HEMATOLOGICAL ABNORMALITIES AND ASSOCIATED FACTORS AMONG PEDIATRICS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS AT FICHE GENERAL HOSPITAL AND KUYYU HOSPITAL, CENTRAL ETHIOPIA: A CROSS-SECTIONAL STUDY

BY: BERHANU ASEFA (MSc Candidate)

A THESIS PEPAR TO BE SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULITY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR A MASTER OF SCIENCE DEGREE IN CLINICAL LABORATORY SCIENCES, SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

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JIMMA, ETHIOPIA

JIMMA UNIVERSITY

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ABSTRACT

Background: *Hematological abnormalities are common in Human Immunodeficiency Virus (HIV) infected individuals particularly, in pediatrics. Hence, this abnormality upsurges the risk of morbidity and mortality among infected individuals. Nevertheless, in Ethiopia little is known about the hematological profiles among HIV- positive pediatrics.*

Objective: *to determine the hematological abnormalities among HIV- positive pediatrics and associated factors in Fitche General Hospital and Kuyyu Hospital, 2023.*

Methods: *Institutional based cross-sectional study was conducted by enrolling a total of 264 HIV- positive pediatrics in 2023, at Fitche General Hospital and Kuyyu Hospital. Data on socio-demographic characteristics of the study participants was collected using a structured pretested questionnaire via face-to-face interview and their clinical conditions were obtained from their follow-up medical records. Hematological parameters (Complete Blood Count) and immunological parameters (CD4+ T cells) was analyzed by Sysmex XN-550 automated hematology analyze and BD FACSPresto™ machine (BD Biosciences, NJ, USA) respectively.. Statistical analysis of the data was done using SPSS v-25 statistical software. P-value < 0.05 was taken as statistically significant.*

Results: *Of the 264 pediatric clients recruited in this study, female participants were 137 (51.9.). The overall prevalence of anemia, thrombocytopenia, and leucopenia were 24.6%, 13.3%, and 17% respectively. Of the 65 (24.6%) participants with anemia, 81.5 % (53/65) presented with normocytic-normochromic anemia, 10.8% (7/65) with microcytic-hypochromic anemia, and 7.7% (5/65) with macrocytic-normochromic anemia. CD4+ T-cell counts, residence, marital status of care giver, and family size were associated with anemia.*

Conclusion and recommendation: *Anemia was the commonest hematological abnormality in HIV infected children. Normocytic normochromic anemia was the commonest type. Early diagnosis and treatment of anemia is essential in these patients and longitudinal studies to be carried out on serum iron and other tests to determine the actual etiology of anemia in HIV infected.*

Key Words: *HIV, Hematological profile, HAART, Pediatrics, Fitche*

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LISTS OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AIHA	Autoimmune Hemolytic Anemia
AOR	Adjusted odds ratio
ART	Anti-Retroviral Therapy
CBC	Complete Blood Count
CD	Cluster of Differentiation
COR	Crude odds ratio
EDTA	Ethylenediaminetetraacetic acid
FGH	Fitche General Hospital
HAART	Highly Active Anti-Retroviral Therapy
HCT	Hematocrit
HGB	Hemoglobin
HIV	Human Immune Deficiency Virus
KH	Kuyyu Hospital
MNA	Macrocytic Normocytic Anemia
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MHA	Microcytic Hypochromic Anemia
NNM	Normocytic Normochromic Anemia
RDW	Red Cell Distribution Width
SPSS	Statistical Package for Social Science
UNAIDS	Joint United Nations Program on AIDS
WBC	White Blood Cell
WHO	World Health Organization

OPERATIONAL DEFINITION

Pediatrics: individuals less ≤15 years.

Immunosuppression and anemia are defined based on WHO (1) criteria as follows

Anemia: hemoglobin level <11.9g/dl.

Mild anemia: hemoglobin level between 10 and 11.9g/dl.

Moderate anemia**:** hemoglobin level between 7.0 and 9.9 g/dl.

Severe anemia**:** hemoglobin level <7.0 g/dL.

Mild immunosuppression**:** CD4+ T lymphocyte counts between 350 and 499 cells/μL; ***advanced immunosuppression***: CD4+ T lymphocyte counts between 200 and 349 cells/μL; ***severe immunosuppression***: CD4+ T cell count <200 cells/μL (2).

Leucopenia:(1) White blood cell count < 4000 cells/µl

Thrombocytopenia: platelet count less than 150×10^3 cells/µl

CHAPTER ONE: INTRODUCTION

1.1. Background

The global Human Immunodeficiency Virus (HIV) infection remains a great challenge and has an intense impact on disease progression and mortality(3). There were approximately 36.7 million people worldwide living with HIV/ Acquired Immunodeficiency Syndrome (AIDS) of which 2.1 million were children below the age of 15 years and 70% (1.5 million) of them were found in sub-Saharan Africa according to the joint United Nations Programme on HIV/AIDS (UNAIDS) report at the end of 2016 (4) .

In Ethiopia there is roughly 65,100 HIV- infected children and nearly about 3200 AIDS-related deaths of child occurring annually(5). HIV infection is described by progressive destruction to the body's immune system which results in a number of opportunistic infections, and immunological and hematological complication.(6)

Human Immunodeficiency Virus is a retrovirus that infects cells of the immune system, destroying or impairing their function, which leads to the occurrence of opportunistic infections, immunological and hematological complications(7). It enters into the host immune system mainly through interactions between cluster of differentiation (CD4) cells and the presence of chemokine co- receptors CCR5 and CXCR4 (7,8). HIV replicates not only in CD4+ lymphocyte cells but also in macrophages and dendritic cells (9) this replication results in immune system depression. Hematological complications like mild-to-severe anemia are highly associated with HIV disease progression (10).

Acquired Immunodeficiency Syndrome is a systemic disorder caused by HIV and characterized by severe impairment of the immune system and progressive damage of both cellular and humoral immune responses(11). Hematological abnormalities have been recognized as strong autonomous predictors of morbidity and mortality in HIV-infected individuals(12).

Hematological abnormalities have been known to be the second most common cause of morbidity and mortality in HIV-positive individuals and manifested with cytopenias that comprise anemia, leucocytopenia, and thrombocytopenia (13). Hematological abnormalities, which can involve all lineages of blood cells and include anemia, thrombocytopenia as well as leucopenia, are the most common complications of HIV infection(14)

Anemia is the most common hematological abnormalities in HIV- infected individuals with prevalence ranges from 1.3% to 95% depending on the stage of HIV infection, sex, age, and presence of opportunistic infections (15,16). Several factors play a role in the development of anemia in patients with HIV including, chronic disease, opportunistic infections, parasitic infection, changes in cytokine production with subsequent effects on hematopoiesis; decreased erythropoietin concentrations and parvovirus B-19; administration of chemotherapeutic agents. Other mechanisms for HIV-associated anemia, although uncommon, includes autoimmune destruction of red blood cells and certain nutritional deficiencies (15,17)

The consequences of untreated anemia may lead to multisystem disabling symptoms, and fatigue, exhaustion, increased risk of HIV dementia, poor quality of life (15,17). Different studies showed that normocytic-normochromic anemia is the dominant type of anemia(13,15).

Thrombocytopenia is the second most common complication of HIV and associated with a normal or increased number of megakaryocytes in the bone marrow and raised levels of platelet-associated immunoglobulin. In addition to this, causes of thrombocytopenia in HIV infection include infectious or neoplastic conditions that involve bone marrow suppression, and medications that cause generalized myelosuppression that can result in thrombocytopenia (18).

Thrombocytopenia is associated with low CD4 cell count and common in older age. The possible mechanisms that have been reported are immune-mediated destruction of platelets by antibodies, cross-reacting antibodies that are directed toward HIV proteins, predominantly gp120 and p-24, resulting in immune thrombocytopenic purpura(ITP) that is characterized by very low platelet counts but, with normal hematocrit and white blood cell count (3,18)

HIV infection suppresses the bone marrow and leads to a decreased levels of granulocyte colony-stimulating factor, resulting in leukopenia(13). Another potentially fatal complication of HIV is autoimmune hemolytic anemia (AIHA) which reveals normocytic erythrocytes, erythrocyte agglutination, and spherocytes (19). Short term studies have shown that the basic laboratory abnormalities associated with ART are hematological changes that may lead to increased prevalence of anemia, neutropenia and thrombocytopenia in HIV-1 infected children(20). Antiretroviral drug toxicities may have both acute and long-term consequences on the hematological and nutritional indices of HIV-infected persons(21).

1.2. Statement of the Problem

It is not a surprising fact that the HIV/AIDS pandemic is a serious burden on the economic, social, and political issues of the world in general, and in developing countries in particular. Close regular follow-up is important since these children are at risk of morbidity and mortality. Mortality estimates in Africa show that without treatment 35.2% of HIV- infected children die in their first year of acquiring the infection and 52.5% by age two of acquiring. These issues bring the importance of timely antiretroviral treatment-care and -support(22)

Hematological abnormalities have been thought to be the prominent manifestations of HIV infection(23). This hematological disorder could result from the effect of the virus itself otherwise from other opportunistic infections in which malignancies are the major cause of hematological abnormalities(13).

Anemia, leukopenia, neutropenia, thrombocytopenia are the major hematological manifestations in HIV- infected individuals(16,24). Anemia is a condition in which hemoglobin (Hb) concentration and/or red blood cell (RBC) numbers are lower than normal and insufficient to meet an individual's physiological needs(25).

Hematological complications have been documented to be the second most common cause of morbidity and mortality among HIV positive children (26). Anemia among HIV infected children is an emerging public health problem. A quarter of the global population(1.8 billion) suffer from anemia, including 293 million (47%) children younger than 5 years whereby more than 100 million of these anemic children live in Africa (27).

According to EDHS 2016 reports, 57% of children age 6–59 months suffered from some degree of anemia (hemoglobin levels below 11 g/dl). Twenty-five percent of children are classified with mild anemia, 29% with moderate anemia, and 3% with severe anemia. Unhappily, the trend of anemia prevalence among Ethiopian children declined from 54 to 44% from 2005 to 2011, but increased to 57% in 2016 (28).

Anemia is a global public health problem that affects both developing and developed countries with a high impact on human health, social and economic growth. The effects of anemia can range from mild to severe and include fatigue and decreased productivity, poor cognitive and motor development in children, poor birth outcomes, and increased morbidity and mortality in women and children with severe anemia(29–32) .

In general, multi-factorial causes contribute to the increase in the burden of anemia among HIV patients. Some include opportunistic infections, change in immune system adaptability, nutritional deficiencies and side effects of HAART drugs including bone marrow suppression by HAART. Other common causes of anemia in HIV are chronic diseases, and hemolytic anemia induced by oxidant drugs.

