# EFFECT OF MALARIA ON HEMATOLOGICAL PROFILES OF PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS ATTENDING GAMBELLA HOSPITAL, SOUTHWEST ETHIOPIA

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# ABSTRACT

***Background:*** *Malaria and human immunodeficiency virus are the two most devastating global health problems of our time, causing more than two million deaths each year. Hematological abnormalities such as anemia, thrombocytopenia and leucopenia are the common complications in malaria and HIV co-infected individuals.*

***Objective:*** *To determine the effect of malaria on hematological profiles of people living with HIV attended Gambella Hospital ART clinic, Southwestern Ethiopia.*

***Methods:*** *A facility based comparative cross-sectional study was conducted from May 25 to November11, 2014 in Gambella Hospital. A total of 172 adult people living with HIV, 86 malaria infected and 86 malaria non-infected participants were included in the study. Demographic and data were collected by using questionnaire, anthropometric data were collected by measuring height and weight and clinical data were collected carefully from the existing ART logbook. Venous blood sample was collected for blood film preparation, complete blood count (CBC) and $CD_4^+$ lymphocyte count. Stool specimen was collected for intestinal parasite examination. The blood films were examined using light microscope for malaria detection. The whole blood sample was analyzed using CELL DYN 1800 for CBC and BD FACS counter for $CD_4^+$ lymphocyte count. The data were analyzed using SPSS, Version-20. All variables with p-value <0.05 were considered as statistically significant.*

***Results:*** *The prevalence of anemia, thrombocytopenia and leucopenia in malaria and HIV co-infected participants were 60.5%, 59.3%, and 43.0%, respectively. A significant difference was observed in prevalence of anemia (P=0.022) and thrombocytopenia (P<0.001) between the two groups. The comparison in mean value of hemoglobin, hematocrit, platelet, lymphocyte and neutrophil count between the two groups were statistically significant (P<0.05). Resident (AOR= 4.67 95% CI: 1.44 - 15.14), malaria infection (AOR=2.42 95% CI: 1.16 - 5.04) and $CD_4^+$ count were predictors for anemia. Predictor for thrombocytopenia was malaria infection (AOR= 9.79 95% CI: 4.33-22.17). Malaria parasitic density (AOR= 0.13 95% CI: 0.03 - 0.57) and $CD_4^+$ count (AOR= 4.77 95% CI: 1.23-18.45) were predictors of leucopenia.*

***Conclusion:*** *Prevalence of anemia and thrombocytopenia were higher in malaria and HIV co-infected study participants than HIV mono-infected study participants. Mean value of hemoglobin, hematocrit, lymphocyte, neutrophils and platelet counts were significantly different in two groups. Malaria infection increases the rate of being anemic and thrombocytopenic. Further longitudinal researches are required by including additional associated factors that elaborate the research.*

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# TABLE OF CONTENTS

CONTENT PAGE

## LIST OF TABLES

## LIST OF FIGURES

## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **AIDS** | Acquired Immunodeficiency Syndrome |
| **ART** | Anti-Retroviral Treatment |
| **BMI** | Body Mass Index |
| **CBC** | Complete Blood Count |
| **$CD_4^+$** | Cluster of Differentiation 4 positive |
| **EDTA** | Ethylene Diamine Tetra Acetic Acid |
| **FACS** | Fluorescent Activated Cell Sorter |
| **HAART** | Highly Active Antiretroviral Therapy |
| **HCT** | Hematocrit |
| **Hgb** | Hemoglobin |
| **HIV** | Human Immunodeficiency Virus |
| **MCH** | Mean Cell Hemoglobin |
| **MCHC** | Mean Corpuscular Hemoglobin Concentration |
| **MCV** | Mean Cell Volume |
| **PLWHA** | People Living With HIV/AIDS |
| **RBC** | Red Blood Cell |
| **WBC** | White Blood Cell |
| **WHO** | World Health Organization |

# CHAPTER ONE: INTRODUCTION

## 1.1 Background

Hematological profiles are those parameters of the blood forms (red blood cells, white blood cells and platelets) with normal range or values as reference for any value to know whether they are normal or not (1). Hematologic abnormalities indicates the derangement of full blood count that can cause potentially life-threatening complications (2).

Anemia, as one of the hematological abnormalities is defined as a decrease in the oxygen-carrying capacity of the blood. All oxygen-carrying capacity of the blood is due to the binding of oxygen to hemoglobin (Hgb) contained uniquely in red blood cells (RBCs). For practical purposes, any of the three concentration measurements are used to establish the presence of anemia: Hgb level (g/l), hematocrit (v/v), RBC number ($10^{12}$/L), but the best indicator of anemia is red cell mass, low red cell mass or red cell mass below the limit of normal reference range suggest the presence of anemia (3, 4). Other hematological cell is white blood cell (WBC) that contains several types of commonly circulating nucleated cells, including granulocytes (mature neutrophils, basophils, and eosinophils), lymphocytes, and monocytes. An increase to the normal physiologic level in the WBC count, termed as leukocytosis. Likewise, a decrease in the WBC count, termed leucopenia (5) and the other one is platelets which play a crucial role in hemostasis (6). Quantitative defects of platelets, either too many (thrombocytosis) or too few (thrombocytopenia), can lead to the clinical complications of thrombosis or bleeding, respectively and there is also qualitative defect in platelet that cause functional abnormality (7).

Hematological abnormalities are the common complications in malaria and they play a major role in malaria pathophysiology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes and the abnormalities such as anemia, thrombocytopenia and leukocytosis or leucopenia (8). In malaria endemic regions, chronic anemia due to nutritional deficiencies, intestinal helminthes, HIV and hemoglobinopathies may be confounded by the effects of malaria (9). Pathogenesis of anemia in malaria patients include one or more of the following mechanisms: the clearance and/or destruction of infected

RBCs, the clearance of uninfected RBCs, and erythropoietic suppression and dyserythropoiesis these are the common cause of anemia in malaria patients (10). Mild or moderate thrombocytopenia is often seen in acute malaria. The platelet lifespan is reduced during malaria infection which is mainly due to increased sequestration in the presence of palpable splenomegaly and circulating immune complexes. It has been found that malaria patients have elevated levels of platelet bound immunoglobulin G (IgG), leading to increased peripheral destruction (11). WBC counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leucocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion (12).

On the other hand, hematological abnormalities such as anemia (13), neutropenia (14), and thrombocytopenia (15) are commonly reported abnormalities associated with HIV infection. It is also a potential means by which HIV affects disease course and outcome in other infections, such as malaria (16).

Through immunosuppression HIV infection, affects the acquisition and persistence of immune response to malaria (17) and malaria infection also has an impact on HIV disease progression (18), this is associated with strong $CD_4^+$ cell activation and up-regulation of pro-inflammatory cytokines, providing an ideal microenvironment for the spread of the virus among $CD_4^+$ cells and thus for rapid HIV RNA replication (19). Availability of insecticide-treated bed-nets (ITNs), the use of cotrimoxazole (CTX) prophylaxis and antiretroviral therapy provide a protective effect from malaria in HIV infected population (20, 21).

## 1.2 Statement of the problem

Malaria and HIV are the two most devastating global health problems of our time, causing more than two million deaths each year (22, 23) and greatest medical challenges facing Africa today (24). Both malaria and HIV are diseases of poverty and contribute to poverty by affecting young people who are the work force and contribute to the local economy (16).

An interaction between HIV infection and malaria could work in either direction. HIV infection might reduce immunity to malaria; conversely malaria might enhance the progression of HIV infection to clinical AIDS (25). Studies on the effect of malaria in hematological profiles of HIV infected persons noticed a significantly lower in blood parameters such as hemoglobin concentrations, packed cell volume, platelet count, differential lymphocyte counts and $CD_4^+$ counts in malaria co-infected patients compared to patients with single infection (26 – 28).

Both malaria and HIV can cause hematological abnormality independently, those abnormalities anemia, thrombocytopenia and leucopenia have been documented as strong independent predictors of morbidity and mortality in HIV infected individuals having malaria when compared with those malaria non infected HIV positive individuals. Beside this HIV infection is associated with a twofold higher risk of severe malaria in adults, and a six to eightfold increase in the risk of death (29).

According to WHO report of 2014, 198 million cases of malaria occurred globally and the disease led to 584,000 deaths. The disease burden is high in the African Region, where an estimated 90% of all malaria deaths occur in this region and in children aged under 5 years, who account for 78% of all death (30).

Malaria is one of the leading causes of morbidity and mortality in Ethiopia. Approximately 57.3 million (68%) of population live in malaria risk areas. On average, 59.2% of malaria cases have been due to *P. falciparum*, with the remainder caused by *P. vivax* (31).

Hematological abnormalities are considered as a hallmark for malaria, and reported to be most pronounced in *P. falciparum* infection, probably as a result of higher levels of parasite number found in these patients. There are common hematological change that occur highly

incidence in malaria infected patients had anemia (94%), thrombocytopenia (70%), monocytosis (17%) and lymphopoenia (12%) (32).

At the end of 2014 Joint United Nations Program on HIV/AIDS (UNAIDS) reported that there were on average 34 million people living with HIV, 2.1 million people newly infected per year and 1.5 million people dying in AIDS related cases. HIV infection is the leading cause of mortality among adults aged 15 – 59 in the world, about 24.7 million people living with HIV are found in sub-Saharan region and prevalence of HIV in Ethiopia adults in 2013 reach 1.2 % (33).

Immunological complications are common in HIV infection, besides these hematological abnormalities have been documented as strong independent predictors of morbidity and mortality in HIV-infected individuals (34). Although from hematological abnormalities cytopenias are the most frequent one but rare in the early stages of HIV infection (35). Occurrence of anemia and leucopenia correlates with disease progression of HIV infection as measured by $CD_4^+$ count (36). Anemia is a frequent complication that occurs in range 70-78% of HIV infected persons (37 – 39), neutropenia (neutrophil count $\leq 1.5 \times 10^9$/L) is common in the advanced stages of AIDS and it may occur in 10 - 31% of HIV patients (14, 40, 41) and thrombocytopenia is found in 4 - 17 % of individuals with HIV infection (40, 42, 43). These hematological profiles could positively or negatively affect by antiretroviral drugs depending on the choice of combination used (44).

Although malaria and HIV are known to be the most common public health problems in Ethiopia (45), yet limited study has been done which evaluates the extent of hematological changes in malaria and HIV co-infections particularly in this study area. Therefore, this study was intended to determine the impact of malaria on the hematological profiles in people living with HIV by comparing hematological profiles of malaria infected and non-infected adults living with HIV who attend Gambella Hospital ART Clinic.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Hematological abnormalities in HIV and Malaria co-infection

HIV and malaria are the most distressing scenario in developing countries, especially in Africa in terms of number of deaths and morbidity (46). In addition, malaria continues to be a major health problem in some of the most populated areas of the world and one of the important causes of febrile illnesses (47). Hematological changes play a major role in malaria pathophysiology. This change involve the major cell lines such as red blood cells, leucocytes and thrombocytes (8).

A study conducted in India at 2012 indicated that from 460 HIV infected participants 45 (9.8%) of them had malaria infected and majority of the infections were due to *P. vivax* 27 (60%) followed by *P. falciparum* 12 (27%) and mixed infections six (13%) (48). In other way study of Nigeria showed that from 30 malaria and HIV co-infected participants, 28 (93.3%) were positive for *P. falciparum* and two (6.7%) were positive for *P. vivax* (27). Similarly study of Gondar indicates that out of 377 HIV infected adult participants who had acute febrile illness, 73 (19.4%) had confirmed malaria cases. From these 53 (72.6%) were infected with *P. falciparum* and 20 (27.4%) were infected with *P. vivax* (26).

