**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCES**

# Assessment of clinical and hematological alteration among visceral leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019

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This is to certify that the thesis prepared by Gebremedhin Gebremichail, entitled: **Assessment of clinical and hematological alterations among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019** complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Chairman of the Department or Graduate Program Coordinator

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**Abbreviations**

| | |
|---|---|
| AAU | Addis Ababa University |
| CBC | Complete Blood Count |
| CI | Confidence Interval |
| DC | Direct Current |
| DM | Diabetes mellitus |
| DREC | Department Research Ethics Committee |
| HCT | Hematocrit |
| HGB | Hemoglobin |
| HIV | Human Immunodeficiency Virus |
| $K_2$EDTA | Di-Potassium Ethylene Diamine Tetra Acetic Acid |
| KAH | Kahsay Abera Hospital |
| LD | Leishmania donovani |
| MCH | Mean Cell Hemoglobin |
| MCHC | Mean Cell Hemoglobin Concentration |
| MCV | Mean Cell Volume |
| MPS | Mononuclear Phagocytic System |
| MPV | Mean Platelet Volume |
| NCNC | Normocytic Normochromic |
| NLR | Neutrophil lymphocyte ratio |
| PCV | Packed Cell Volume |

| | |
|---|---|
| PDW | Platlete distribution widith |
| PI | Principal Investigator |
| PLR | Platelet lymphocyte ratio |
| QC | Quality Controls |
| RBC | Red Blood Cell |
| RDW | Red Blood Cell Distribution Width |
| SD | Standard Deviation |
| SOP | Standard Operating Procedure |
| SPSS | Statistical Package for Social Sciences |
| TLC | Total Leukocyte Count |
| VL | Visceral leishmaniasis |
| WBC | White Blood Cells |
| WHO | World Health Organization |

**Abstract**

**Background:** Hematological abnormalities are common in visceral leishmaniasis patients, which is one of the major public health problems worldwide. The most common hematological abnormalities are anemia, leucopenia and thrombocytopenia.

**Objective:** To asses clinical and hematological alteration among visceral leishmaniasis patients attending Kahsay Abera and Mearg Hospitals,Western Tigrai, Northern Ethiopia , 2019

**Method**: Institutional based comparative cross sectional study was conducted from November 2018 to March 2019 at Kahsay Abera and Mearg hospitals, Western Tigrai, Northern Ethiopia. A total of 100 Visceral Leishmaniasis patients and 100 healthy control groups were included in this study. Blood was collected and analyzed by sysmex KX-21N hematology analyzer. Data was entered and analyzed using SPSS Version 23. Student independent t-test was used for data analysis. P value <0.05 was considered as statistically significant at 95% confidence level.

**Result**: From the total 100 visceral leishmaniasis patients the following abnormalities were reported: 96(96%) anemia, 95(95%) leucopenia, 92(92%) neutropenia, 73(73%) Lymphopenia, 45(45%) eosinopenia and 97(97%) had thrombocytopenia. Splenomegally and fever was seen in all visceral leishimaniasis patients. Red blood cell, hemoglobin, hematocrit, red cell indices and platelet were significantly lower ($p<0.05$) in visceral leishmaniasis patients compared with the control groups. Similarly the total WBC, neutrophil, lymphocyte, eosinophil and basophil count were significantly lower ($p<0.05$) in visceral leishmaniasis patients compared with the control groups.

**Conclusion:** The principal changes in peripheral blood of patient with visceral Leishmaniasis are reduced number of red blood cells, reduction in leukocytes and decreased platelet count. VL patients presented with splenomegaly, fever, bleeding, anemia, leucopenia and thrombocytopenia. This finding indicates that visceral leishmaniasis causes alterations of hematological parameters.

**Key words**: Amastigote, Anemia, Hematological Parameters, Promastigote, Visceral leishmaniasis, Ethiopia

# 1. Introduction

## 1.1. Background

Blood is a specialized body fluid composed of a liquid called *plasma* and of cellular elements, including white blood cells , platelets, and red blood cells **.** The normal adult body composed of about 6 liters blood, which accounts 7% to 8% of the total body weight. Plasma consists of 55% of the blood volume, whereas about 45% of the volume is composed of red blood cells and 1% of the volume is composed of white blood cells and platelets. Hematology is primarily the study of the formed cellular blood elements. Blood has different functions like respiration, circulation, excretion, osmotic balance and transport of metabolic substance and it delivers necessary substances to the body's cells. Deviation of in the quantity of these blood elements are often the first indicator of disease occurring in body tissue[1].

Changes in the hematological parameters are common in patients having Visceral Leishmaniasis(VL).This chronic disease is characterized by irregular fever, hepatosplenomegaly and pancytopenia ,progressive weakness and which can cause death if left untreated [2].

From VL associated hematological changes, anemia is the most common abnormality. The mechanism of this anemia is due to multiple factors: this may be due to sequestration and destruction of red blood cells (RBC) in enlarged spleen, opportunistic infection, chronic disease**,** certain nutritional deficiencies, immune mechanism and alterations in RBC membrane permeability have been implicated [3].

Leucopenia is another hematological change seen in VL. The absolute number and percentage of neutrophils in VL are usually decreased, and there is a shift towards left in case of juvenile neutrophils. The mechanism of this neutropenia is thought to be hypersplenism. While absolute number of lymphocytes is mildly decreased, there is relative lymphocytosis by increased the percentage of lymphocyte. The eosinophilis number is significantly decreased, or completely disappear from peripheral blood [4].

Thrombocytopenia is a relatively late manifestation. Splenic sequestration and immune-mediated mechanisms are mainly thought to be responsible for decreased in platelet count. Because of decreased platelet count, bleeding manifestations are very common[4].

VL is a systemic infection of the reticuloendothelial system caused by protozoa Leishmania donovani (LD) which belongs to the genus Leishmania. Leishmania donovani complex are the causative agents of *VL* in Ethiopia [5].It is transmitted by female Phlebotomine sandflies as a flagellated, metacyclic promastigote, which is phagocytized by host macrophages and then differentiates into the non-flagellated, replicative amastigote **[6]**.

The amastigote form exists and multiplies in the mononuclear phagocytic system (MPS), mainly spleen, liver and bone marrow. This leads to hyperplasia of the MPS with resultant disturbances in phagocyte bearing of organs and producing hematological manifestations. Hence, the spleen, in particular becomes massively enlarged[3].

VL is usually diagnosis by combination of clinical and laboratory findings. The diagnosis of VL is confirmed by microscopic demonstration of amastigote in spleen or bone marrow biopsies. Serological tests, such as rK39 dipsticks and polymerase chain reaction can also use for the diagnosis of VL[6].

## 1.2. Statement of the Problem

Visceral leishmaniasis is a potentially fatal human disease with an estimated incidence of at least 0.2 to 0.4 million cases worldwide, causing 20,000-40,000 deaths annually [6]. VL is primarily distributed in East Africa, South Asia, South America, and Mediterranean Region, with an estimated 50,000 to 90,000 new VL cases each year. More than 90% of reported VL cases occur in Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan [7]. VL has been occurred in 88 countries and about 500 000 new cases has been reported each year [8, 9]. In Africa at least 4,000 deaths are reported annually. The worst epidemic VL was recorded which leads to death of 100,000 people in western upper Nile area of Southern Sudan[10]. In Ethiopia 4,000 VL cases was reported annually and 60% of cases were reported from the most endemic areas in Metema and Humera lowlands of Northwest[11].

Visceral leishmaniasis is causes different hematological abnormalities such us hepatosplenomegaly, anemia, leucopenia and thrombocytopenia. Normocytic normochromic anemia is common significant feature of VL and Hb level of 7-10g/ dl were commonly found. Hemolysis is the major cause of anemia, though there may also be plasma volume expansion associated with massively enlarged spleen [3].

Thrombocytopenia is also one of the hematological changes in VL patients where platelets counts can adversely affected after long duration of illness , because the duration of illness is significantly longer in thrombocytopenic patients compared to non-thrombocytopenic. Reports confirm that VL disease decrease platelet production and lead to thrombocytopenia [3].

Leucopenia is an important abnormality seen in VL. It occurs early in the course of the disease. As early as in the beginning of twentieth century, researches stated that, clinicians should suspect kala-azar if the proportion of leukocykes to red blood cells is decreased to around `1:1500. In the two large studies conducted in the past, the total leucocyte count was 2800/cm3 and 4000/cm3 respectively. The leucopenia in VL is mainly due to neutropenia. The percentage and absolute number of neutophlls in VL are remarkably decreased, and there is a shift towards left in case of juvenile neutrophils. The main cause of neutropenia is thought to be hepersplenism. While absolute number of lyphocytes is mildly decreased, there is relative lymphocytosis. The number of eosinophilis is decreased significantly, or they completely disappear from peripheral

blood. While the percentages of monocyes are increased, there is no consensus among various authors about the absolute number of lymphocytes in VL [4].

Many studies have reported pancytopenia in the late stages of VL. There is variation in the frequency of pancytopenia reported by various researchers. The cause of pancytopenia is thought to be due to sequestration of blood cells in the spleen. The presence of reticulocytes and immature blood cells in peripheral blood helps in differentiating pancytopenia due to VL from aplastic anemia. The clinical picture in these patients can mimic leukemia, especially in the presence of fever, hepatomegaly and lymphadenopathy. In those cases bone marrow examination is very useful for making accurate diagnosis [4]. While several studies pointed to the hematological profile of visceral leishmaniasis, there are limited studies in this regard in our country, particularly there are no published studies in the study area.

So the aim of this study is to asses the clinical and hematological alteration of visceral leshimaniasis patients attending in Kahsay Abera and Mearg hospital, western Tigrai, Northern Ethiopia.

### 1.3. Significance of the study

Hematological abnormalities cause life threatening consequence in VL patients that required a rapid and specific treatment. However, in our setting, the hematological profiles among VL infected individuals are not well documented. Therefore, the finding of this study is important to provide substantial information for policy makers and health administrators to implement appropriate interventions in combating visceral leishmaniasis associated and hematological complication. Also the finding of this study is used to provide information to clinicians about the hematological abnormalities, which may contribute in improving the management of individuals infected with VL through early diagnosis and preventing hematological complications. Besides; the finding of the study may serve as a reference data for future researchers who have interested in similar topics.

## 2. Literature Review

A descriptive analytic study was conducted in Yemen on Hematological Characteristics of Yemeni Adults and Children with Visceral Leishmaniasis. A total of 47 (32 males and 15 females) Patients and 51 non-VL subjects (control group) was included in the study. The result showed that all patients with VL had anemia, 41 (87%) leukopenia, 42 (89%) neutropenia, 44 (94%) thrombocytopenia, 42 (89%) eosinopenia, 25(53.2%) lymphopenia.The mean values of the peripheral blood counts of VL patients versus control subjects were (14.19±22.20 versus 88.10±108.84 ($x10^6$/L) for Eosinophil's, 992.67±773.71 versus 1347.76±630.26 ($x10^6$/L) for lymphocytes and 2097.87±1304.59$x10^6$/L versus 2703.92±826.55$x10^6$/L for WBC. Comparison of VL patients with the control group showed significantly more frequent peripheral blood eosinopenia counts (p value 0.000) and lymphopenia (p value 0.014). There was no significant difference between adults and children in any of the hematological features [12] .

A cross sectional study was conducted on 40 leishmania donovani bodies' positive cases over the period of one year in Dharan, Nepal the age ranged from 2-60 years. Male (55%) affected predominantly than females (45%). The result revealed that the most common symptoms was splenomegaly (82.5%), hepatomegaly (65%), and pallor (75%).The laboratory result showed that Anemia was present in 90%, leucopenia in 67.5% and thrombocytopenia in 72.5% cases. normocytic normochromic blood picture was predominantly seen in the peripheral examination of RBC morphology. Hepatomegaly, neutropenia, anemia and lymphocytosis were statistically significant to parasite load (p-value <0.05) [13].

A retrospective study was conducted in North Bihar India in a total number of 43 cases were diagnosed as Kalaazar (VL). In this study all 43 VL cases presented with fever as the commonest symptom whereas splenomegaly was the commonest clinical sign present in each and every case (100%). 39 cases (90.7%) of the VL cases presented with pallor during clinical examination and all 43 cases (100%) had splenomegaly. Hepatomegaly was seen in 26 cases, jaundice 9 (20.3%), generalized weakness 93%, bleeding manifestation (9.3%). *L*ymphocytosis was the most common (100%) hematological abnormalities in this study. Leukopenia was observed in 60.4%, Pancytopenia 60.5 % and thrombocytopenia was seen in 83.7% cases. Thrombocytopenia was the cause of bleeding manifestation in this study. On peripheral blood smear microcytic hypochromic blood picture was the most common finding seen in 26 cases (60.46%) followed by

normocytic normochromic blood picture in 20.9% and macrocytic blood picture in 11.6% cases [14].

