BLOOD CELLULAR HEMATOLOGICAL CHANGES IN PATIENTS WITH VISCERAL LEISHMANIASIS ATTENDING KIMALEL SUB-COUNTY HOSPITAL, BARINGO COUNTY, KENYA

LUKAS SEREMANI AGURA

MASTER OF SCIENCE

(Medical Laboratory Sciences)

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Blood Cellular Hematological Changes in Patients with Visceral Leishmaniasis Attending Kimalel Sub-County Hospital, Baringo County, Kenya

Lukas Seremani Agura

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature…… Date……...

Lukas Seremani Agura

This thesis has been submitted for examination with our approval as university supervisors

Signature…….. Date……...

Dr. Michael Kahato, PhD

JKUAT, Kenya

Signature…….. Date……...

Kibet Shikuku MBChB, MMED (Human Path)

UoN, Kenya

DEDICATION

I dedicate this thesis to my family Sarah Kwache, Carlene Chepngetich, Jackson Obiko, Mercy Kwamboka, Levy Omambia, Julian Agura, Omari Gabriel, Abel Agura, Emma Nyarango and Laby Mochache who have supported me in many ways to complete this study. May God bless you.

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ABBREVIATIONS AND ACRONYMS

APTT	Activated Partial Thromboplastic Test
CBC	Complete Blood Count
DAT	Direct Agglutination Test
DDT	Dichloro-Diphenyl-Trichloro-ethane
DNA	Deoxyribonucleic Acid
E	Eosinophils
EDTA	Ethylene Diamine Tetra Acetic Acid
ERC	Ethical Review Committee
ESR	Erythrocyte Sentimentation Rate
FPC	Finite Population Correction
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
Hb	Hemoglobin
HCT	Hematocrit
HLH	Hemophagocyticlymphohistiocytosis
ICT	Immunochromatographic Test
IFA	Immunofluorescent Antibody
IgG	Gamma Immunoglobin
IL-10	Interleukin-10

ITNs	Insecticide Impregnated Materials
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
L	Lymphocytes
LD	Leishmania Donovani
LYMPH	Lymphocytes
M	Monocytes
MCH	Maternal Child Health
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MPS	Mononuclear Phagocytic Systems
MPV	Mean Platelet Volume
N	Neutrophils
NNN	Novy-McNeal, Nicholle
NTD	Neglected Tropical Diseases
PI	Principal Investigator
PLT	Platelets

PT	Prothrombin Time
PTI	Prothrombin Time Index
Pts Intls	Patients Initials
Pts	Patients
RBCs	Red Blood Cells
RDT	Rapid Diagnostic Tests
Rk39	Recombinant 39amino Acid Antigen
SPSS	Statistical Package for Social Sciences
TAT	Turn around Time
Th1/Th2	T-helper1/T-Helper2
TNF-α	Alpha Tumor Necrosis factor
VL	Visceral Leishmaniasis
WBCs	White Blood Cells

ABSTRACT

Blood cellular changes in visceral leishmaniasis (VL) are common. This study sought to find a balanced view between the significance of the blood cellular changes and their clinical utility in diagnosis of VL. The VL disease causes various hematologic manifestations in humans. Usually, anaemia, leucopenia, and thrombocytopenia are most common. VL disease affects people of all age groups that are exposed to infected sand flies and is characterized by fever, hepatomegaly, splenomegaly, weight loss, and enlarged lymph nodes. However, other common tropical illnesses manifest with similar clinical features. The study dealt with two presumptions: that the diagnosis of VL was not based on symptoms and signs alone; and that the existing definitive procedures of diagnosis were invasive and necessitated safe laboratory options. Due to these challenges, blood cellular parameter changes were used as a unique indicator for VL and to reduce Turnaround Time (TAT) and subsequent management. To achieve its objective of determining the unique blood cellular changes that can be used to aid in early diagnosis of visceral leishmaniasis, a descriptive cross-sectional study was undertaken at Kimalel Sub-County Hospital Baringo County, Kenya. A total of seventy-four (74) splenic aspirates and whole blood samples were collected for analysis. Splenic aspirates stained with Giemsa stain were examined for Leishmania donovani (LD) bodies. AcT10 Coulter Counter hematology analyzer was used to run complete blood cell count. Peripheral blood films were stained with Giemsa and examined for cell morphology, characterization and variations using a Daly cell counter. Amastigotes were microscopically identified in splenic aspirates smears. The study results revealed a decrease in Hb: 3.8-9.8g/dl, RBCs: 1.19-3.85 for males and Hb of 3.6-10.2g/dl, RBCs: 1.13-3.83 for females. There was also a decrease in WBCs: 0.6-3.3,neutrophils:29-71,lymphocytes:26-70,monocytes:0-5,platelets 21-225 for males and 0.6-3.0 Neutrophils: 32-73,lymphocytes:24-66,monocytes:0.5,platelets: 19-405 for females. RBC indices were reduced (except MCV which was normal in all cases) with decrease also in white blood cells and Platelets counts using automated electronic cell counter. The differential count and microscopic examination showed a complete absence of eosinophils. All cells were affected but eosinophils were mostly affected in the study. The study demonstrated that there were no unique blood cellular changes which could inform early diagnosis or detection of visceral leishmaniasis. More research should be conducted on various test methodologies to ascertain the simplest, cheap and appropriate laboratory diagnosis for VL.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

In Africa, VL is mainly reported in Sudan, South Sudan, Ethiopia, Kenya, and Somalia. East Africa is second only to India in the number of human cases of VL. (Alvar et al., 2012) and has been challenging regarding leishmaniasis control and elimination. The ongoing outbreak of VL in Kenya is a significant setback to the World Health Organization's (WHO) 2021–30 NTD roadmap for the global elimination of visceral leishmaniasis. In addition, it has stretched the fragile healthcare infrastructure in Kenya. Amongst the 47 counties of Kenya, nine counties, including Marsabit, Garissa, Kitui, Baringo, West Pokot, Mandera, Wajir, Tharaka Nithi and Isiolo, have reported a total of 2037 cases of confirmed and assumed cases of VL since 2020 of which ten patients (case fatality rate, CFR, of 0.5%) succumbed to the disease (WHO, 2024). The outbreak is presently active in four counties of Wajir, Kitui, Isiolo, West, and Pokot.There have been numerous outbreaks of VL in Kenya in the past many years in the counties of Isiolo, Marsabit, Wajir and Baringo Counties in the years 2008, 2011, 2013, 2014, 2017 and 2019 .(Makau-Barasa et al., 2022).

In addition, the Marsabit county of Kenya has consistently shown an increase in cases for the last few years. For instance, in 2014, 136 cases of VL were validated with CFR 9.6% (de Souza et al., 2022)

In 2017, 437 were reported, which was followed by a sharp increase in the number of cases to 2338 in 2019.There was also an increase in the cases amongst the younger population (mean age 15.3 in 2019 vs 17.6 in 2017) and an increase in the proportion of cases in females and children less than ten years of age. It was hinted that this rise could be because of the more serious number of people being tested because of the availability of new testing facilities. (de Souza et al., 2022).

Commonly, VL was considered more prevalent in arid counties of eastern and northern areas. The migrating and expanding of human population for trade or survival, mostly the refugees and pastoral communities, the interaction of humans with the animal population, environmental changes, and social unrest may be some of the factors playing a role in the relocation of the disease foci to the earlier non-endemic areas. There are many challenges in the control of VL in Kenya which have led to a persistent increase in the number of cases as well as the geographical move of the disease. The interventional strategies of diagnosis, management and control of the disease have not proven effective; nevertheless, they have remained unchanged even in the face of the changing epidemiology of VL.

The symptoms of VL include prolonged fever, anaemia, pancytopenia, splenomegaly, and weight loss. These symptoms are often mimicked by malaria, typhoid fever, schistosomiasis, brucellosis, tuberculosis or other hematological malignancies. (Safavi et al., 2020).

. Therefore proper, accurate, simple and affordable diagnostic methods for VL must be available, for the community that is plan for. Since majority of cases are asymptomatic and even symptomatic cases may not be recognized if proper diagnosis is not done. In general, the use of multiple diagnostic approaches is recommended to increase the likelihood of a positive result. (Aronson et al., 2016) ;(Varani et al., 2017) In general, the use of multiple diagnostic approaches is recommended to increase the chances of getting a positive result (Aronson et al., 2016); (Varani et al., 2017).