Both HIV and malaria can cause hematological abnormality independently; those abnormalities such as anemia, thrombocytopenia and leucopenia were frequent one. A case control study conducted in Nigeria at 2006 shows that occurrence of anemia, thrombocytopenia, neutropenia and leucopenia was significantly higher in malaria parasitized HIV infected subjects compared to non-parasitized controls. The incidence of anemia was two times higher in parasitized subjects compared to non-parasitized controls (66.7% versus 32.9%). The incidence of thrombocytopenia is significantly higher (60%) in malaria parasitic subject than non-parasitic subject (42.9%). Moreover, this study compared the mean values of hematological parameters in the groups and showed a statistical significant difference in mean value of hemoglobin, platelet and erythrocyte sedimentation rate (ESR) of malaria parasitized and non-parasitized controls (27).

A comparative study conducted in Nigeria at 2013 reported that there were statistically significant difference in the mean values of RBC, Hgb, HCT, MCHC and MID among malaria infected and malaria non-infected PLWHA. While the differences in the mean values of WBC, MCV, MCH, Platelet, lymphocyte and neutrophil count among the groups was found to be statistically insignificant (28).

A study conducted in Ghana at 2012 describe that from 34 (15.5%) HIV and malaria co-infected participants only one (2.9%) had normal Hgb, whereas 79 (42.5%) of the HIV mono-infected participants had normal Hgb. None of the HIV mono-infected participants had severe anemia compared with six (18.2%) severely anemic HIV and malaria co-infected participants (49). Similarly, study of Benin showed that anemia was significantly associated with asymptomatic malaria infection among HIV infected patients (50).

Another study conducted in Cameroon at 2012 indicated that the respective mean (±SD) of hematological parameters in malaria infected and non-infected people living with HIV were compared and the difference was less than 10%. Red blood cell count and platelet count were very similar between malaria infected and non-infected participants. The hemoglobin level, white blood cell count, lymphocyte and $CD_4^+$ count were slightly lower in malaria and HIV co-infected participants although the difference did not achieve statistical significance (51).

In addition study of Gondar conducted in 2013, also showed that occurrence of anemia was higher in HIV and malaria co-infected participants than HIV mono-infected participants. Those who had hemoglobin level of < 8 g/dl were 8.3 times more likely to be co-infected than individuals who had hemoglobin level of >12 g/dl. The mean hemoglobin value of HIV mono-infected participants was 12.7±2 g/dl and malaria and HIV co-infected participants had mean hemoglobin value of 11.8± 2.2 g/dl this difference was statistically significant (26).

## 2.2 Hematological abnormalities in people living with Human immunodeficiency virus (PLWHA)

Acquired immunodeficiency syndrome (AIDS) is a systemic disorder caused by HIV, and characterized by severe impairment and progressive damage of both cellular and humoral immune responses (52). In addition to this hematological abnormalities are common complications of HIV infection (53).

A study done in India in 2009, also reported that anemia as the most common presentation of HIV infection, among 200 HIV infected participant 131 (65.5%) were found to be anemic, thrombocytopenia was seen in 7% (14/200) cases and there was no patient having absolute neutrophil count < 0.8 $x10^9$/l (53). Likewise a cross-sectional study conducted in Rwandan in 2012 showed that anemia was the most frequent hematological abnormality than neutropenia and thrombocytopenia in HIV-infected Rwandan women (54). These two studies showed that anemia in HIV patients can be a good clinical indicator to predict and access the underlying immune status.

Another study conducted in India in 2012 to assess hematological changes in people living with HIV and correlate them with $CD_4^+$ counts of three groups ($CD_4^+ \geq 500/\mu l$, 200 to 499/µl and < 200/µl); showed that occurrence of anemia, lymphopenia and leucopenia increase as $CD_4^+$ count decreases and those parameter showed significant difference between three groups with differing $CD_4^+$ counts. Though there was a difference in mean platelet count between these three groups, it was not statistically significant. This indicates occurrence of thrombocytopenia is independent of disease progression (36).

A cross sectional study conducted in Brazil in 2011 reported that there was association between $CD_4^+$ count with hemoglobin level, neutrophil count, and platelet count. A total of 701 HIV infected out patients involved in the study among these 37.5% had anemia: with 61.1% in the low $CD_4^+$ (< 200/µl) and 29.4% in the high $CD_4^+$ (> 200/µl). The study also reported that the mean neutrophil count was 2.610 $x10^9$/l and 3.204 $x10^9$/l in low $CD_4^+$ and high $CD_4^+$ count, respectively. Mean platelet counts were 218.639 $x10^9$/l and 234.807 $x10^9$/l for the low $CD_4^+$ and the high $CD_4^+$, respectively (55).

Study of Uganda in 2014 showed that anemia was associated with female sex and decreasing BMI. Being female had increased the probability of anemia by 2.24 times. The prevalence ratio of having anemia decreased with increasing BMI. Thrombocytopenia was associated with sex and HAART status. The only factor associated with the presence of leucopenia was $CD_4^+$ count (56).

Other cross-sectional study conducted in Ghana in 2011 indicated that the incidence of anemia and lymphopenia in HAART naïve participants were significantly higher compared to participants on HAART. Although the relative risk of developing thrombocytopenia in all the study participant is the same, the mean platelet counts of participant on HAART was significantly lower compared to their HAART naïve (57). Similarly study conducted in Gondar University Hospital, Ethiopia in 2014 indicated that prevalence of anemia was high in HAART naïve participants while leucopenia and neutropenia prevalence were higher in participants on HAART and their prevalence increased as the $CD_4^+$ count decreased (44).

## 2.3 Significance of the study

HIV and malaria are among the two most important global health problems, in developing countries especially in Africa. This study will provide information about the change in hematological profiles due to malaria and HIV co-infection which may contribute in improving the management of patient. On the other way, this study also provides information for the clinicians whether HIV positive patients need frequent malaria test and anti-malarial drug therapy. In addition, it can be used as a base line data for those who are interested in the area for further research.

# CHAPTER THREE: OBJECTIVES

## 3.1 General objective

- To determine the effect of malaria on hematological profiles of people living with HIV attended Gambella Hospital ART Clinic.

## 3.2 Specific objectives

- To determine hematological profiles of HIV and malaria co-infected patients
- To compare the mean hematological profiles of malaria infected and malaria non-infected people living with HIV
- To determine the factors associated with abnormal hematological profile of people living with HIV

# CHAPTER FOUR: METHODS AND MATERIALS

## 4.1 Study area and period

The study was conducted in Gambella Hospital from May 25 to November 11, 2014. Gambella Hospital is located in Gambella Town which is located 777 Km Southwest of Addis Ababa, Ethiopia. The town is characterized by hot and humid climate. Based on the 2007 Ethiopian national and housing census, the population in the town is projected to be about 38,994 with male 20,766 and female 18,228 (58). The city has a latitude and longitude of 8°15′N 34°35′E and an elevation of 526 meters above sea level. Gambella Hospital is the only hospital in the region and gives service for nearly 200,000 people. A total of 4,466 individuals were registered in Gambella Hospital ART Clinic among these 1,203 started HAART.

## 4.2 Study design

A facility based comparative cross sectional study was used.

## 4.3 Populations

### 4.3.1 Source population

All HIV positive individuals who attended Gambella Hospital ART Clinic

### 4.3.2 Study population

HIV positive adults attending Gambella Hospital ART Clinic for routine follow up and/or treatment during the study period were considered as study population.

## 4.4 Eligibility criteria

### 4.4.1 Inclusion criteria

- HIV positive adults willing to participate in the study

### 4.4.2 Exclusion criteria

- Pregnant women
- Person on any anti-malarial treatment during the study period.

## 4.5 Sample size

A total of 172 HIV positive participants were included in the study from this 86 were malaria positive and 86 were malaria negative individuals. The sample size was calculated by using statistical formula for comparison of two populations mean (59).

- Figures use for calculation the mean value and standard deviation of Hgb that obtained from previously conducted study in Gondar University Hospital, Ethiopia (26).

$$n = \frac{(u+v)^2 \times (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

$$= \frac{(0.84+1.96)^2 \times ((2.2)^2 + (2.0)^2)}{(11.8 - 12.7)^2}$$

$$= 86$$

n = 86 HIV positive participants in each group

Total sample size became 172 HIV positive participants

Where, $\mu_1$- mean of the first category (11.8)

$\mu_2$- mean of the second category (12.7)

$\delta_1$- standard deviation of first category (2.2)

$\delta_2$- standard deviation of second category (2.0)

u - Power (1-β), i.e.; 80%. = 0.84

v -Value of standard normal deviate at level of significance 1.96 in 95% CI, α=0.05

## 4.6 Sampling technique

All study participants who came to the ART Clinic during the study period were included consecutively.

## 4.7 Study variables

### 4.7.1 Dependent variables

- Hematological abnormalities

### 4.7.2 Independent variables

- Sex
- Age
- Marital status
- Resident
- Monthly income
- BMI
- Malaria infection
- $CD_4^+$ count
- Status of antiretroviral therapy
- Current opportunistic infection
- Intestinal parasites
- Other clinical illness
- Drugs other than HAART

## 4.8 Data collection procedures

### 4.8.1 Questionnaire

The participant socio-demographic and anthropometric data were collected using structured questionnaire and clinical data of each participant were collected carefully from the existing ART logbook by the clinicians who work in ART clinic.

### 4.8.2 Sample collection and laboratory method

Four (4) ml of venous blood sample was collected using EDTA containing Vacutainer tube from each participant for laboratory investigation. For the detection of malaria both thick and thin blood films were prepared, stained with 10% Giemsa solution and examined with light microscope (Annex-III). The malaria parasitic density was calculated by counting the number of parasites in thick blood film against 200 or 500 WBCs. If after counting 200 WBCs, 100 or more parasites are found; record the results in terms of the number of parasites per 200 WBCs but less than 100 parasites are found after counting 200 WBCs, parasite quantification should be continued until 500 WBCs are counted and multiply with the actual total WBC (Annex-III). The number of parasites per µl of blood were calculates as follows (60):

$$\text{Parasites/µL} = \frac{\text{Parasites counted}}{\text{WBCs counted}} \times \text{WBCs / µL}$$

The remaining blood sample was used for hematological analysis (CBC) and for $CD_4^+$ lymphocyte count. Hematological parameters: red blood cell count (RBC), hemoglobin (Hgb), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), total white blood cell count (WBC), differential neutrophil (NEUT), MID (Eos, Bas and Mon), differential lymphocyte count (LYM) and platelet count (PLT), were determined using the automated blood cell analyzer CELL DYNE 1800® (Abbott Laboratories Diagnostics Division, USA) (Annex-I). $CD_4^+$ lymphocyte count was assayed using the BD FACS® COUNT (Becton Dickenson California, USA) (Annex-II). The reference used to classify hematological abnormalities such as anemia,

thrombocytopenia and leucopenia: Where, Hgb <12 g/dl for female and Hgb <13g/dl for male (61), platelet count < 150 $x10^9$/l and WBC count < 4.0 $x10^9$/l, respectively (62). Stool specimen was collected and examined directly using both wet mount smear preparations and formol-ether concentration technique for the intestinal parasite examination (Annex-IV).

### 4.9 Quality assurance

To get reliable data training was given for clinicians on data collection procedures, on issues related to the way of respondents approach, how to fill questionnaires, and how to record data. To assure the quality of the data generated by laboratory test the senior laboratory technologist strictly follow standard operating procedure during pre-analytical, analytical and post-analytical phase of laboratory procedures. Reagents used were checked for their expiry date and prepared according to the manufacturer's instruction. Quality control procedures were performed daily according to the laboratory's protocol and controls materials were used for hematology analyzer and for FACS count run control beads with whole blood specimen from healthy donor run daily. Malaria slides were checked by two experienced laboratory technologist.