Another cross-sectional study was conducted in India on hematological parameters in Visceral Leishmaniasis on 30 patients (18 Patients were male and 12 patients were female). The mean age of patients was 35.4 ±16.3 with male: female ratio 1.5:1. In this study the Hematological parameters studied showed that mean hemoglobin (Hb) was 6 ± 1.3 gm/dl (Range 5-7.1gm/dl). Leucopenia was present in 25 patients. Mean value of the total Leucocyte count (TLC) was 3.6 ± 2.4 x $10^9$/L (Range 2.3-3.6 x $10^9$/L). Thrombocytopenia was seen in 22 patients and mean platelet count was 88.7 ± 62.2 x $10^9$/L (Range 43-152 x $10^9$/L). Pancytopenia was noted in 20 patients [15].

A cross sectional observation study was conducted at North Bengal Medical College, West Bengal, India over a two year period. From a total of 36 cases of VL; the male to female ratio of the cases was 1.6:1 and the mean age was 20.1±11.1 years. Splenomegaly and fever were the most common symptoms fond in all 36 cases. Weakness, abdominal pain, bleeding, and hepatomegaly were seen in 63.9%, 27.8%, 8.3% and 58.3% of cases, respectively. Pancytopenia, Bicytopenia, leukopenia and thrombocytopenia were seen in 58.3, 41.7, 61.1 and 83.3% of cases, respectively [16].

A clinico-haematological profile of childhood visceral leishmaniasis was conducted in a single-center study from Islamabad, Pakistan. The study included a total of 32 cases of childhood Visceral Leishmaniasis. Out of these, 20 cases were observed in males and 12 cases in females. Fever, pallor and abdominal distention were seen all cases. Among the hematological features, pancytopenia in the peripheral blood was present in 20(62.5%) cases. The values of peripheral blood counts were not significantly associated with the presence or absence of lymphadenopathy **[17]**.

A study was conducted in Yemeni on Clinical and hematological manifestations of visceral leishmaniasis in children. A total of 64 cases were diagnosed as childhood VL out of these 33 patients were female and 31 were male. Clinical examination of patients with VL revealed that fever was seen in 100% of children with duration before diagnosis of 56 days. Splenomegaly was present in all cases and hepatomegaly in 84.4%. The laboratory blood results revealed that

hemoglobin concentration ranging from 2.4 to 10 g/dl (mean: 6.6±1.7 g/dl). The red blood cell count ranged from 0.8 to 3.5x1012/L (mean: 2.4±0.6x1012/L. Total white blood cell count ranged from leukopenia of 1.1x109/L to near normal count of 8.5 x 109/L (mean: 3.5±1.6x109/L). The mean absolute neutrophil count was 0.78±0.56x109/L (range: 0.032 to 2.31x109/L). The platelets also showed a count ranging from thrombocytopenia of 5x109/L to normal range of 188x109/L (mean: 71.7±41x109/L).On peripheral morphology microcytic hypochromic blood picture 32(50%), macrocytic normochromic 20 (13%) observed on VL patients. The presence of peripheral pancytopenia was noticed in 45 patients (70.3%) with VL, while the remaining patients had anemia plus either leukopenia or thrombocytopenia [18].

Clinical and Epidemiological Features of Visceral Leishmaniasis among Children was also investigated in a similar study in Yemen. A total of 106 children were included in this study, 94 patients (88.7%) were from rural settings and (11.3%) from Sana'a city. Sixty-eight children (64.2%) were males and 38 (35.8%) were females ($p < 0.00$). Clinical examination of the patient revealed that fever was constantly present in all patients; other features included splenomegaly (96.2%), pallor (90.5%), hepatomegaly (71.7%). Various degree of anemia was evident in all patients with mean hemoglobin concentration (7.0 ± 1.49 g/dl). Leucopenia, neutropenia and thrombocytopenia were found in 62.2%, 73.5%, and 87.7%, respectively. Anemia, leucopenia and thrombocytopenia had no significant association with parasite load [19].

Another descriptive study was conducted to observe clinical and hematological findings of visceral leishmaniasis patients from Northern areas of Pakistan. A total of 70 cases were included in the study out of these, 49 (70.0%) were males and 21 (30%) were females. Age of the patients ranged from 1.6 to 25 years with a mean of 03.51+3.56 years. Fever and splenomegaly were present in all the patients and pallor, hepatomegaly and abdominal distention in majority. Hemoglobin ranged from 03 to 12 grams per drci liter with a mean of 6.42±2.19. Mean RBC count was 3.41±0.87 X $10^{12}$ per liter, mean WBC count 5.15±2.64 X 109 per liter, and mean platelet count 49.5±45.8 X 109 per liter[20].

A retrospective study was conducted at a tertiary care center serving the kumaon region of Uttarakh. In this study 20 LD positive cases and 20 LD negative cases was evaluated. Splenomegaly was the most common sign present in 17 cases (85%) and 15 (75%) of LD negative cases. Blood examination of the patients' states that anemia was observed in both LD

positive and negative cases 100%. Leucopenia with relative lymphocytosis was more observed in LD positive cases than LD negative cases. Pancytopenia was seen in 85% LD positive and 70% of LD negative cases, Bicytopenia in 15% LD positive and 30% LD negative cases while thrombocytopenia was seen 85% LD positive and 90% LD negative cases. Peripheral blood smear examination revealed microcytic hypochromic blood picture as the most common finding in 11 cases (55%) followed by normocytic normochromic (NCNC) blood picture in 7 cases (35%) and macrocytic blood picture in 2 cases (10%) of LD positive cases [21].

A total of 34 children with confirmed VL through 2004-2011 were included in a study conducted on Clinical Features and Laboratory Findings of Visceral Leishmaniasis in Children Referred to Children Medical Center Hospital, Tehran, Iran. The mean age of these patients were 26.9 ± 18.9 months (range from 6 to 92 months) and 91.2% of them were under the age of 5 years. The male to female ratio was 0.8 and there was no statistically significant difference in gender over time. The most prevalent symptoms were fever (97.1%), pallor and weakness (97.1%) and appetite loss (61.8%).The most frequent signs at admission were splenomegaly (97.1%) and hepatomegaly (88.2%). The mean and standard deviation of the laboratory results WBC (/mm3) (4681.7± 3521.3) ,Hemoglobin (%) (7.25± 1.44), and Platelets (103/mm3) (95.5± 97.9). The most frequent laboratory abnormalities were hematological including anemia (97.1%), thrombocytopenia (91.2%) and leukopenia (67.6%) **[22]**.

From similar study conducted on Clinico-Hematological Analysis of 380 Visceral Leishmaniasis case in Southwestern Iran, 217 (57.1%) were male and 163 (42.9%) were female. The majority of the cases (91.5%) were 5 years old. Bone-marrow aspiration detected Leishmania amastigote only in 26.6% of cases. Fever (98.1%), abdominal protrusion (65.1%) and hepatosplenomegaly (63.7%) were the most common clinical presentations of the patients. Among the hematological abnormalities pancytopenia was observed in 43.1%, anemia in 87.3% and thrombocytopenia in 64% of cases [23].

Clinical and Hematological Features of visceral leishmaniasis was investigated in 17 patients in Sudan. In this study all 17 patients were males and aged 10 to 48 years (mean 24 ± 8 years). They were all farmers or laborers who came from endemic areas around Gadaref (15 patients), Darfur (one patient), and Malakal (one patient). Symptoms had lasted from 15 days to 6 months (average, 3 months). Fever, weight loss, and signs of anemia were found in all cases .The

hemoglobin concentration was low in all patients (mean, 77 ± 12 g/L). The peripheral blood films showed anisopoikilocytosis with a preponderance of macrocytes in nine patients. Slight to moderate polychromasia was common. All patients were leukopenic, with neutropenia in 15 (88%) and Lymphopenia in 16 (94%). Thrombocytopenia was present in all patients. The mean platelet count was 51 ± 41 × $10^9$/L. Counts below 50 × 109/L were found in 11 (65%) patients, and the 8 patients with epistaxis also had severe thrombocytopenia [24].

From similar study conducted on hematological profile of patients with visceral leishmaniasis at Al-Gaderf State – Sudan. Most affected pediatric patients with age less than 10 years. Among adult patients the most frequent age group ranged between 21 and 30 years. Both Males and females were infected, but males showed higher frequency. Significant decrease was observed in Hb and RBC of pediatric patients (7.2 ± 1.6 g/dl and 3.6 ± 0.7×$10^6$ µl respectively compared to control group (12.3 ± 1.1 g/ dl and 4.7 ± 0.4 ×$10^6$ µl respectively). Total WBC count and neutrophil percent decreased significantly (p-value=0.000). Thrombocytopenia was also observed in patients in both groups (p-value= 0.001) [25].

A cross sectional study was conducted in Gonder on Hematological Abnormalities in Visceral Leishmaniasis Patients. From the total 414 Visceral Leishmaniasis patients, 405(97.8%) were males and 9(2.2%) were females. The overall magnitude of anemia, leucopenia, thrombocytopenia, neutropenia and Lymphopenia was 94.4%, 95.4%, and 90.1%, 90.1% and 37.9% respectively. There was a significant association between age and prevalence of anemia, neutropenia and thrombocytopenia. The finding of the study shows with predominant existence of anemia, leucopenia and thrombocytopenia [26].

Humera, a town in northern Ethiopia, is one of the areas where visceral leishmaniasis is very common. However, published studies are lacking from the area to support evidence based management of patients. This study is trying to address this gap.

## 2.1. Conceptual Frame Work

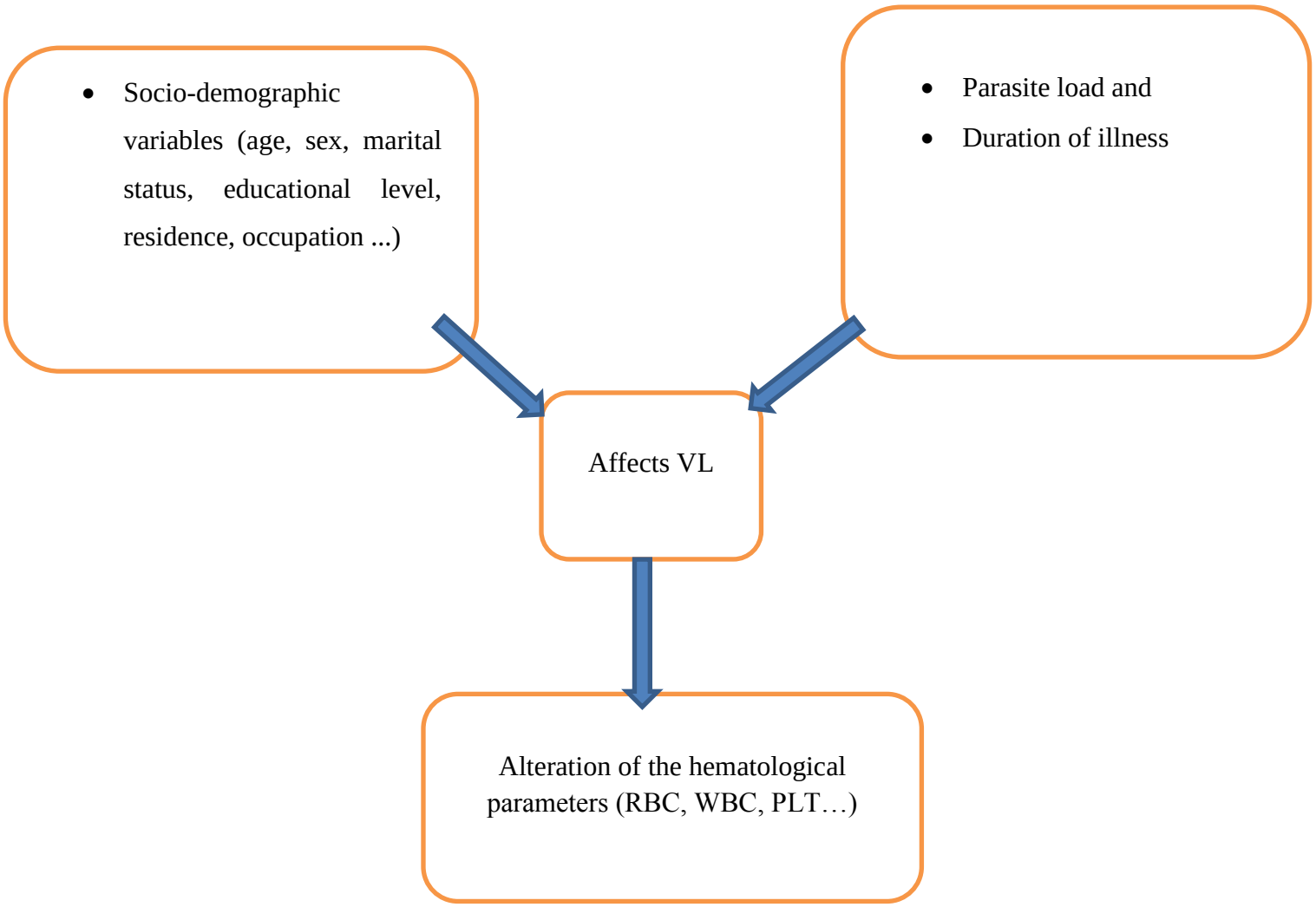


**Figure 1: Conceptual frame work**

## 3. Objectives

### 3.1. General objective

To assess clinical and hematological alteration among visceral leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019.