Spleen aspirates, which are considered the gold standard, have an incidence of hemorrhage of up to 1/1000 procedures (Sundar et al., 2002) The methods requiring invasive sampling are costly and tedious, thus neither efficient nor adaptable.(Alvar et al., 2021) and are routinely performed only in eastern Africa and the Indian subcontinent (Van Griensven et al., 2019) ; The specimen for smears and cultures are obtained by aspiration of the organs known to harbor the parasites e.g. spleen, bone marrow and lymph nodes. The sensitivity of microscopy varies, being higher for spleen (93–99%) than for bone marrow (53–86%) or lymph node (53–65%) aspirates

(Babiker et al., 2007). Splenic aspirates are an abundant source of Leishmania parasites, but their examination conform to a high risk of bleeding in unskilled hands (Barrett & Croft, 2012).

The use of culture, though sensitive is a method that requires specialized laboratory settings, it is laborious and takes about two to four weeks before detection .Other diagnostic methods have been developed for these reasons i.e. rk39, the lateral flow strips for rK39 antigens are available commercially for detection of visceral leishmaniasis, but their performance is still not optimal (Welch et al., 2009); (Solano-Gallego et al., 2011).Indirect immunological methods of diagnosis which include the antibody based direct agglutination test (DAT), and Immunochromatographic test (ICT). Although these tests are readily available, they are not the standard diagnostic tests due to their deficient sensitivity and specificity.

Molecular methods; benefits of molecular methods are elevated specificity and sensitivity, the likelihood of using bone marrow and peripheral blood (Lindoso et al., 2014). Positivity of PCR differs, keeping in mind the use of whole blood (83–98%) and bone marrow (93–100%) (Cota et al., 2012). PCR analyses depending on the amplification of kinetoplast are the most analytical method to detect Leishmania DNA (Cota et al. 2013; Lindoso et al. 2016). MK1F/R primer for targeting the kDNA sequences were used (Khatun et al. 2017) and found the sensitivity and specificity to be 98% and 100%.

The latex agglutination test KAtex (KALON biological, UK) detects a heat-stable low molecular weight carbohydrate antigen in urine. Its specificity is high (93%), but sensitivity is low (64%), thus, it is rarely used in clinical practice (Van Griensven et al., 2019).

More recent urine antigen tests, based on ELISA techniques, show better sensitivity (Vallur et al., 2015;(Ghosh et al., 2016).The antigen has been characterized and found to be a low molecular mass (5–20 kD) glycoconjugate (Sarkari et al., 2002).Animal experiments and early results recommend that it is a promising diagnostic tool, and its results correlate well with the splenic parasite rating. Preliminary studies using urine of infected animals and patients have confirmed the

presence of leishmanial antigen in active VL, and its disappearance after successful treatment (Sundar et al., 2005).

Consequently, efforts have been made to develop noninvasive, simpler, and sensitive techniques. Therefore there is need to simplify diagnostic procedures by use of a noninvasive hematological techniques to identify leishmania using blood. This study aimed at researching the possibility of using the specific blood cellular changes that can provide unique characteristics of identifying VL infection and Unique cellular characteristic that can aid in early and accurate diagnosis of VL .Its success will permit more studies to evaluate and confirm the tests in diagnosing VL. This will eliminate the invasive and tedious risks associated with splenic and bone marrow aspirates and other diagnostic methods which have low sensitivity and specificity of identifying visceral leishmaniasis.

1.2 Statement of the Problem

Visceral leishmaniasis is a systemic disease that is potentially fatal if untreated. Diagnosis of the disease rests on demonstration of amastigotes from the spleen or bone marrow using traditional microscopy which remains the gold standard which has a senstifity and specificity of 93-99% for splenic aspirates and 60-80% for bone marrow aspirates.Ther is also a chance to use cultures to improve senstifity. These procedures are invasive and require skilled clinicians to undertake them. Splenic aspiration carries the added risk of intraperitoneal bleeding. The rk39 rapid diagnostic test (RDT) and direct agglutination test (DAT) were introduced into use for VL diagnosis in Kalaazar endemic areas of Kenya but were severely constrained with limited sensitivity (Chappuis et al., 2006). Studies have recently (2010-2012) been done in VL endemic areas of Baringo and North Pokot counties of Kenya using serum for RDT kit DiaMed-IT LEISH (DiaMed AG Switzerland) and Signal KA (Span Diagnostic, India).The sensitivity and specificity of these RDT kits were between 80% to 95% respectfully. Nevertheless, the cold chain requirement of the Signal KA (storage at 2-8^{0}C) is a major obstacle to field use in most VL endemic areas. Prolonged exposure to very high temperatures > 45^{0}C may also have a negative impact on the accuracy of the DiaMed IT LEISH (Cunningham et al., 2012).

The major limitations of these serological tests is that though antibodies decrease after successful treatment (Kumar et al., 2001), they remain detectable up to several years after cure (De Almeida et al., 2006; Hailu, 1999). This has led to the continuous use of the current invasive diagnostic methods. Therefore noninvasive and simple tests may be needed to replace the invasive tests for diagnosis of visceral leishmaniasis.

1.3 Justification

Due to the fact that diagnostic as well as clinical symptomatology is non-specific in VL, the diagnostic mechanisms utilized are both invasive and time consuming. Therefore noninvasive and simple techniques need to be developed to easy VL diagnosis.

Several methods of diagnosis have been developed i.e. parasitological method which remain the gold standard for diagnosing VL (de Vries et al., 2015) and are crucial in eco-epidemiological studies. Diagnosis in the laboratory can be achieved by direct examination of amastigotes in giemsa –stained smears. This method is invasive with low sensitivity and specificity except splenic aspirates which have high sensitivity (93-99%) (Srivastava et al., 2011).Immunological methods were also developed (Singh and Sundar 2025) which includes, ELISA in which the sensitivity of this test depends on the assay and its methodology while specificity depends on the antigen than the serological format used. DAT test is depended on the agglutination of 8promastigotes of *Leishmania* (Elmahallawy et al., 2014).The sensitivity and specificity ranges from 70.5 -100% and 53-100% (Mondal et al., 2010) DAT has even more stringent requirements necessitating its deployment in health facilities only with trained technicians and availability of laboratory infrastructure. Polymerase Chain Reaction (PCR) This method serves as a supplement as well as an alternative method of diagnosis. The application and encouraging outcomes have led to continues approval of these methods (de Paivan-Cavalcanti et al., 2015).PCR is rarely used in resource limited settings mainly because of its high costs. This technique is more used in research and advanced laboratories.

VL diagnosis in Kenya requires the deployment of the recommended rK39 rapid diagnostic tests (RDTs) (Safavi et al., 2020) which is required only in health facilities with the appropriate refrigeration for adequate storage conditions. This limitation prevents the rollout of rK39 RDT in all health facilities in VL endemic counties. Therefore a simple and more appropriate method need to be developed to replace these techniques.

Using hematological blood cell parameter changes as a unique indicator of VL will pave way for fast Turnaround Time (TAT) for the disease as well as ease management in an endemic environment. Patients who tested VL positive were done both a complete blood count (CBC) including a differential count and a peripheral blood film for evaluation and subsequent comparisons. The findings were then evaluated to determine their potential utility in aiding the diagnosis and management of the patients.

1.4 Hypothesis

1.4.1 Null Hypothesis

Visceral leishmaniasis infection is not associated with blood cellular hematological changes

1.5 General Objectives

To determine hematological blood cellular changes in visceral leishmaniasis infection in patients attending Kimalel sub-county hospital.