### 4.10 Data analysis and interpretation

The data were checked for cleanness and completeness before analysis. Statistical analysis was performed using statistical package for social sciences (SPSS) version 20 (IBM corporation Chicago, USA) software for windows. The descriptive statistics was used to see the distribution of the socio-demographic, anthropometric and clinical characteristics of the participants. For the continuous variable mean, standard deviation and 95% confidence interval were determined in each groups, Student's t - test was used to compare the mean value of hematological parameters between malaria infected and non-infected participants. To see the relationships between the groups Chi square ($X^2$) was used. Multivariable logistic regression was used to test the degree of association between dependent and independent variables. The variables with p-value $\leq 0.25$ in bivariable analysis were nominated for multivariable analysis. All variables with p-value $< 0.05$ were considered as statistically significant.

## 4.11 Ethical consideration

Ethical clearance was obtained from Jimma University, Ethical Review Committee. Letter of permission to conduct the study was obtained from Gambella Regional Health Bureau and Gambella Hospital Management Committee. Written informed consent was obtained from each study participants after clearly describing the purpose, benefits and risks of the study. Any information concerning the participants was kept confidential and the specimens collected from the participants were only analyzed for the intended purposes. The result of participant with hematological abnormalities and malaria positive were given the treatment and medical consultation by the physicians, as early as possible.

## 4.12 Dissemination of the result

The result will be given to Gambella Regional Health Bureau and Gambella Hospital administration. It will be also being presented to Jimma university scientific community in thesis defense. The research finding will be presented in different meeting and conferences. Beside this great efforts will be made for publication

## 4.13 Operational Definitions

**Adult**: Individuals age between 18 - 50 years.

**Hematological profiles**: Includes red blood cell count, hemoglobin, hematocrit, red cell indices, white blood cell count, differential lymphocyte count, differential neutrophil count and platelet count

**Anemia:** Hemoglobin values of less than 12 g/dl for women and less than 13g/dl for men.

**Thrombocytopenia:** Platelet count less than 150 x $10^9$/l.

**Leucopenia:** White blood cell less than count 4 x$10^9$/l.

# CHAPTER FIVE: RESULT

## 5.1 General characteristics of study participants

A total of 172 people living with HIV, categorized in to two groups; 86 malaria infected and 86 malaria non-infected participants were involved in this study, the age ranged from 18-50 years. The mean age of the study participants was 31.95 ± 7.59 years. From the total participants, 104 (60.5%) were females (Table 1).

Table 1. Socio-demographic and socio-economic characteristics of PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Variable | | Malaria infected n (%) | Malaria non-infected n (%) | Total n (%) |
|---|---|---|---|---|
| Age | 18-29 | 34 (56.7) | 60 (34.9) | 26 (43.3) |
| | 30-39 | 42 (50.6) | 83 (48.2) | 41 (49.4) |
| | 40-49 | 8 (34.8) | 23 (13.4) | 15 (65.2) |
| | ≥50 | 2 (33.3) | 6 (3.5) | 4 (66.7) |
| Sex | Female | 59 (56.7) | 104 (60.5) | 45 (43.3) |
| | Male | 27 (39.7) | 68 (39.5) | 41 (60.3) |
| Residence | Urban | 80 (52.3) | 153 (89.0) | 73 (47.7) |
| | Rural | 6 (31.6) | 19 (11.0) | 13 (68.4) |
| Marital status | Single | 24 (57.1) | 42 (24.4) | 18 (42.9) |
| | Married | 51 (47.7) | 107 (62.3) | 56 (52.3) |
| | Divorced | 5 (55.6) | 9 (5.2) | 4 (44.4) |
| | Widowed | 6 (42.9) | 14 (8.1) | 8 (57.1) |
| Income in birr | ≤ 500 | 46 (63.9) | 72 (41.8) | 26 (36.1) |
| | 500-3000 | 36 (38.7) | 93 (54.1) | 57 (61.3) |
| | ≥ 3000 | 4 (57.1) | 7 (4.1) | 3 (42.9) |

Among total study participants, 118 (68.6%) had normal BMI. About 142 (82.6%) were on HAART and 30 (17.4%) were HAART naïve. On the other hand from the total study participants 30 (17.4%) were taking drug other than HAART (Table 2).

Table 2. Anthropometric and clinical characteristics of PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Variables | | Malaria infected n (%) | Malaria non-infected n (%) | Total n (%) |
|---|---|---|---|---|
| BMI | < 18.5 | 19 (43.2) | 44 (25.6) | 25 (56.8) |
| | 18.5-24.99 | 63 (53.4) | 118 (68.6) | 55 (46.6) |
| | > 25 | 4 (40.0) | 10 (5.8) | 6 (60.0) |
| $CD_4^+$ (cell/µl) | ≤ 200 | 18 (69.2) | 26 (15.1) | 8 (30.8) |
| | 200-499 | 40 (47.6) | 84 (48.8) | 44 (52.4) |
| | ≥ 500 | 28 (45.2) | 62 (36.1) | 34 (54.8) |
| HAART status | Yes | 62 (43.7) | 142 (82.6) | 80 (56.3) |
| | No | 24 (80.0) | 30 (17.4) | 6 (20.0) |
| OI | Yes | 9 (56.2) | 16 (9.3) | 7 (43.8) |
| | No | 77 (49.4) | 156 (90.7) | 79 (50.6) |
| Other drug | Yes | 24 (80.0) | 30 (17.4) | 6 (20.0) |
| | No | 62 (43.7) | 142 (82.6) | 80 (56.3) |
| Chronic illness | Yes | 8 (66.7) | 12 (7.0) | 4 (33.3) |
| | No | 78 (48.8) | 160 (93.0) | 82 (51.2) |
| IP | Yes | 11 (84.6) | 13 (7.6) | 2 (15.4) |
| | No | 75 (47.2) | 159 (92.4) | 84 (52.8) |

*BMI-Body mass index, $CD_4^+$-cluster of differentiation 4, HAART-Highly active antiretroviral therapy, other drug (cotrimoxazole, INH, pyridoxine & fluconazole), chronic illness (Epilepsy, hemorrhoid and asthma), IP-Intestinal parasite and OI-Opportunistic infection

Among malaria and HIV infected participants 81 (94.2%), 3 (3.5%) and 2 (2.3%) of study participants were infected with *P. falciparum, P. vivax* and mixed infection, respectively. The malaria parasitic density ranges from 110 - 179,705 with a median of 4134 parasites/µl.

Among the total study participants, 16 (9.3%) had opportunistic infections with the slightly higher 9 (10.5%) proportion on malaria infected participants than malaria non-infected 7 (8.1%) participants. From these; four participants had tuberculosis, three participants had Pneumocystis pneumonia and other three participants had herpes zoster virus while the remaining six study participants had oesophageal candidiasis, tinea capitis, cryptococcal meningitis each accounts for two participants. Majority of the study participants 66 (46.5%) were taking TDF/3TC/EFV HAART regimen followed by ZDV/3TC/NVP regimen 32 (22.5%).

Stool specimen was examined and intestinal parasites were detected in 13 (7.6%) of participants (Table 2). The most frequent intestinal parasite was *Giardia lamblia* ten (76.9%) followed by *Entamoeba histolytica/dispar* one (7.7%), Hook worms one (7.7%) and *Ascaris lumbricoides* one (7.7%).

## 5.2 Hematological profiles of HIV and malaria co-infected participants

Out of the total number of study participants, 89 (51.7%) had anemia, 61 (35.5%) had thrombocytopenia, and 68 (39.5 %) had leucopenia. Among malaria and HIV co-infected study participants 52 (60.5%), 51 (59.3%), and 37 (43.0%) had anemia, thrombocytopenia and leucopenia, respectively. From malaria non-infected study participants 37 (43.0%), 10 (11.6%) and 31 (36.0%) had anemia, thrombocytopenia leucopenia, respectively. A statistically significant difference was observed in prevalence of anemia ($P = 0.022$) and thrombocytopenia ($P < 0.001$) between the two groups (Figure 1).

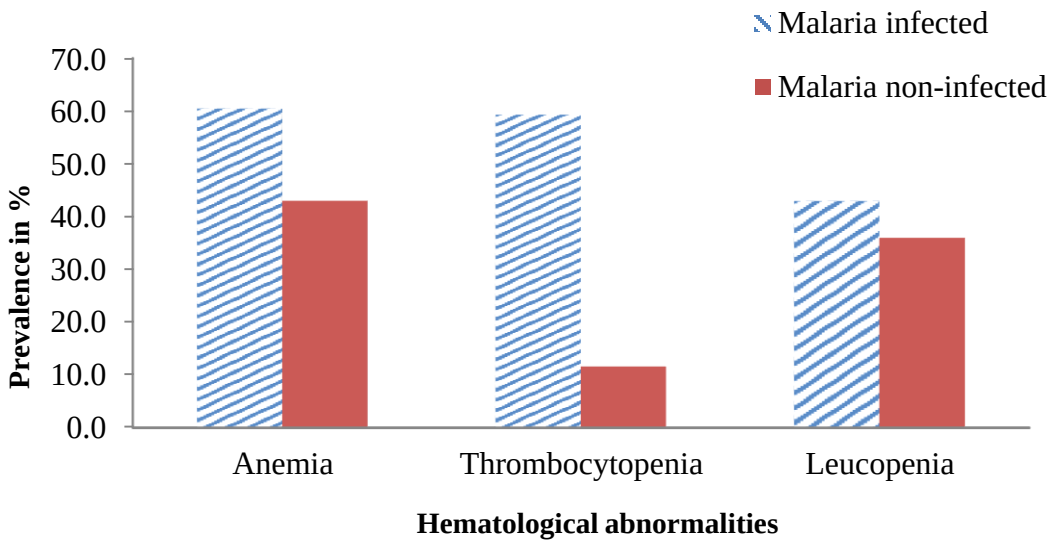


Figure 1. Prevalence of hematological abnormalities in PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

The mean hematological profiles of malaria infected and malaria non-infected PLWHA were compared and there was a difference in all mean hematological profiles of the group. There was a significant difference with respect to Hgb, HCT, lymphocyte, neutrophil, and platelet values. However, no significant difference observed in values of RBC, MCV, MCH, MCHC, WBC, MID and $CD_4^+$ count (Table 3).

Table 3. Comparison of hematological profiles between malaria infected and malaria non-infected PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Hematological Profiles | Malaria infected Mean ±SD | Malaria non- infected Mean ±SD | t- value | P-value |
|---|---|---|---|---|
| RBC ($\times 10^{12}$/L) | 3.88±0.69 | 4.08±0.66 | -1.914 | 0.057 |
| Hgb (g/dL) | 11.77±1.80 | 12.69±2.03 | -3.120 | 0.002 |
| HCT (%) | 34.60±5.44 | 37.23±5.59 | -3.116 | 0.002 |
| MCV (FL) | 88.99±10.86 | 91.10±12.33 | -1.190 | 0.236 |
| MCH (pg) | 30.53±3.38 | 31.36±4.12 | -1.443 | 0.151 |
| MCHC (g/dL) | 34.08±1.38 | 33.74±3.08 | 0.921 | 0.359 |
| WBC ($\times 10^{9}$/L) | 4.66±2.19 | 5.09±3.57 | -0.944 | 0.346 |
| LYM (%) | 35.76±14.24 | 48.17±12.95 | -5.974 | <0.0001 |
| MID (%) | 14.42±7.81 | 13.89±22.80 | 0.204 | 0.838 |
| NEUT (%) | 49.11±17.41 | 40.34±12.03 | 3.842 | <0.0001 |
| PLT ($\times 10^{9}$/L) | 148.72±77.91 | 236.88±75.02 | -7.559 | <0.0001 |
| $CD_4^+$ (cell/µl) | 434.36±292.41 | 460.63±230.24 | -0.655 | 0.513 |

*SD- Standard deviation , RBC-Red blood cell, HCT-Hematocrit, Hgb-Hemoglobin, MCV-Mean cell volume, MCH-Mean cell hemoglobin , MCHC-Mean cell hemoglobin concentration, WBC-White blood cell, LYM-Lymphocyte , MID - Average of basophils, eosinophil and monocyte , NEUT-Neutrophil, PLT- Platelets and $CD_4^+$-cluster of differentiation

## 5.3 Factors associated with abnormal hematological profiles in PLWHA

Bivariate and multivariate analyses were performed to identify factors associated with abnormal hematological profiles in PLWHA. Accordingly, participants who live in rural area were 4.67 times more likely to be anemic than those who live in urban area. Those malaria infected study participants were two times more likely to be anemic than malaria non-infected study participants (AOR= 2.42 95% CI: 1.16 - 5.04) and $CD_4^+$ count in range 200 - 499 cells/µl also predictor for anemia (AOR= 0.37 95% CI: 0.17 - 0.77) (Table 4). Study participant who were malaria infected had ten times more likely to be thrombocytopenic than malaria none infected study participants (AOR= 9.79 95% CI: 4.33 - 22.17) (Table 5). Malaria parasitic density ≥10,000 parasites/µl (AOR= 0.13 (95% CI: 0.03 - 0.57) and $CD_4^+$ count ≤ 200 cells/µl (AOR= 4.77 95% CI: 1.23 - 18.45) were predictors of leucopenia (Table 6).