### 3.2. Specific objectives

- To assess clinical features of visceral leishmaniasis patients
- To determine hematological parameters among visceral leishmaniasis patients as compared with controls
- To determine peripheral blood abnormalities of the visceral leishmaniasis patients

## 4. Hypothesis

Ho- There is no significance difference of hematological parameters among VL patients and control groups.

## 5. Materials and methods

### 5.1. Study area

The study was conducted in Kahsay Abera and Mearg Hospitals, western Tigrai, Ethiopia. Western zone of Tigray is the biggest in terms of geographic and territorial possessions that stretches along the border of Sudan in the North West, the Amhara Adminstativeregion in the south and the Eritrea border in the north where people live in clusters over a wide range of areas. It is one of the agriculture surplus areas in Ethiopia known for producing sorghum and exportable products like sesame which makes it a net contributor to the national economy. Humera is located in north western Ethiopia 984 km far from the capital city Addis Ababa.Western Tigrai located at longitude and latitude 14$^{o}$18'N 36 $^{o}$37'E with an elevation of 585 meters above the sea level. Kahsay Abera Hospital is a district hospital in a visceral leishmaniasis endemic region in north Ethiopia with about 210 beds and an estimated 742,000-catchment population including workers. Mearg hospital is a district hospital which is found in VL endemic area in Tsegedia Woreda western Tigray which have an estimated 299,594 catchment population migrants and have 134 beds at emergency, medical, surgical and gynecology and pediatrics ward [27].

### 5.2. Study design and period

An institutional based comparative cross-sectional study was conducted from November, 2018 to March, 2019.

### 5.3. Population

#### 5.3.1. Source population

Case group

- All VL suspected patients who visiting the Kahsay Abera and Mearg Hospitals during the study period.

Control group

- All staff members of Kahsay Abera and Mearg Hospitals who were avalilable during the study period.

### 5.3.2. Study population

Case group

- All VL patients confirmed at Kahsay Abera and Mearg hospital laboratories during the study period.

Control Group

- Apparently healthy individuals of Kahsay Abera and Mearg hospitals workers who were match with cases in age and sex without having VL.

## 5.4. Inclusion and exclusion criteria

### 5.4.1. Inclusion criteria

Case group

- All VL patients confirmed at the Kahsay Abera and Mearg Hospital Laboratories at the study period and willing to participate in the study.

Control Group

- All staff of Kahsay Abera and Mearg hospitals that had the similar age range to study participant without having visceral leishmaniasis and willing to participate in the study. The control groups were selected by physicians based on the WHO guide line for the diagnosis of VL and rk-39 test was used to screen the controls.

### 5.4.2. Exclusion criteria

Case group

- Patient already on anti- VL treatment
- Patients who have history of hematological malignancies , HIV, malaria and other chronic diseases

Control group

- Individuals who have history of hematological malignancies , HIV, malaria and other chronic diseases

### 5.5. Study variables

#### 5.5.1. Dependent variables

- Hematological parameters
    - RBC parameters
    - WBC parameters
    - PLT parameters

#### 5.5.2. Independent variables

- Age
- sex

### 5.6. Sample size calculation and Sampling method

#### 5.6.1. Sample size calculation

The sample size for this study was calculated using sample size calculation for comparison of two independent means of 95% confidence level and 80% power. From research done in Yemen on hematological characteristics of yemeni adults and children with visceral leishmaniasis eosinophil and lymphocyte count of study and control group with the following result was used to calculate sample size [12].

**Table 1: Mean and standard devation of the VL patients and control subjects**

| Mean and standard deviation of the VL patients and control subjects in Yemen [12] | | | | |
|---|---|---|---|---|
| no | Blood count | Mean and SD of the VL patients | Mean and SD of control subjects | Calculated sample size |
| 1 | Eosinophil | 14.19±22.20 | 88.10±108.84 ($x10^6$/L) | 18 |
| 2 | Lymphocyte | 992.67±773.71 | 1347.76±630.26 ($x10^6$/L) | 62 |

$$n = \frac{(s_1^2+s_2^2) * (Z\alpha+Z\beta)^2}{d^2}$$

Where, **n**= desired sample size, **S**= Standard deviation (s1/study group=773.71, s2/ control group=630.26) from previous study, Zα=1.96, Zβ = 0.84, **d**= effective size /difference between two means = |u1-u2 |= |992.67-1347.76|=355.09 from previous study.

$$n = n = \frac{(s_1^2+s_2^2)*(Z\alpha+Z\beta)^2}{d^2} = \frac{(773.71^2+630.26^2)*(1.96+0.84)^2}{(355.09)^2} = 61.9 \text{ round to } 62$$

Non response rate (10%) = 62 *0.1= 6.2 round to 7

Minimum sample size =62+7= 69. Therefore the minimum sample size calculated for this study was 138 which consist of 69 VL cases and 69 control group. But the sample size was increased to 200 which consist of 100 VL cases and 100 control groups to gain greater power to detect the difference.

### 5.6.2. Sampling Method

A total number of 100VL patients and100 controls participants were included in this study using convenience sampling method.

## 5.7. Measurement and Data collection

### 5.7.1. Data collection procedure

All confirmed VL patients and control groups were interviewed for socio demographic characteristics by using pre tested semi structured translated questionnaire. The questionnaire mainly consists of closed and open ended questions focusing on socio-demographic data, age, gender, occupation. The clinical data of the VL patients was obtained from their medical record log sheet.The VL cases and control groups were screened by exprianced physians for any chronic diseases. Seventy one study participants were selected from Kahsay Abera hospital and 29 study participants selected from Mearg hospital.Thin and thick blood film was also done for screening of the VL cases and control for identification of hemo-parasite.

### 5.7.2. Laboratory analysis

#### 5.7.2.1. Specimen Collection and Processing

About 3 mL venous blood sample was collected on vacutainer tube containing EDTA after cleaning with 70% ethanol. As soon as the sample was collected and labeled, it was transported to the hematology working area to be analyzed. Blood analysis was done for the hematological parameters using sysmex KX-21N hematology analyzer and peripheral blood morphology was examined.

#### 5.7.2.2. Complete blood count (CBC) analysis

The complete blood count (CBC) is one of the most common blood test used. It consists of the three major types of cells in blood: red blood cells, white blood cells, and platelets. The CBC analyzer counts these cells, measures hemoglobin (the oxygen-carrying molecule in red blood cells), estimates the red cells' volume, and sorts the white blood cells into five subtypes or three types depending on the type of hematology analyzer.

#### 5.7.2.3. Sysmex KX-21N hematology analyzer

The Sysmex KX-21N is a quantitative automated hematology analyzer for *in vitro* diagnostic use. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The KX-21 processes approximately 60 samples per hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and platelets, along with data of 17 parameters, as the analysis results. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM #, MIXED #and NEUT #. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT% [28].

**Detection Principle**

The KX-21N analyzer counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection method. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin is converted to methemoglobin, and read photometrically at 555 nm. White blood cells (WBC) are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophilis [28].

#### 5.7. 2.3.Peripheral blood morphology

Peripheral blood smears were prepared from all participants for investigation of red blood cell morphology, white blood cell and platelets abnormalities.

### Peripheral blood film preparation and examination

A properly prepared blood film is essential for accurate assessment of cellular morphology. Blood film was prepared on glass slide and air dried. The blood film was fixed to the glass slide by the methanol. After drying the film, Giemsa stain was added to stain the cells. Examination of the blood film was a multistep process. Begin the film examination with a scan of the slide using the 10x or low-power objective. This step was necessary to assess the overall quality of the film, including abnormal distribution of RBCs, suggesting the presence of rouleaux or autoagglutination, and/or the presence of a disproportionate number of large nucleated cells such as monocytes or neutrophils at the edges of the film. The next step in film evaluation was to perform the WBC, RBC and platelet. finally examined using the 100x oil immersion objective [29]. 100 white blood cells /HPF was counted by using hemocytometer and sorted in to five differentials by relative percentage of each cell type. The absolute differential count was calculated by multiplying the percentage of each counted cell type by the total WBC (in thousands/µL).

## 5.8. Data Quality Assurance

The quality of any research depends on the quality of data that are used as input for that research. Therefore the quality of the blood and the participant information was ensured by collecting and processing using a standard operating procedure.

### 5.8.1. Pre analytical

To collect the socio-demographic data, age, gender, job, and health status and getting any medications the questioner was translated to local language Tigrigna/Amharic to make easily understandable the questions at the time of interview. Pretest was conduct among of the study population one week before the actual data collection, to ensure clarity, length, wordings, logical sequence and skip patterns of the questions. Before the actual data collection questionnaire was pre tested on 5% participants of VL patients in Sheraro town which is found 172 Km far from Humera town.

Concerning sample collection, transportation and processing the principal investigator together with senior laboratory technologist assembled blood sample collection materials. SOPs were to assure that sample was collected on EDTA tube, labeled with participant identification number, and checked for hemolysis, clot, correct volume etc. Before participants sample were analyzed

availability and expiry dates of reagents were and they were brought to room temperature before use.

### 5.8.2. Analytical

The reliability of the study finding especially the analytical part was guaranteed by running three levels of commercially prepared hematology cell controls (Normal, Low and High). Analysis was performed by following standard operating procedure (SOP) after running and passing of these levels of controls. The peripheral morphology smear was re-examined by trained and experienced laboratory personnel.

### 5.8.3. Post analytic

For avoiding any clerical error printout results that were generated by the analyzer were used. No results from the screen of the analyzers were recorded by hand. Results for peripheral morphology were registered with correct value. Data was entered using double entry method to trace data entry errors which has strong negative effect on study results and conclusions.

## 5.9. Data analysis and interpretation

Data was entered and analyzed using SPSS Version 23. Table and graphs were used for descriptive data. The cut-off points for different parameters were defined according to the laboratory standard defined by the manufacturer instruction.Independent t-test was used to evaluate the compression between VL patients and controls subjects regarding of the hematological parameters (HB, WBC, PLT...) Percentage and frequency was used to evaluate the age, gender, between VL and control subjects. P value less than 0.05 was considered as statistically significant at 95% of confidence level.

## 5.10. Operational definitions

- **Visceral leshimanisis**–A chronic and potentially fetal parasitic disease of the viscera (the internal organ, particularly the liver spleen, bone marrow and lymph nodes) due to infection by the parasite called Leishmania donovani.
- **Anemia-** hemoglobin concentration less than 13 g/dL in men and 12 g/dL in women indicates anemia
- **Thrombocytopenia** - platelet count below 150×103 cells/µl indicates thrombocytopenia
- **Cases groups** -are visceral leishmaniasis patients confirmed at Kahsay Abera and Mearg Hospitals

- **Control groups -** are apparently healthy workers of Kahsay Abera and Mearg hospitals who had the same age to study participant without having visceral leishmaniasis.
- **Leucopenia -** total white blood count <4000 cells/µl classified as leucopenia
- **Anemia severity** - Hgb<8 g/dl have termed as sever for both sex, Hgb =8 – 9.9g/dl (moderate) for females and Hgb =8 – 10.9g/dl for male (moderate) and Hgb =10 – 11.9g/dl for females (mild) and 11-12.9 (mild).
- **Neutropenia** – Neutrohpil count <1500 cell/µl classified as Neutropenia
- **Lymphopenia**- lymphocyte count <600 classified as Lymphopenia
- **Red blood cell Prameters** – are the combination of hemoglobin, hematocrit, MCV, MCH, MCHC, and RDW
- **White blood cells parameters**- are the combination of neutrophil, eosinophil, basophil, monocyte, lymphocyte, and neutrophil to lymphocyte ratio.
- **Platlet parameters**- are the combinations of MPV, PDW, and PLR

### 5.11. Ethical considerations

Before starting the study, ethical clearance was obtained from the ethical review committee of the department of Medical Laboratory Sciences of Addis Ababa University. Further permission was also obtained from Tigrai Regional Health Bureau and from Administrators of selected hospitals. Furthermore, after explaining the importance of the study, an informed written consent was obtained from study participants.The confidentiality of the information collected was maintained by using code numbers for participants. For those participants who had haematological abnormality associated with visceral leishmaniasis, farther diagnosis was done and appropriate treatment was prescribed by physicians.About 40% of the study participants who have any hematological abnormalities was get medication.