Therefore, while there is a wide variation in the prevalence of anemia among HIV/AIDS patients in different studies all over the world, there is paucity of information on the prevalence and associated risk factors of anemia among pediatric HIV patients in Ethiopia. Therefore, this study is intended to determine hematological profiles and associated factors where information is limited in my study area.

1.3. Significance of study

The local health planners, health administrators, authorities, study participants, and stakeholders at a different level will be benefited from the recommendation drawn from this study. Study participants with abnormal result were benefited from the study through means of treatment. Above all, this study will provide information for the clinicians on the management of the various hematological manifestations observed in HIV- positive children. It will also provide health planners and other stakeholder evidence-based intervention. It also serves as information for researchers to conduct further study.

CHAPTER TWO: LITERATURE REVIEW

2.1. Literature Review

Infection with HIV has been associated with a broad range of clinical outcomes because it involves the hematopoietic system. All hematopoietic cell lineage are prone to the effect of HIV infection leading to a wide range of hematological abnormalities, such as peripheral blood cytopenias, bone marrow, and as well as coagulation pathways(33).

Clinically important hematological abnormalities are very common in children with HIV/AIDS. Impaired hematopoiesis, immune-mediated cytopenias, and altered coagulation mechanisms have all been described in HIV infected individuals, and tend to be more severe in advanced stages of the disease(34,35). Anemia is a common feature of HIV infection, happening in approximately 35% of patients who initiate antiretroviral treatment (ART) in Europe and North America(36).

A cross-sectional study conducted (from November 2011 to November 2012) at Regional Pediatric ART center, Medical College and Hospital, Kolkata, India, showed that (69%) of the study population was anemic and the distribution of anemia at WHO stage 1, 2, 3 and 4 was 47.37%, 66.67%, 71.43%, and 93.10% respectively(23).

According to a study conducted (2013) in Telangana, there was a 77%, 21%, and 5% prevalence of anemia, leucopenia and thrombocytopenia respectively. The magnitude and severity of hematological abnormality, and other parameters were found to be more in patients not on ART when compared to patients on ART. Likewise, the magnitude and severity of most hematological abnormalities were inversely proportional to the CD4 count in non-ART cases but not with cases on ART(13).

A Cross-Sectional Study conducted in Iran (2008), revealed that the likelihood of anemia increases with progressive immunologic deterioration, and with the advancement of HIV-related disease, and the prevalence of Macrocytic anemia, Normocytic normochromic anemia, and microcytic Hypochromic anemia was 11%, 41.1% and 47.9% respectively. Anemia was associated with female sex, stage of HIV infection were the most important factors associated with anemia in HIV-infected patients in the study(37).

According to a study conducted(2019) among HIV-infected children and adolescents aged 2 years to less than 18 years receiving ART at the Dalhatu Araf Specialist Hospital, Lafia , Nigeria, the prevalence of anemia, leukopenia, and thrombocytopenia were 32.7%, 11.4%, and 3.04%, respectively. In this study, about 75% of the participants was on a zidovudine-based regimen, and there is an association between anemia and the use of zidovudine-based ART regimen and female patients are more prone to anemia than the male patient(38).

In a study conducted (between July 2005 and March 2013) at the pediatric HIV clinic of the Jos University Teaching Hospital, Nigeria, The prevalence of anemia, thrombocytopenia and leukopenia among children before ART was 6.4%, 7.0%, and 8.6% respectively. After initiation of ART Median haemoglobin (Hb) levels improved from 10 g/dL (9–11 g/dL) at baseline to 11 g/dL (10–12 g/dL), and 11 g/dL (10–12 g/dL) at 6 and 12 months of ART respectively(39).

According to a cross-sectional clinic-based study conducted (between2015 and 2016) at a tertiary hospital in Tanzania, Anemia, leucopenia, and thrombocytopenia account for 58.4%, 23.6%, and 14.4%, respectively, and cytopenias are highly strongly related to advanced HIV infection (40).

According to the study conducted (from 2007 to 2009) in Mbarara, Uganda on HIV- infected pediatrics, the prevalence of Anemia was 57.6%, out of which, 62.2% were with mild anemia, 32.0% with moderate anemia, and 4.8% with severe anemia. Children with more advanced HIV disease revealed lower mean hemoglobin (Hgb) level and Microcytic-hypochromic anemia (44.9%) was the commonest type of anemia in this study (41)

A cross-sectional study conducted in Gondar (2012) on HIV positive treatment naïve and individuals on HAART positive adult study subjects showed that prevalence of anemia, leucopenia, thrombocytopenia, and neutropenia was 11.7%, 35.9%, 4.1%, and 28.3% in patients on HAART respectively (42).

An institution-based study conducted (from2013 to 2018) in Debre Berhan Referral Hospital, North Showa, Amhara, Ethiopia, the overall estimated survival rate after initiation of antiretroviral therapy was 81.7% (95% CI=75.36–86.54%) at 72 months of follow-up and the independent predictors of mortality were clinical stage IV Among Adult HIV/AIDS Patients Initiating HAART (43).

A cross-sectional study (2013) conducted among pediatric HIV/AIDS patients of Zewditu Memorial Hospital (ZMH), Addis Ababa, Ethiopia revealed that the total prevalence of anemia was 22.2%. This showed that there was a significant increase in severity and prevalence of anemia in those with CD4+ T cell counts less than 350 cells/μL, intestinal parasitic infections, being HAART (44).

In Cross- sectional study conducted in Gondar (2015) among HIV-positive children on pre and post-treatment, the prevalence of anemia was 42.8% before and 18.9% after ART initiation. 38.3% of participants were at stages 1 and 2, whereas 61.7% of participants were in clinical stages 3 and 4 (16).

A study (from 2007 to 2010) conducted in Hiwot Fana Specialized University Hospital, Harar, Ethiopia, on children on ART followed up revealed that the prevalence of anemia (54.4%) and growth failure were common in the study area. Conversely, the prevalence of anemia was declined to (39.2%) after initiation of ART (45).

A study conducted (from 2007 to 2009) at Felege-Hiwot Referral Hospital, Bahir-Dar, Ethiopia, revealed the prevalence of anemia among HIV-infected children after ART initiation was 65%. A significant impact on the survival of children in the study area was associated with baseline hemoglobin level, WHO clinical stage, and age (46).

A cross- sectional study conducted (from May 2012 to February 2013) among children who had been on HAART for a maximum of twelve months at Yekatit 12 Hospital, Addis Ababa, Ethiopia, revealed that the prevalence of anemia was 18.9%, 12.3%, and 10.4% at baseline, at six months, and twelve months after initiation of ART, respectively. Patients who were taking AZT-based regimen were more likely to develop anemia than those on D4T-based regimen, (OR=4.5, p-value <0.05). The prevalence of thrombocytopenia at baseline was 8.5%, but it was lowered for both at six and twelve months by 5.7% (47).

According to a hospital-based cross-sectional study conducted (2019) on HIV-infected children receiving HAART at Hawassa University comprehensive specialized Hospital, the prevalence of anemia, thrombocytopenia, Leucopenia, Pancytopenia, and neutropenia was 11.4, 4%, 14.3%, 2.9%, and 18.3%, respectively and All forms of hematological abnormalities were highly prevalent in children with a CD4-cell percentage <15% of the normal(48).

A cross-sectional study conducted (2007) at Jimma University medical center, Ethiopia, on Hematological abnormalities among children on HAART, revealed that the prevalence of anemia, thrombocytopenia and neutropenia among the study children was 21.9%, 7.8%, and 4.7%, respectively(49).

A cross-sectional study conducted (2020) on HIV- infected children attending at University of Gondar Specialized Referral Hospital, Northwest Ethiopia revealed that the prevalence of Anemia, leukopenia, lymphopenia, thrombocytopenia were 21.2%, 12.2%, 11%, and 1.6% respectively(50).

2.2 contributing factors of hematological abnormalities

The associated factors of hematological abnormalities among HIV-infected children are similar with slight differences in different geographic areas. Some studies revealed that hematological abnormalities are positively associated with socio-demographic characteristics, nutritional status, and parasitic infection, type of HAART used, and WHO clinical stages of HIV-infected individuals.

Anemia is one of the most commonly detected hematological abnormalities and an independent prognostic marker of HIV disease progression in people living with HIV. The prevalence of anemia in HIV- positive people has been estimated to be 63% to 95% in different study settings(51).

A cross-sectional study (2013) conducted among pediatric HIV/AIDS patients of Zewditu Memorial Hospital (ZMH), Addis Ababa, Ethiopia revealed that intestinal parasitic infections (AOR =2.7, 95% CI, 1.1–7.2), and lower CD4+ T cell count (AOR = 3.8, 95% CI, 1.6–9.4) were identified as significant predictors of anemia(44).

A cross-sectional study was conducted in Jimma university specialized hospital (JUSH) from February 1 to March 30, 2012 which showed in Stool samples of 231 participants intestinal parasites were detected in 53 (22.9%) of participants and 58.5% of these were detected from HAART experienced participants. *Ascaris lumbricoids* 28(43.1%) was the most prevalent parasite detected, followed by *Trichuris trichiura* 26 (40%), and *Hook worm* 5(7.7%). The study revealed that HAART regimen and duration of HAART for HAART experienced groups were remained independently and significantly associated with anemia(52).

A cross-sectional study conducted (2020) on HIV- infected children attending at University of Gondar Specialized Referral Hospital, Northwest Ethiopia revealed that children in the age group of 2–10 years (AOR = 5.38, 95%CI 2.33–12.46), AZT based regimen (AOR = 5.44, 95%CI: 2.24–13.21), no eating green vegetables (AOR = 2.49, 95% CI: 1.26–4.92) and having plasma viral load >1000 copies /ml (AOR = 5.38, 95%CI: 2.22–13.03) showed significant association with anemia(50).

A study was conducted on 350 HIV-infected children on HAART attending Hospitals of North Wollo Zone (2019). This study revealed that associated factors of anemia were parasitic infections, history of opportunistic infections, being malnourished, having poor adherence to ART, caregivers living in the rural area, and eating non-diversified foods(53).

From literature review I summarize that hematological abnormality in HIV –positive patients, particularly anemia range from 1.3% to 95% depending different predictors of anemia. Anemia is associated with CD count, history of opportunistic infections, residence of study participants, history of parasitic infection, WHO clinical stage, age, gender, HAART duration, nutritional status , ART regimen type, and educational level. Most of the studies fail to address type of anemia based on peripheral morphology finding. Therefore, in our study we tried to fill this gap.