Table 4. Predictors of anemia in PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Variables | Anemia | | COR (95% CI ) | P-value | AOR (95% CI) | P-value |
|---|---|---|---|---|---|---|
| | Yes n (%) | No n (%) | | | | |
| Age | | | | | | |
| 18-29 | 35 (58.3) | 25 (41.7) | 1 | | 1 | |
| 30-39 | 39 (47.0) | 44 (53.0) | 0.63 (0.32 - 1.24) | 0.181 | 0.58 (0.27 - 1.23) | 0.157 |
| 40-49 | 11 (47.8) | 12 (52.2) | 0.65 (0.25 - 1.72) | 0.390 | 0.73 (0.25 - 2.12) | 0.563 |
| ≥50 | 4 (66.7) | 2 (33.3) | 1.43 (0.24 - 8.41) | 0.693 | 1.79 (0.23 - 13.68) | 0.571 |
| Sex | | | | | | |
| F | 55 (52.9) | 49 (47.1) | 1.12 (0.61 - 2.07) | 0.711 | | |
| M | 34 (50.0) | 34 (50.0) | 1 | | | |
| Resident | | | | | | |
| Urban | 75 (49.0) | 78 (51.0) | 1 | | 1 | |
| Rural | 14 (73.7) | 5 (26.3) | 2.91 (1.00 - 8.48) | 0.050 | 4.67 (1.44 - 15.14) | 0.010 |
| Income in birr | | | | | | |
| ≤ 500 | 39 (54.2) | 33 (45.8) | 2.95 (0.54 - 16.23) | 0.213 | 3.24 (0.48 - 21.64) | 0.225 |
| 500-3000 | 48 (51.6) | 45 (48.4) | 2.66 (0.49 - 14.44) | 0.255 | 4.24 (0.62 - 28.63) | 0.138 |
| ≥ 3000 | 2 (28.6) | 5 (71.4) | 1 | | 1 | |
| BMI | | | | | | |
| < 18.5 | 23 (52.3) | 21 (47.7) | 0.73 (0.18 - 2.95) | 0.659 | | |
| 18.5-24.99 | 60 (50.8) | 58 (69.2) | 0.69 (0.18 - 2.57) | 0.580 | | |
| > 25 | 6 (60.0) | 4 (40.0) | 1 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| HAART status | | | | | | |
| Yes | 70 (49.3) | 72 (50.7) | 1 | | 1 | |
| No | 19 (63.3) | 11 (36.7) | 1.77 (0.79 - 4.00) | 0.165 | 1.87 (0.71 - 4.91) | 0.203 |
| Opportunistic infection | | | | | | |
| Yes | 7 (43.8) | 9 (56.2) | 0.70 (0.25 - 1.98) | 0.503 | | |
| No | 82 (52.6) | 74 (47.4) | 1 | | | |
| Drugs other than HAART | | | | | | |
| Yes | 16 (53.3) | 14 (46.7) | 1 | | | |
| No | 73 (51.4) | 69 (48.6) | 0.93 (0.42 - 2.04) | 0.848 | | |
| Chronic illness | | | | | | |
| Yes | 4 (33.3) | 8 (66.7) | 0.44 (0.13 - 1.52) | 0.196 | 0.28 (0.07 - 1.12) | 0.072 |
| No | 85 (53.1) | 75 (46.9) | 1 | | 1 | |
| Intestinal parasite | | | | | | |
| Yes | 8 (61.5) | 5 (38.5) | 1.54 (0.48 - 4.91) | 0.465 | | |
| No | 81 (50.9) | 78 (49.1) | 1 | | | |
| Malaria infection | | | | | | |
| Yes | 52 (60.5) | 34 (39.5) | 2.02 (1.10 - 3.72) | 0.023 | 2.42 (1.16 - 5.04) | 0.018 |
| No | 37 (43.0) | 49 (57.0) | 1 | | 1 | |
| Parasitic density of malaria | | | | | | |
| 1-999 | 12 (57.1) | 9 (42.9) | 1 | | | |
| 1000-9999 | 26 (60.5) | 17 (39.5) | 0.76 (0.22 - 2.59) | 0.664 | | |
| ≥ 10,000 | 14 (63.6) | 8 (36.4) | 0.87 (0.30 - 2.53) | 0.804 | | |
| $CD_4^+$ count (cells/µl) | | | | | | |
| ≤ 200 | 18 (69.2) | 8 (30.8) | 1.62 (0.61 - 4.30) | 0.328 | 1.34 (0.45 - 3.94) | 0.596 |
| 200-499 | 35 (41.7) | 49 (58.3) | 0.52 (0.26 - 1.00) | 0.051 | 0.37 (0.17 - 0.77) | 0.009 |
| ≥ 500 | 36 (58.1) | 26 (41.9) | 1 | | 1 | |

*COR-Crud odd ratio, AOR-Adjusted odd ratio, CI-Confidence interval, 1-indicator and Statistical significant at $p< 0.05$

Table 5. Predictors of thrombocytopenia in PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Variables | Thrombocytopenia | | COR (95% CI) | P-value | AOR (95% CI) | P-Value |
|---|---|---|---|---|---|---|
| | Yes n (%) | No n (%) | | | | |
| Age | | | | | | |
| 18-29 | 19 (31.7) | 41 (68.3) | 1 | | | |
| 30-39 | 32 (38.6) | 51 (61.4) | 1.35 (0.67 - 2.72) | 0.397 | | |
| 40-49 | 8 (34.5) | 15 (65.5) | 1.15 (0.42 - 3.17) | 0.786 | | |
| ≥50 | 2 (33.3) | 4 (66.7) | 1.08 (0.18 - 6.41) | 0.933 | | |
| Sex | | | | | | |
| F | 37 (35.6) | 67 (64.4) | 1.05 (0.56 - 1.99) | 0.868 | | |
| M | 24 (35.3) | 44 (64.7) | 1 | | | |
| HAART status | | | | | | |
| Yes | 45 (31.7) | 97 (68.3) | 1 | | 1 | |
| No | 16 (53.3) | 14 (46.7) | 0.41 (0.18 - 0.90) | 0.027 | 1.46 (0.59 - 3.59) | 0.407 |
| Opportunistic infection | | | | | | |
| Yes | 5 (31.2) | 11(68.8) | 0.81 (0.27 - 2.45) | 0.712 | | |
| No | 56 (35.9) | 100(64.1) | 1 | | | |
| Chronic illness | | | | | | |
| Yes | 5 (41.7) | 7 (58.3) | 1.33 (0.40 - 4.37) | 0.642 | | |
| No | 56 (35.0) | 104 (65.0) | 1 | | | |
| Malaria infection | | | | | | |
| Yes | 51 (59.3) | 35 (40.7) | 11.07 (5.04-24.33) | <0.001 | 9.79 (4.33-22.17) | <0.001 |
| No | 10 (11.6) | 76 (88.4) | 1 | | 1 | |
| Malaria parasitic density | | | | | | |
| 1-999 | 12 (57.1) | 9 (42.9) | 1 | | | |
| 1000-9999 | 26 (60.5) | 17(39.5) | 1.15 (0.39 - 3.30) | 0.799 | | |
| ≥10000 | 13 (59.1) | 9 (40.9) | 1.08 (0.32 - 3.64) | 0.897 | | |
| $CD_4^+$ count (cells/µl) | | | | | | |
| ≤ 200 | 16 (61.5) | 10 (38.5) | 3.36 (1.29 - 8.71) | 0.013 | 2.69 (0.90 - 8.05) | 0.076 |
| 200-499 | 25 (29.8) | 59 (70.2) | 0.89 (0.44 - 1.80) | 0.747 | 0.79 (0.33 - 1.80) | 0.589 |
| ≥ 500 | 20 (32.3) | 42 (67.7) | 1 | | 1 | |

*COR-Crud odd ratio, AOR-Adjusted odd ratio, CI-Confidence interval, 1-indicator and statistically significant at p< 0.05

Table 6. Predictors of leucopenia in PLWHA at Gambella Hospital, Southwest Ethiopia, May 25 to November 11, 2014

| Variables | Leucopenia | | COR (95% CI) | P-value | AOR (95% CI) | P-Value |
|---|---|---|---|---|---|---|
| | Yes n (%) | No n (%) | | | | |
| Sex | | | | | | |
| F | 41 (39.4) | 63 (60.6) | 0.98 (0.53 - 1.84) | 0.970 | | |
| M | 27 (39.7) | 41 (60.3) | 1 | | | |
| Age | | | | | | |
| 18-29 | 23 (38.3) | 37 (61.7) | 1 | | | |
| 30-39 | 35 (42.2) | 48 (57.8) | 1.17 (0.59 - 2.31) | 0.645 | | |
| 40-49 | 7 (30.4) | 16 (69.6) | 0.70 (0.25 - 1.97) | 0.504 | | |
| ≥50 | 3 (50.0) | 3 (50.0) | 1.61 (0.23 - 8.65) | 0.580 | | |
| HAART status | | | | | | |
| Yes | 56 (39.4) | 86 (60.6) | 1 | | | |
| No | 12 (40.0) | 18 (60.0) | 1.02 (0.46 - 2.28) | 0.954 | | |
| Opportunistic infection | | | | | | |
| Yes | 5 (31.2) | 11 (68.8) | 0.67(0.22 - 2.02) | 0.479 | | |
| No | 63 (40.4) | 93 (59.6) | 1 | | | |
| Drug other than HAART | | | | | | |
| Yes | 14 (46.7) | 16 (53.3) | 1 | | | |
| No | 54 (38.0) | 88 (62.0) | 0.70 (0.32 - 1.55) | 0.381 | | |
| Chronic illness | | | | | | |
| Yes | 6 (50.0) | 6 (50.0) | 1.58 (0.48 - 5.12) | 0.445 | | |
| No | 62 (38.8) | 98 (61.2) | 1 | | | |
| Malaria infection | | | | | | |
| Yes | 37 (43.0) | 49 (57.0) | 1.34 (0.73 - 2.47) | 0.350 | | |
| No | 31 (36.0) | 55 (64.0) | 1 | | | |
| Malaria parasitic density (parasite/µl) | | | | | | |
| 1-999 | 13 (61.9) | 8 (38.1) | 1 | | 1 | |
| 1000-9999 | 20 (46.5) | 23 (53.3) | 0.53 (0.18 - 1.55) | 0.250 | 0.55 (0.18 - 1.69) | 0.303 |
| ≥ 10000 | 4 (18.2) | 18 (81.8) | 0.14 (0.34 - 0.55) | 0.005 | 0.13 (0.03 - 0.57) | 0.007 |
| $CD_4^+$ count (cells/µl) | | | | | | |
| ≤ 200 | 16 (61.5) | 10 (38.5) | 7.12 (2.58 - 19.61) | <0.001 | 4.77 (1.23 - 18.45) | 0.023 |
| 200-499 | 25 (29.8) | 59 (70.2) | 3.11 (1.47 - 6.57) | 0.003 | 2.85 (0.94 - 8.62) | 0.064 |
| ≥500 | 20 (32.3) | 42 (67.7) | 1 | | 1 | |

*COR-Crud odd ratio, AOR-Adjusted odd ratio, CI-Confidence interval, 1- indicator and statistically significant at p< 0.05

# CHAPTER SIX: DISCUSSION

This study was aimed to determine the impact of malaria on the hematological profiles of PLWHA. Therefore, the prevalence of anemia, thrombocytopenia and leucopenia were higher in malaria infected participant than malaria non-infected participants. In addition, the mean hematological profiles were lower in participants with malaria infection than without malaria infection. The difference in mean values of Hgb, HCT, platelet, neutrophil and lymphocyte were statistically significant. Residence, malaria infection and $CD_4^+$ count were identified as predictors for anemia. Malaria infection was significantly associated with thrombocytopenia, whereas malaria parasitic density and $CD_4^+$ count were significantly associated with leucopenia.