### 5.12. Dissemination of the result

Findings of this study will be presented to the scientific community in the Addis Ababa University, department of Medical laboratory science, College of Health Sciences.The result will be disseminated to the study health facilities, weredas and zonal health administrations, Tigrai regional health bureau. Finally it will be submitted to peer reviewed local and international journals for publication.

**6. Work flow**

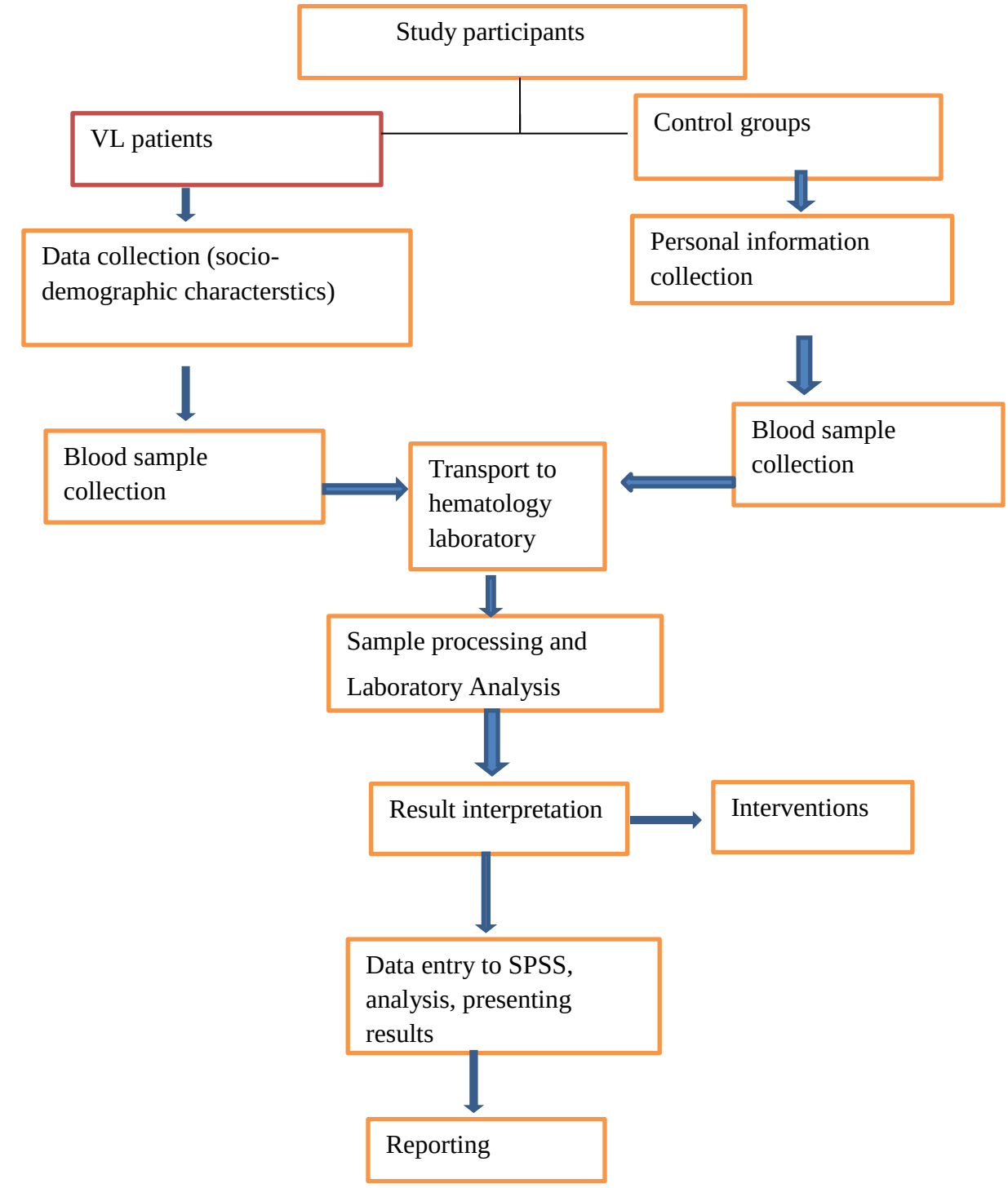


Figure 2: Work flow of the study

## 7. Result

### 7.1. Socio demographic Characteristics of study participants

The study included 200 study participants that comprise 100 VL patients with mean age 27.98±9.634 years and 100 controls with mean age 27.64±4.758 years. Ninety one (91%) was males in case group and 90(90%) were males in control group. Forty nine percent of the VL cases and 55% of the control groups were single. Most of the VL cases were primary school which accounts 51% while 85% of the controls were diploma and above .The majority of the VL cases were from rural residence 94% but all of the controls were urban residents. The detail Sociodemographic characteristics were summarized in table 2.

Table 2: Socio demographic characteristics of study participants in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March, 2019 (n=200)

| Variables | Case group (n=100) | Control group (n=100) |
|---|---|---|
| **Gender** | | |
| Male n (%) | 91(91%) | 90(90%) |
| Female n (%) | 9(9%) | 10(10%) |
| **Age in year n (%)** | | |
| 15-20 | 28(28%) | 4(4%) |
| 21-25 | 20(20%) | 31(31%) |
| 26-30 | 23(23%) | 42(%) |
| 31-35 | 9(9%) | 15(%) |
| >35 | 20(20%) | 8(%) |
| Mean ±SD | 27.98±9.634 | 27.64±4.758 |
| **Marital status** | | |
| Single n (%) | 49(%) | 55(55%) |
| Married n (%) | 37(%) | 42(42%) |
| Divorced n (%) | 13(%) | 3(3%) |
| Widowed n (%) | 1(1%) | 0 |
| **Educational status** | | |
| Illiterate | 29(29%) | 0 |
| Primary (1-8) | 51(51%) | 0 |
| Secondary (9-12) | 20(20%) | 15(15%) |
| Diploma and above | 0 | 85(85%) |
| **Occupational status** | | |
| Students | 15(15%) | 0 |
| Farmers | 24(24%) | 0 |
| Daily laborer | 60(60%) | 0 |
| Merchant | 1(1%) | 0 |
| Government employers | 0 | 100(100%) |
| **Residence** | | |
| Rural | 94(94%) | 0 |
| Urban | 6(6%) | 100(100%) |

In this study the main clinical signs and symptoms presented at the initial evaluation were: fever (100%), splenomegaly (100%), general weakness (85%), skin mucosal pallor (72%), bleeding (67%), weight loss (65%), anorexia (52%) and hepatomegaly (36%) (Table-3).

Table 3: The frequency of clinical features of visceral leishmaniasis patients in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March, 2019 (n=100)

| Sign and symptom | No (%) |
|---|---|
| Splenomegaly | 100(100%) |
| Hepatomegaly | 36(36%) |
| Fever | 100(100%) |
| Weight loss | 65(65%) |
| Jaundice | 21(21%) |
| Skin mucosal pallor | 72(72%) |
| Diarrhea | 15(15%) |
| Anorexia | 52(52%) |
| Abdominal pain | 17(17%) |
| General weakness | 85(85%) |
| Bleeding | 67(67%) |

### 7.2. Hematological abnormalities among VL patients

In this study the most common hematological abnormalities were thrombocytopenia, anemia leucopenia, neutropenia and pancytopenia (Table 4).

Table 4: Frequency of hematological abnormalities of VL patients in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March , 2019 (n=100)

| Hematological abnormalities | N (%) |
|---|---|
| Anemia | 96 |
| leucopenia | 95 |
| neutropenia | 92 |
| Lymphopenia | 73 |
| Eosinopenia | 45 |
| Thrombocytopenia | 97 |
| Pancytopenia | 89 |

The red blood cell morphological characteristics of VL showed that normocytic normochromic ,microcytic hypochromic, dimorphic cells and macrocytic normochromic RBC cells were the

most common red blood cell morphological findings which were present in 60 (60%), 35 (35%), 4(4%) and 2 (2%) respectively (Figures 3).

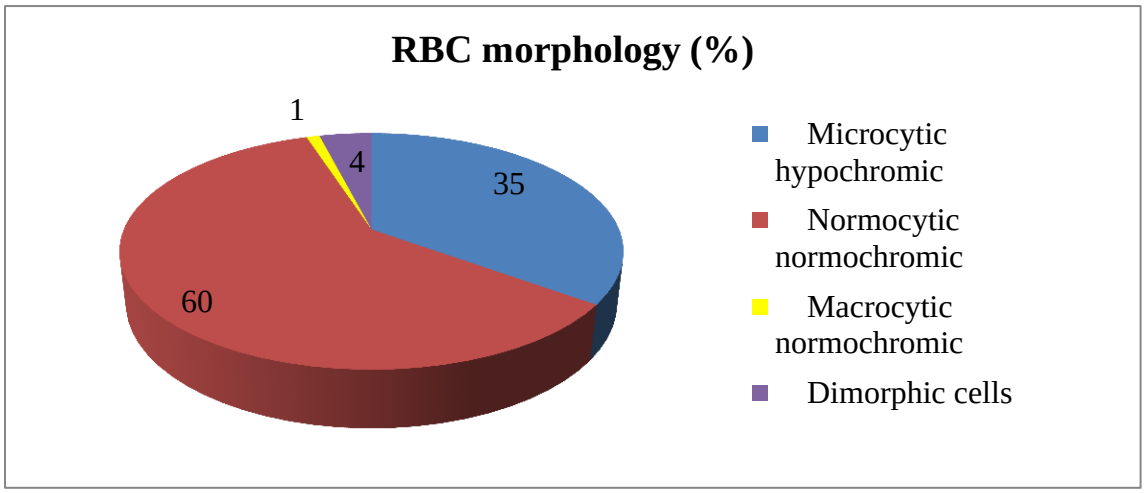


Figure 3: Morphological classification of anemia among VL patients in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March, 2019 (n=100)

Anemia severity

The severity of anemia depends on the hemoglobin level where Hgb<8 g/dl have termed as sever for both sex , Hgb =8 – 9.9g/dl (moderate) for females and Hgb =8 – 10.9g/dl for male (moderate) and Hgb =10 – 11.9g/dl for females (mild) and 11-12.9 (mild). Based on this classification, from the 96 anemic patients, majority of them 52 (54.17%) shows severe anemia. Mild anemia was seen in only 4 patients (4.17%) (Figure -4).