1.5.1 Specific Objectives

1) To determine the specific blood cellular changes that can provide unique characteristics of identifying the visceral leishmania infection
2) To determine the unique cellular characteristic that can aid in early and accurate diagnosis of visceral leishmania
3) To determine the most affected hematological blood cellular parameters by visceral leishmaniasis infection

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of Visceral Leishmaniasis

Visceral leishmaniasis (VL), is one of the most important neglected tropical diseases (NTD). *Leishmania donovani complex* protozoa cause this disease. More than 95% of the global VL cases are reported from 10 countries in South Asia, Africa, and South America. Visceral leishmaniasis in Africa is mainly reported in Sudan, South Sudan, Ethiopia, Kenya, and Somalia. East Africa is second only to India in the number of human cases of VL (Alvar et al., 2012).It is being considered mainly as a disease of developing countries, and is included among the neglected diseases (Pacheco-Fernandez et al., 2021, Yomey, 2002). Access to health care can be difficult in most cases and drugs are often not affordable, which certainly make the death rate higher (Murray, 2002). About 13,000 cases of VL occurred in 2020 according to the World Health Organization (WHO) (Ruiz-Postigo et al., 2021). VL is endemic in over 70 countries spread through all continents, except Antarctica and Australia, with population of 200 million people estimated to be at-risk (Meredith et al., 2024). Nevertheless the distribution of VL is mostly in seven countries, that is., India, Brazil, Ethiopia, Somalia, Kenya, South Sudan, and Sudan, where more than 90% of the worldwide VL cases are reported (Meredith et al., 2024) Annually, 500,000 new cases of VL estimated and 50,000 deaths occur which are thought to be underestimated(Murray, 2002), (Van Griensven & Digo, 2012).The pattern of VL infection noticed around the world increased between 1998 and 2005, was constant globally between 2005 and 2007, and remarkably decreased between 2007 and 2009, with a stead reduction in the South-East Asia zone. VL cases in the South-East Asia Region globally decreased after 2011(Mondiale de la Sante, WHO, 2016). Comparing the three main endemic areas. The number of cases in Eastern Africa elevated from 2015 to 2016 while the Indian subcontinent exhibited a reducing trend in the same two-year period, at the same time in Brazil cases of VL remained consistent in 2015 and 2016 Mondiale de la Sante,(WHO, 2016). *Leishmania donovani* and *Leishmania infantum*, are the main causes of VL the latter also called

Leishmania chagasi in South America. VL due to *Leishmania donovani* occurs in Southeast Asia, specifically India, Nepal, Bangladesh, and in Eastern Africa, particularly Sudan, Ethiopia, Kenya, and Somalia (Pacheco-Fernandez et al., 2021, Meredith et al., 2024), (Van Griensven & Digo, 2012). It can affect 2). VL present cyclical patterns of occurrence: in particular, the incidence increases o people of all ages and its prevalence even if in endemic areas is higher among children due to the acquired immunity of adults (Van Griensven & Digo, 201ver 2 to 5 years in both regions with a peak and then drops for some years (Mondiale de la Sante & WHO, 2016). Ethiopia, Sudan, and South Sudan, reported more than 14,000 cases in 2014, and in India and Bangladesh about 10,000 cases were declared (Mondiale de la Sante, WHO, 2016).

A female sand-fly is the vector responsible for Leishmania transmission which is a hematophagous, noiseless, 2–3 mm long arthropod whose color ranges from white to black (Martinez-Lopez et al., .2018). Some sandflies are active outdoors from dusk to dawn, while others can bite indoors and in the daylight (Martinez-Lopez et al., .2018). The Leishmania parasite needs a mammalian reservoir for survival, In the case of *Leishmania donovani*, the reservoir is characterized by infected humans, so that in the Asian and African areas transmission occurs anthropologically. The reservoir for *Leishmania infantum*, is represented by dogs, wild rabbits, and other wild mammals, in the Mediterranean region and in Latin America, VL is a zoonotic (Bern et al., 2008, Jimenez et al., 2014).

The extracellular promastigote within the sand-fly and the intracellular amastigote in the monocyte-macrophage cells of the mammalian are the two major morphological forms of the protozoan in the host (Martinez-Lopez et al., .2018). The amastigotes are able to replicate inside the infected cells until they are released by cell lysis so that they can infect other macrophages. These parasite forms are relevant to diagnostic purposes, in terms of direct visualization in different tissue specimens.

The most important transmission foci in Kenya remain Baringo, Isiolo, Marsabit, Pokot, Turkana, Wajir West Pokot, Kitui, Meru, and Machakos counties The disease

foci may change to areas previously not known to be endemic as a result of climate change and population movements.

In Kenya, the causative agent of visceral leishmaniasis is Leishmania donovani, which is transmitted by two different sandfly species depending on the region: Phlebotomus martini and Phlebotomus orientalis. Infection starts with the bite of an infected female sandfly that injects parasites into a susceptible host.The reservoir host is rock hyrax.

Status of Endemicity of Visceral leishmaniasis Worldwide, 2018

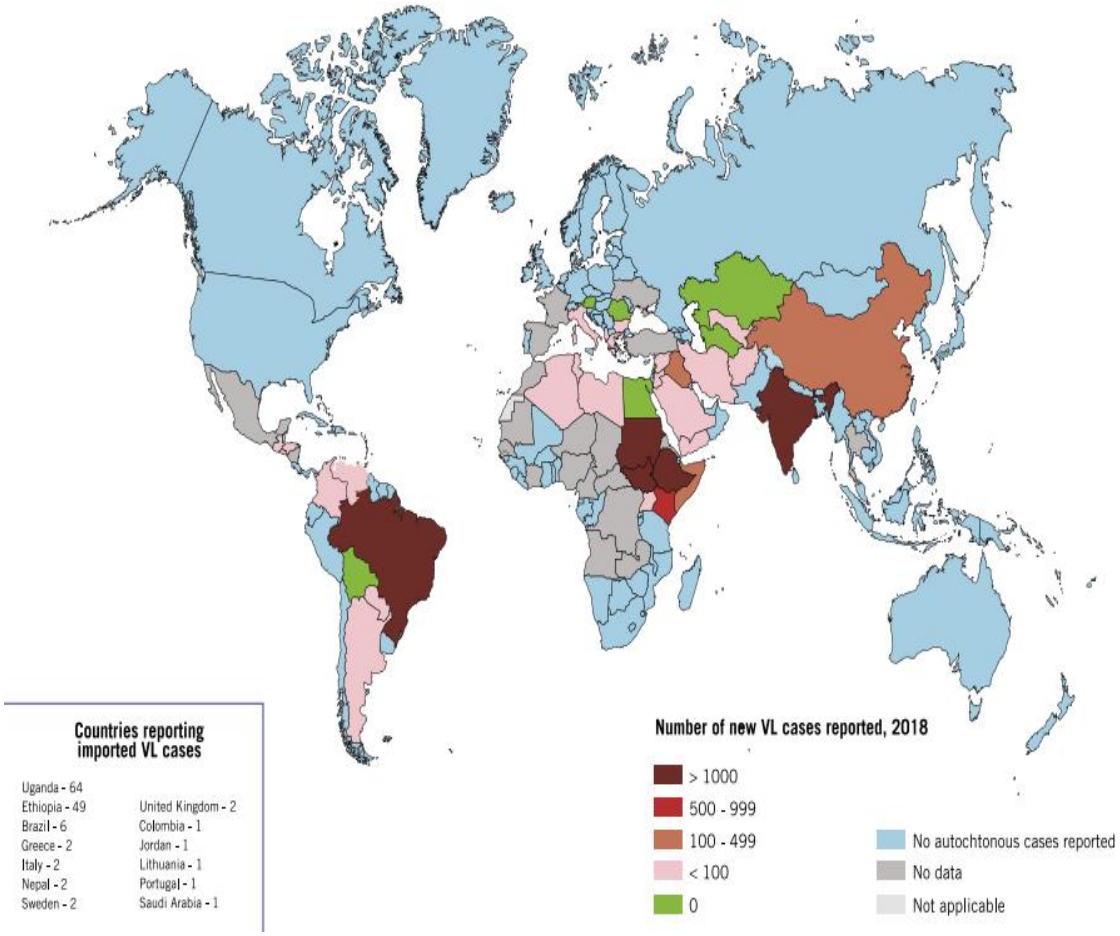


Figure 2.1: Distribution of Visceral Leishmaniasis Worldwide, (Source: WHO, 2018)

VISCERAL LEISHMANIASIS BURDEN IN KENYA, 2017- 2020.