In this study the prevalence of anemia was higher (60.5%) in malaria and HIV co-infected participants than those HIV mono-infected participants (43.0%). This finding was in agreement with other studies (26, 27, 49, 50). However, prevalence of anemia in malaria and HIV co-infected participants was lower as compared to studies conducted in Ghana (2012), Nigeria (2006) and Gondar (2013) which reported 97.1%, 66.7% and 71.3%, respectively (26, 27, 49). The difference might be due to variation in methods according to sample size they use small sample size and clinical condition like HAART status and immune status of the participants. Anemia due to malaria infection can occur through different mechanisms; include RBC lysis, organ sequestration, phagocytosis of uninfected and infected RBCs, and dyserythropoiesis (10).

The second prevalent hematological abnormality in malaria and HIV co-infected study participants was thrombocytopenia (59.3%) and in HIV mono-infected study participants (11.6%). This shows there is a significant association between malaria infection and thrombocytopenia, which is supported by study done in Nigeria (60%) (2006) (27). The possible causes of thrombocytopenia in malaria infection was increased sequestration and highly elevated levels of platelet bound immunoglobulin that lead to increased peripheral destruction (11).

Among malaria and HIV co-infected participants 43.0 % had leucopenia and 36.0 % had leucopenia in HIV mono-infected participants. This shows there is difference among the groups since it is not statistically significant. In contrary to this study of Nigeria (2006) reported that occurrence of leucopenia were more than two times higher in malaria and HIV co-infected participants than HIV mono-infected participants (27). This difference might be due to variation in method used for investigation of hematological profiles, Nigeria study manually determine hematological parameters and reference value they used for the leucopenia was $\leq 3x10^9/l$. Sometimes reduction in the leukocyte counts is attributed to hypersplenism or sequestration in the spleen rather than actual depletion (12).

Our result showed that the prevalence of *Plasmodium falciparum* (94.2%) was higher than prevalence of *Plasmodium vivax* (3.5%) in malaria and HIV co-infected participants. It is consistence with the studies conducted in Gondar (2013) and Nigeria (2006) (26, 27) but contradict to a study done in India (2012) (48). This difference might be due to geographical and climatic variations of the study area. *Plasmodium falciparum* is highly prevalent in Sub-Saharan country than Asia and Latin America (63).

The mean value of Hgb, HCT, platelet and lymphocyte count were lower in malaria and HIV co-infected participants than malaria non-infected participants. This finding is consistent with the previous studies done in Nigeria at (2006 and 2013) (27, 28). However, this result contradict to the study from Cameroon in 2012 (51). This difference might be due to Cameroon study use small sample size than our study sample size.

In contrary, this study also showed there was no significant difference in the mean value of RBC, MCV, MCH and MCHC in malaria infected and non-infected PLWHA. This finding is supported by study done in Cameroon (2012) (51). This might be due to normocytic normochromic (NCNC) nature of anemia in both malaria and HIV infections.

Multivariable logistic regression analysis of this study showed that predictors for hematological abnormalities in PLWHA. Those participants live in rural area were more anemic than those participants live in urban. It might be due to the type of food the rural community use, lack of frequent follow up and other factors which could cause anemia. Malaria infected PLWHA were two times more likely to be anemic than malaria non-

infected PLWHA. This result is consistence with the study done in Nigeria (2012) (50). Likewise, malaria infected study participants were ten times most likely to be thrombocytopenic than malaria non-infected PLWHA.

Participants who had $CD_4^+$ count ≤ 200 cells/µl were nearly five times more likely to have leucopenia than who had $CD_4^+$ count ≥ 500 cells/µl PLWHA. This finding is consistent with other studies done in India (2012), Brazil (2011) and Uganda (2014) (36, 55, 56). Other predictor of leucopenia in this study was malaria parasitic density. Those study participants who had higher malaria parasitic density were 13% less likely to be leucopenic than those having the lowest parasitic density. However, in contrast to this a study from Cameroon (2012) reported that parasitic density is not significantly associated with any hematological parameters (51).

Malaria and HIV are highly prevalent infection in the developing countries especially in sub-Saharan Africa. They cause hematological abnormalities which decrease the immune response and increase adult mortality. Therefore, assessment of hematological abnormalities in malaria HIV co-infection individuals has remarkable benefit to prevent HIV malaria co-morbidity (64).

## Limitation of the study

- Since the study is cross-sectional it cannot show cause and effect relationship between different factors with outcome variables
- This study used unmatched comparable group according to sex, HAART status and like

# CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

## 7.1 Conclusion

- The result of this study showed that prevalence of anemia and thrombocytopenia were higher in malaria and HIV co-infection than HIV mono-infection.
- There was significant difference in mean values of Hgb, HCT, lymphocyte, neutrophils and platelet counts.
- Residence, malaria infection and $CD_4^+$ count were found to be predictors of anemia. Malaria infection also found to be predictor for thrombocytopenia. On the other hand malaria parasite density and $CD_4^+$ count were found to be predictors of leucopenia.

## 7.2 Recommendation

- The effect of malaria infection on the decrease in hematological parameters should be considered during the management of HIV patients co-infected with malaria.
- This study also highlights the clinician need of regular screening of malarial for those HIV-infected people particularly living in malaria endemic areas.
- Further longitudinal researches are required on hematological profiles of malaria and HIV co-infected individuals by including additional associated factors that elaborate the research.

## REFERENCES


1. Hoffbrand A.V & Petit J.E. Clinical Haematology. 3rd ed. Mosby, London.; 2000. p. 56.
2. Kidney Disease: Improving Global Outcomes (KDIGO). Hematological Complications. *Am J Transplant*. 2009;9(Suppl 3):97–101.
3. Briges KR, A.Pearson H, editors. Principles of anemia evaluation. Anemias and other Red cell disorders. 1st ed. McGraw-Hill Companies; 2007. p. 4.
4. Glader B. Anemia: General Considerations. In: John P. Greer, John Foerster JNL, editor. Wintrobe ' s Clinical Hematology. 11th ed. Lippincott Williams & Wilkins Publishers; 2003. p. 1888–90.
5. Beck N. Diagnostic hematology. Pretoria,South Africa: Springer; 2009. p. 24 & 317.
6. Sharathkumar AA, Amy S. Platelet Function Disorders. Treatment of hemophilia. Canada; 2008.
7. Lichtman M, Beutler AE, Kipps TJ, Seligsohn U, editors. Williams Hematology. Sevanth Ed. McGraw-Hill; 2007.
8. Maina NR, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, et al. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. *Malar J*. 2010;9(3):1–11.
9. Crawley J. Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. *Am J Trop Med Hyg*. 2004;71(Suppl 2):25–34.
10. Lamikanra AA, Brown D, Potocnik A, Casals-pascual C, Langhorne J, Roberts DJ, et al. Malarial anemia : of mice and men. *Blood*. 2007;110(1):18–28.
11. Iqbal W. Hematological manifestations in malaria. *Haematol Updat*. 2010;35–7.
12. Mckenzie FE, Prudhomme WA, Magill AJ, Forney JR, Lucas C, Jr RAG, et al. White blood cell counts and malaria. *J Infect Dis*. 2005;192(2):323–30.
13. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ward JW, Sullivan BPS. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. *Blood*. 1998;91(1):301–8.

14. Kuritzkes DR. Neutropenia, neutrophil dysfunction, and bacterial infection in patients with human immunodeficiency virus disease : the role of granulocyte colony-stimulating factor. *Clin Infect Dis*. 2000;30(2):256–60.
15. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ciesielski CA. Surveillance for Thrombocytopenia in Persons Infected With HIV: Results From the Multistate Adult and Adolescent Spectrum of Disease Project. *J Acquir Immune Defic Syndr*. 1996;14(4):374–9.
16. Hochman S, Kim K. The impact of HIV and malaria co-infection: what is known and suggested venues for further study. *Interdiscip Perspect Infect Dis*. 2009;2009:1–8.
17. Flateau C, Le Loup G, Pialoux G. Consequences of HIV infection on malaria and therapeutic implications a systematic review. *Lancet Infect Dis*. 2011;11(7):541–56.
18. Idemyor V. Human Immunodeficiency Virus (HIV) and malaria interaction in Sub-Saharan Africa: the collision of two titans. *HIV Clin Trials*. 2007;8(4):246–53.
19. Orlov M, Vaida F, Finney OC, Smith DM, Talley AK, Wang R, et al. P .falciparum enhances HIV replication in an experimental malaria challenge system. *PLoS One*. 2012;7(6):1–8.
20. Van Geertruyden J. Interactions between malaria and human immunodeficiency virus anno. *Clin Microbiol Infect*. 2014;20:278–85.
21. Mermin J, Ekwaru JP, Liechty CA, Were W, Downing R, Ransom R, et al. Effect of co-trimoxazole prophylaxis , antiretroviral therapy , and insecticide-treated bednets on the frequency of malaria in HIV-1-infected adults in Uganda : a prospective cohort study. *Lancet*. 2006;367:1256–61.
22. World vision and Roll Back malaria. The link between malaria and HIV and AIDS [Internet]. [cited 2015 Mar 30]. Available from: http://gyroconference.event123.no/RodeKors/OMC2011/pop.cfm?FuseAction=Doc&pAction
23. World Health Organization (WHO). Malaria in HIV/AIDS patients [Internet]. 2013 [cited 2015 Mar 30]. Available from: http://www.who.int/malaria/areas/high_risk_groups/hiv_aids_patients/en/
24. World Health Organization (WHO). Malaria and HIV interactions and their implications for public health policy. Geneva, Switzerland; 2004.

25. Chandramohan D, Greenwood BM. Is there an interaction between human immunodeficiency virus and Plasmodium falciparum? *Int J Epidemiol*. 1998;27(2):296–301.
26. Wondimeneh Y, Ferede G, Atnafu A, Muluye D. HIV-Malaria Co-infection and their immunohematological profiles. *Eur J Exp Biol*. 2013;3(1):497–502.
27. Erhabor O, Babatunde S, Uko KE. Some haematological parameters in Plasmodial parasitized HIV-infected Nigerians. *Niger J Med*. 2006;15(1):52–5.
28. Etusim PE, Ihemanma CA, Nduka FO, P.E. M, Ukpai O. Comparative study on the haematological characteristics of malaria infected and malaria non-infected persons referred to Art/HIV laboratory, Abia State University Teaching Hospital, Aba, Abia State. *J Sci Multidiscip Res*. 2013;5(1):100–12.
29. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. HIV infection as a cofactor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *AIDS*. 2004;18(3):547–54.
30. World Health Organization (WHO). World malaria report 2014. Geneva, Switzerland; 2014.
31. President ' S Malaria Initiative (PMI). Malaria Operational Plan FY 2014. Addis Ababa,Ethiopia; 2014.
32. Akhtar S, Gumashta R, Mahore S. Hematological changes in malaria : A comparative study. *J Pharm Biol Sci*. 2012;2(4):15–9.
33. Joint United Nations Program on HIV/AIDS (UNAIDS). The Gap Report. Geneva, Switzerland; 2014.
34. Kirchhoff F, Silvestri G. Is Nef the elusive cause of HIV-associated hematopoietic dysfunction ? *J Clin Invest*. 2008;118(5):1622–5.
35. Coyle TE. Hematological complications of human immuno deficiency virus infection and the acquired immuno deficiency syndrome. *Med Clin North Am*. 1997;81(2):449–70.
36. Parinitha SS, Kulkarni MH. Haematological changes in HIV infection with correlation to $CD_4$ cell count. *Australas Med J.* 2012;5(3):157–62.

