, I agreed to give blood sample for the stated purpose and I have no any objection if this sample also used for similar research in the future. Participating in this study is purely voluntarily; I am very happy and I have informed this to the consent offering personnel.

For this, I declared with my signature.

Consent acceptor signature (no name) _____witness signature ____Person Obtaining Consent__

Date _____ Date_____ Date_____

Researchers address
Metsihet Mohammed; Hawassa University
Tel. 09-11-56-27-69

ANNEX VIII: CONSENT FORM AMHARIC VERSION

ስለስምምነቱማረጋገጫ

የሚስጥርቁጥር_____

እኔውልተቀባይየዩሪክአሲድበደምዉስጥመብዛትጉዳትየሚያስከትልመሆኑንእኔበሚገባኝቋንቋተነግሮኝተረድቻለሁ:: የዚህጎመምስርጭትበበቂመጠንእንደማይታወቅናበዚህምምክኒያትበሽታውንበተመለከተይህንንጥናትበማካሄድየበሽታውንስር ጭትመጠንለመቀነስየሚደረግጥረትአጋዥ እንደሆነበሚገባተረድቻለሁ ::

ስለዚህምለተለያዩምርመራዎችየደምናሙናመስጠትእንዳለብኝናለአምስትደቂቃኢንተርቪውመደረግእንዳለብኝተነግሮኛል:: በተጨማሪምየኔስምናአድራሻበዚህጹሁፍምይሁንበሌላበሚደረግሪፖርትእንደማይገለጹለምጠየቀውምጥያቄያለመመለስበፈል ግምበምርምሩመሳተፍወይምአለመሳተፍመብቴየተጠበቀመሆኑንናላለመሳተፍብወስንምንምአይነትተፅዕኖእንደማይኖረውተረ ድቻለሁ::

እንዲሁምናሙናውለተባለውአላማእንደሚውልበሚደረገውነፃምርምራአገልግሎትተጠቃሚልሆንእንደምችልናበውጤቱምመሰ ረትከተመደበውሀኪምየተለመደውንሙያዊእርዳታእንደማገኝተረድቻለሁ ::

ስለዚህየዉሉንአላማበሚገባበመረዳትየሰጠሁትንየደምናሙናለተባለውምርምርናበተጨማሪምለወደፊቱናሙናዉለተመሳሳይ ምርምርቢያውሉትተቃውሞእንደሌለኝ፣በጥናቱመሳተፌበሙሉፍቃደኝነትእንደሆነ፣በፍቃደኝነትበመተባበሬዐኔናወገኖቼንልረ ዳበመቻሌደስተኛመሆኔንለውልሰጪውገልጫለሁ ::

ስለዚህምበፊርማዬአረጋግጣለሁ ::

የውልተቀባይፊርማ (ስምአይፃፍም)_____የምስክርፊርማ_____የውልሰጪፊርማ_____

ቀን_____ ቀን_____ ቀን_____

የተመራማሪውአድራሻ

መዕሄትሞሀመድ፣ሀዋሳዩኒቨርሲቲ

ስልክቁጥር09-11-56-27-69

Annex IXASSENT FORM in English

Purpose of the study a research is a way of finding out new information about something. I am doing a research to find out anemia and its factor in HIV positive children. We are asking children to be part of the study. When you accept to be part of the study, we shall request you to respond to some questions concerning your health. A doctor will then examine and also draw small blood from you using a needle so that we can test it. During the process of drawing blood, you will feel some pain as the needle enters your body but this will not last long and after the needle is out you will not feel pain again. We shall also give you one container so that you put a small part of your stool. You are free to decline to participate in this study or withdraw from the study at any time and this will not affect your management in any way. You are free to ask any questions now or if you get any questions later, you can call or ask your parent to call Metsihet +251911562769.

Annex XASSENT FORM in Amharic

የስምምነትውል

የጥናቱአላማስለአንድነገርመረጃማግኘትነው፡፡እኔይህንጥናትየማደርገውኤችአይቪ፣ዝቲቭበሆኑህፃናትላይያለውንየደምማነስእ
ናምክኒያቱንስለሆነህፃናተየጥናቱአካልእንዲሆኑእጠይቃለሁ፡፡የጥናቱአካልመሆንህን/ሽንከተቀበልክ/ሽስለጤናህ/ሽየሚመለከት
ህን/ሽንጥያቄትመልስልኛለህ/ሺልኛለሽ፡፡በመቀጠልምህኪምካየህ/ሽቡሃላለምርመራየሚሆንትንሽየደምናሙናመርፌበመጠቀም
የወሰዳል፡፡ይህትንሽህመምይኖረዋለ፣ግንአይቆይም፡፡በተጨማሪትንሽሰገራለምርመራታመጣለህ/ጫለሽ፡፡የናሙናአቃይሰጣል፡፡
በማኒኛውምሰአትበጥናቱላይያሎትንተሳትፎማቁዋረጥይችላሉ፡፡ይህምበርሶየጤናከትትልላይምንምአይነትተፅኖኤይኖረውም፡፡
ማንኛውኒምጥያቄበአሁኑሰአትዌይምከመጠይቁቡሃላመጠየቅከፈለጉየጥናቱንባለቤትመፅሄትንበስልክቁጥር 09-11-56-27-
69 ደውለውመጠየቅወይምቤተሰብማስጠየቅይችላሉ፡፡