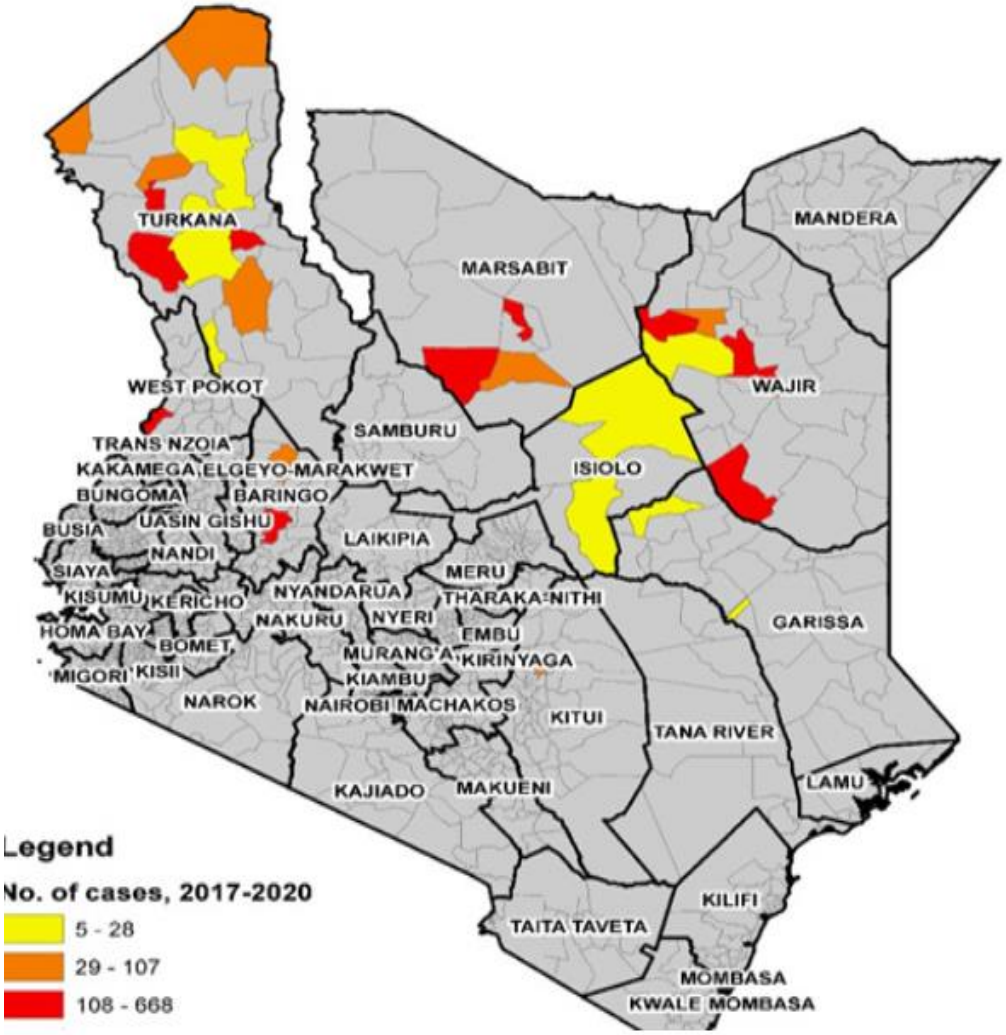


Figure 2.2: Map Showing Visceral Leishmaniasis Cases Reported in Kenya at Ward Level in Each of the Endemic Counties 2017-2020. (Source DHIS 2 data)

2.2 Biology and Life Cycle

Leishmania live as extracellular, flagellated promastigotes, (Fig.2.3), in the guts of female phlebotomus sandflies. The parasite stages in sandflies vary from rounded forms to elongated, highly motile, metacyclic promastigotes. Most are in the range of 15-26µm in length and 2-3µm in width. The flagellum extends from the anterior pole. Promastigotes grow at ambient temperatures ranging from 22ºC to 26ºC. (Pearson & Sousa, 1996)

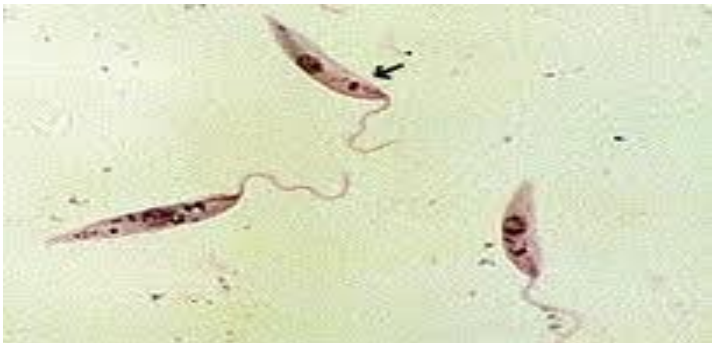


Figure 2.3: *Leishmania Donovani* Promastigotes

(Source:www.msu.edu/zol/316/jpg)

Leishmania exist within the mononuclear phagocytes of mammals as oval, intracellular amastigotes that are 2-3μm in diameter (Fig.2.4) The amastigotes have a relatively large, eccentrically located nucleus and a bar shaped specialized mitochondrial structure, the kinetoplast that contains extra nuclear DNA in the form of catenated mini-and maxi circles. Amastigotes are adapted to mammalian body temperature and the acid environment of the macrophage phagolysosome where they reside (Pearson, & Sousa 1996).

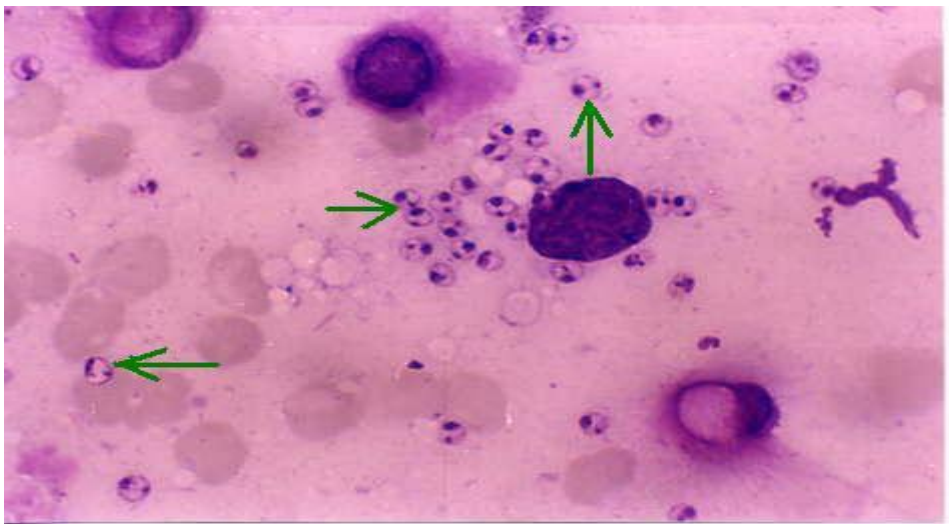


Figure 2.4: *Leishmania Donovani* Amastigotes Inside Macrophage (Source: www.msu.edu/zol/ 326/idonprom.gpg)

The sand-fly vector becomes infected when feeding on the blood of an infected individual or an animal reservoir host. The *Leishmania* parasites live in the macrophages as round, non-motile amastigotes (3-7 micrometers in diameter). The macrophages are ingested by the fly during the blood-meal and the amastigotes are released into the stomach of insect. Almost immediately the amastigotes transform in to the motile, elongated (10-20 micrometers), flagellate promastigote form. The promastigotes then migrate to the alimentary tract of the fly, where they live extracellularly and multiply by binary fission. Four to five days after feeding the promastigotes move forward to the esophagus and the salivary glands of the insect. When the sand-fly next feeds on a mammalian host, its proboscis pierces the skin and saliva containing anti-coagulant is injected into the wound to prevent the blood from clotting. The *Leishmania* promastigotes are transferred to the host along with the saliva. Once in the host, the promastigotes are taken up by the macrophages where they rapidly revert to the amastigote form. The *Leishmania* are able to resist the micro biocidal action of the acid hydrolases released from the lysozymes and so survive and multiply inside the macropha ges, eventually leading to the lysis of the macrophages. The released amastigotes are taken up by additional macrophages and so the cycle continues. Ultimately all the organs containing macrophages and phagocytes are infected, especially the spleen, liver and bone marrow in VL (Pearson & Sousa, 1996).