37. Obirikorang C, Yeboah FA. Blood haemoglobin measurement as a predictive indicator for the progression of HIV / AIDS in resource-limited setting. *J Biomed Sci.* 2009;16(102):1–7.
38. Alem M, Kena T, Baye N, Ahmed R, Tilahun S. Prevalence of anemia and associated risk factors among adult HIV patients at the anti-retroviral therapy clinic at the University of Gondar Hospital, Gondar, Northwest Ethiopia. doi:104172/scientificreports662. 2013;2(3):602.
39. Johannessen A, Naman E, Gundersen SG, Bruun JN. Antiretroviral treatment reverses HIV-associated anemia in rural Tanzania. *BMC Infect Dis*. 2011;11(1):190.
40. Satya Attili SV, Singh VP, Rai M, Varma DV, Gulati AK, Shyam S. Hematological profile of HIV patients in relation to immune status - a hospital-based cohort from Varanasi , North India. *Turk J Hematol*. 2008;25(1):13–9.
41. Levine AM, Karim R, Mack W, Gravink DJ, Anastos K, Young M, et al. Neutropenia in Human Immunodeficiency Virus Infection. *Arch Intern Med*. 2006;166(4):405–10.
42. Wondimeneh Y, Muluye D, Ferede G. Prevalence and associated factors of thrombocytopenia among HAART naive HIV positive patients at Gondar university hospital , northwest Ethiopia. *BMC Res Notes*. 2014;7(5):1–4.
43. Akinbami A, Oshinaike O, Adeyemo T, Adediran A, Owolabi Dosunmu, Mobolaji D, et al. Hematologic Abnormalities in Treatment-Naïve HIV Patients. *Infect Dis Treat*. 2010;3:45–9.
44. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital , Gondar , Northwest Ethiopia. *BMC Hematol.* 2014;14(1):8.
45. Kassa D, Petros B, Messele T, Admassu A, Adugna F, Wolday D. Parasito-haematological features of acute Plasmodium falciparum and P . vivax malaria patients with and without HIV co-infection at Wonji Sugar Estate , Ethiopia. *Ethiop J Health Dev*. 2005;19(2):132–9.
46. Dolores Herrero M, Rivas P, I Rallón N, Ramírez-olivencia G, Puente S. HIV and Malaria. *AIDS Rev*. 2007;9:88–98.

47. Jairajpuri ZS, Rana S, Hassan MJ, Nabi F, Jetley S. An analysis of hematological parameters as a diagnostic test for malaria in patients with acute febrile illness: an institutional experience. *Oman Med J.* 2014;29(1):12–7.
48. Bharti AR, Saravanan S, Madhavan V, Smith DM, Sharma J, Balakrishnan P, et al. Correlates of HIV and malaria co-infection in Southern India. *Malar J.* 2012;11(306):2–5.
49. Tagoe DN, Boachie J. Assessment of the impact of malaria on $CD_4^+$ T Cells and haemoglobin levels of HIV-malaria co-infected patients. *J Infect Dev Ctries*. 2012;6(9):660–3.
50. Akinbo FO, Omoregie R. Plasmodium falciparum infection in HIV-infected patients on highly active antiretroviral therapy (HAART) in Benin City, Nigeria. *J Res Heal Sci*. 2012;12(1):15–8.
51. Tchinda GG, Atashili J, Achidi EA, Kamga HL, Njunda AL, Ndumbe PM. Impact of malaria on hematological parameters in people living with HIV/AIDS attending the Laquintinie Hospital in Douala, Cameroon. *PLoS One. 2012*;7(7):e40553.
52. Rudnicka D, Schwartz O. Intrusive HIV-1-infected cells. *Nat Immunol*.2009 ;10(9):933–4.
53. Dikshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. *BMC Blood Disord*. 2009;9(5):1–6.
54. Munyazesa E, Emile I, Mutimura E, Hoover DR, Shi Q, Mcginn AP, et al. Assessment of haematological parameters in HIV-infected and uninfected Rwandan women : a cross-sectional study. *BMJ Open*. 2012;2(6):e001600.
55. De Santis GC, Brunetta DM, Vilar FC, Brandao RA, de Albernaz Munizb RZ, de Lima GMN, et al. Hematological abnormalities in HIV-infected patients. *Int J Infect Dis*. 2011;15(12):808–11.
56. Kyeyune R, Saathoff E, Ezeamama AE, Löscher T, Fawzi W, Guwatudde D. Prevalence and correlates of cytopenias in HIV-infected adults initiating highly active antiretroviral therapy in Uganda. *BMC Infect Dis*. 2014;14(496):1–10.

57. Owiredu WK, Quaye L, Amidu N, Addai-Mensah O. Prevalence of anaemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. *Afr Heal Sci*. 2011;11(1):2–15.
58. Federal Democratic Republic of Ethiopia Population Census Commission. Summary and Statistical Report of the 2007 population and Housing Census. Addis Ababa, Ethiopia; 2008.
59. Rosner B. Fundamentals of Biostatistics. Fifth edit. Duxubury; 2000.
60. Ethiopian health and nutrition research institute (EHNRI). Manual for the Laboratory Diagnosis of Malaria. first edit. Ethiopia federal ministery of health (EFMOH). Addis Ababa; 2012. 40 p.
61. World Health Orgnization (WHO). Worldwide prevalence of anaemia 1993-2005. Benoist B de, McLean E, Egli I, Cogswell M, editors. 2008.
62. Hoffbrand AV, Catovsky D, Tuddenham EG, Green AR, editors. Postgraduate hematology. Sixth edit. Blackwell Publishing Ltd; 2011. p. 956.
63. Quintero JP, Siqueira AM, Tobón A, Blair S, Moreno A, Arévalo-herrera M, et al. Malaria-related anaemia : a Latin American perspective. *Mem Inst Oswaldo Cruz*. 2011;106(1):91–104.
64. Berg A, Patel S, Aukrust P, David C, Gonca M, Berg ES, et al. Increased severity and mortality in adults co-infected with malaria and HIV in Maputo , Mozambique : A prospective cross-sectional study. *PLoS One*. 2014;9(2):e88257.

# ANNEXES

## ANNEX-I: Standard operating procedures for Cell-Dyne 1800

### Purpose

The purpose is to evaluate anemia, leukemia, reaction to inflammation and infections, peripheral blood cellular characteristics, state of hydration and dehydration, polycythemia, hemolytic disease of the new born, inherited disorders of red cells, white cells, and platelets; manage chemotherapy decisions; determine qualitative and quantitative variations in white cell numbers and morphology, morphology of red cells and platelet evaluation.

### Principle

During each instrument cycle, the sample is aspirated, diluted, and mixed before each parameter is measured.

The CELL-DYN 1800 uses two independent measurement methods; they are:

- Electrical impedance method for determining WBC, RBC, and PLT data
- Modified methemoglobin method for determining Hgb

### Electrical Impedance Measurement

Electrical impedance is used to count and size blood cells. This method is based on the measurement of changes in electrical resistance produced by a particle suspended in a conductive diluent as it passes through an aperture of known dimensions.

An electrode is submerged in the liquid on each side of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes occurs, producing a measurable electrical pulse. The number of pulses generated indicates the number of particles that pass through the aperture. The amplitude of each pulse is essentially proportional to the particle volume.

Each pulse is amplified and compared to internal reference voltage channels. These channels are delineated by calibrated size discriminators to accept only pulses of certain amplitude. Thus, the pulses are stored into various size channels according to their amplitude.

**Specimen Required**

- Collected in EDTA anticoagulant. Follow the manufacturer's guidelines regarding collection and stability.
- Mixed well before processing.
- Fresh whole blood specimens are recommended (process within eight hours after collection). WBC size distribution can shift if specimens are tested within the first 20 minutes following collection or more than eight hours after collection. A minimum of 50µL must be collected for micro-collection specimens. This ensures an adequate amount of blood for the 30 µL aspiration.

**Supplies and Materials required**

- CELL-DYN 1800
- Waste reservoir
- CELL-DYN 1800 Reagents
- CELL-DYN 1800 Controls
- Bio safety materials
- EDTA (K3) VACUTAINER Blood collection tube (Lavender top, liquid EDTA).
- Calibrator

**Reagents**

- Cyanide-Free Diff Lyse Reagent, Detergent, Diluents and Enzymatic Cleaner.
- Reagent must be stored at room temperature except Enzymatic Cleaner which should be stored between 2 and 8 degree centigrade. Do not use reagents that have been frozen.

**Procedure**

- Entering and running patient specimen

  **Note**: prior to running patient specimens, perform daily start-up procedures
- When the **ready** message is displayed on the run screen, the instrument is ready to run specimens.

Entering specimen

- From **run** screen, press **[specimen type**]
- In the specimen type screen, press **[patient specimen**]
- The cursor is placed in the **<next id #>** entry field. Use the alphanumeric keys on the pc keyboard to enter a specimen id of up to 16 characters.

Running patient specimen

To run patient specimens, proceed as follows:

1. With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times.
2. Remove the cap from the pre-mixed specimen tube.
3. Place the tube under the aspiration probe and raise tube so that the end of the probe is deeply immersed in the specimen.
4. Press the touch plate to aspirate the run.
5. When the sample has been aspirated from the tube, the probe will move up through the wash block. Remove the specimen tube and replace the cap.
6. After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the Data Log.
7. If automatic graphics printout has been specified in the setup menu, a report is printed according to the parameters selected during the setup procedure.
8. If automatic graphics printout has not been specified in the setup menu, press **[print report**] to obtain a copy of the results.

**Note**: if a system has been idle for 15 minutes or more, a normal background should be run immediately prior to running patient specimens.

**Quality Control**

Quality control checks should be performed daily according to the laboratory's protocol. Commercial controls materials should be properly warmed and mixed according to the manufacturers' recommendations patient controls should be handled according to the laboratory's protocol.

The **quality control** (QC) menu is accessed from the **main menu** screen. The **quality control** screen displays the following (soft keys) that take the user to related submenus for performing specialized tasks:

[XBFILE][LOWCONTROL][NORMALCONTROL][HIGHCONTROL][REPLICATES]
[MAIN] [HELP/ERROR]

- The **[X-B FILE]** key is used to display and print data and graphs for the MCV, MCH, and MCHC parameters, including date and time for each batch.
- **[LOW CONTROL]** is used to display the Low Control file names and the number of specimens in each file. It also displays detailed QC information, such as limits, standard deviation, and coefficient of variation for each parameter for each lot number when the **[VIEW QC LOG]** key is pressed.
- **[NORMAL CONTROL]** is used to display the Normal Control file names and the number of specimens in each file. It also displays detailed QC information, such as limits, standard deviation, and coefficient of variation for each parameter for each lot number when the **[VIEW QC LOG]** key is pressed.
- **[HIGH CONTROL]** is used to display the High Control file names and the number of specimens in each file. It also displays detailed QC information, such as limits, standard deviation, and coefficient of variation for each parameter for each lot number when the **[VIEW QC LOG]** key is pressed.
- **[REPLICATES]** is used to display the replicated file names and the number of specimens in each file. It also displays detailed QC information, such as limits, standard deviation, and coefficient of variation (CV %) for each parameter for each replicate ID/lot number when the **[VIEW QC LOG]** key is pressed.

**Performing a QC Run**

If the system has been idle for fifteen minutes or more, run a background prior to running any control specimens. Be sure to prepare the control product according to directions on the package insert.