2.3. Conceptual frame work

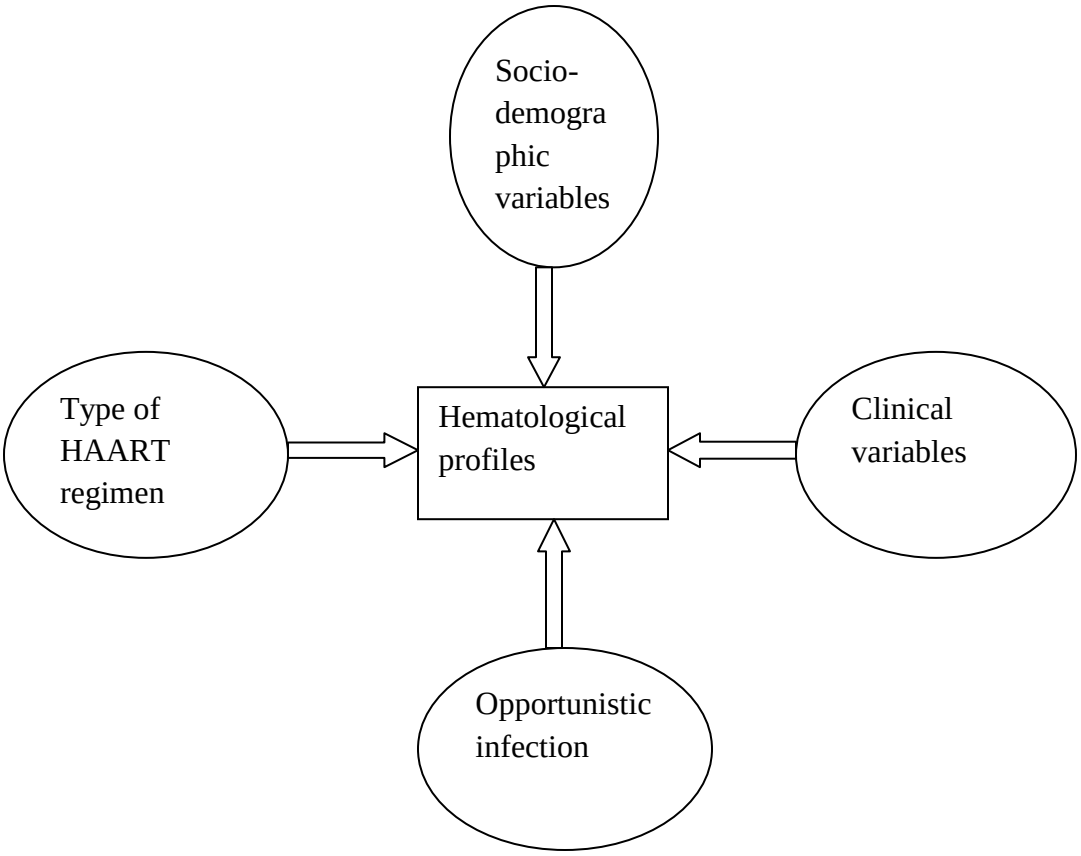

Figure 1 : Conceptual frame work of hematological parameters and associated factors among HIV infected pediatrics, 2023.

CHAPTER THREE: OBJECTIVES

3.1. General Objective

- To determine hematological parameters and associated factors among HIV-infected pediatrics at fitche general Hospital and Kuyyu Hospital , Central Ethiopia from April 30 to June 30, 2023.

3.2. Specific Objectives

- To determine the prevalence of anemia, leukopenia, and thrombocytopenia among HIV-infected pediatrics at FGH and KH, 2023
- To assess associated factors related to Hematological parameters among HIV-infected pediatrics at FGH and KH, 2023.
- To determine the type of anemia based on peripheral morphology findings at FGH and KH, 2023.

CHAPTER FOUR: METHODS AND MATERIALS

4.1. Study Setting

This study was conducted in Fitche General Hospital and kuyyu Hospital, North Showa Zone, Oromia Region, Ethiopia. Study setting was located on about 114 and 154 km away from Addis Ababa to the north direction, respectively. Fitche General Hospital was established in 1999 G.C as a primary Hospital and shifted to General Hospital after 15 years. It delivers services for about 1.5 million peoples and the Hospital started delivering ART service in 2006 G.C, currently, there are 1682 HIV-positive individuals enrolled in chronic follow-up care of which 269 of them were pediatrics. Information Obtained from the zonal health department revealed that there are 14 woredas and 291 kebeles in the zone. The population size of the zone in 2011 EFY based on the projection from the 2007 population and housing census is estimated to be 1,639,587 with 78,700 houses holds.

4.2. Study Design and Period

An Institutional based cross- sectional study was conducted from April 30 to June 30, 2023.

4.3. Population

4.3.1. Source population

All HIV-positive pediatrics those who attending ART clinic at Fitche General Hospital and Kuyyu Hospital during the study period

4.3.2. Study Population

All HIV infected pediatrics those fulfill inclusion criteria and selected for the study.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All pediatrics receiving care at the ART clinic during the study period those who were volunteer to take part in the study and uses HAART for 3 months and more

4.4.2. Exclusion criteria

- Children on medication (antimicrobial therapy, vitamin supplements, and tuberculosis) during sampling.
- Pediatrics who were newly transfused in the last 4 months
- Pediatrics with malaria.
- Pediatrics those who had a traumatic injury or surgical procedure which may result in blood loss.

4.5. Sample size determination and Sampling Technique

4.5.1. Sample size determination

The sample size was computed using the single population proportion formula by considering the following assumption.

P = **22.2 %** proportion of anemia among HIV infected children from previous study conducted at Zewditu memorial hospital, Ethiopia (44)

Zα/2 = standard deviate at 95% confidence level = 1.96

d = level of precision at 5% or Margin of error 5%

n = required sample size if population is greater than 10,000

nf = required sample size (with population < 10,000)

Therefore, $n= Z^2 p (1-p)/d^2$

$n= (1.96)^2 x0.222 (1-0.222) / (0.05)^2$=**264**

Therefore, the final sample size was **264** HIV positive pediatric

4.5.2. Sampling Technique

A consecutive convenient sampling technique was used to recruit participants who meet the inclusion criteria as they visited the clinic until the calculated minimum sample size was reached

4.6. Variables

4.6.1. Dependent variable

Hematological value/profiles

4.6.2. Independent Variables

4.6.3. Socio-demographic variables

- Age, Sex, Residence, Educational status of both children and family, Family size, HIV status of care giver, Parental status, Family occupation, Relationship to child

4.6.4. Clinical variables

- Type of HART regime
- WHO clinical stage
- CD4 count
- History of Parasitic infection
- History of opportunistic infection

4.7. Data Collection Technique / Recruitment procedure

4.7.1. Socio-demographic and clinical data collection

A structured pre- tested interviewer-administered questionnaire and checklist for medical records review was used to collect socio-demographic and clinical data of the study participants with the aid of two professional nurses. History of parasitic infection and opportunistic infection was obtained from medical records. The questionnaire, first prepared in English language was translated into local language, and then it was translated back to English language to ensure its consistency. The questionnaires were administered after giving the parents/caregivers adequate information about the study and informed consent with or without assent.

4.7.2. Specimen Collection and processing

About 4 ml of venous blood was collected from antecubital fossa of the forearm by a senior laboratory professional and pediatrician for peripheral blood film and complete blood count through an aseptic technique in a tri-potassium ethylene diamine tetra-acetic acid (K3-EDTA) test tube and gently mixed to prevent blood clotting. The specimen was labeled and analyzed for CBC within 4 hours using Sysmex XN-550 (Sysmex Europe GMBH, Germany) hematological analyzer machine based on direct current principle for cells and spectrophotometric principle for Hgb. CD4 + T cells count was analyzed using BD FACSPresto^{TM} machine **(**BD Biosciences, NJ, USA) based on flow cytometry principles.

Hence this parameter was used in this study to define anemia in HIV-infected children. In this study, anemia was defined as Hgb value <11.9g/dL and between 10 and 11.9g/dl as mild anemia; between 7.0 and 9.9 g/dl as moderate anemia and < 7 as Severe anemia.

Two peripheral blood smears was prepared and stained by Wright's stain to appreciate morphological characteristics of anemia. The smears were examined by a senior laboratory technologist. .

4.8 Quality Assurance

To assure the quality of the data, all laboratory tasks were done by strictly following manufacturer's instructions and standard operating procedures (SOP) was strictly followed in each step to maintain the quality of the laboratory results. All reagents used were checked for their expiry date and prepared according to the manufacturer's instructions. The quality Control of both CBC and CD4+ T cell analyzer were daily before patient sample analysis and the collected data was checked for validity, completeness, and internal consistency by quality officer daily.

In addition, all flagged specimens were subjected to manual differential and morphologic examination to confirm the results. Training was given for the data collectors on data collection tools, methodology and maintaining quality to minimize technical and observer biases by principal investigator, and pre-testing was done on patients before the study, and modification was done accordingly.

4.9. Data Processing and Statistical Analysis

The data obtained from questionnaires and laboratory results was cleaned, coded, and double-entered using EpiData version 4.6 and exported to SPSS-version 25 software for analysis. Descriptive statistics (mean and standard deviation) was used. Proportions and percentages were calculated and statistical analysis of continuous variables was dichotomized using defined cut-off values into hematological abnormalities. The result was presented using tables and text.

The bivariate analysis was done to see the association of each independent variable with the outcome variable. Those variables having P-value less than 0.25 was entered into a multivariate logistic regression model to identify the effect of each independent variable with the dependent variable and to avoid confounding variables. The independent variables was taken as significant at $P < 0.05$ in the multiple logistic regression analysis

4.10. Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board of the Institute of Health of Jimma University; Permission to carry out the study was obtained from Fitch General Hospital and Kuyyu Hospital. Written informed consent prepared in the local language (Afaan oromo and amharic) was provided by the parents and additional verbal assent was taken for children between 10 and 15 years of age. All participants were informed of their right to withdraw from the study at any stage or to restrict their data from analysis; the interview took place in a separate room to ensure their privacy. The abnormal results were timely reported to the clinicians for appropriate interventions.

4.11. Plan for Dissemination and Utilization of Results

The result of this study will be presented to Jimma University then it will be summated to the research directorate of JU as partial fulfillment of the degree of Master of Science in Hematology and immunohematology and it will be submitted to Fitche General Hospital, Kuyyu Hospital, and others who will be concerned at the regional or federal level. Finally, efforts will be made to publish the research on local or international reputable peer reviewed journals.

CHAPTER FIVE: RESULTS

5.1. Socio-demographic characteristics of the study population

A total of 264 pediatric clients were recruited for this study. The mean age of the study population was 10.5 ± 4.03 years (range: 2 to 15 years). More than half of study participants were 137 (51.9%). The mean Hgb were 12.5mg/dL±2.34 (SD). Children within the under-five age bracket were 19 (7.2%), those aged 5–10 years were 104 (39.4%), while those aged above 10 years were 141 (53.4%), respectively. Most of the participants were from rural areas (59.1%). Regarding educational status of the study participants, 194 (73.5%), had no formal education while, 70 (26.5%), were in primary and secondary education. Majority of the study participants, 214 (81.1%), were in WHO clinical stage I. The biological parent was the primary caregiver for the majority of children 170(64.4%), and approximately one-thirds of primary caregivers were married 80(30.3%). This study showed that most of the caregivers 183 (69.3%) were HIV-positive and the HIV status of 6 (2.3%) caregivers were unknown (Table 1).