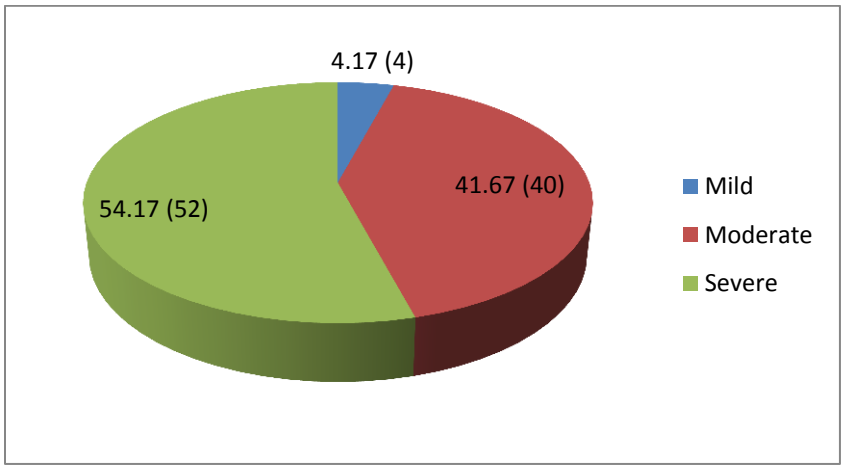


Figure 4: Severity of anemia among Visceral Leishmaniasis patient in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March, 2019 (n=96)

### 7.3. Hematological parameters tests of Visceral Leishmaniasis patients and healthy control groups

In this study the means and standard deviations of hematological parameters of VL cases and controls are presented in Table 5. Based on the analysis, the absolute mean number of RBC ($10^{12}$/L) (3.14± 0.89 vs. 4.76 ±0.50, p=0.001), level of hemoglobin (g/dl) (7.99 ±2.21vs 14.4± 1.487, p=0.001), the percentage of hematocrit (%) (24.8 ±6.6 vs. 41.9± 3.2, p=0.001),), the mean cell volume (fl)( 79.8 ±6.9 vs. 85.8 ±4.9, p=0.001),the mean cell hemoglobin MCH (pg) (25.5±3.1vs 29.9 ±1.9, p=0.001), mean cell hemoglobin concentration MCHC (g/dl) (32.1 ±2.3vs 34.8 ±1.3,p=0.001), and the absolute platlate count ($\times 10^9$/L) (70.8±36.8vs 228.9± 62.5, p=0.001), were significantly lower in VL cases compared with the control groups. Similarly the absolute count WBC ($10^9$/l), neutrophil count, lymphocyte count and eosinophil count were significantly lower in VL cases compared with the control groups with means and standard deviations of (2.12 ±1.0 vs. 6.7± 1.8, p=0.001), (1.0 ± 0.6vs 4.1 ±1.5, p=0.001), (0.9 ±0.5 vs. 1.9± 0.7 , p=0.001), and (0.032±0.033 vs. 0.21±0.14, p=0.001), respectively. Also neutrophil lymphocyte ratio (NLR) and platlate lymphocyte ratio (PLR) were significantly lower in VL cases compared with the control groups with means and standard deviations was (1.37±0.92 vs. 2.65±1.97, p=0.001), (101.83±76.5 vs. 141.2±83.1, p= 0.001). The RDW-CV, lymphocyte percentage, monocyte percentage and monocyte count was significantly higher in VL cases compared with the control groups with means and standard deviations of (17.6±3.53 vs. 13.8± 2.94, p=0.001), (42.7± 13.40 vs. 30.66± 10.73, p=0.001), and (7.9± 5.01vs 6.52± 2.77, p= 0.017), respectively. No statistically significant difference was observed on mixed percentage(%) ,basophile percentage (%) and Mean Platlate volume MPV(fl) with means and standard deviations of (10.26± 6.93 vs. 8.8± 5.15, p=0.094) , (1.46± 1.35 vs. 1.31± 1.22, p= 0.411) and (9.437 ±1.29 vs. 9.72 ±1.03, p= 0.092), respectively.

Table 5: hematological parameter tests for VL patients and control groups in in Kahsay Abera and Mearg hospitals, Tigrai, Northern Ethiopia, November, 2018 to March, 2019 (n=200)

| Parameters | VL cases(n=100) (mean±SD) | Controls (n=100) (mean±SD) | Test of significance (95% confidence interval) | |
|---|---|---|---|---|
| | | | T | p-value |
| **RBC ($\times10^{12}$/L)** | 3.14± 0.89 | 4.76 ±0.49 | -15.86 | 0.001 |
| **HGB (g/dl)** | 8 ±2.21 | 14.4± 1.5 | -24.08 | 0.001 |
| **HCT (%)** | 24.8 ±6.6 | 41.95± 3.16 | -23.4 | 0.001 |
| **MCV (fl)** | 79.8 ±6.9 | 85.76 ±4.85 | -7.07 | 0.001 |
| **MCH (pg)** | 25.5±3.13 | 29.87 ±1.9 | -11.8 | 0.001 |
| **MCHC (g/dl)** | 32.0 ±2.34 | 34.79 ±1.27 | -10.1 | 0.001 |
| **RDW CV (%)** | 17.6±3.53 | 13.8± 2.94 | 8.3 | 0.001 |
| **WBC ($\times10^{9}$/L)** | 2.1 ±1.0 | 6.7± 1.7 | -22.6 | 0.001 |
| **Lympho (%)** | 42.7± 13.4 | 30.6± 10.7 | 7.0 | 0.001 |
| **NUETR (%)** | 47.4± 13.6 | 60.3 ±13.9 | -6.6 | 0.001 |
| **Mixed (%)** | 10.26± 6.93 | 8.8± 5.15 | 1.684 | 0.094 |
| **Eosino (%)** | 1.5± 1.2 | 2.9± 1.8 | -7.5 | 0.001 |
| **Baso (%)** | 1.5± 1.4 | 1.31± 1.22 | 0.825 | 0.411 |
| **Lympho ($\times10^{9}$/l)** | 0.88 ±0.5 | 1.9± 0.7 | -12.4 | 0.001 |
| **Neutrophil ($\times10^{9}$/l)** | 1.0 ± 0.61 | 4.1 ±1.5 | -19.0 | 0.001 |
| **Mixed (x$10^{9}$/l)** | 0.211± 0.155 | 0.593± 0.374 | -9.437 | 0.001 |
| **Eosino ($\times10^{9}$/l)** | 0.032±0.032 | 0.2±0.14 | -11.8 | 0.001 |
| **Platelet ($\times10^{9}$/L)** | 70.8±36.8 | 230± 59.4 | -21.8 | 0.001 |
| **MPV (fl)** | 9.4 ±1.3 | 9.7 ±1.0 | -1.7 | 0.092 |
| **PDW** | 14.6±4.2 | 12.5±2.2 | 2.14 | 0.001 |
| **NLR** | 1.37±0.92 | 2.65±1.97 | -1.28 | 0.001 |
| **PLR** | 101.8±76.5 | 141.2±83.1 | -39.3 | 0.001 |

*RBC-red blood cell, HGB-hemoglobin, HCT-hematocrit, MCV-mean cell volume, MCH-mean cell hemoglobin, MCHC-mean cell hemoglobin concentration , RDW-red cell distribution width, WBC-white blood cell, Lympho - lymphocyte, NUETR-neutrophil, Mono-monocyte, Eosino-eosinophil, Baso-basophil, MPV-mean platelet volume, PDW-platlate distribution width, NLR-neutrophil lymphocyte ratio, PLR-platlate lymphocyte ratio, SD-standard deviation, g/dl-gram per deciliter: fl,-fomtolitter , pg-picogram*

**8. Discussion**.

Visceral leishmaniasis is a potentially fatal human disease with an estimated incidence of at least 0.2 to 0.4 million cases worldwide, causing 20,000-40,000 deaths annually [6].In the current study, the main clinical symptoms and signs of VL patients were splenomegaly ,hepatomegaly ,fever ,weight loss ,jaundice ,skin mucosal pallor ,diarrhea ,anorexia ,abdominal pain ,general weakness and bleeding , similar to studies conducted Nepal [13] ,India [14],Yemen [15],Pakistan [20],Kuman [21],Iran [22, 23].

The present study showed that anemia, leucopenia, neutropenia, lymphopenia, thrombocytopenia, and pancytopenia were the most common hematological problem present in VL patients. These findings are in line with studies reported from Yemen [12], Nepal [30], India [16], Iran [22, 23], Sudan [24, 25], Gonder, Ethiopia [26].

In present study significantly decreased mean Hb, RBC, HCT and RBC indices values was reported in VL patients compared to control groups, similar to report in Sudan [25]. The 96% anemia observed in VL patients in this study, is conisstent with studies done in Iran (97.1%) [22] ,Yemen (100%) [12] India (100%) [15], Sudan (100%) [24],and Gondar, Ethiopia (94.4%) [26]. However, the prevalence of anemia in this study was higher than other studies done in Nepal (90%) [30] and Iran (87.3%) [23]. The cause of anemia in these VL patients may be multifactorial: sequestration and destruction of red blood cells (RBC) in enlarged spleen, immune mechanism and alterations in RBC membrane permeability, plasma volume expansion. Moreover, hypersplenism, nutritional deficiencies of iron, folate and vitamin B12 may also have some additional role. Other suggested causes include increased sensitivity to complement, inhibition of erythrocyte enzymes, production of hemolysin by the parasites and presence of cold agglutinins [3, 4].

Most of the RBC morphology of VL in this study showed that normocytic normochromic cells followed by microcytic hypochromic cells similar to studies conducted in Nepal [30],India [3, 4].The finding of this study is in contrast with previous studies conducted in India [14], Yemen [18] and Kumaon [21] , which showed that microcytic hypochromic cells was the predominant blood cell morphology followed by normocytic normochromic cells.

Total white blood cell count and neutrophil count of VL patients significantly decreased compared to controls , similar with the study conducted in Sudan [25].The prevalence of leucopenia in this study was 95% which is similar with a study done in Gonder, Ethiopia (95.4%) [26]. The prevalence of leucopenia in this study was higher than studies done in Yemen (87%) [12], Nepal (67.5%) [30], India (83.3%) [15], Iran (67.6%) [23].The cuase of leucopenia is may be due to delayed presentation to hospital which was attributing to hypersplenism causing leucopenia. Neutropenia was the most common abnormality seen in 92% in this study, which was similar with a study done in Gonder (90.1%) [26], but which was higher than studies in Yemen (73.5%) [19] and Sudan (88%) [24].This increases of neutropenia may be due to destroyed premature white blood cell (especially Neutrophils) by the parasite [4].

Similarly the prevalence of Lymphopenia in this study was 73%, which was higher than in studies done in Yemen (53.2%) [12] and Gonder ,Ethiopia (37.9%) [26].The prevalence of Lymphopenia in this study was lower than study done in Sudan (94%) [24]. Also eosinopenia was observed in VL patients in this study. The result of this study is in line with pervious study conducted in India [4, 14], Yemen [12], and Sudan [24].The suggested mechanism for development of this leucopenia is due to hypersplenism [3].

Platlate count of VL patients in this study is significantly decreased compared to control groups.The result of this study is consistent with a study conducted in Sudan [25]. The prevalence of thrombocytopenia in this study was 97%, which is consistent with studies done in Yemen (94%) [12], Sudan (100%) [24] and Gonder ,Ethiopia (90.1%) [26]. However this study showed slightly higher prevalence than studies done Nepal (72.5%) [13], India (83.3%) [16], Kumaon (85%) [21], Iran (91.2 %) [22]. Splenic sequestration and immune mediated mechanisms are mainly thought to be responsible for development of thrombocytopenia [4]. Pancytopenia is the most common haematological abnormality seen in 89% VL patients in this study, similar to a studies conducted in India [15, 16], Pakistan [17], Yemen [18], Iran [23]. The reason for pancytopenia could be due to long duration of symptoms and splenomegaly before presentation leading to increased peripheral destruction of blood cells [3].

Mean platelet volume (MPV), is one of the parameters used in the evaluation of platelet size. MPV has been recently getting increased attention as biomarker in different disease like cardio vascular disease, cerebral stroke, respiratory disease, chronic renal failure and rheumatoid

disease [31]. From a study conducted in Turkey no significant difference in MPV level in patients with cutaneous leishmaniasis and control groups was observed [32]. Similarly in the present study there is no statistically significant difference between VL patients and control groups in terms of MPV values. Platelet Distribution widith (PDW) another platelet parameter directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology. PDW is increased in the presence of platelet anisocytosis. PDW was reported to be significantly higher in the acute cholecystitis group when compared to the control group from study conducted in Turkey [33]. Also PDW was significantly higher in cutaneous leishmaniasis than control groups form study conducted in Turkey [32]. Consistent with the Turkish finding, in the present study PDW is significantly higher in VL patients compared control groups. This finding suggests that PDW value can be used as a biomarker for screening of VL patients.

Neutrophil lymphocyte ratio (NLR) is the ratio of the number of neutrophils by the number of lymphocyte counts. NLR has been reported as important biomarker for the diagnosis of different diseases such as diabetes mellitus, atherosclerosis, hypertension, metabolic syndrome, cancers of the lung , ovaries and stomach as an indicator for systemic inflammatory status [34]. In the study conducted in Turkey higher neutrophile count, NLR and lower lymphocyte count were detected in the patients with psoriasis than the control groups [35]. Another study in Turkey reported that NLR value was higher in patients with cutaneous leishimaniasis [32]. In the present study neutrophil count is lower in the VL patients than the control group, and as a result, NLR is significantly lower in the VL patients.

Platelet lymphocyte ratio (PLR) is the ratio of the number of platelets by the number of lymphocytes. PLR is another new biomarker suggested for the diagnosis of cancers and cardiovascular diseases. From a study conducted in Turkey the PLR values were higher in patients with cutaneous leishmaniasis compared to the control groups [32]. In the present study, that platelet count was lower in VL patients and PLR values were also significantly lower in the VL patients.