To perform a QC run, proceed as follows:

- From the **MAIN MENU** screen, press **[RUN]**.
- From the **RUN** menu, press **[SPECIMEN TYPE]** followed by **[QC TYPE]**.
- Select the desired level of control (**Low, Normal, High or Replicates**).

**NOTE:** Prepare a permanent record (printed copy) of any files to be deleted or purged, as required, according to your laboratory's protocol. You can also copy the QC Log before you purge it.

1. Using the [↑] and [↓] arrow keys, select the desired control file.
2. Remove the cap from a well-mixed control specimen tube and place the open tube under the Sample Aspiration Probe. Raise the tube so that the end of the probe is deeply immersed in the specimen.
3. Press the Touch Plate to activate the run.
4. When the well-mixed control has been aspirated from the tube , and the probe moves up through the Wash Block, remove the specimen tube and replace the cap.

**NOTE:** If a flow error, clog, or other fault message appears on the display screen during **RUN** cycle, press **[CLEAR ORIFICE]** or refer to **Operating Manual:** ***Troubleshooting and Diagnostics*** and repeat the run.

1. Verify that control results are within your laboratory's acceptable limits.
2. If the control results fall within acceptable limits, review the data for shifts or trends, record the results, and begin to process patient specimens.
3. After analysis is complete and results are displayed, press **[RETURN]** to return to the **RUN** menu.
4. Press **[MAIN]** to return to the **MAIN MENU** screen.
5. Press **[PRINT]** if a report of printed results is desired.

## ANNEX-II: Standard operating procedure for FACS count machine

### Purpose

This machine used to determine T-lymphocyte phenotype & enumeration, it have been used to evaluate the immune statues of patients with, or suspected of developing immune deficiencies such as Acquired immune deficiency syndrome (AIDS). The $CD_4^+$ Antigen is the receptor of human immune deficiency Virus (HIV). The absolute number of $CD_4^+$ T-lymphocyte is the cellular parameter most closely associated with HIV disease progression and patient Prognosis.

### Principle

A single test requires one convenient, ready-to-use reagent tube. When whole blood is added to the reagent, fluorochrome-labelled antibodies in the reagent bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tube, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count cells. In addition to containing the antibody reagent, the tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantification standard for enumerating the cells.

### Specimen collection and storage

- Use whole blood specimen collected by venipuncture in evacuated blood collection K3-EDTA or K2EDTA containers.
- Samples must be transported as soon as possible after collection at 18–22ºC, and the tests should be performed within 48 hours but no later than 72 hours after the blood specimen (kept at room temperature) is drawn.

### Requirements

Equipment

- BD FACS count instrument
- Automatic electronic pipette and tips
- Vortex mixer
- Coring station
- Cleaning tubes
- FACS count workstation
- Disposable clothing
- Biohazard waste container or bag
- Safety Cabinet class II (optional)

Reagents

- BD FACS count reagent kit
- BD FACS count control kit
- BD multicheck control
- BD FACS count sheath fluid
- BD FACS count rinse
- BD FACS count clean

**Procedures**

1. Label the tab of one reagent tube with patient laboratory number.
2. Vortex the reagent tube upside down for 5 seconds, then upright for 5 seconds.
3. Open the reagent tube with the coring station.
4. Transfer the reagent tube from the coring station to the workstation, keeping the tubes upright.
5. Close the workstation cover to protect the reagents from light.
6. Mix the whole blood by inverting the BD Vacutainer tubes five times.
7. Pipette 50 µL of blood into reagent tubes. Change the tips between each tube.
8. Cap the reagent tube and vortex upright for 5 seconds.
9. Replace the reagent tube in the FACS count workstation, close the cover to protect reagent from light, and incubate for 30–45 minutes at room temperature (20–25ºC).

10. After the incubation step is complete, uncap the tubes and pipette 50 µL of fixative solution into each reagent tube. Change tips between tubes.
11. Seal the reagent tube with new caps and vortex upright for 5 seconds. (Fixed samples can be held up to 12 hours before adding the control beads.)
12. Run the tubes on the FACS count instrument within 2 hours of adding Control beads to the reagent tubes.
13. Store samples at room temperature in the workstation until they are run on the instrument. Vortex upright for 5 seconds immediately before running and run on the BD FACS count instrument following the instructions in the user's manual.

**Quality Control**

- Commercially available BD multicheck controls or fresh peripheral blood from blood donors must be run every morning to verify both the reagents and methodology.
- Control specimens must be tested in the same manner as patient samples.
- Control samples that fall out of range need to be investigated, and patient results from the same test run are suspect until the reason for the control sample failure is resolved.
- All control data must be documented and the results verified for acceptability before reporting results.

**Results**

Important immunological evaluation includes:

- $CD_4^+$ absolute count
- Percentages of $CD_4^+$ in special laboratories, especially for pediatric and pregnant patients

**Reference ranges**

- Normal values range from 500–1,500 cells/µL of blood.

**Interpretation of Results**

- The lower the $CD_4^+$ count, the more the disease has progressed. Treatment with ARVs will be initiated when counts are below 500cells/µL (For Ethiopia policy is below 200cells/µL).
- AIDS is diagnosed when $CD_4^+$ cell counts are below 200cells/µL.

**Tests Limitations and Sources of Error**

Upon receipt, check for:

- Clots
- Gross haemolysis
- Lipaemic samples for rejection

## ANNEX-III: Standard operating procedures for malaria blood film examination

### Procedure for preparation of the thin film

- Working quickly, obtain a second clean and polished slide (spreader) and place in front of the small blood drop at a 30º - 45º angle. Pull back the slide and hold until the blood is evenly spread along the edge of the slide. Do not delay between applying and spreading the drop.
- Rapidly push the slide forward in a single, smooth, continuous motion. Avoid hesitation or jerky motions when spreading the blood. (A feathered end of the film should have red blood cells that are lying individually without overlapping and relatively evenly distributed).

### Procedure for Preparation of thick blood film

- With one corner of the spreader slide, in a circular motion, spread the blood out to make a circle with approximately 1cm (1/3 inch) in diameter, finishing off at the center.
- The ideal thickness of the smear should allow for printed text to be readable when it is placed on it.
- Discard the spreader into an appropriate slide container and DON'T re-use it for another patient's blood sample.
- Allow both blood films to air dry in a horizontal position on a slide tray . Slow drying prevents cracking. Avoid using a fan or blow dryer to dry these slides.

### Quality Control

Monitor the quality of the preparation of thick and thin smears

- Follow proper collection procedures.
- Glass slides must be clean and free from grease.
- Thick films and thin films must be prepared properly while drying protects blood films from dust, flies and insects.
- Do not dry expressed to direct sun light.

- Too thin a film may not have adequate quantity of blood for detection of parasite.
- Blood film spread unevenly on a greasy slide makes examination difficult.
- Thin film too long, leaves less space for thick film.
- When fixing the thin film, be careful not to let methanol touch the thick film.
- Wet slides are wrapped together and the slides stick to one another.

**SOP for preparation of Giemsa working solution**

**Purpose**

This SOP provides instructions for preparation of Giemsa working solutions from Giemsa stock.

**Principle**

Light microscopy, usually applying the Giemsa staining technique, is the established method for the laboratory diagnosis of malaria.

Giemsa is a Romanowsky stain used for staining blood films. Romanowsky stains contain Eosin, an anionic acidic dye, and Azure, a cationic basic thiazine dye obtained by oxidation of methylene blue. When the dyes are diluted in a buffer, the anionic dye stains the acidic components (nucleus) of cells red, and the cationic dye stains the basic components (cytoplasm) of cells blue.

**Materials and Reagents**

- Giemsa stock solution
- Buffered Distilled water
- Measuring cylinder 10 and 100ml capacity
- Filter paper
- Funnel

**Special Safety Precaution**

- highly flammable with flash point $12^0$c and Keep away from sources of ignition
- Avoid inhaling fumes and contact with skin

- Procedure of Preparing 10% Giemsa working solution
- Pour 90 ml of buffered water (pH 7.0 – 7.2) into the measuring cylinder.
- Add 10 ml of filtered Giemsa stock into the measuring cylinder
- Mix well before using
- Procedure of Preparing 3% Giemsa working solution
- Pour 97 ml of buffered water (pH 7.2) into the measuring cylinder.
- Add 3 ml of filtered Giemsa stock into the measuring cylinder.
- Mix the stain well before using.

**Quality Control**

Check the staining quality using known QC slides for every batch of Giemsa stain solution.

**SOP for examination of malaria blood films and estimation of parasitemia**

**Purpose**

This SOP provides instructions for the proper detection, identification and quantification of malaria parasites in Giemsa-stained MBFs.

**Principle**

Examination of both thick and thin blood film is used to detect & identify malaria parasite respectively and estimation of parasitemia.

In the thick blood film the red blood cells (RBCs) are lyses and dehemoglobinized while the malaria parasites are left intact and concentrated and used as a screening test to detect the presence of malaria parasite.

In the thin blood film, when fixed with absolute methanol, enables the RBCs to retain their original morphology with malaria parasites, if present, visible inside the RBCs, is used to identify the species and stages of malaria parasites.

**Materials, Reagents and Equipment**

Materials

- Patient Register
- Pen
- Lens paper
- Reagents
- Immersion oil
- Lens cleaning solution (80/20 Ethyl Ether solution)

Equipment's

- Binocular microscope
- Tally counter(s) / Differential counter
- Slide boxes

**Procedure for Focusing and scanning blood films**

1. Place the MBF on the microscope stage, switch on the light and adjust the light source optimally by looking through the ocular and the 10X/40X objectives.
2. Place a drop of immersion oil on the dry stained slide. To avoid cross contamination, ensure that the tip of immersion oil dropper never touches the slide.
3. Slowly change to the oil immersion objective, and a thin film of oil will form between the slide and the lens.
4. Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the100X objective and use the fine adjustment knob to focus the field; the lens should not be allowed to touch the slide.
5. Examine the slide in a systematic fashion. Start at the left end of the thick 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.

**Procedure for examining the thick blood film**

1. Scan the thick film under oil immersion objective (x100) and ascertain whether a smear is positive or negative.

2. Use the “WHO Bench Aids in the Diagnosis of Plasmodium Infections” for the characteristics and illustrations of Plasmodium species.
3. If positive, determine all species and stages present.
4. Read a minimum of 200 oil immersion fields before declaring the slide as negative. If time permits, scan the whole thick film.

**Procedure for examining the thin blood film**

1. If the blood film is positive for malaria parasite on the thick blood film a careful examination of the parasite morphology should continue on the thin blood film for verification of species.
2. If different species are observed, all types should be recorded.

**Procedure for Estimating Parasite density**

- Parasites/µl of blood by counting parasites against 200 WBCs in the thick film
- Select a part of the thick film, under oil immersion objective, where the white cells are evenly distributed and the parasites are well stained.
- Using a piano-type tally counter (or 2 single tally counters), count parasites while simultaneously counting WBCs in each field covered.
- Count asexual parasites on the thick film against 200 or 500 WBCs.
- Stop counting after counting 200 WBCs if the asexual parasites counted are greater than 100.
- Continue counting up to 500 WBCs if parasites are less than 100 after 200 WBCS have been counted.
- All parasites in the final field must be counted even if a count of 200 or 500 WBCs has been exceeded. Record actual number parasites and WBCs counted.

**Quality control**

- Before reading the slide, examine the thick and thin films grossly under 40 x objectives to check the quality of the slide as follows and ensure the following:
- Thick film is >90% intact and red cells should be completely lysed, except around the edges.
- WBCs in the thick and thin films are properly stained (i.e., purple granules visible within the cytoplasm of the neutrophils).
- RBCs in the thin film do not appear pink to red.
- Thin film has RBCs that are in one single, distinctive layer.
- Thick or thin films have no significant debris.