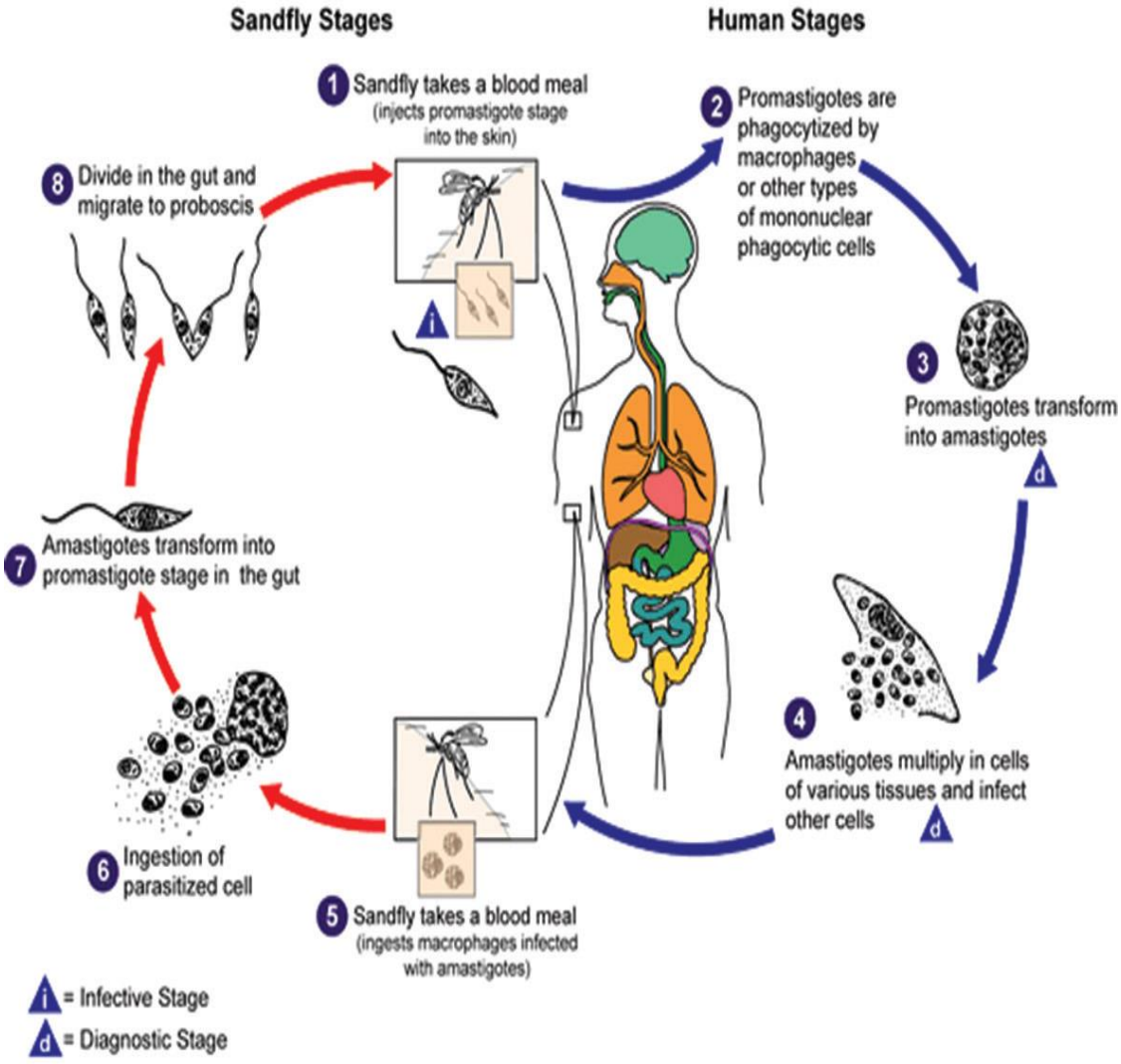


Figure 2.5. Life Cycle of *Leishmania* Parasite Courtesy of the United States Centers for Disease Control and Prevention

2.3 Clinical Manifestations of Visceral Leishmaniasis

Visceral Leishmaniasis encompasses a broad range of manifestations of infection. Infection remains asymptomatic or subclinical in many cases or can follow an acute, sub-acute, or chronic course (Herwaldt, 1999). Classical VL or kala-azar is characterized by fever, malaise, cough, weight loss, hepatomegaly and splenomegaly, lymphadenopathy, pancytopenia, and hypergammaglobulinimea. Thrombocytopenia may cause uncontrollable epistaxis or bleeding from other sites

(Guerin et al., 2002). On physical examination, the spleen, which is enlarged to a greater extent than the liver is typically appreciated 5-15cm below the left coastal margin: however, it should be noted that splenomegaly may be absent in 5% of cases (Hashim et al., 1994). Although ocular complications are rare they sometimes manifest as retinal hemarroges, keratitis, and central retinal vein thrombosis and many more. Hemoglobin concentration of 5-9g/dl, WBC counts of 2000-2004/m3 and platelet counts of 100,000-200,000/m3 are typical, although lower, more abnormal values can be observed in cases of more extensive disease (Berman, 1997). The differential diagnosis of VL is broad when patients present with subacute or chronic infection. VL must be differentiated from histoplasmosis, lymphoma, and other myeloproliferative disorders, milliary tuberculosis, brucellosis, hepatosplenic schistosomiasis, subacute bacterial endocarditis, infectious mononucleosis, or prolonged salmonella bacteremia Massive splenomegaly like that observed in patients with VL, is also seen in patients with topical splenomegaly syndrome that is associated with chronic malaria. Acute VL must be differentiated from malaria, typhoid fever, acute Chagas' disease, acute schistosomiasis, amebic liver abscess, and a number of bacterial and viral pathogens that cause febrile disease (Pearson & Sousa, 1996). Visceral leishmaniasis is a well-recognized opportunistic disease in persons with AIDS. They can classically present with fever, weight loss and organomegaly but atypical presentations are also common (Russo, et.al, 2003). There may be absence of splenomegaly. Amastigotes have been identified in macrophages in the pleural effusions and lungs as well as in the esophagus oral mucosae, stomach, small intestine and skin. Amastigotes were found in the bone marrow of HIV-infected persons who in several cases presented with aplastic anemia. Leishmanial infections have also been reported in asymptomatic patients with concurrent infections (Russo et al., 2003). *Leishmania tropica* which causesViscerotropic leishmaniasis is typically thought to be derma tropic and was parasitologicaly confirmed in 12 U.S. soldiers who participated in Operation Desert Storm in the Persian Gulf in the early 1990s. Persons affected had light parasite burdens and manifestations of visceral infection were nonspecific (e.g. gastrointestinal symptoms, fever, fatigue,) (Magil et al., 1993). In Africa and India, Post kala-azar Dermal Leishmaniasis (PKDL) develops after therapy in a subset of persons with visceral

leishmaniasis(Van Griensven et al., 2012; Das, 2020). It is characterized by macular, papular or nodular lesions on the face, trunk or extremities that may be mistaken for leprosy (Van Griensven et al., 2012; Das, 2020). Macrophages have amastigotes present in the lesions. PKDL appears shortly after VL symptoms subside in East Africa leishmania Donovani focus (0-6months), the interval in India is 1 to 2 years, with up to 30 years being reported in exceptional cases (Zijlstra et al., 1995). In India and Sudan, the main loci of PKDL, 50% and 5-10% of VL treated patients respectively; develop PKDL (Zijlstra et al., 2003).