Table 1: Demographic characteristics of the study population at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264)

Variables	category	Frequency n (%)
Sex	Female	137 (51.9)
	Male	127 (48.1)
	Total	264 (100)
Age groups (years)	Under 5	19 (7.2)
	5-10	104 (39.4)
	>10	141 (53.4)
Residence	Rural	156 (59.1)
	Urban	108 (40.9)
WHO clinical stage	I	214 (81.1)
	II	40 (15.2)
	III	9 (3.4)
	IV	1 (0.4)
Educational status of children	Primary education	194 (73.5)
	No formal education	70 (26.5)
Child caregivers	mother	128 (48.5)
	female caregiver	43 (16.3)
	father	42 (15.9)
	male caregiver	51 (19.3)

5.2. Hematological parameters of the study population

A total of 264 clients were enrolled. Of these, 24.6% (65/264) were anemic (95% CI 0.19-0.28), 13.3% (35/264) had thrombocytopenia while 17% (45/264) had leucopenia, and from anemic children 24 (36.9%), 34 (52.3%), and 7 (10.8%) were mild anemia, moderate anemia, and severe anemia respectively. Of the 65 (24.6%) participants with anemia, 81.5 % (53/65) presented with normocytic-normochromic anemia (MCV range: 81-100fl) with morphological examination revealed some spherocytes, fragmented cell, and polychromasia, 10.8% (7/65) presented with microcytic-hypochromic anemia (MCV <80fl) with morphological examination revealed cigar cells, some target cells, and 7.7% (5/65) presented with macrocytic-normochromic anemia (MCV>100fl) with morphological examination revealed some ovalocytes, and hyper segmented neutrophil. Normocytic normochromic blood picture was most commonly found in stage I disease .There was no one with pancytopenia or bicytopenia (Table 2).

Table 2: Hematological parameters of HIV- positive pediatrics at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264)

variable	**category**	**Male, n (%)**	**Female, n (%)**	**Total, n (%)**
Anemia	normal	82 (41.2)	117 (58.8)	199 (75.4)
	Mild anemia	16 (66.7)	8 (33.3)	24 (36.9)
	moderate	24 (70.6)	10 (29.4)	34 (52.3)
	Severe anemia	5 (71.4)	2 (28.6)	7 (10.8)
	Total anemia	45 (69.2)	20 (30.8)	65 (**24.6**)
WBC	leucopenia	17 (37.8)	28 (62.2	45 (**17**)
	normal	106 (50.5)	104 (49.5)	210 (79.5)
	leucocytosis	4 (44.4)	5 (55.6)	9 (3.5)
platelet	thrombocytopenia	18 (51.4)	17 (49.6)	35 (**13.3**)
	normal	108 (47.4)	120 (52.6)	228 (86.4)
	Thrombocytosis	1(100)	0	1(0.3)

5.3. Relationship of CD4 Counts and anti-retroviral regimen with anemia

CD4 percentage was obtained by absolute CD4 count and Values were categorized according to WHO guideline as not significant (normal CD4 cells), mild immunodeficiency, advanced immunodeficiency, severe immunodeficiency. Forty two (**16.7%**) of the study population had CD4 counts of <200 cells/μL (Table 3).

Majority of the study population (62.9%) had CD4 counts between 350 and 499 cells/ μL. But, only **7.9** % of study population had CD4 counts ≥500 cells/ μL. From a total of 65 (24.6%) anemic pediatric patients, Thirty one **(47.7%)** had CD4 count<200 cells/μL .Increased levels (percentage) of Anemia were observed in HIV positive pediatric patients whose CD4 count was <200 cells/μL (Table 3).

In this study there were significantly more anemic patients 60 (51.7%) on zidovudine based (AZT/3TC/NVP) than on other treatment regimens used. At the time of this study about 116 (43.9%) of study population were on zidovudine based regimens, of which sixty of them developed anemia. Most of the anemia caused by zidovudine based regimen was moderate anemia 34 (56.7%). From a total of study participants 64 (24.2%) discontinued ART, of which 26 (40.6%) developed anemia (Table 3).

Table 3: Relationship of CD4 Counts and anti-retroviral regimen with anemia among HIV-positive pediatrics at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264)

immunosuppression	**CD4 category**	**Anemic, n (%)**	**Non-anemic, n(%)**	**Total, n (%)**
Severe immunosuppression	<200 cells/ μL	31 (73.8%)	11 (26.2%)	42 **(15.9%)**
Advanced immunosuppression	200-349 cell/ μL	16(45.7%)	19(54.3%)	35 (**13.3%**)
Mild immunosuppression	350-499 cell/ μL	13(7.8%)	153(92.2%)	166 **(62.9%)**
Normal	>500 cell/ μL	5(23.8%)	16(76.2%)	21 (**7.9%**)
Anti-retroviral regimen	Zidovudine based	60(51.7%)	56 (48.3%)	116(**43.9%**)
	Others	5 (3.4%)	143(96.6%)	148(**56.1%**)
ART status	discontinue	26(40.6%)	38 (59.4%)	64(**24.2**%)
	Not discontinue	39(19.5%)	161(80.5%)	200(**75.8**%)

Others= ABC/3TC/EFV, TDF/3TC/LPV/r, and ABC/3TC/LPR

3TC: Lamivudine, ABC: Abacavir, EFV: Efavirenz, LPV/r: Lopinavir/

5.4. Relationship between hematological parameters and WHO clinical stage

Table 4: Relationship between hematological parameters and WHO clinical stage at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264)

Hematological parameters	Classification	WHO clinical stage				Total
		Stage I	Stage II	Stage III	Stage IV	**N (%)**
Hemoglobin%	Anemic	49(75.4%)	13 (20%)	3(4.6%)	0	65(**24.6**%)
	Normal	165(82.9%)	27 (13.6)	6 (3%)	1	199(75.4%)
RBC pictures based on indices	NNA	41 (77.4%)	7(13.2%)	5(9.4%)	0	53(81.5%)
	MHA	3 (42.8%)	2(28.6%)	2(28.6%)	0	7(10.8)
	MA	3 (60%)	2 (40%)	0	0	5 (7.7%)
Total WBC Count	Leucopenia	36 (80%)	2 (4.4%)	7 (15.6%)	0	45 (17%)
	Normal	171(81.4%)	37(17.6%)	1 (0.5%)	1	210(79.6%)
	Leucocytosis	7 (77.8%)	1(11.1%)	1(11.1%)	0	9 (3.5%)
Platelet	Low	28 (80%)	7 (20%)	0	0	35 (13.3%)
	Normal	185(81.1%)	33(14.6%)	9 (3.9%)	1	228(86.4%)
	High	1(100%)	0	0	0	1 (0.3%)
Immunological level	Severe	33 (75%)	6(13.6%	5(11.4%)	0	44 (16.7%)
	Advanced	17 (41.5%)	23(56.1%)	1 (2.4%)	0	41 (15.5%)
	Mild	154(92.2%)	10 (6%)	3 (1.8%)	0	167(63.3%)
	Normal	10 (83.4%)	1 (8.3%)	0	1(8.3%)	12 (4.5%)

Regression analysis on factors associated with hematological profiles among HIV-positive children

To determine risk factors of hematological profiles among HIV infected pediatric patients, bivariate and multivariate logistic regression was used. In bi-variable logistic regression analysis, age of the child, residence of child, marital status of the caregiver, parental status of the care giver, HAART discontinue status, family size, children education level, CD4 count, family educational level, and presence of opportunistic infection were taken as candidate variables for multivariable logistic regression.

Then, these variables were entered into a multivariable logistic regression to control the confounding effect of one variable on the other predictor variable. In the final model of the multivariable logistic regression analysis, residence, marital status of care giver, family size, and, CD4 count were the significant factors associated with anemia among HIV-positive children. But there was no significant association between anemia and HIV status of care giver, presence of opportunistic infection, parental status, family education level, children education status, HAART status, and, child age (Table 5).

Based on this, CD4 count (AOR 0.02; 95 % CI 0.01, 0.08), family size (AOR 0.10; 95 % CI 0.03, 0.31), Residence (AOR 0.34; 95 % CI 0.13, 0.88), and marital status of care giver being married (AOR 0.12; 95% CI 0.02-0.53), and widowed (AOR 0.22; 95% CI 0.0, 0.97) were associated with anemia (Table 5). Leucopenia was associated with ART regimen type (AOR 6.7; 95% CI 2.62, 17.4) and HAART discontinue status (AOR 0.07; 95% CI 0.03, 0.17) (Table 6).

CHAPTER SIX: DISCUSSION

Clinically significant hematological abnormalities are common in children with HIV/AIDS. These abnormalities may occur as a result of HIV infection itself, as sequel of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and other associated conditions. In our study anemia, leucopenia, and thrombocytopenia were common findings. This was also documented in different studies done in different year (24,38,49,50) .

6.1. Prevalence of Anemia in HIV-Infected Children

The overall prevalence of anemia in this study was 24.6% (65/264). Several study results reported from Jimma, Addis Ababa, Gondar, Ethiopia (21.9%)(49), (22.2%)(44), (21.2%)(50), respectively, Tanzania (20.9%)(54) were consistent with this study result. The similarity may due to the similarity in the study design (cross-sectional study).

Conversely, this study result was higher than the study conducted in Gondar, Addis Ababa Ethiopia 11.7% (42) and 12.3% (47), respectively. Sample size variations and duration on ART differences could be the cause of this discrepancy. In the present study, 264 study participants were included, whereas only 145 and 106 study participants were included to a study from Gondar and Addis Ababa respectively. Regarding duration on ART, the study conducted from Addis Ababa determined anemia at six months post treatment while this study determined anemia at any time post treatment. The finding in the current study is also higher than the study conducted in North West Ethiopia (18.9%) among children who are at least 6 months on ART (16). The discrepancy may be due to the nonstratification of our study population based on years on ART.

On the other hand, the magnitude of anemia (24.6%) in the current study was lower than the previously published study from Harar, Ethiopia (39.2%)(41), Addis Ababa (34.6%)(55), Lafia, Nigeria (32.7%)(38), Uganda (57.6%) (41), South-East China which was 55.15% (56), Hyderabad, India(77%) (13), and Sao Paulo, Brazil 37.5% (24) . The difference may be due to the study population which is HIV-infected children lower than 15 years compared to the Harar study which was among HIV-infected children lower than 14 years.