## 9. Strength and limitation of the study

### 9.1. Strength of the study

The strength of the study is participants were tested for malaria , rk-39 test for VL to screening of the controls as well as VL cases. Moreover biomarkers like MPV, PDW, NLR and PLR were done which were not assesed in previous studies. Also the finding of the study may serve as base line data in the study area for future researchers who have interest in similar topics.

### 9.2. Limitation of the study

Limited published studies are available making it difficult for comparison of this study with other findings. Also being a cross-sectional study by design, it cannot observe prospectively and thus cannot associate causal relationships between the factors under study.

# 10. Conclusion and Recommendation

## 10.1. Conclusion

The principal changes in peripheral blood of patient with visceral leishmaniasis were reduced number of red blood cells, reduction in leukocytes and decreased platelet count. VL patients presented with splenomegaly, fever, bleeding, anemia, leucopenia and thrombocytopenia. This finding indicates that visceral leishmaniasis causes alterations of hematological parameters.

## 10.2. Recommendation

Based on the above finding the following recommendation are forwarded

- Panytopenia with neutropenia and high RDW in combination with clinical features can be used for proper management of visceral leishmaniasis patients.
- Further large scale cohort study is recommended to find out associated risk factors of hematological abnormalities of VL.

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## 12. Annexes

### Annex I. Participant Information Sheet

### A.English Version

**Principal investigator**: Gebremedhin Gebremichail Gebreslasse

**Institution:** Addis Ababa University, College of health Science, Department of Medical Laboratory Science

**Title of the project:** Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019

**Introduction:** Dear study participants I am MSc student of Addis Ababa University, Department of Medical laboratory Sciences. You are invited to participate on the study of Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019.

**Purpose of the study:** The purpose of the study is to assess clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019

**Duration**: the duration of this study depend on the availability of study subjects and it can take about 3-4 months.

**Procedures to be carried on**: dear study participant if you agree to participate in the study, you will provide 3-5 ml of blood sample to assess your hematological profile tests.

**Risks and Discomfort:** during blood samples collection there will be minor discomfort or feel pain. During collection of samples from your hand appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be given to you if needed.

**Expected Benefit:** There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. The result will be given to you and if your result is clinically significant, it will help you for further diagnosis and treatment.

**Confidentiality:** Any information that that i am going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study.

**Withdrawal from the study:** Your participation in this study is completely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You may withdraw at any time without giving reason if you are uncomfortable. You can ask any questions regarding to this study and you have a right to get a laboratory results for free.

If you have any question regarding to this study please contact the following address:

Principal Investigator: Gebremedhin Gebremichail (BSc)

Mobile Phone: +251914255686

Email: gmedhinmichail86@gmail.com

Advisors: Aster Tsegaye (MSc, PhD)

Mobile Phone: Tel: +251911696085

E-mail: tsegayeaster@yahoo.com

## B. Amharic Version

**ለተሳታፊዎች መረጃ መስጭያ ሰነድ**

**የአጥኝው ስም:** ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

**የተቋሙ ስም:** አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

**የጥናቱ ርእስ፡** በካሕሳይ ላበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ሙሉ የደም ምርመራ ጥናት ማድረግ ።

**መግቢያ:**የተከበሩ የጥናቱ ተሳታፊ እኔ የአ.አ.ዩ. የላበራቶሪ ሳይንስ ትምህርት ክፍል የማስተርስ ዲግሪ ተማሪ ነኝ.እርሶ በካሕሳይ ላበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ሙሉ የደም ምርመራ ጥናት ማድረግ በሚለዉ ርእስ የተዘጋጀ የመመረቂያ ጥናት ፅሁፍ ላይ እንዲሳተፉ ተጋብዛል።

**የጥናቱ ጊዜ፡** የጥናቱ ግዜ በምናገኘዉ የካላኣዛር በሽተኞች ብዛት የሚወሰን ሲሆን ከ3 እስከ 4 ወር ለወስድ ይችላል፡፡

**የጥናቱ ሂደት፡** የተከበሩ የጥናቱ ተሳታፊ የሚከተሉትን ነገሮች ስለጥናቱ ከተገነዘቡ ና ፍቃደኛ ከሆኑ በኋላ ለጥናቱ የሚዉል 3 እስከ 5 ሚ.ሊ ደም እንወስዳለን።

**ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት**: የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም ። ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ልያስከትል ይችላል ። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ባላቸዉ ባለሞያ ስለሚመደብና አስፈላጊውን ጥንቃቄ እርምጃ ስለሚወስድ የህመም ስሜት አይኖርም።

**ከጥናቱ የሚያገኙት ጥቅም**

ይህንን ጥናት በማስተርስ ድግሪ መመሪቅያ እንደ መሆኑ መጠን በዚህ ጥናት በመካፈሎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም በጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ኖዎት። ለጥናቱ በተወሰደ ናሙና ላይ የሚገኘውን ውጤት በነፃ ያገኛሉ። በተጨሪም በውጤቱ ላይ ለውጥ ካለው ከህኪሞችን እንዲገናኙና እንዲመረመሩ ይደረጋል።

**የተሳታፊዎች ምስጢር ስለመጠበቅ ፡** በዚህ ጥናት ስለእርስዎ የምንሰበስበው ማንኛውም መረጃ ሚስጥራዊነቱ ይጠበቃል፡፡ለጥናቱ ሲባል በመጠየቂያው ወረቀት ሊይ ያለውን የእርስዎ ስምም ሆነ ማንነት ወደ ሚስጥራዊ ቁጥር ይቀየራል፡፡ እንዲሁም የሰጡት ናሙናም ሆነ መረጃ ከዚህ ጥናት ውጪ ለሌላ ኣላማ ጥቅም አይውልም፡፡

**ከጥናቱ ስለማቋረጥ፡** በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብትዎን የተጠበቀ ነው። ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡የደም ናሙና ያለመስጠት መብቶ የተጠበቀ ነው። ከዚህ በተጨማሪ ጥናቱን በተመለከተ ማንኛውምን ኢይነት ጥያቄ የመጠየቅ ወይም ገለፃ የማግኘት መብት አሎት።

**ጥያቄ ካለዎት፡** ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ ካለዎት በሚከተሀዉ አድራሻ ይጠቀሙ፡፡

ጥናቱን የሚያከሀደው ሰው ስም: ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

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ሞባይል: +251911696085: ኢሜል፤ tsegayeaster@yahoo.com

## C. Tigrigna Version

**ናይ መፅናዓይ ሽም:** ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

**ናይ ትካል ሽም:** ኣዲስ ኣበባ ዩኒቨርሲቲ ጥዕናን ሕክምና ሳይንስን ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

**ናይቲ መፅናዕቲ ርእሲ፡-** ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ምርመራ ምፍላጥን ምጽናዕን፡፡

**መእተዊ:** ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት ኣነ ናይ ኣድስ ኣበባ ዩኒቨርስቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ብማስተርስ ድግሪ ተምሃራይ እየ።ንሶም ወይ ንሰን ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ምርመራ ምፍላጥን ምጽናዕን ኣብ ዝብል ናይ መመረቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኾኑ ተዓድሞም አለዉ።

**ናይቲ መፅናዕቲ ዋና ዓላማ፡-** ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ምርመራ ምፍላጥን ምጽናዕን ይምልከት፡፡

**እቲ ጽንዓት ዝካየደሉ እዋን** ፡ ናይቲ ጽንዓት እዋን ዝዉሰን በቶም ንረክቦም ናይ ካላኣዛር ሕሙማት እንትከዉን ካብ 3 ክሳብ 4 ወርሒ ክወስድ ይክእል እዩ፡፡

**ቐደም ሳዒብ እቲ ጽንዓት፡** ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት እዞም ዝስዕቡ ናይዚ ጽንኣት ምስ ተረድኡ እና ፍቃደኛ እንድሕር ኮይኖም ነዚ ጽንዓት ዝከዉን ካብ 3 ክሳብ 5 ሚ.ሊ ዝከዉን ደም ክንወስደሎም ኢና።

**ምስቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡-** ንምርመራ ዝከዉን ደም ኣብ ዝህብሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም ኣየጋጥሞምን። ነገር ግን ደም ኣብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉይ ኽእል እዩ። ይኹን ዳኣምበር ደም ንምስብሳብ ልምዲ ብዘለዎም ባዓል ሞያታት ስለ ዝምደቡን ኣድላዩ ዝኾነ ጥንቃቐን ስለዝውሰድ ናይ ምሕማም ስምዕት ኣይህሉን።

**ካብቲ መፅናዕቲ ዝረክብዎ ጥቅሚ፡** እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቕ ፅሕፍ ከም ምኻኑ መጠን ኣብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቅሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቃሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት ብነፃ ይረኽቡ እዮም። ብተወሳኺ ኣብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኸቡን ንኽምርመሩን ይግበር እዩ።

**ናይ ሕክምና መረዳእታ ብምስጥር ምሕላዉ ዝምልከት ፡** ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክቦ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም ንሕዘለኩም ነፍልጥ፡፡ ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሽጥር ክንቐይሮ ኢና፡፡ብተወሳኪ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን፡፡

**ካብቲ መፅናዕቲ ስለምቁራፅ:-** ኣብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመስረተ ኮይኑ ኣብ ማእከል ምቑራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኽእሉ/ላ እዮም/የን:: ኣብዚ መፅናዕቲ ዘለዎም/ወን ሕቶን/ ርኢቶን ኣብ ዝኾነ ይኩን ግዜ ክሓቱ/ታ ይክእሉ/ላ::

ንተወሳኺ ሓበሬታ ነዞም ዝስዕቡ ኣድራሻ ይጠቀሙ::

**ናይ መፅናዓይ ሽም:** ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

ሞባይል:+251914255686 ኢሜል; gmedhinmichail86@gmail.com

**ኣማካሪት** : ዶ/ር ኣስቴር ፀጋዬ

ሞባይል: +251911696085: ኢሜል፤ tsegayeaster@yahoo.com

## Annex II: Consent Form (study participants)

### A.English version

**Principal investigator**: Gebremedhin Gebremichail

**Research title**: Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures and risks of this research project as described within it. I understand that at any time I may withdraw from this study without giving a reason. I have had an opportunity to ask questions and I am satisfied with the answers I have received. I know that no special payment for being participating in the study. I freely agree to participate in this study, as described. I understand that I was given a signed copy of this document to keep.

Name of participant._____ Age____Address_____Signature_____Date_____

Interviewer's name_____Signature_____

Principal investigator Name_____ Signature_____

### B. Amharic version

**የፍቃደኝነት ማረጋገጫ ቅጽ**

**ጥናቱን የሚያከሀደው ሰው ስም** :ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

**የጥናቱ ርእስ፡** በካሕሳይ ላበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል  የሚገኙ የካላኣዛር ሕሙማን  ሙሉ የደም ምርመራ ጥናት ማድረግ ።

እኔ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ልመሆን ስወሰን የጥናቱ ዓላማዎች ኣሳራሮችና ቾድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ  ተሳታፋይነት ፈቓደነኝነቴን በማነኛዉም ግዜ የማስወገድ መብቴን በመራጋገጥ ነዉ።ሰለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማየ እየራጋገጥኩ ይህንን ስውስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነኛዉም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው። እነዚህ መረጃዎች ሁሉ በሚገባ  በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፈርማየ ኣጋግጣለሁ።

የተሳታፊው ስም ……

ፊርማ…… ቀን …….