Calvo et al. (1994) studied the effect of parasitaemia on bone marrow ultra-structure but found no significant correlation between degree of parasitaemia and both the rate and structural abnormalities of bone marrow in patients with VL. Ineffective hematopoiesis and splenic sequestration are the main likely etiopathogenic factors in the emergence changes of bone marrow and peripheral cytopenias (Marwaha et al., 1999). A study by (Tripathi et al., 2007) provided new insights in the basic immunological mechanism which controls leishmaniasis and indicated a crucial role of IL-10 Leishmania parasitized macrophages produce in the disease early stages and progression. The Th1/Th2 paradigm of resistance/susceptibility may be an oversimplification of a far more complicated network of regulatory/counter-regulatory interactions seen in these patients (Tripathi et al., 2007). The understanding of immune response to the parasite would pave the way for development of prophylactic and therapeutic strategies against it. This review summarizes the associations of VL with both common and uncommon conditions that are of interest to hematologists to provide guidance to the appropriate means of investigation to aid in identifying VL in a timely manner (Tripathi et al., 2007).

2.4 Hematological Changes Seen in VL Infection

2.4.1 Anemia

The clinically frequent significant feature of VL.is normochromic normocytic anemia with the hemoglobin levels of 7–10g/dl. The reported hemoglobin levels in two large series of patients were 8.3 and 7.8 g/dl (Varma et al., 2010). (Al-Jurrayan et al., 1995) examined 94 patients who had VL and found that all were anemic.

(Marwaha et al., 1999) reviewed 23 patients infected with VL and confirmed that they had moderate to severe anemia (Hb = 4.3–8.1 gm/dl). Children were found to have slightly lower hemoglobin values (mean = 6.4 vs. 7.3 g/dl) Studies of Ferro kinetic and red cell survival have indicated that hemolysis is the great cause of anemia in VL(Varma and Naseem, 2010; Neki & Singh, 2017) though there may also be plasma volume expansion associated with massively enlarged spleen. However Ferro kinetic studies have shown very little evidence of ineffective erythropoiesis. Reduced reticuloendothelial hyperplasia is accompanied by abnormal iron retention by macrophages; typical of anemia of chronic diseases (Pippard et al., 1986).This may limit the marrow response to hemolysis. In Mediterranean population a very rapid onset of anemia with hemolysis is commonly observed (Livotti et al., 1980). Occasionally both IgG and complement components are found on red cells, but this finding is not consistent and its significance remains to be determined. However, in most instances there is no evidence of immune hemolysis, and it appears that non sensitized red blood cells are destroyed in the macrophages that are recruited to the spleen and liver as part of inflammatory response to parasite. Hypersplenism is another primary pathogenetic mechanism although nutritional deficiencies of iron, folate and vitamin B12 may play further contributory role (Varma et al., 2010). Other mechanisms suggested include increased sensitivity to complement inhibition of erythrocyte enzymes (Neki & Singh.2017), hemolysin production by the parasites and cold agglutinins presence (Neki & Singh, 2017).

2.4.2 Leucopenia

Leucopenia is an early and striking manifestation of VL. There is relative lymphocytosis the presence of significant numbers of eosinophils rules out the diagnosis of VL. The mean Total Leucocyte Count reported in two large series is 2.8×10^9/l and 4×10^9/l respectively (Cartwright et al., 1948). However, a lower Total Leucocyte count (2.4×10^9/l) was reported in a series of VL patients studied at their Centre (Naveed et al.2011). About 75% patients with VL have been shown to have leucopenia in various studies (Boyer et al., 2019).The main cause for its development has been attributed to hypersplenism.

2.4.3 Thrombocytopenia

Platelets counts are usually affected after long duration of illness. (Neki et al., 2017) reported in their study that the average duration of illness was significantly longer in thrombocytopenic patients as compared to non-thrombocytopenic (9.2 ± 3.4 vs. 4.2 ± 1.8 months). Mean platelet count of $109 \pm 82.3 \times 10^9$/l and an incidence of 55–65% were recorded in other studies (Al-Jurray et al., 1995; Marwaha et al., 1999). Splenic sequestration is perhaps the main factor contributing where immune mechanisms are said to be non-contributory as anti-platelet antibodies which have not been recorded in any VL studies. Deranged platelet function studies on patients infected with VL were reported by (Dube et al., 1995). They conducted platelet function studies on 25 Indian cases with positive VL matched with 25 age and sex health controls. Variable degrees of Thrombocytopenia were found in 92% patients and 44% of patients had platelets less than 60,000 mm^3. The platelet adhesive index was less than 30% in 70% of patients with VL (normal 31–60%).Adenosine diphosphate and adrenaline was abnormally prolonged compared to the controls in Platelet aggregation time. (Dube et al., 1995) 40% of cases available were poor platelet factor III. They found that there was atleast degree of correlation between platelet adhesiveness and platelet factor III in these patients: half of the patients with poor platelet adhesiveness showed reduction in platelet factor III availability (Dube et al., 1995).

2.4.4 Pancytopenia

The varying degree of frequency and severity has been reported by several groups of workers (Agrwal et al., 2013: Varma et al., 2013). Prolonged presence of illness shows pancytopenia It occurs due to splenic sequestration of cellular blood elements. The peripheral blood picture resembles a plastic anemia in such cells; however the presence of reticulocytes and young white blood cells shows on words regeneration of blood which helps to differentiate from aplastic anemia. Where pancytopenia is associated with fever, lymphadenopathy and hepatosplenomegaly examination of bone marrow differentiates the two from clinical pictures of Leukemia (Varma et al., 2010).

2.4.5 Change in Bone Marrow

Increased plasma cells and intracellular parasites in mononuclear phagocytes and erythroid hyperplasia are Common findings (Al-Jurrayan et al., 1995).Variable degrees of granulomatous reaction (25%), leukophagocytosis and erythrophagocytosis (46%) could be seen. Mathur et al. (2007) reported a case of a 4 year old child with fatal hemophagocytosis secondary to VL. Effective treatment was emphasized in patients diagnosed with hemophagocytic syndrome, where diligent search for the etiologic agent including LD bodies should be made. (Rajagopala et al., 2008; Skinner et al., 2019; Daher et al., 2015) showed hemophagocyticlymphohistiocytosis (HLH) in patients who had Visceral leishmaniasis and explained that VL related HLH is always under-recognized because of overlapping features with HLH and negative marrow evaluation at the onset, leading to high mortality rates. Recently (Varma et al., 2008) encountered a VL patient showing profound histiocytic hyperplasia, producing syncytium like arrangement on BM examination. (Cotterell et al., 2000) Sought to identify the factors associated with *L.donovani* infection in VL, which regulate hematopoiesis, by studying the interaction between this intracellular pathogen and stromal cells responsible for regulating hematopoietic colony formation. Their results indicated that stromal macrophages are a target for *L.donovani* infection in vivo and in vitro and as a consequence of the selective induction of granulocytic-macrophage colony stimulating factor (GM-CSF) and alpha tumor necrosis factor (TNF-α) production, infected stromal macrophages preferentially support increased levels of myelopoiesis. The severity of the hematological changes generally depends on the duration of the disease and the size of the spleen rather than on the number of parasitized mononuclear cells (Cotterell et al., 2000).

2.4.6 Coagulation Abnormalities

Liver dysfunction with jaundice, ascites and deranged coagulation may occur in late stages and has a poor prognosis. Liver dysfunction may be caused directly by protozoa itself or indirectly to the effect related to the immune response of the parasites. (Varma et al., 2010) recorded coagulation abnormalities in 10 (11%) of the

94 VL patients in their study, in the form of prolonged prothrombin time (PT) and activated partial thromboplastic test (APTT), with 4 (36%) of these having disseminated intravascular coagulation.

2.4.7 Erythrocyte Sedimentation Rate (ESR)

This is a type of laboratory test that measures how quickly red blood cells settle at the bottom of a test tube containing a blood sample. Normally red blood cells settle slowly. When red blood cells settle faster than normal rate may indicate inflammation in the body which can be a reaction to an infection or injury and may also be a sign of a chronic disease or other medical condition. ESR does not specifically diagnose any disease but it can provide information about whether or not there is inflammation in the body. Increase of erythrocyte sedimentation rate is regularly noted in VL infected patients which is probably due to acute phase proteins release. (Costa et al., 2023).