Our study population is HIV-infected children on ART drug who were on clinic follow-up compared to the China study which was among hospitalized HIV-infected adults and the Ugandan study among children yet to be launched on ART. Compared to our study the Indian study (77%) was conducted on all age group and 120 HIV positive cases including both patients on ART and not on ART. In contrast to our study (n=264) the study conducted in Sao Paulo Brazil (37.5%) used large sample size (n=701). In addition to this, it is also lower than the study conducted in India 69% (56).The difference may be due to the higher sample size in this study compared to the relatively lower sample size of 100 in their study.

In this study normocytic normochromic Anemia (NNA) was the commonest form (81.5%) of anemia. This is supported by different studies in which normocytic normochromic is the commonest form of anemia in HIV infected patients(42,57,58). An increasing number of this type of anemia was observed in stage I of the disease, probably due to direct effect of HIV/AIDS. Another studies also found that the prevalence of NNA was more in the children with advanced and severe immune suppression than those with mild and no immune suppression(59).

About 10.8% of study population had Microcytic Hypochromic Anemia (MHA). Microcytic Hypochromic Anemia found almost equally in stage I, II, and III. This type of blood picture was probably due to iron deficiency and inhibition of iron utilization in inflammatory condition but iron deficiency was very common in this part of the world (60)..

6.2. Prevalence of leucocytopenia in HIV-Infected Children

In this study, leucopenia was found in 45 (17%) of the study population. This study prevalence is similar to the 14.3% reported in Ethiopia (61) and 21% reported in India (13). However, this study result is higher than the 12.2% reported in Gondar, Ethiopia(62) and 11.4% in Nigeria (59) while lower than the study conducted in Ethiopia 35.9% (57). This difference may be due to variation in study populations, clinical conditions and study design methods.

6.3. Prevalence of thrombocytopenia in HIV-Infected Children

One of a common hematological disorder of HIV/AIDS-infected patients is thrombocytopenia (64), which is not affected by age group or gender difference(14). Thrombocytopenia was present in 13.3% of study population and it had no association with stage of disease.

This study prevalence (13.3%) is comparable to the 11% reported in India(65) and 14.4% in Tanzania (40) but higher than the study conducted in Ethiopia,4.1%, (57) and India,5%, (13). This difference may be due to variation in sampling techniques, study population, clinical conditions, and study design methods.

6.4. Factors Associated with anemia

The present study established that CD4 count, family size, marital status of care giver and residence of study participants were significant predictors of anemia among HIV-positive children on HAART. The prevalence of anemia was found to be higher among male, even though gender was not an independent predictor of anemia unlike a study done in Gondar (66) where gender was associated with significantly increased anemia.

The risk of developing anemia in HIV-positive children on HAART who had < 350 CD4 count were 2 % times higher than those who had CD4 count of >350 (AOR 0.02; 95 % CI 0.01, 0.08). In agreement with our finding, a study conducted at Benishangul Gumuz, Northwest Ethiopia and Zewditu memorial hospital reported that patients who had a CD4 count of <350 had a high risk of developing anemia(44,67), respectively. The well-known mechanism of CD4+T cell depletion in HIV patients is related to apoptosis in which the dying cells exceed the number of HIV-infected cells(68,69). Another study conducted on this area indicates that disease progression and severe immunosuppression are associated with cytokine-mediated myelosuppression resulting in multiple hematologic abnormalities, including anemia (70).

The risk of developing anemia in patients with family size >5 was 10% (AOR 0.10; 95% C I 0.03, 0.31) higher likelihood of being anemic than with family size <5. This might be due to nutritional deficiency as family size increase.

The odds of being anemic among children with married (AOR 0.12; 95% CI 0.02, 0.53) and widowed caregiver (AOR 0.22; 95% CI 0.05, 0.97) were 12% and 22% higher likelihood of being anemic than children with single caregiver respectively. This might be due increase in family size in married caregiver.

Odds of being anemic among HIV-positive children those lived in rural area were 34% (AOR 0.34; 95 % CI 0.13, 0.88) higher than their counterparts. Similar to other study conducted in Jimma(49), the prevalence of anemia was significantly associated with rural residence but different from a study conducted in Zewditu memorial hospital where rural residence was not significant predictor of anemia (44). This higher prevalence of anemia in rural residents might be due to the fact that those participants residing in rural areas might not have adequate information about nutrition and other factors that could cause anemia.

Table 5: Bivariable and Multivariable Binary Logistic Regression Analysis of Factors Associated with Anemia among HIV positive pediatric patients at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264)

Characteristics of study participants		Anemic	Non-anemic	COR(95%Cl)	AOR(95%Cl)	P-value
Age of children	>10	34(24.1%)	107(75.9%)	1	1	0.391
	5-10	20(19.2%)	84 (80.8%)	1.33(0.71-2.48)	1.58(0.55-4.55)	
	<5	11(57.9%)	8 (42.1%)	0.23(0.08-0.62)	0.49(0.07-3.15)	
Residence	Urban	22(20.4%)	86(79.6%)	1	1	**0.026**
	Rural	43(27.6%)	113(72.4%)	0.67(0.37-1.21)	0.34(0.13-0.88)	
Educational status of children	No formal education	53(27.3%)	141(72.7%)	0.55(0.27-1.10)	0.43(0.14-1.26)	0.125
	Primary school and above	12(17.1%)	58(82.9%)	1	1	
HAART discontinued	Yes	26(40.6%)	38(59.4%)	0.35(0.19-0.65)	0.42(0.14-1.25)	0.140
	No	39(19.5%)	161(79.5%)	1	1	
HIV status of the Care giver	negative	9(12%)	66(88%)	1	1	0.566
	Not known	2(33.3%)	4(66.7%)	0.27(0.04-1.70)	0.74(0.03-15.5)	
	positive	54(29.5%)	129(69.5%)	0.32(0.15-0.70)	0.61(0.11-3.29)	

CD4	>350 cell/ μL	17(9.1%)	169(90.9%)	1	1	**0.001**
	<350 cell/ μL	48(61.5%)	30(38.5%)	0.06(0.03-0.12)	0.02(0.01-0.08)	
Presence of OPI	yes	5(41.7%)	7(58.3%)	0.43(0.13-1.42)	1.18(0.66-21.1)	0.908
	no	60(23.8%)	192(76.2%)	1	1	
Educational status of care giver	College / University	5(21.7%)	18(78.3%)	1	1	0.060
	Secondary school	7(11.5%)	54(88.5%)	2.14(0.60-7.59)	3.38(0.67-17.0)	
	Primary school	8(8%)	92(92%)	3.19(0.93-10.8)	4.62(0.97-22.0)	
	No formal education	45(64.3%)	35(35.7%)	0.21(0.07-0.63)	0.60(0.12-2.81)	
Parental status	Both live	7(9.9%)	64(90.1%)	1	1	0.179
	Father dead	15(19.2%)	63(80.8%)	0.45(0.17-1.20)	0.71(0.14-3.42)	
	Mother dead	10(35.7%)	18(64.3%)	0.19(0.06-0.59)	0.35(0.07-1.76)	
	Both dead	33(37.9%)	54(62.1%)	0.17(0.07-0.43)	0.28(0.03-1.83)	
Marital status of the care giver	single	7(12.7%)	48(87.3%)	1	1	
	married	22(27.5%)	58(72.5%)	0.38(0.15-0.97)	0.12(0.02-0.53)	**0.005**
	widowed	12(21.4%)	44(78.6%)	0.53(0.19-1.48)	0.22(0.05-0.97)	**0.046**
	divorced	24(32.9%)	49(67.1%)	0.29(0.11-0.75)	0.26(0.06-1.15)	0.077
Family size	1-5	23(11.4%)	178(88.6%)	1	1	**0.001**
	>5	42(66.7%)	21(33.3%)	0.07(0.03-0.12)	0.10(0.03-0.31)	

6.5. Factors associated with leucocytopenia

The cause of HIV-associated leucopenia is multifactorial, including a direct consequence of HIV infection, autoimmune disorders, malignancies, and drugs used to treat HIV (71). In our study leucopenia was associated with ART regimen type and HAART discontinue status. The prevalence of leucocytopenia was found to be higher among female (62.2%), even though gender was not an independent predictor of leucocytopenia.

The risk of developing leucocytopenia in HIV-positive patient those discontinued HAART was 7% (AOR 0.07; 95% CI 0.03, 0.17) higher likelihood of being leucocytopenic than those not discontinued HAART.

The odds of being leucocytopenic among HIV-positive children those on zidovudine based therapy was 6.7 times higher than those on other therapies (AOR 6.7; 95% CI 2.62, 17.4). In agreement with our finding, a study conducted at Debre Berhan Referral Hospital (DBRH), North East Ethiopia reported HIV-positive children those were on zidovudine based therapy had high risk of developing leucocytopenia than those on other therapies (72). This may be due to the suppression of bone marrow by Zidovudine-based therapy(42).

. 6.6. Factors associated with thrombocytopenia

The overall prevalence of thrombocytopenia in HIV –positive children was 35(13.3%). Our study revealed that patients on an AZT-based ART regimen had a higher prevalence of thrombocytopenia compared with those on other regimen type. But, there was no significant association between the presence of thrombocytopenia and types of ART-based regimen. This finding is similar with study conducted at Debre Berhan Referral Hospital, North East Ethiopia(73). The high prevalence of thrombocytopenia observed in zidovudine-based therapy could be due to destruction of both platelets and mega karyocytes by an immune-mediated reaction which can occur in a zidovudine containing ART-regimen(42).

Table 6: Bivariable and Multivariable Binary Logistic Regression Analysis of Factors Associated with leucocytopenia among HIV positive pediatric patients at FH and KH, Central Ethiopia from April 30 to June 30, 2023 (n = 264).

Characteristics of study participants		Anemic	Non-anemic	COR(95%Cl)	AOR(95%Cl)	P-value
ART Regimen type	Others	5(3.4%)	143(96.6%)	1	1	**0.001**
	Zidovudine based	60(51.7%)	56 (48.3%)	2.84(1.37-5.90)	675(2.62-17.4)	
HAART discontinue status	Not discontinued	26(40.6%)	38(59.4%)	1	1	**0.001**
	discontinued	53(27.3%)	141(72.7%)	0.11(0.59-0.24)	0.07(0.03-0.17)	

NB: table was constructed only for associated factors.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

Anemia was the commonest hematological abnormality in HIV infected children. Normocytic normochromic anemia was the commonest type. There is a significant association between anemia and CD4 count, educational level of care giver, residence, and family size. HIV patients with low CD4 count are more likely to have anemia while those on AZT-based regimen are more likely to have leucopenia. Based on our findings, we recommend that health care professionals routinely investigate and should treat leucopenia**.** Despite HAART use, anemia is still highly prevalent among HIV positive children. In summary, this study has shown that regular checkup of CD4 count might have benefit in reducing anemia in HIV positive pediatric patients.