የአጥኚው ስም ……. ፊርማ

…… ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

## C.Tigrigna version

**ናይ ፍቓደኛነት መረጋገፂ ቅጥዒ**

**ናይ መፅናዓይ ሽም:** ገብረመድሂን ገብረሚካኤል ገብረሰላሴ

**ናይቲ መፅናዕቲ ርእሲ፡-** ኣብ ካህሳይ ላበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት  ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም  ምርመራ ምፍላጥን ምጽናዕን፡፡

ኣነ ኣብዚ ታሕቲ ተገሊጹ ዝኣሆ መጽናዕቲ ተሳታፋይ ንምኳን  እንትዉስን እንተለኩ ናይዚ ጽንዓት ዘድልዩ ነገራት ብምርዳእ እና ካብዚ ጽንዓት እዚ ምስታፍ ኣብ ዝኮነ ይኩን እዋን ከም ዘግልል ብምርግጋጸይ እዩ።ስለዚ ኣብዚ ጸንዓት ተሳታፋይ ምኳነይ በፈረማይ የራጋግጽ።እዞም ኩሎም መረደእታታት ብዝግባእ ብዝሩድኡኒ ቋንቋ ዝተገለጹለይ ምኳኖም በፈረማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት መለለዪ ቑፅሪ ---------- ፊርማ ---------- ዕለት----------

ሓበሬታ ዝኣከበ በዓል ሞያ ሽም ---------- ፊርማ ----------ዕለት----------

## Annex III: Parental consent form

### A.English version

I_____ parent, after being fully informed about the purpose of this study on the Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019 .I, the undersigned, have been told about this research. I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

| | | |
|---|---|---|
| _____ | _____ | ____/____/___ |
| Name of parent | signature | Day/month/year |
| _____ | _____ | ____/____/___ |
| Witness (Illiterate) | | Day/month/year |
| _____ | _____ | ____/____/___ |
| Name of the researcher | Signature | Day/month/year |

### B.Amharic version

**የስምምነት መጠየቂያ ቅጽ**

እኔ----------የልጄ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ በካሕሳይ ኣበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ሙሉ የደም ምርመራ ጥናት ማድረግ በሚለው ርእስ በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና መስጠት ምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጄ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊ ስም……

ፊርማ…… ቀን ……

የአጥኚው ስም …… ፊርማ

…… ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

## C.Tigrigna version

**ናይ ፍቓደኛነት መረጋገፂ ቅጥዒ**

ኣነ---------- ናይ ዉላደይ መላዓሊ እንትከዉን ናይዚ ጽንዓት ዓላማ ተረዲአ ኣለኩ፡፡ ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ምርመራ ምፍላጥን ምጽናዕን ብዝብል ርእሲ ዉላድይ ከም ዝሳተፍ/ትሳተፍ እቲ ድልየት ናተይ ከም ዝኾነ ነጊሩኒ/ነጊራትኒ ኣሎ/ኣላ፡፡ናሙና ምሃብ ኣብ ዉላደይ ምንም ዓይነት ሽግር ከም ዘይብሉ ተነጊሩኒ ኣሎ፡፡ዝኾነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲአ ኣለኩ፡፡ ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቅሚ ከም ዘይረክብ እዉን ተረዲአ ኣለኩ፡፡ናይ ዉላደይ ኣብዚ ጽንዓት ምስታፍ ብናተይ ድልየት ዝዉሰን ከም ዝኾነና ኣብዚ ጽንዓት ብዘይምስታፉ/ፉ ምንም ዓይነት ተጽዕኖ ክም ዘየምጽእ ተርዲአ ኣለኩ፡፡ካብዚ ብተወሳኪ ናይ ውላዲ ድልየት ከም ዘድሊ ተረዲኣ ኣለኩ፡፡ ብናተይ ድልየት ዉላደይ ኣብዚ ጸንዓት ከም ዝሳተፍ ብፈርማይ የራጋግጽ፡፡

ናይ ተሳታፊ/ፊት ሽም ---------- ፊርማ ---------- ዕለት----------

ሓበሬታ ዝኣከበ በዓል ሞያ ሽም ---------- ፊርማ ----------ዕለት----------

ናይቲ መጽናዓይ ሽም---------- ፊርማ ----------ዕለት----------

ስለ ዝተሓባበሩኒ የቐንየለይ!

## Annex IV: Assent form for children aged 12-17 years

### A.English version

I fully informed about the purpose of this study on the Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019 . I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my test results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent to give my blood for the study.

Name of participant signature Day/month/year

_____ _____ ____/____/___

Witness (Illiterate)

Parents phone number_____

Name of the researcher Signature Day/month/year

_____ _____ ____/____/___

### B.Amharic Version

በካሕሳይ ኣበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ሙሉ የደም ምርመራ ጥናት ማድረግ በሚለው ርእስ ዋና ዓላማዉ ተነግሮኛል.ናሙና ብሰጥ ምንም ዓይነት ችግር እንድሌለዉም ተነግሮኛል። በጥናቱ ወቅትም የኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጀ በግሉ የገኘዉ ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። በዚህ ጥናቱ ለመሳተፍ የኔ ፍላጎት እንዳለ ሁኖ ወላጆቼ ከፈቀዱልኝ ተስማምቻለሁ።

የተሳታፊ ስም……

ፊርማ…… ቀን …….

የአጥኚው ስም ……. ፊርማ

…… ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

## C.Tigrigna Version

ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ምሉእ ኣብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ምርመራ ምፍላጥን ምጽናዕን ብዝብል ርእሲ ኣብ ዝብል ርእሲ ዋና ዓላምኡ ተነግሪኒ ኣሎ፡፡ ናሙና ምሃብ ምንም ዓይነት ሽግር ከም ዘየምጸአለይ ተነጊሩኒ ኣሎ፡፡ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲአ ኣለኩ፡፡ ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቅሚ ከም ዘይረክብ እዉን ተረዲአ ኣለኩ፡፡ ኣብዚ ጸንዓት እዚ ምስታፍ ናተይ ድልየት ከም ዘሎ ኮይኑ ወለደይ እንድሕር ፈቂዶምለይ ንክሳተፍ ተስማዕሚዐ ኣለኩ፡፡

ናይ ተሳታፊ/ፊት ሽም ---------- ፊርማ ---------- ዕለት----------

ሓበሬታ ዝኣከበ በዓል ሞያ ሽም ---------- ፊርማ ----------ዕለት----------

ናይቲ መጽናዓይ ሽም---------- ፊርማ ----------ዕለት----------

ስለ ዝተሓባበሩኒ የቐንየለይ!

## Annex V: Structured Questionnaire for visceral leishmaniasis patients and control groups

**Research Title:** Assessment of clinical and hematological alteration among visceral Leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia, 2019

### A.English Version

Identification: Name of facility _____

Region _____ Zone _____ Woreda _____ Tel: ----------

**Note:** Please Encircle or Write the appropriate answer on the provided space.

| No. | Variable | Response |
|---|---|---|
| 1 | Sex | 1. Male 2. Female |
| 2 | Age | _____ |
| 3 | Marital Status | 1. Single 2. Married 3. Divorced 4. Widowed |
| 4 | Education level | 1. Illiterate 2. Primary 3.Secondary 4.Diploma and above |
| 5 | Residence | 1. Rural 2. Urban |
| 6. | Occupation | 1. Student 2. House wife 3. Government employer 4. Private employers 5. Farmer 6. Merchant 7. Daily labor 8. Other |
| 7 | Do you have history of hypertension | 1. yes 2. No |
| 8 | Do you have history of HIV/AIDS? | 1. Yes 2. No |
| 9 | Do you have history tuberculosis for the past two years? | 1.Yes 2. No |
| 10 | Do you have history of DM? | 1. Yes 2.No |
| 11 | Do you have habit of smoking? | 1. Yes 2. No |
| 12 | If yes to Q # 13, how often do you smoke? | 1. every day 2. two or three times per week 3. Other |

| | | |
|---|---|---|
| 13 | Do you have alcohol drinking habit | 1. yes 2. No |
| 14 | If yes to Q#16, how often do you take? | 1. one per day 2. More than two per day<br>3.two or three times per week 4. some times(holiday) |
| 15 | Do you take any medication within three months? | 1. Yes 2. No |
| 16 | If your answer is yes for Q#15, what type of drug do use? | 1. Anti-protozoa 2. Anti-bacterial 3. Anti TB 4. other |
| 17 | Do you have history of chronic liver disease? | 1. Yes 2. No |
| 18 | Do you have history of chronic kidney disease? | 1. Yes 2. No |
| 19 | Do you have history of cancer? | 1. Yes 2. No |
| 20 | Do you have history of chronic heart disease? | 1. Yes 2. No |

I thank you for your cooperation

## B. Amharic version

መለያ: የተቋሙ ስም _____

ክልል_____ ወረዳ_____ ቀበሌ_____ ስልክ: ----------

ማሳሰብያ: እባክዎ ትክክለኛውን መልስ ያክብቡ ወይም ይፃፉ።

| ተ.ቁ | ጥያቄዎች | መልስ |
|---|---|---|
| 1 | ጾታ | 1. ወንድ 2. ሴት |
| 2 | እድሜ | |
| 3 | የጋብቻ ሁኔታ | 1. ያላገባ/ች<br>2. በትዳር ላይ<br>3. የፈታ/ች<br>4. በሞት ምክንያት የተለያዩ |
| 4 | የትምህርት ደረጃ | 1. ያልተማረ<br>2. ኣንደኛ ደረጃ(1-8)<br>3. ሁለተኛ ደረጃ (9-12 )<br>4.ዲፕሎማ/ድግሪና ከዛ በላይ |
| 5 | መኖርያ | 1.ገጠር 2.ከተማ |
| 6 | ስራ | 1.ተማሪ 2.የቤት እመቤት 3.የግል ሰራተኛ 4.ገበሬ 5.ነጋዴ 6.የቀን |

| | | |
|---|---|---|
| | | ሰራተኛ 7.ሌላ |
| 7 | በደሙ ዉስጥ የድም ግፊት ኣለበት | 1. ኣዎ 2. የለኝም |
| 8 | በደሙ ዉስጥ የኤች ኣይቪ በሽታ ኣለበት | 1. ኣዎ 2. የለኝም |
| 9 | የቲቪ በሽታ ታካሚ ኖት | ኣዎ 2. የለኝም |
| 10 | በደሙ ዉስጥ የስኳር በሽታ ኣለበት | 1. ኣዎ 2. የለም |
| 11 | ሲጋራ የመጨስ ልምድ ኣለበት | 1. ኣዎ 2. የለኝም |
| 12 | ለተራ ቁጥር 11 መልስዎ ኣዎ ከሆነ ለምን ያክል ግዜ ያጨሳሉ | 1.በየቀኑ 2.ሁለት ወይም ሰሶት ግዜ በሳምንት 3.ሌላ |
| 13 | ኣልኮል የመጠጣት ልምድ ኣለቦት | 1. ኣዎ 2. የለም |
| 14 | ለተራ ቁጥር 13 መልስዎ ኣዎ ከሆነ ምን ያክል ይጠጣሉ | 1. 1.በየቀኑ 2. .ሁለት ወይም ሰሶት ግዜ በሳምንት 3.ኣንዳ ኣንድ ግዜ(በባኣል ግዜ) |
| 15 | በዚ ሶስት ወር ወስጥ መድሓኒት ወስዶ ያዉቃሉ | 1. ኣዎ 2. የለም |
| 16 | ለተራ ቁጥር 15 መልስዎ ኣዎ ለምን ኣይነት በሽታ | 1.ለ ፕሮቶዝዋ 2. ለባክቴሪያ 3.ለቲቪ 4.ሌላ |
| 17 | ለብዙ ግዜ የቆየ የጉበት በሽታ ኣለቦት | 1. ኣዎ 2. የለም |
| 18 | ለብዙ ግዜ የከላሊት በሽታ ኣለቦት | 1. ኣዎ 2. የለም |
| 19 | የካንሰር በሽታ ኣለቦት | 1. ኣዎ 2. የለም |
| 20 | ለብዙ ግዜ የቆየ የልብ በሽታ ኣለቦት | 1. ኣዎ 2. የለም |

ስለ ትብብርዎ ኣመሰግናለሁ!