2.4.8 Platelet Function Studies

Deranged platelet function studies were reported by Dube et al. (1995) on patients with VL. Further they conducted platelet function studies on 25 parasitological positive cases of Indian VL and 25 age and sex matched health controls. Variable degree of thrombocytopenia was found in 92% of patients, platelets were less than 60,000mm2 in 44% of patients. There was less than 30% platelet adhesive index in 70% of patients with VL. Platelet aggregation time with ADP and adrenaline was abnormally prolonged compared to the controls. In 40% of cases platelet factor 111 availability was poor. They found that there was a fair degree of correlation between platelet adhesiveness and platelet factor 111 availability in these patients 50% of patients with poor platelet adhesiveness showed reduced platelet 111 availability. Never the less further studies on platelet function are needed to corroborate their findings. However, further studies on platelet function are needed to corroborate their findings.

2.5 Diagnosis

Recommendations for serological studies are made as the initial diagnostic tests in suspected leishmaniasis. Parasites can be found in phagocytic cells, bone marrow, spleen, lymph nodes and rarely in blood in advanced stages of the disease. Early morphological identification provides specific and cost-effective diagnosis. Bone marrow culture is a more sensitive diagnostic technique than microscopy. Specimen aspirations are collected aseptically and cultured in Schneider's Drosophila medium supplemented with calf serum or in Novy–MacNeal–Nicolle medium (Reithinger et al., 2007). The promastigotes in cultures usually begins to show in 2-5 days. Leishmania is microscopically diagnosed in the hematology laboratory by direct visualization of the amastigotes (which is referred to as LD bodies -Leishman Donovan). A thin smear buffy coat preparation of peripheral blood or aspirates from spleen, lymph nodes, bone marrow or skin lesions should be spread on a slide and stained with Leishman or Giemsa stain for 20min. A mastigotes are small, round bodies 2-4 um in diameter with indistinct cytoplasm, a nucleus and a small rod – shaped kinetoplast and are seen within monocytes or less commonly, in neutrophils in the peripheral blood and in macrophages in bone marrow aspirates extracellular free lying LD bodies may at many times also be seen (after they have been released from the disrupted cells).Various tests for diagnosis of VL was evaluated On the basis of bone marrow aspirate positivity, the sensitivity and specificity of DAT was 100% while that of rk39 strip test and ELISA was 100 and 87%, respectively. (Mondal et al., 2010).

2.5.1 Microscopic and Culture Methods of Parasitic Diagnosis

The most accurate method available and commonly used for diagnosis of VL is the demonstration of parasites in the relevant tissue such as spleen, liver, bone marrow, lymph nodes, or the buffy coat of peripheral blood. Diagnosis is based on finding amastigotes in macrophages. The analysis is done using a special grading scale ranging from 0 (indicating no parasite in 1000 microscopic fields) to +6 (indicating more than 100 parasites per field). Spleen and bone marrow are the two most commonly used tissues. The sensitivity of this method using spleen is more than

95%; the bone marrow is about 60-85% making direct detection of parasites as the most powerful technique and the spleen the most sensitive tissues to detect Leishmania (Santarem and da Silva 2007). Splenic and bone marrow aspirate collections are invasive, painful and risky techniques and the use of microscopy in the diagnosis of VL, like all microscopic procedures also suffers from variability of detecting sensitivity and therefor the need for an expert microscopist (Santarem & da Silva, 2007). Strains of leishmania can be isolated and maintained as promastigotes in artificial media. (Novy-McNeal, Nicolle (NNN) medium and Tobias medium), are biphasic culture media which may be used, for conversion of amastigotes into promastigotes, while monophasic medium (Schneider's insect medium, M199, or Grace's medium) is preferred for amplifying parasite number (Srivastava et al., 2019.).The culture technique also, suffers from the deficiencies as the microscopic technique, and the, time consuming nature and the high cost are prohibitive and thus, except in dedicated research laboratories, it is seldom used for clinical diagnosis. The correct identification of parasites requires trained and skilled personnel. This sort of special approach to diagnosis makes it unfit for field work as it is time consuming and requires expert personnel.

2.5.2 Serological Diagnosis

Serological methods which are comparatively reliable and non-invasive are more suited, for diagnosing VL in endemic regions. Many antibody detection-tests have been developed for field diagnosis of VL, but all have two major limitations. First, serum antibody levels decrease after successful treatment and remain detectable after several years of cure, and VL relapses cannot be diagnosed by serology. Second, a significant number of people living in endemic areas with no history of VL are positive for anti-leishmanial antibodies owing to asymptomatic infection (Chappuis et al., 2007). In healthy populations sero-prevalence varies from <10% in low to moderate endemic areas to >30 in high transmission foci or in house hold contacts .Antibody-detection tests must therefore always be used in combination with a standardized clinical case definition for VL diagnosis. Complement fixation test, gel diffusion immunoelectrophoresis, indirect haemagglutination test and counter-current immunoelectrophoresis which are Conventional serological methods have limited

diagnostic accuracy or feature for field use. The needs for a fluorescence microscope restricts indirect fluorescence test (IFA) which showed acceptable estimates for sensitivity and specificity (77%-100%) their use to reference laboratories (Boelaret et al., 2007). Enzyme linked immunoserpentassay (ELISA) has been used in the serodiagnosis of VL. Sensitivity and specificity depends upon the antigen used. Most promising results are shown by antigen rk39 with sensitivity and specificity of 100% and 96% respectively. The antibody titer to this antigen directly correlates with active disease and have potential in monitoring chemotherapy and in predicting the clinical relapse (Kumar et al., 2012). In addition rk39 ELISA has a diagnostic and prognostic utility in HIV infected patients (Houghton et al., 1998). Elisa is not used in the endemic regions for the diagnosis of VL due to the requirements of skilled personnel, sophisticated equipment and electricity.

Immunoblotting test provides detailed antibody responses to various leishmanial antigens and is more sensitive than IFAT and ELISA, but it is expensive, time consuming, and requires considerable skill and is therefore sparingly used in the diagnosis of VL. (Srivastava et al., 2011). The DAT using freeze dried antigen and the rk39 immunochromatographictest (ICT) are two antibody detection tests that have been extensively evaluated for field use.

In 1986, EL-Harith and his co-workers at the KIT developed the DAT for the detection of anti-leishmanial antibodies in the serum samples from humans and dogs suspected for visceral Leishmaniasis (EL- Harith et al., 1986). The DAT is semi-quantitative test and uses microtitre plates with V-shaped wells in which increasing dilutions of patients' serum or blood eluted from filter paper are incubated with comassie brilliant blue stained and killed *L.donovani* promastigotes. Agglutination is observed after 18 hours if antibodies are present (Chappuis et al., 2007). The test gives antibody titers ranging from 1/50 (usually1/100) to 1/102,400 or even higher. The test is the first line diagnostic tool in many countries (EL-Harith et al., 1988). DAT is used to confirm diagnosis of VL and initiate treatment in patients that strictly meet the clinical case definition in Ethiopia (MOH, 2006). The DAT's problem is the limited stability of the used aqueous antigen for which cold storage is required (EL-Harith et al., 1988). To circumvent this problem, a freeze dried antigen that remains

stable at ambient temperature (20oC-45oC) compares well with the standard aqueous antigen under laboratory and field conditions has been developed (Meredith et al., 1995; Zijlstra, et al., 1997; Oskam et al., 1999). A recent meta-analysis of the DAT gave sensitivity and specificity estimates of 94.8% (95%confidence interval (CI) 92.7-96.4) and 97.1% (95% CI 93.9-98.7), respectively (Chappuis et al., 2006).The performance of the DAT was not dependent on the region nor on the Leishmania species (Boelaert et al., 2007). However, the major disadvantage of the DAT is the need of multiple pipetting, relative long incubation time, high cost of antigen and limited production facility of quality controlled antigens in two European countries (Schallig et al., 2001). rk39 ICT is a VL RDT test. A RDT is a simple, test that can be used at all levels of the health care services, it does not require highly skilled laboratory staff and results can be read easily and within 15-20 minutes. Rapid tests expedite the initiation of treatement. (WHO/TDR, 2008).Rk39 is a 39-amino acid repeat that is part of a kinesin-related protein in *Leishmania chagasi* and which is conserved within the *Leishmania donovani* complex. The rk39 ICT is composed of antigen immobilized on a piece of nitrocellulose membrane. Above it we find anti protein A. Half a drop of blood, serum or plasma is placed on the lower part of the strip, after the addition of phosphate buffered saline the mixture flows along the strip by capillarity dragging the conjugate (protein A colloidal gold) that was dried on the strip. Antibodies against the antigen are present in blood/ serum of kala-azar patients and they will bind to the recombinant antigen that is bound to the test strip yielding a pink/purple band. The final outcome of a positive test for the rk39 and the anti-protein band control is the presence of two bands. (Santarem & da-Silva 2007; WHO/TDR, 2008). A recent meta-analysis of rk39 studies gave sensitivity and specificity estimates of 93.9% (95%confidence interval (CI) 87.7-97.1) and 95.3% (95% CI 88.8-98.1), respectively (Chapais et al., 2006). The rk39 strip has been shown to be less accurate in East Africa but accurate in diagnostic performance in India and Nepal. After cure of VL the disadvantage of rk39 ICT is that a significant proportion of health individual in endemic regions remain positive for long periods. Antigen detection tests should be more specific than antibody detection tests as they avoid cross reactivity and can distinguish active from past infections, and is particularly practical in the case of HIV/VL co-infection where deficient antibody