7.2. Recommendation

This study results showed that majority of HIV infected children in Central Ethiopia who are anemic have normochromic anemia and the prevalence is related to being having CD4<200 cells/ μL and HAART discontinue status. Therefore, early diagnosis and treatment of anemia is essential in these patients. We recommend longitudinal studies to be carried out on serum iron, folate, cobalamin level, erythropoietin level and viral load determination to determine the actual etiology of anemia in HIV infected. We recommend a larger study to be carried out and that as a basis of critical care and management, immune thrombocytopenia should be considered in our study population. Furthermore, patients with advanced HIV should be given special attention, as they are more likely to be affected by cytopenias.

Limitation of the study

This study had a number of limitations. One limitation of the study is that the anthropometric measurement was not done due to the non-functionality of weight scale at the time of data collection. Another limitation of our study was that the data which allow the classification of the causes of anemia such as reticulocyte counts, iron level, and erythropoietin levels were not measured. This study classifies anemia as Hgb < 11.9 g/dl which does not consider the age-specific value level of hemoglobin. Finally, since the study was a hospital-based study, the findings may not necessarily be generalizable.

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ANNEX

ANNEX ONE: Questionnaires (English version)

Dear respondents, you are kindly requested to give correct information accordingly.

Thank you for your time and participation.

Participants code number_____

Data collection Questionnaire's

Title of the study: hematological profiles and associated factors among HIV-positive pediatrics at Fitche General Hospital, and Kuyyu Hospital, Central Ethiopia.

Overview of the study: This study intended to determine the hematological profiles and associated factors among HIV- positive pediatrics that is considered as the main cause of morbidity and morbidly, and deliver a recommendation on possible prevention and controlling mechanism and assist the stakeholder for effective intervention plan in the future.

Since participation in this study is volunteer bases, you have the right to participate or not to participate, even to withdraw from participation under any condition and the confidentiality of the information gathered will be kept and only used for this study. The result of the laboratory finding will be transferred to your physician or caregiver.

Part one: **Socio-demographic characteristics of children**

1. Children's gender Male ☐ Female ☐
2. Children's age: _____
3. Children educational status: No formal education ☐ Primary School ☐ secondary ☐
4. Residence: Rural ☐ Urban ☐

Part –II: Socioeconomic /demographic characteristics of family/caregiver.

1. Age of care giver: -_____
2. Gender: - Male ☐ Female ☐
3. Marital status: - Single ☐ Married ☐ Separated ☐ Widowed ☐ Divorced ☐

4. Family occupation: - Privately employed ☐ Gov't employee ☐ Merchant ☐

   House wife☐ other_____

5. Family educational status: - No formal education ☐ Primary School ☐

   Secondary school ☐ College/ University ☐

6. Parental status: - Both live ☐ Both dead ☐ Mother dead ☐ Father dead ☐

7. Relationship to child: Mother ☐ Female caregiver☐ Father ☐ Male caregiver ☐

   other _____

8. The HIV status of care giver: - positive ☐ negative ☐ not known ☐

9. Family size: No_____

Part III -Children's food habit

10. Have you eaten dark green leafy vegetables like cassava, kale, spinach, cabbage?
    Never ☐ per day _____ per week_____ per month_____
11. Have eaten meat and milk product (Beef, lamb, goat, chicken or other organ meats, milk, cheese, yogurt or other milk products)?
    Never ☐ per day _____ per week_____ per month_____

PART- IV: -Clinical and anthropometric characteristics

1. What was his/her WHO clinical staging? I ☐ II ☐ III ☐ IV ☐
2. Is there any opportunistic infection? Yes ☐ No ☐
3. If so, yes what type of infection? _____
4. Current regimen type? _____
5. HAART discontinue Yes ☐ No
6. Is the children develop diarrhea? Yes ☐ No ☐
7. If so yes, type of diarrhea Acute ☐ Chronic ☐

I thank you!!!

ANNE TWO: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላትና ቃል ለመስጠት ስለተባበሩን እናመሰግናለን።

መለያ ቁጥር_____

ክፍል -1: የህፃኑ ማህበረሰባዊ ባህርያቶች (Socio-Demographic Characteristics)

1. የህፃኑ ጸታ፡- ወንድ ☐ ሴት ☐
2. የህፃኑ ዕድሜ፡- _____
3. የህፃኑ የት/ት ደረጃ:- መደበኛ ት/ት ያለተማረ ☐ አንደኛ ደረጃ ☐ ሁለተኛ ደረጃ ☐
4. የመኖሪያ ቦታ፡- ገጠር ☐ ከተማ ☐

ክፍል፡-2 የቤተሰብ /አሳዳጊ ማህበረሰባዊ ና ኢኮኖሚያዊ ባህሪያቶች

1. የቤተሰብ /አሳዳጊ ጸታ፡- ወንድ ☐ ሴት ☐
2. የቤተሰብ /አሳዳጊ ዕድሜ፡- _____
3. የቤተሰብ /አሳዳጊ የጋብቻ ሁኔታ፡- ያገባች ☐ ያላገባች ☐ የተለያዩ☐ የሞተባት☐
4. የቤተሰብ/አሳዳጊ የስራ ዓይነት፡- የግል ተቀጣሪ ☐ የመንግስት ተቀጣሪ ☐ ነጋዴ ☐ ሌላ_____
5. የቤትስብ/አሳዳጊ የት/ት ደረጃ ፡- መደበኛ ት/ት ያለተማረ ☐ አንደኛ ደረጃ ☐ ሁለተኛ ደረጃ ☐ ኮሌጅ/ዩኒቨርሲቲ ☐
6. የቤተሰብ ሁኔታ፡- እናት/አባት በህይወት አሉ ☐ አባት ብቻ አለ ☐ እናት ብቻ አለች ☐ እናት/አባት በህይወት የሌሉ ☐
7. ከህፃኑ ጋር ያላቸው የዝምድና ሁኔታ፡-እናት ☐ የሴት አሳዳጊ ☐ አባት ☐ የወንድ አሳዳጊ ☐
8. በደምወ ውስጥ HIV ቫይረስ አለ፡- አለ ☐ ለም ☐ አላውቀም ☐
9. ቤት ውስጥ ያለ የቤተሰብ ብዛት_____

ክፍል፡-3 የህፃኑ የአመጋገብ ሁኔታ

1. ለምን ያህል ጊዜ ቅጠላቅጠል(ጎመን፤ሰላጣ፤ካሮት፤ቆስጣ) ይመገባል፡

   በቀን_____ በሳምንት_____በወር_____ አልጠቀምም ☐

2. ለምን ያህል ጊዜ የእንሰሳት ተዋፅኦ (የከብትስጋ፣የበግስጋ፣የፍየል ስጋ፣የዶሮ ስጋ እነዚሁም ወተት፤አይብ፣እርጎ የመሳሰሉትን ይመገባል፡-

   በቀን____ በሳምን____ በወር_____ አልጠቀምም ☐

ክፍል 4፡- ከህክምናና ከልኬታ ጋር የተያያዙ ባህርያቶች

1. በዓለም አቀፍ የጤና መስፈረት መሰረት የታማሚው የበሽታ ደረጃ፡- I☐ II☐ III☐ IV☐
2. በአሁኑ ሰአት ከኤድስ በተጨማሪ ሌላ ጥገኛ በሽታ አለበት፡- አለ ☐ የለም ☐
3. በተራ ቁ2 መልስዎ አለ ከሆነ የታየበት ጥገኛ በሽታ ስም፡- _____ እና _____
4. የሚጠቀመው የመድሃኒት አይነት _____
5. መድሃኒት አቐርጿል፤ አወ ☐ የለም ☐
6. ታማሚው ተቅማጥ አለበት አወ ☐ የለበትም ☐
7. መልስወ አወ ከሆነ ምን አይነት ነው፤ አጣዳፊ ☐ አጣዳፊ ያልሆነ☐

አመሰግናለሁ!!!!!!!!

ANNEX THREE: Questionnaires (Afaan Oromoo version)

Kabajamtoota Hirmaattota keenya gaafilee armaan gadiif deebii sirrii akka nuuf laattan kabajaan isin gaafanna.

Hirmannaa keessaniif guddaa galatooma!!!

Lakkoofsa addaa----------

Kutaa 1^{ffaa}: gaafii sosho-dimoograffikii daa'immanii

1. Saala daa'immanii 1) dhalaa 2) dhiira

2. Umrii daa'immanii ----------

3. Sadarkaa barnoota isaanii 1) barnoota bu'uraa kan hin baranne 2) sad. 1ffaa 3) sad. 2ffaa

4. Iddoo jireenya isaanii 1) Baadiyyaa 2) magaala

Kutaa 2^{ffaa}: gaafii sosho-dimoograffikii maatii ykn gudiftootaa

1. Saala maatii ykn gudiftootaa 1) dhalaa 2) dhiira

2. Umrii maatii ykn gudiftootaa ----------

3. Haala fuudhaf heeruma maatii 1) kan heerumte 2) kan hin heerumne
   3) kan hiikte 4) kan jalaa du'e

4. Goosa hojii maatii 1) hojii dhunfaa 2) hojjataa motummaa
   3) Daldalaa 4) Haadha manaa

5. Sadarkaa barnootamaatii 1) barnoota bu'uraa kan hin baranne 2) sad. 1ffaa
   3) sad. 2ffaa 4) kolleejii/ Yunivarsiiti

6. Haala maatii 1) Haadha fi abbaan jiru 2) abbaa qofatu jira
   3) Haadha qoofatu jira 4) lachuu lubbuun kan hin jirre

7. Haariroo firummaa da'imma waliin qaban

1) Haadha 2) Dubartii guddistuu 3) abbaa 4) dhiira guddisaa

8. Haala HIV maatii ykn guddistootaa

1) HIV ni qabu 2) HIV hin qaban 3) hin beekamu

9. Baay'ina maatii----------

Kutaa 3ffaa: Haala nyaataa da'immanii

1. Gosoota nyaataa kan akka Raafuu, kaarotii, salaxaa yeroo hangamiin fayyadamu?

1) guyya guyyan 2) torbeen 3) ji'aan 4) hin fayyadamu

2. Bu'aawwan beeladaa kan akka foon loonii, foon hoolaa, foon ra'ee fi foon lukkuu akkasumas Aannan, baaduu fi ittittuu yeroo hangamiin fayyadamu?