## C. Tigrigna version

መለለዪ: ናይቲ ተቋም ሽም:_____ ናይቲ ተቋም ኮድ:_____

ክፍለ ከተማ_____ ወረዳ_____ ጣበያ_____ ስልኪ: ----------

መተሓሳሰቢ: ትኽክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፅሓፉ።

| ተ.ቑ | ሕቶታት | መልሲ |
|---|---|---|
| 1 | ፆታ | 1. ተባዕታይ 2. ኣንስታይ |
| 2 | ዕድመ | |
| 3 | ኹነታት ሓዳር | 1. ዘይተመርዓወ/ወት<br>2. በዓለ ሓዳር<br>3. ዝፈትሐ//ሐት<br>4. ብሞት ምኽንያት ዝተፈላለዩ |
| 4 | ናይ ትምህርቲ ደረጃ | 1. ዘይተምሃረ |

| | | |
|---|---|---|
| | | 2.ቐዳማይ ደረጃ(1-8)<br>3. ካልኣይ ደረጃ (9-12)<br>4. ዲፕሎማ/ድግሪንልዕሉኡን |
| 5 | መንበሪ | 1.ገጠር 2.ከተማ |
| 6 | ስራሕ | 1.ተምሃራይ/ት 2.ናይ ገዛ ሰራሕተኛ<br>3.ናይዉልቀ ስራሕ 4.ሓረስታይ 5.ነጋዳይ<br>6.መዓልታዊ ሰራሕተኛ 7.ካሊእ |
| 7 | ኣብ ደመም ዉሽጢ ኣለዎም ዶ? | 1.እወ 2.የለን |
| 8 | ኣብ ደሞም ዉሽጢ ኤች ኣይቪ ኣለዎም/ን? | 1. እወ 2. የብለይን |
| 9 | ናይ ቲቪ ሕማም ተሓካሚ ድዮም? | 1. እወ 2. የብለይን |
| 10 | ኣብ ደሞም ዉሽጢ ናይ ሽኮርያ ሕማም ኣለዎም/ን? | 1. እወ 2. የብለይን |
| 11 | ሽጋራ ናይ ምትካክ ልምዲ ኣለዎም /ን? | 1. እወ 2. የብለይን |
| 12 | ንተራ ቁጥር 11 መልሶም ኣዎ እንተኮይኑ? | 1.በቢማዓልቱ 2.ክልተ ወይ ሰለስተ ግዜ ኣብ ሰሙን 3.ካሊእ |
| 13 | ኣልኮል ናይ ምስታይ ልምዲ ኣለዎም /ን? | 1. እወ 2. የብለይን |
| 14 | ንተራ ቁጥር 14 መልሶም ኣዎ እንተኮይኑ? | 1.በቢማዓልቱ 2.ክልተ ወይ ሰለስተ ግዜ ኣብ ሰሙን 3.ካሊእ |
| 15 | ኣብዉሽጢሰለስተወርሒዝኮነዓይነትመድሓኒትንዝኮነዓይነትሕማም ወሲዶምዶይፈልጡ ? | 1.እወ 2.የለን |
| 16 | ንታራቁፅሪ15 መልሶም እወ እንተኾይኑ ኣየናይዓይነት መድሓኒት እዮም ወሲዶም? | 1. ፀረ-ፕሮቶዝዋ 2. ፀረ-ባክቴሪያ 3 ፀረ-ቲቢ 4. ካለእ |
| 17 | ናይ ጸላም ከብዲ ሕማም ኣለዎም /ን ዶ? | 1.ኣወ 2.የለን |
| 18 | ናይ ከላሊት ሕማም ኣለዎም /ን ዶ? | 1.ኣወ 2.የለን |
| 19 | ናይ ካንሰር ሕማም ኣለዎም /ን ዶ? | 1.ኣወ 2.የለን |
| 20 | ናይ ልቢ ሕማም ኣለዎም /ን ዶ? | |

ሰለ ዝተሓባበሩና ነመስግን

## Annex-VI: standard operating procedures for blood collection

**Equipment**

- 21 gauge needle for each participant with closed vacutainer system
- Blood collection tubes for each participant
- Tourniquet
- Box of nitrile /vinyl gloves
- 70% Alcohol wipes
- Cotton balls/swabs
- Bandages
- Pillow/pad for raising arm to comfortable elevation
- Apple/orange juice and snacks for fasting participants
- Disposable, single use materials or equipment are to be used whenever possible
- Any reusable materials or equipment must be cleaned and disinfected with

  Alcohol-based sanitizers before use with another participant

**Safeguards /safety procedures**

• A new pair of disposable latex/vinyl gloves is used with each participant. Gloves are for single-procedure use only. Gloves should always be removed using a glove-to-glove or skin-to-skin technique which will prevent contaminating the hands.

• The use of gloves does not replace the need for hand hygiene. Hands should be properly washed before the gloves are put on and after the gloves are removed. Hand hygiene is also needed before and after the replacement of gloves during a procedure or in between tasks.

• Participants are reminded to do no heavy lifting for 24 hours.

**Procedure for drawing blood**

Steps 1; Assemble equipment

Collect all the equipment needed for the procedure and place it within safe pack which is simple for transport to collection site and place easy reach on a flat surface table ensuring that all the items are clearly visible.

Step 2; Identify and prepare the participants and allow to sit comfortably preferably be stretching his/her arm

Step 3; Perform hand hygiene and put on gloves

Step 4; Select the site of injection

Step 5; apply the tourniquet

Step 6; Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol.

Step 7; insert the needle properly into the vein

Step 8; draw the required amount of blood

Step 9; Fill the laboratory sample tubes and mix properly

- When obtaining multiple tubes of blood, use vacunated tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.
- If a syringe or winged needle set is used, best practice is to place the tube into a rack before filling the tube. To prevent needle-sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of hemolytic.

- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper. DO NOT remove the stopper because it will release the vacuum.
- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of hemolysis. DO NOT recap and remove the needle.
- Before dispatch, invert the tubes containing additives for the required number of times (as specified by the local laboratory).

Step 10; Draw samples in the correct order and label the sample using unique code of participants

Step 11; Clean contaminated surfaces and complete patient procedure

Step 12; Prepare samples for transportation

Step 13; Clean up spills of blood or body fluids

## Annex VII: SOP for Sysmex KX-21N hematology analyzer

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The KX-21 processes approximately 60 samples an hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and platelets, along with data of 17 parameters, as the analysis results. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM#, MIXED# and NEUT#. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%.

**DETECTION PRINCIPLE**

The KX-21N counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to methemoglobin, and read photometrically at 555 nm. White blood cells (WBC) are analyzed by

direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils**.**

**DC Detection Method**

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

**Non-Cyanide Hemoglobin Analysis Method**

To analyze hemoglobin by automated methods, the Cyanmethemoglobin method or Oxyhemoglobin method have so far been the main stream. Cyanmethemoglobin method was recommended as the international standard method in 1966 by ICSH (International Committee for Standardization in Hematology). This method, however, is so low in hemoglobin conversion rate that it cannot be said an appropriate method in the automated process in which multi-sample processing is the pre-condition. In addition, this method uses the reagent of cyanide compound which is a poisonous substance and requires waste processing; thus, it can hardly be called an environmentally favorable method. At present, this method cannot be said suitable for a fully-automated instrument which is required to handle a large amount of waste. The Oxyhemoglobin method, on the other hand, is faster in hemoglobin conversion rate; in fact, blood hemoglobin is converted instantaneously into oxyhemoglobin. Also, it does not contain poisonous substance as cyanmethemoglobin method, making the method suitable for automation. This method, however, is unable to convert methemoglobin into oxyhemoglobin. Consequently, when a great amount of methemoglobin is contained as in control blood, lower-than-real values result, although usual human blood poses no problems.

Non-cyanide hemoglobin analysis method utilizes the advantages of both of the above methods. Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing methemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

**Reagents**

**Cell Pack:** - is ready to use for impedance and photoelectrical analysis of whole blood and its ingredients are: sodium chloride, boric acid, sodium tetra borate, and K2EDTA.

**Stromatolyzer WH:** - is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC free and easy to count; whole blood sample by resistance measurement and photometric measurement and its ingredients are: nonionic surfactant, organic quaternary ammonium salt.

**Cell Clean**: - is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex analyzer.

**Reagents preparation:** Reagents are commercially prepared.

**Reagents stability and storage:** All reagents are stable at room temperature up to their expiry date.

**Equipments:**

- Sysmex KX-21N
- Electrical blood mixer
- Electrical power stabilizer (500 or 1000 w)

**Sample**

- Whole blood specimen collected in K2EDTA anticoagulant tube.

**Amount required**

¾ of the lavender collection tube (3 ml)

**Transport and Storage:** 2-8 0C

**Aspiration**

- Whole blood mode- Approximately 50µl
- Pre-diluted mode-Approximately 20µl

**Dilution**

In pre-dilution mode a sample is diluted in to 1:26 before analysis.

**Instrument dilution**

In whole blood mode

- The dilution for HGB and WBC 1:500
- The dilution for RBC 1:25000

In pre-dilution mode

- The dilution for HGB and WBC is 1:1000
- The dilution for RBC is 1:25000

**CBC Analysis using sysmex** Sysmex KX-21N

**WBC/HGB Analysis procedure**

In WBC and HGB analysis, the volume of WBC and hemoglobin in the blood are measured. The flow of WBC/HGB analysis is described below:

**Whole Blood Mode**

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 6 ul of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/HGB lyse is added to prepare 1:500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.
3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.

4. 500 ul of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

**Pre-diluted Mode**

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.
2. 78 ul of diluted blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.922 mL of diluent. At this time, 1.0 mL of WBC/HGB lyse is added to prepare 1:1000 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.
3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.
4. 500 ul of sample in the WBC transducer chamber is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

**RBC/PLT Analysis procedure**

In RBC/PLT analysis, RBC and platelet count in the blood are measured. The flow of RBC/PLT analysis is described below:

**Whole Blood Mode**

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 4.0 ul of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 mL of diluent and brought to the mixing chamber as diluted sample. (1st step dilution)
3. Out of the 1:500 dilution sample, 40 µL is measured by the sample rotor valve, diluted into 1:25000 with 1.960 mL of diluent, then transferred to the RBC/PLT transducer chamber. (2nd step dilution)
4. 250 ul of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT are counted by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

**Pre-diluted Mode**

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.
2. 2.08 ul of diluted blood measured by the sample rotor valve is transferred in 1.99792 mL of diluent to the RBC/PLT transducer chamber, and is made into 1:25000 dilution sample.
3. Of the sample in the RBC/PLT transducer chamber, 250 µL is aspirated through the aperture. At this time, RBC and PLT are calculated by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

**Calculation of RBC Constant**

RBC constant (mean RBC volume, mean RBC hemoglobin, mean RBC hemoglobin concentration) is calculated from RBC, HGB, and HCT.

1. Mean RBC Volume (MCV)
   Calculation is made from RBC and HCT by the formula below:
   $$\text{MCV (fL)} = \frac{\text{HCT (\%)}}{\text{RBC } (x10^6/\text{ul})} \times 10$$
2. Mean RBC Hemoglobin (MCH)

Calculation is made from RBC and HGB by the formula below:

$$MCH\ (pg) = \frac{HGB\ (g/dL)}{RBC\ (x10^6/ul)} \times 10$$

4. Mean RBC Hemoglobin Concentration (MCHC)

Calculation is made from HCT and HGB by the formula below:

$$MCHC\ (g/dL) = \frac{HGB\ (g/dL)}{HCT\ (\%)} \times 100$$

**General Analytical procedure**

1. Mix the sample sufficiently

2. Remove the plug while taking care not to allow blood scatter

3. Set the tube to the sample probe and in that condition, press the start switch

4. The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing,"remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.

5. When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures. To analyze the sample in Pre diluted Mode, first switch the analyzer to Pre diluted mode and follow the procedure as Whole Blood analysis.

**Adult Reference Range**

| **PARAMETER** | **REFERENCE RANGE** |
|---|---|
| Red Blood Cell Count (RBC) | |
| Men | $4.5 – 5.5 \times 10^{12}/l$ |
| Women | $3.8 – 4.8 \times 10^{12}/l$ |
| Haemoglobin (Hb) | |
| Men | 13 – 17 g/dl |
| Women | 12 – 15 g/dl |
| Haematocrit (HCT) | |
| Men | 40 – 50 % |

| | |
|---|---|
| Women | 36 – 45 % |
| Mean Cell Volume (MCV) | |
| Men | 83 – 99 fl |
| Women | 83 – 99 fl |
| Mean Cell Haemoglobin (MCH) | |
| Men | 27 – 32 pg |
| Women | 27 – 32 pg |
| Mean Cell Haemoglobin Concentration (MCHC) | |
| Men | 32 – 36 g/dl |
| Women | 32 – 36 g/dl |
| Red Cell Distribution Width (RDW) | 11.6 – 14.0 % |
| White Blood Cell Count (WBC) | 4-10 x $10^9$/l |
| Differential White Cell Count (Diff) | |
| Neutrophils | 40 – 80 % (2 - 7 x $10^9$/l) |
| Lymphocytes | 20 – 40 % (1 – 3 x $10^9$/l) |
| Monocytes | 2- 10 % (0.2 – 1.0 x $10^9$/l) |
| Eosinophils | 1 – 6 % (0.02 – 0.5 x $10^9$/l) |
| Basophils | < 1- 2 % (0.02 – 0.1 $10^9$/l) |
| Platelet Count | 150 – 400 x $10^9$/l |
| MPV | |
| Female | 7.2-10.4 FL |
| Male | 7.5-11.5 FL |
| PDW | 9-14 FL |

**Quality Control**

The reliability of this instrument and reagents is monitored by quality control. By use of control blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

**Control material**

The control materials, EIGHTCHECK-3WP-N (Normal), EIGHTCHECK-3WP-L (Low level) and EIGHTCHECK-3WPH (High level) are used. These are equivalent to Low, Normal and High level.

**Declaration**

**Assurance of Principal Investigator**

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

**Name of the student:** Gebremedhin Gebremichail Gebreslasse

Date _____ Signature _____

**Approval of Advisors:**

**Aster Tsegaye (MSc, PhD)**

Date_____ Signature _____