## ANNEX IV: Standard operating procedure for parasitological investigation

### A. Direct examination of feacal specimens /wet mount smear preparations

**Procedure**

1. Place one drop of 0.85% NaCl on the slide.
2. Take a small amount of faecal specimen and thoroughly emulsify the stool in saline
3. Slide a 22mm cover slip at an angle in to the edge of the emulsified faecal drop. Push the cover slip across the drop before allowing it to fall into place.
4. Systematically scan the entire 22mm cover slip with overlapping fields with the 10x Objective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs or Protozoa.

### B. Formol-Ether Concentration Technique

**Procedure**

1. Using a stick, emulsify an estimated 1g of faeces in about 4ml of 10% formol water contained in a screw –cap bottle or tube.
2. Add further 3-4ml of 10% formol water, cap the bottle and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical tube and add 3-4 ml of diethyl ether.
5. Stopper the tube and mix for 1 minute.
6. With a piece of wrapped around the top of the tube, loosen the stopper.
7. Centrifuge immediately at 3000 rpm for 1 minute.
8. Using a stick, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, fecal debris and formol water.
9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to re-suspend and mix the sediment.
10. Transfer the sediment to the slide, and cover with cover glass. To assist the identification of cysts run a drop of iodine under the cover glass.
11. Examine the preparation microscopically using the 10x objective with the condenser closed sufficiently to give good contrast. Use 40 x objectives to examine cysts.

## ANNEX-V: Information sheet

**Title of the project**: impact of malaria on hematological profiles of people living with HIV/AIDS in Gambella hospital.

Name of the principal investigator: Tsion Shale

**Name of the organization**: Jimma University (Medical laboratory science and pathology department)

**Purpose**

The aim of this study to assess the effect of malaria on hematological profile of the HIV positive patients in comparison with malaria negative and malaria positive HIV patients in Gambella hospital. In addition, it will also indicate the prevalence hematological abnormalities in HIV and malaria co-infected patients and the factors that cause hematological abnormalities.

**Procedures**

If you are willing to participate in the study and sign a consent form after that the following procedures will be undertaken

- ✓ Your medical history will be reviewed
- ✓ You will provide us at 5 minutes interview
- ✓ 4ml of blood sample will be collected
- ✓ stool sample will be taken
- ✓ The sample will be analyzed for malaria, hematological profile, immunological profile and intestinal parasite.

**Risks and discomforts**

During sample collection we will follow Standard operational procedures. The blood drawing may cause minor pain, at the place where blood is taken. However, this pain will disappear in few hours

**Benefits**

There is no direct financial benefit you get by participating in this study but the test result will be delivered timely and appropriate intervention will be pointed.

**Confidentiality**

Any information obtained during this study will be kept confidential. This is assured by avoiding use of any identifier and information will be recorded with code number.

**Voluntary participation**

Participation on this study is voluntary and you have the right to refuse participation at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put at risk any present or future medical care or other benefits to which you otherwise entitled.

You may ask questions now and in the future if you do not understand something that is being done. Here are addresses of individuals who you can contact:

Tsion sahle, phone no- +251911895173, Email address- sahle_tsion@yahoo.com

Lealem Gedefaw, phone no- +251913024541, Email address- lealew07@gmail.com

Dr. Tilahun yemane, phone no- +251917804067, Email address- yemanetilahun@yahoo.com

የጥናቱተ ሳታፈዎች መረጃ ቅፅ

**የጥናቱ ርዕስ፡** ከኤች ኣይ ቪ ቫይረስ ጋር የሚኖሩ ወገኖች በወባ በሽታ ቢያዙ በደም ህዋሳቶች ላይ የሚያመጣወን ለወጥ

•

የዋና ተመራማሪ ስም:ጽዮን ሣህሌ

የድርጅቱ ስም፡- ጀማ ዩኒቨርስት

ይህ የመረጃ ቅፅ የተዘጋጀው ከላይ በተጠቀሰው ጥናት ለሚሳተፉ የኤችኣይቪ ህሙማን ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሂዳቸው ስለፈለግናቸው ጉዳዮች እና ስለጥናቱ ጠቅላላ ማብራር ይሰጣል ☐ህም በመሆኑ በጥናቱ የሚሳተፉት በራስዎ ፍላጐት ብቻ መሆኑን በትህትና እንገልፃለን፡፡

የጥናቱ ኣላማ

☐ጥናት የሚካሃደው ከቫየረሱ ጋር በሚኖሩ ወገኖች በወባ በሽታ ቢያዙ በደም ህዋሳቶች ላይ የሚያመጣወን ለወጥ ለማየት ወባ የተያዙትን ካልተያዙትጋር በማነፃፀር ለውጦቹን መመልከት.

የጥናቱ ሂደት ዝርዝር

በጥናቱ ለመሳተፍ ከተስማሙ የሚከተሉትን መረጃዎችና ናሙና እንወስዳለን፡

- ✓ የህክምና መዝገብዎ ይታያል ::
- ✓ የ 5 ደቂቃ ቃለ መጠየቅ ይደረግሎታል፡:
- ✓ 4 ሚሊ ሊትር ያክል የደም ናሙና ይወስዳል፡:
- ✓ የ**ሰገ**ራ ናሙና ያ**መ**ጣሉ;;
- ✓ የተወሰደዉ ናሙና ኣስፈላጊዉ ምርመራይ ደረግበታል።

ስጋት ወይም ጉዳት

ህክምናው የሚያስገድደውን የአሰራር ሂደት ስለምንከተል ሊያጋጥሙ የሚችሉ የህመም ስሜት በጣም አነስተኛ ነዉ :: ቢሆንም የደም ናሙና በሚወስድበት ጊዜ ትንሽ የህመም ስሜት ሊያጋጥም ይችላል ፡፡ ነገር ግን ይህ ህመም በአጭር ጊዜ ይጠፋል፡፡

ሊያስገኛቸው የሚችሉት ጥቅሞች

በዚህ ጥናት ውስጥ በመሳተፍዎ በጥሬ ገንዘብ የሚደረግ የካሳ ክፍያ አይኖርም ::ነገር ግን የምርመራዉ ዉጤት በወቅቱ የሚሰጥ ሲሆን በምርመራዉ ዉጤት መሰረት ኣስፈላጊዉ የህክምና እርዳታ ይጠቆማል።

የጥናቱ ምስጢራዊነት

ማንኛውም በጥናቱ የሚገኙ መረጃዎች በምስጢር ይጠበቃሉ:: የጥናቱ መረጃዎች በሙሉ የሚቀመጡት ከእርሶ ስም ጋር ሳይሆን ለጥናቱ ተብሎ በሚሰጠው ስውር ቁጥር ሲሆን ጥናቱን ከሚያስከሄዱት ባለሙያዎች በስተቀር ማንም ሊያውቅ አይችልም፡;

በፍቃኝነት □ከመሠተ□ መበት

በዚህ ጥናት ውስጥ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ይሆናል :፡ በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብትዎ ሙሉ በሙሉ የተጠበቀ ነው:: በጥናቱ ባለመሳተፍዎ ወይም ከጥናት በመገለልዎ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታ ላይ ተፅዕኖ አይኖረውም ፡ከዚህ በፊት ሲያገኙ ከነበሩት ጥቅሞች አንዳች ነገር አይጐሎቦትም፡ጥናቱን የሚያከናውነው አካል ወይም ድጋፍ ሰጭ አካል ከእራስዎ ጥቅም ሲባል በጥናቱ እንዳይሳተፉ ሊከለከል ይችላል፡፡

ስለ ጥናቱ ማንኛውንም ጥያቄ ወይም እርስዎ በዚህ ጥናት ውስጥ ለሚኖርዎት ድርሻ ፣አሳሳቢ ጉዳት ወይም ቅሬታ ካለዋት የሚከተሉትን ስልኮች ወይም ኢሜል አድራስ መጠቀም የጥናቱን ባለቤቶች ማነጋገር ይችላሉ፡፡

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ለጥናታችን ስኬት ትክክለኛዉን መረጃ እንዲሰጡ በኣክብሮት እንጠይቃለን።

## ANNEX-VI: Consent form

Participant Code Number_____

Participant full name _____

I am understood aim of above mentioned research because informed fully in my own language and am willing to participate in the study on impact of malaria on hematological parameter of people living with HIV/AIDS. I have been informed that medical history, blood samples will be taken and there will be minimal risk during sample collection. In addition I have been told all the information collected throughout the research process will be kept confidential.

Code of participant: _____

Signature_____

Date: _____

Name of the witness _____

Signature_____

Date: _____

የስምምነት ቅፅ

የተሳታፊው መለያ ቁጥር _____

የተሳታፊው ስም _____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ ከኤችኣይቪ ቫይረስ ጋር የሚኖሩ ወገኖች በወባ በሽታ ቢያዙ በደም ህዋሳቶች ላይ የሚያመጣወን ለወጥ በሚል ርዕስ በሚቀርበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ ስላገኘው ጥናቱ ላይ ለመሳተፍ ተስማምቻለሁ::የህክምና መረጃና የደም ናሙና ምንም አይነት ጉዳት በማያደርስ መልኩ እንደሚወሰድ ተረድቻለሁ :: በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጥር እንደሚያዙ ተነግሮኛል::

የታካሚ/ የተሳታፊ

ስም _____

ፊርማ ----------

ምስክሮች

1. ስም ----------

   ፊርማ ----------

2. ስም ----------

   ፊርማ----------

## ANNEX-VII: Questionnaire

A research questionnaire

Date of data collection: _____

PART 1:

1. Are you pregnant?
2. Are you taking malaria treatment at this time?

❖ If the answer of above questions is NO continue part two

PART-2: Socio-demographic data

**1.** Unique ART nº_____

2. Ages _____
3. Sex:
   - ☐ Male
   - ☐ Female

1. Residence
   - ☐ Urban
   - ☐ Rural
2. Marital status:
   - ☐ Single
   - ☐ Married
   - ☐ Divorced
   - ☐ Widow(er)

PART 3: Clinical data collection

1. Weight (KG)_____
2. Height(cm)_____
3. Are you on antiretroviral treatment?

   ☐ Yes

   ☐ No

4. Type of HAART regimen

   ☐ ZDV/3TC/NVP

   ☐ TDF/3TC/ NVP

   ☐ D4T/3TC/ NVP

   ☐ ZDV/3TC/EFV

   ☐ TDF/3TC/ EFV

   ☐ D4T/3TC/EFV

   ☐ other _____

5. Is there any current opportunistic infections?

   ☐ yes, Specify_____

   ☐ no

6. Do you use any therapeutic drugs other than HAART?

   ☐ yes, Specify_____

   ☐ no

7. Is s/he has any chronic illness?

   ☐ Yes, specify_____

   ☐ No

PART- 4: Laboratory data results register

Immunohematological test results

| **Test values** | **Current test result** |
|---|---|
| RBC×$10^{12}$/L | |
| Hgb g/dl | |
| HCT% | |
| MCV fl | |
| MCH pg | |
| MCHC g/dl | |
| WBC×$10^{9}$/L | |
| NEUT% | |
| LYM% | |
| MID% | |
| PLT×$10^{9}$/L | |
| $CD_4^+$ cell/µl | |

1. Blood film examination_____

   Malaria: P.F ☐ P.V ☐ Mixed ☐

2. Stool examination ……..

❖ Thank you for giving your time///

## Declaration sheet

I, the undersigned declare that this thesis is my own work and that all sources of materials used for the thesis have been fully acknowledged.

**Principal investigator**

Tsion Sahle (BSc, MSc Candidate; Jimma University)

Signature _____

Date_____

**Place of submission:** Jimma University College of Health Sciences Department of Medical Laboratory Sciences and pathology.

Examiner

Wondemagege Addisu (BSc, MSc)

Signature----------

Date ----------

**Advisors**

1. Mr. Lealem Gedefaw (BSc, MSc)

   Signature _____

   Date_____

2. Dr. Tilahun yemane (MD, MSc)

   Signature_____

   Date_____