1) guyya guyyan 2) torbeen 3) ji'aan 4) hin fayyadamu

Kutaa 4ffaa: Amaloota Qorannoo yaalii fi safarrii waliin waal qabatan

1. Sadarkaa WHO meeqarra jirtaa? (Kaardii dhukubsataairraa)

1) I 2)II 3)III 4)IV

2. Yeroo amma dhukuba HIV ala dhukuba dabalataa kan biraa qabdaa? 1) qaba 2) hin qabu

3. Deebiin gaaffii 2ffaa keessan yoo nan qaba jettan maqaa dhukkubicha dabalataa---------- fi----------

4. Akaakuu qoricha dhibee Eedsii fayyadamtanii (kardi irraa)----------fi----------

5. Qoricha dhibee Eedsii addaan kuttanii? 1) eeyyen 2) miti

6. Dhukuba garaakasaa qabdu? 1) eeyyen 2) miti

7. Deebin keessan eeyee yoo ta'e dhukuba garaakasaa kan akkami?

1) arifachiisaa 2) arifachiisaa kan hin taane

ANNEX-FOUR: LABORATORY PROCEDURES

A. *COMPLETE BLOOD COUNTING BY SYSMEX XN-550 AUTOMATION HEMATOLOGY ANALYZER*

PRINCIPLE: The Sysmex XN-450 and the XN-550 are multi-parameter quantitative automated hematology analyzesr for in vitro diagnostic use in determining whole blood diagnostic parameters. Examination of the numerical and/or morphological findings of the complete blood count by the physician are useful in the diagnosis of disease states such as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections.

The devices perform hematology analyses based on the hydrodynamically focused impedance measurement, the flow cytometry method (using a semiconductor laser) and the SLS-hemoglobin method.

The device counts and sizes red blood cells (RBC) and platelets (PLT) using hydrodynamic impedance counting (sheath flow DC method). At the same time the hematocrit (HCT) is measured as a ratio of the total RBC volume to whole blood via the RBC pulse height detection method.

The Data Innovations Instrument Manager (DI) is a Data Innovations computer program which is used to manage data coming from the XN-450 and 550 analyzers and sends that data to the Laboratory Information System (LIS). Sets of WBC, RBC, and PLT rules determine how positive parameter flags are handled by the instrument and/or operator and which samples are auto-validated by the DI. Manual differentials and RBC/Platelet morphology is entered via the DI. Pending Orders are checked via the Outstanding List in EPIC.

Specimen type: Whole blood collected in EDTA tube. A minimum of 1 ml must be collected for micro collection specimens. This ensures an adequate amount of blood for the 25µl aspiration at open mode.

Supplies:

- EDTA anticoagulant tube(Vacutainer tube)
- Dry gauze
- Cotton Swab
- Vacutainer needle with holder
- 70% Ethanol alcohol
- Tourniquet
- Glove

Reagent required:

1. CELLPACK DCL of 20L/10L
2. SULFOLYSER of 5L
3. Lysercell WDF of 2 x 4L
4. Fluorocell WDF of 2 x 42m

CELLPACK DCL: - Whole blood diluent for use in hematology analyzers

Sulfolyser (SLS):- Reagent for the automated determination of hemoglobin concentration of Blood. Sulfolyser is a lysing reagent that releases the hemoglobin to be measured by the SLS Hemoglobin method.

Lysercell WDF:- Reagent product to be combined and used with Fluorocell WDF. By hemolyzing red blood cells with Lysercell WDF and dying the white blood cell component With Fluorocell WDF< the counts and percentages of neutrophils, lymphocytes, monocytes, Eosinophils and basophils are analyzed.

Fluorocell WDF: - Used to stain the leukocytes in diluted and lysed blood samples for determination of differential count in blood.

CELLCLEAN AUTO: Detergent for fully automated hematology analyzers. To be used as a strong alkaline detergent to remove lysing reagents, cellular residuals, and blood proteins remaining in the hydraulics of the analyzer on XN Series/XN-L Series automated hematology analyzers.

Specimen stability: A well-mixed whole blood specimen, collected in EDTA anticoagulant and run within eight hours after collection, provides the most accurate results for hematological parameters.

XN-L CHECK (Commercial Control Material)

i. Manufactured by Streck, available as a tri-level package.

ii. Whole blood commercial control used to monitor performance of the XN analyzers.

iii. Formulation

i. Consists of human red and white blood cells with a platelet component

suspended in fluid medium.

ii. Each vial contains 3 mL of control material.

iv. Storage

i. Store vials at 2-8oC

ii. Do not freeze or expose to excessive heat.

Calibrators

1. XN CALTM: for use in calibrating the analyzer for WBC, RBC, HGB, HCT, PLT.
2. XN CAL Storage: Store the calibrator in a dark refrigerator at 2-8oC
3. XN CAL Stability: Unopened and properly stored, XN CAL is stable until the expiration date printed on the unopened vial. Open vial stability is 4 hours.

START UP PROCEDURE:

A. Start-up Procedure

1. Checks prior to turning on:

a. Visual inspections of analyzer / system / reagents

i. If applicable, verify waste container is empty

ii. Verify network / host connections are properly working.

iii. Verify sufficient reagent supply is nearby

b. Turning ON the entire system

1. Verify that all power switches for the device is in the ON position

2. Press the GREEN power button on the front of the XN-L to power ON the entire system

c. Log on to the XN-450/550

1. When the logon dialog box appears, enter user name and password

d. Analyzers self-checks

1. XN-450/550: Initialization of the mechanical parts; Rinse; Temperature stabilization; Background Check (up to 3 times).

XN-L Acceptable Background Count	
Parameters	**Acceptable Limit**
WBC	0.10 x 103 / uL
RBC	0.02 x 106 / uL
HGB	0.1 g/dL
PLT-I	10 x 103 / u

QUALITY CONTROL

Utilizing the BeyondCare Quality Monitor (BCQM)

Quality control is performed in order to monitor an analyzer's performance over time. XN-L CHECK is the material used to monitor the performance of the XN-450/550 analyzer. Quality control should be run in accordance with regulatory agency requirements. For the BeyondCare Quality Monitor program, a minimum of 2 levels of controls are needed to be run at least once every 24-hours. It should be noted that for troubleshooting purposes, additional control runs may be necessary. The BeyondCare Quality Monitor program is a toll that will help you determine when troubleshooting is necessary and dynamic screen prompts will guide the end user for the next action. All troubleshooting actions are logged in the Activity Log. (Reference the BeyondCare Quality Monitor User Manual)

A. **XN-L CHECK Commercial Controls Instructions for Use**

1. Remove vials from refrigerator and allow them to come to room temperature (18-25oC), for approximately 15 minutes.
2. Mix vials according to the package insert accompanying the product until the cell button in the bottom of the vial is completely suspended.
3. Perform a close visual inspection of each vial confirming the cell button is completely removed from the bottom of the vial and cellular elements are uniformly suspended with no aggregates.

B. **Frequency of Control use and review.**

1. XN-L CHECK control levels 1, 2, and 3 will be run each morning before reporting patient results.
2. The tech will review QC prior to reporting test results. If repeats are necessary, document corrective action on the log. The lab supervisor reviews data logs and Insight QC reports monthly

C. **Registering and modifying a QC file – lot information input**

1. Select [QC File] icon
2. Select a QC file that does not have a lot registered.
3. Select [Register]
4. Select [Read Assay file]
5. Select the correct QC product, lot number, and level

6. Select [Ok]

7. Verify the QC lot number, level and expiration date matches the QC vials received by the lab.

8. Repeat for each level of XN-L CHECK to be registered.

9. Perform parallel studies between production lot and new lot prior to production lot expiration

D. XN-L CHECK QC Analysis

1. Confirm the analyzer is in a Ready state.
2. If sample tube holder is not ejected, press the sample tube holder open/close switch.
3. Touch [Mode] on the control Menu.
4. Touch the Analysis Mode. Select Whole Blood.
5. Touch OK.
6. Touch the [QC] icon on the Menu screen.
7. Touch the [QC Analysis] icon.
8. From the QC file list, touch the file you want to analyze.
9. Perform Manual analysis on thoroughly mixed vial.
10. Check the analysis results in dialog box. Touch [Accept].

a. If analyzer displays **GREEN**, this indicates QC passed and analyzer is ready to process samples

b. If analyzer displays YELLOW, more information is needed, or QC is overdue. The resolve button becomes active if there is a QC value outside of limits.

c. **RESOLVE** is activated: If a QC error has been detected, resolve button becomes active and dynamic troubleshooting prompt guides the end user to the next course of action. The instructions button gives details on how to perform the troubleshooting action.

d. QC is overdue: End user needs to analyze QC since it exceeds the timeframe from the preferences screen.

e. If analyzer displays **RED**, QC failed analysis and analyzer is determined out of service with a reference to a service call

Source of Error: If the sample is

- Hemolyzed ‘Clotted
- Collected in improper tube
- Not mixed well and Small volume

(Source:-Sysmex XN-550 haematology analyzer user manual)

B. THIN BLOOD FILM BY WRIGHT STAIN

Principle: Wright’s stain is a polychromatic stain consisting of a mixture of **Eosin and Methylene blue**. As the Wright stain is methanol based, it doesn’t require a fixation step prior to staining. However, fixation helps to reduce water artefact that can occur on humid days or with aged stain.

Methanol fixes the cells to the slide. Eosin Y is an acidic anionic dye and methylene blue is basic cationic dye. When diluted in buffered water, ionization occurs. Eosin stains the basic components such as hemoglobin and eosinophilic granules an orange to pink color. Methylene blue stains acidic cellular components such as nucleic acid and basophilic granules in varying shades of blue. The neutral components of the cells are stained by both components of the dye, producing variable colors.

Materials, Reagents &Equipment’s

- Clear microscope slide
- Microscope
- Sterile lancet
- Staining jars (dish)
- Lens paper
- Methyl alcohol
- Immersion oil
- Buffer (ph6.5-6.8)
- Wright stain solution
- Timer
- Drying rack

Sample: One to two drop of whole blood from a capillary puncture or EDTA ant coagulated venous blood.

Evaluation of well-stained thin film:

- The back ground should be clean and free from debris; the color of erythrocytes is a pale green pink.
- Neutrophil leukocytes have deep purple nuclei and well defined granules.

Procedure:

1. Prepare a film of blood or bone marrow on a microscopic slide and allow to air dry.
2. Place the air-dried smear on the slide staining rack, smear side facing upwards.
3. Cover the blood film with undiluted staining solution. The undiluted stain fixes and partially stains the smear.
4. Let stand for 2-3 minutes.
5. Add approximately equal amount of buffered water (pH 6.5). The diluted stain shouldn't overflow. Mix by gentle blowing. A metallic sheen (or green 'scum') should appear on the slide if mixing is appropriate.
6. Leave for 5 minutes.
7. Without disturbing the slide, flood the distilled water and wash until the thinner parts of the film are pinkish red.
8. Allow the slide to dry at room temperature and examine under microscope.

Focusing and scanning the blood film:

- Place the blood film on the microscope stage, switch on the light and adjust the light source optimally by looking through the ocular and the 40x objective.
- Place the drop of immersion oil on the dray stained slide. To avoid cross contamination, ensure that the immersion applicator never touches the slide.
- Slowly change to the oil immersion objective, and a thin film of oil will form between the slide and the lenses.

- Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the 100x objective and use the fine adjustment knob to focus the lens should not be allowed to touch the slide.

- Examine the slide in a systematic fashion. Start at the left end of the film and begin reading at the periphery of the field and finish at the other end. When the field is read, move the slide right to examine adjacent fields.

Quality control

- Follow proper sample collection procedures.

- Glass slides must be clean and free from grease.

- Thin films must be prepared properly while draying protects blood films from dust, flies and insects.

- Do not dry exposed to direct sun light.

- Blood film spread unevenly on a grease slide makes examination difficult.

- Wet slides are warped together and the slides stick to one another.

- Never add a pinch of EDTA powder directly to the sample tubes. High concentration of EDTA leads to shrinking of RBC and destroys the structure of WBC and platelet

- Never add the blood before the EDTA solution is completely dried it will dilute the blood.

- Quality control of wright stain is performed for every batch of the stain prepared and quality control results documented using the quality control form.

- Check pH of buffered water, and add appropriate correcting fluid.

ANNEX FIVE: PATIENTS INFORMATION SHEETS

3.1. Information sheet English version

This information sheet is ready for study subjects who are volunteers to participate in the study. Informed consent is obtained after reading the description that is indicated below.

Title of the study: Hematological profile and associated factors among HIV positive pediatrics in Fitche General Hospital and Kuyyu Hospital, Central Ethiopia

Principal Investigator: Berhanu Asefa

Telephone number: +251922340791

E.mail: berhanuasefa7@gmail.com

Description and Purpose of the study: - The study is aimed at determining the hematological profile has vital significance in reducing the progression of the disease. The findings of the study are central for health planners and caregivers for evidence-based intervention and would serve as baseline information for further studies at a different level. Above all this study offers information for the clinicians to decide whether HIV-positive pediatrics need frequent hematological tests or not. Therefore this study will be conducted to determine the hematological profile in Fitche General Hospital.

General Procedures:-After the confirmation of your willingness you are asked to sign a written consent and the following things will be done.

- You will give as 10 minutes for the interview
- 4 ml of blood will be collected for the study purpose

Voluntary participation: - Involvement in this study is voluntary and you have the right to refuse participation at any time. Your decision will not result in an unfavorable condition or penalty.

Risks and discomforts:- At the time of sample collection standard operational procedures will be followed. The blood draw may cause minor pain, but it is for a short period.

Benefits: - The entire community will be benefited from this study because its success will result in proper clinical decision-making and treatment of patients. So, there is no direct financial benefit that you get by participating in this study but the test result will be issued in a timely for appropriate intervention.

Confidentiality:-Any information obtained during this study will be kept confidential which is assured by recording information using a code number. The result of this study will be released using this code number.

Thank you for your attention!!

3.2. Ibsa Hirmaattota qo’annootif guca guutamu (Afaan Oromoo)

Gucni Kun Kan guutamu warren qo’annaa irratti fedhiin hirmaataniif Kan ooluu fi haallii qo’annaa sirritti erga ibsameefii booda Kan guutamufi Kan mallattaa’udha

Mata-duree qo’annaa:-Qorannon kun kan gaggeeffamu Daa’imman dhibee Eedsii qaban irrattii giddugala Itoopiyaatti dalagama.

Maqaan qorataa:-Birhaanuu Asaffa

Lak.bilbila:- +251922340791

E.mail:- berhanuasefa7@gmail.com

Dhimmi-qu’anicha:-Bu’aan laboratorii warra dhibee Eedsii qaban irraa argamu warra karooraa fayyaa baasanii fi gara fuulduraattii warra akka biyyaattii qorannoo adeemsisaniif akka bu’uuraattii gargaara.

Haala adeemsa qo ’annichaa: - namoonni qo’ aannaa irratti fedhiin hirmaachuu keessan mallattoo keessaniin nuuf mikaneessitan wantoot armaan gadii kanneen nuuf kennitan.

- Daqiiqaa 10 gaaffiilee afaaniitiif
- Dhiiga 4ml ni fudhanna

Fedhaan hirmaachuu- qorannoo irratti hirmaachuun fedhii kee qofa tahuu isaa beektee, yeroo barbaaddeettii qorannoo keessaa bahuu kan dandeessuu fi yeroo keessaa baatulee rakkoo tokkoo kan sirratti hin fidnee fi tajaajila argachuu qanduu hundumaa argachuu kan dandeessu tahuusaa fi qorannoof hirmaachuu keetiif galannii keenya guddaadha.

Sodaa fi miidhaa qabu:-seeraa fi naamusa ogummaa fayyaa hordofuun waan dalagamuuf wanti na sodaachisu hin jiru.Haa tahu malee, dhukkubbiin xixiqqoon yeroo dhiiga fudhatamu namatti dhagahamuu kan yoo xiqqoo turuufi miidhaa osoon hin geessisin kan badu tahu hubadhe.

Faayida qo’anniichaa fi kafaaltii hirmaataaf godhamu- qorannoo irratti hirmaachuuf kafaltii kan hin qabnee fi bu’a qorannoo irraa argamuu fi tajaajila wal ’aansa argachuu ni danda ’u.

Iccitii qo’annichaa-wantootni qorannoo irraa argaman hundinuu icciitiin kan eeggamaniifi ragaaleen argaman hundinuu maqaa keessaniin osoo hin tahiin lakkoofsa addaatiin kan beekkamaniifi odeeffannoon hundinuu iccitiidhan warra ragaa funaanan biratti kan hafu tahuu isaa isiiniif ibsina. **Horaa Bulaa!!**

ANNEX-SIX: CONSENT AND ASSENT FORMS

4.1. Consent forms (English version)

Participant Code Number_____

Participant full name _____

I have been informed fully in the language I understand about the aim of the mentioned study. I understood the purpose of the study titled "*Hematologic profiles among HIV positive pediatrics in Fiche General Hospital, Central Ethiopia*". I have been addressed that, blood samples will be taken from my child and there will be a minimal risk during sample collection. In addition, I have been told all the information collected throughout the research process will be kept confidential. I understood my child current and future medical services will not be affected if I refused to participate or withdraw from the study at any point.

Agree _____ Not agree_____

Therefore, I give my consent freely for my child participation in this study.

Participant's Name _____ signature _____ Date_____

Investigator's name _____ signature _____Date_____

Witness

I have accurately read or witnessed the reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that consent was given freely.

1. Name _____signature _____ date _____

2. Name _____signature _____ date_____

4.2. Assent forms (English version)

Participant Code Number_____

Participant full name _____

I am informed fully in the language I understand about the aim of the mentioned study. I understood the purpose of the study titled "*Hematologic profiles among HIV positive pediatrics in Fiche General Hospital, Central Ethiopia*". I have been addressed that, blood samples will be taken from me and there will be a minimal risk during sample collection. In addition, I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or withdraw from the study at any point.

Agree _____ Not agree_____

Therefore, I give my assent freely for my participation in this study.

Participant's Name _____ signature _____ Date_____

Investigator's name _____ signature _____Date_____

Witness

2. Name _____signature _____ date _____

2. Name _____signature _____ date_____

4.2. የስምምነት ቅጾች (እንግሊዝኛ)

የተሣታፊ ኮድ ቁጥር _____

ተሳታፊ ሙሉ ስም _____

ከላይ ከተጠቀሱት ምርምሮችን ምርምር በተረዳሁበት ቋንቋ ሙሉ በሙሉ ተረድቼአለሁ. / ኤችአይቪ / ከተጋለጡ ግለሰቦች ጋር የተያያዙ ምክንያቶች" የደም ናሙናዎች እና የሱፍ ቁስሉ ከእኔ እንደሚወሰዱ ተነግሮኛል እናም በማጣቀሻ ቅደም ተከተል ወቅት አነስተኛ አደጋ ይኖረዋል. በተጨማሪም በጥናቱ ሂደት ውስጥ የተሰበሰበውን መረጃ በሙሉ በሚስጥር ይያዛል. ለመሳተፍ ወይም ለመጥቀስ ፈቃደኛ ካልሆንኩ የአሁኑ እና የወደፊት የሕክምና አገልግሎቶቼ ምንም አይሆኑም::

ተመስክሮ _____ አልስማማም _____

ስለሆነም, በዚህ ጥናት ውስጥ ለመሳተፍ የእኔን ነፃነት በነፃ ሰጥቼዋለሁ.

የተሳታፊው ስም _____ ፊርማ _____ ቀን _____

መርማሪ ስም _____ ፊርማ _____Date_____

ምሥክር

1. ስም _____ ፊርማ _____ ቀን _____

2. ስም _____ ፊርማ _____ ቀን_____

5.3. UunkaaWalii galtee (Afaan Oromoo)

Lakk.addaa Hirmaattota_____

Maqaan guutuu Hirmaattota_____

Ani hirmaatan maqaan koo armaan olitti ibsame kun bu'aa fi miidhaan isaa erga sirritti natti himame fi miidhaan isaa baay'ee xiqqaa ta'uu isaa ergan hubadhee, booda, samuuda qorannoon laboratoriitiif kan dhiiga akkan kennuu fii Dabalataaniis odeefannoo narraa argaman hunduu icciitiin akka qabaman nattii hiimameera.Akkasumas gaaffileen gaafatamuuf deebii kennuu dhiisuu, hiirmachuu dhiisuu fi yeroon barbaadetti addaan kutuu akkan danda'uu bareen jira. Kana godhuu kiyyaaniis ammas ta'ee fuulduraaf fayyadamummaa tajaajila fayyaa kiyya irratti rakkoon tokkollee akka hin uumamanee huubadheen jira.

Walii galeera_____ ***walii hin gallee_____***

Kanaafuu qorannoo kana irratti fedhiin nan hirmaadha.

Maqaa hirmaataa_____Mallattoo_____Guyyaa_____

Maqaa qoo'ataa_____Mallattoo_____Guyyaa_____

Ragaalee

1. Maqaa_____Mallattoo_____Guyyaa_____
2. Maqaa_____ Mallattoo_____Guyyaa_____

Declaration

I, the undersigned, declare that this thesis proposal entitled," *Hematologic profiles and associated factors among HIV positive pediatrics in Fiche General Hospital and kuyyu Hospital, Central Ethiopia*" is my work and has never been presented in this or any other University and that all source of materials used for the thesis and individual contributed for it have been fully acknowledged

Name of the Principal investigator Signature Date

Berhanu Asefa _____ _____

After I make essential revisions and correction, I assured that this Thesis paper is technically and methodologically legible for submission and submitted with my approval as an advisor:

Approval of the first Advisor

Dr. Tilahun Yemane _____ _____

Approval of the Second Advisor

Wondimagegn Addisu (MSc, Asst. prof) _____ _____

Approval of Internal examiner

Gebeyaw Aragaw (BSc, MSc) _____ _____