production complicates accurate diagnosis of VL (Salam et al., 2011). Kalon Biological limited has introduced a new latex agglutination test (Katex) for detecting leishmanial antigens in the urine of patients with active VL. Katex detects a heat-stable, non-protein, carbohydrate based, disease-specific parasite antigen in the urine of patients with active infections (Sarkari et al., 2002). The test has shown a sensitivity of 68-100% and a specificity of 100 % in some preliminary studies. More recent field studies have however shown a much wider variation in estimates of sensitivity of (36-100%) and specificity of (64-100%).Even though it has low sensitivity, the limitations of the tests are: to avoid false-positive reactions the urine must be boiled and it is difficult to distinguish weakly positive from negative results, which affects the reproducibility of the test (Chappuis et al., 2006, Rijal et al., 2004).

2.5.3 Molecular Diagnosis

The ordinary parasitological and serological methods presents certain limitations to the diagnosis of leishmaniasis (de Paiva-Cavalcanti et al., 2015) and these restrictions have led to the discovery of molecular methods (Tlamcani, 2016). In contrast to normal diagnosis, molecular techniques are recommended more for CL because of their excellent precision and fast speed (Azizi et al., 2012). Molecular techniques serve as a supplement as well as an alternative method of diagnosis. The primary reason for approval of molecular techniques in the routine laboratories worldwide is the feasibility, safety, and reliable molecular tools. Besides, several applications and the encouraging results have led to continued approval of these methods (de Paiva-Cavalcanti et al., 2015). Apparently various molecular techniques of diagnosis have been enhanced for example, pulse-field gel electrophoresis and multilocus enzyme electrophoresis yet, assays based on polymerase chain reactions currently constitute the primary way of molecular diagnosis for health professionals and researchers. Information and knowledge of DNA sequencing have been significantly used for realizing the polymerase chain reaction based analyses for different utilizations in illustrating the parasite and disease (Singh et al., 2005). In a study conducted to differentiate Leishmania species based on internal transcribed spacers (ITS)-PCR, in contrast to parasitological techniques of diagnosing CL, it was observed that ITS-PCR is not only valid for identification of species but also

displayed a high sensitivity and specificity (98.8% and 100%) as compared to microscopy and culture techniques (79.6% and 86.2% sensitivity respectively) (Shahbazi et al., 2008). PCR and its modifications such as quantitative-PCR, semi-nested-PCR, and nested-PCR have remarkably been used for diagnostic assays using various samples and target zones (da Silva Solca` et al., 2012; Reis et al., 2013). Originally, the qPCR method had been used to detect DNA from the causative agent in different samples of human and animals to study host-parasite relations and to quantitatively estimate parasitic load in infected patients (de Paiva-Cavalcanti et al., 2015).

2.6 Visceral Leishmaniasis Control

The use of insecticide impregnated materials for Reservoir and vector control and active case detection and treatment are the control strategies for VL control (Chappuis et al., 2007).

2.6.1 Reservoir Control

Dogs are the main reservoir of *L.infantum* In zoonotic VL. Screening of dogs and killing of sero-positive animals, has become unacceptable and is increasingly being debated. As relapses are frequent treating infected dogs is not an effective strategy. In study conducted in Iran the use of delamethrine treated collars, which reduced the risk of infection in dogs by 54% and in children by 45% (Gavgani et al., 2002).

2.6.2 Vector Control

Sandflies and Anopheles mosquitoes are susceptible to the same insecticides. Following the large scale anti-malaria insecticide (dichloro-diphenyl-trichloro ethane, DDT) spraying campaign that were implemented in the 1950s, VL almost completely disappeared from the Indian sub-continent. The disease re-emerged unfortunately when the spraying campaign was discontinued (Chappuis et al., 2007). VL transmission in East Africa endemic countries occurs mainly outside villages during various activities like shepherding and farming. Indoor residual spraying for disease control is therefore unlikely.

2.6.3 Insecticide Impregnated Materials (ITNs)

ITNs could concomitantly prevent VL and vector borne diseases such as malaria. VL protection using bed nets has limited evidence

Other insecticide-impregnated materials such as curtains and blankets should be evaluated for use in VL prevention depending on the sleeping traditions of the population and the biting habits of the local vector (Chappuis et al., 2007).

2.6.4 Treatment

Sodium stibogluconate, meglumine antimonite (Antimonial compounds) form the traditional treatment for leishmaniasis. Amphotericin B is the most common alternative since resistance to antimonial is prevalent in some parts of the world. The Institute of One World Health has funded for the production of Paromomycin which is an inexpensive alternative with fewer side effects than amphotericin as an orphan drug for use in the treatment of leishmaniasis starting in India. Response to treatment occurs as stated below (Manson-Bahr PEC, Bell DR, 1987).

Generally within a day of starting treatment symptomatic improvement occurs Hematological improvement is noted within a week. Complete hematological response occurs in 4–6 weeks. Clearance of parasites in the Spleen occurs in 2–3 weeks Splenomegaly reduction occurs within 2 weeks. Very large spleen may take several months to reduce to normal size, but small spleen may become impalpable within a month.

Immunoglobulin levels and serological reactions revert to normal over a period of six months.

Monitoring of treatment and follow up:

Daily monitoring of temperature and weekly assessment of spleen size clinically

To monitor the clearance of parasites from the spleen by doing weekly aspirates

To assess hematological response by doing weekly hemograms

3 and 12 monthly follow up after course of drugs to detect any relapses

Test of Cure (to discontinue treatment) (Manson-Bahr PEC, Bell DR, 1987) defined as absence of parasites from two successive splenic aspirates taken 1 week apart. Patients to indicate as cured, should have absence of fever, hematological and clinical improvement, and reduction in spleen size and zero score of splenic aspirate. Patients in whom response to treatment is delayed with appropriate therapeutic agents complicating illnesses, such as AIDS and pulmonary tuberculosis should be looked for in all.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in Kimalel Sub-county Hospital in Baringo County. It is located 265Km from Nairobi. It is a Kenyan government hospital which is managed by Kenya Medical Research Institute (KEMRI) and the kala-azar diagnostic and treatment Centre. It has a bed capacity of 40. Kimalel Sub county hospital offers a wide range of services such as: Laboratory, Mother and Child Health (MCH), Pharmacy, and Maternity, minor theater and In-patient.

Other study sites were Marigat county hospital, Sabor dispensary, Koriema dispensary, Kibingor dispensary, Roboi dispensary and Kamp Samaki Health centre. These sites are located in endemic areas of VL. The health workers within these health facilities were contacted and informed about this study that later referred patients on basis of clinical signs and symptoms of VL to Kimalel Sub-County Hospital where screening and recruitment to the study was taking place.

3.2 Study Design

This was a cross sectional prospective study to determine hematological blood cellular changes in VL infection in patients attending Kimalel sub-county hospital, Baringo County, Kenya.

Seventy four (74) patients aged between 1- 60 year who were selected during the study period and recruited into the study after fulfilling inclusion criteria.

3.3 Study Population

The study population consisted of VL case-patients aged 1year to 60years.

3.3.1 Inclusion Criteria

Patients who gave consent or assent to the study

Patients who were between 1 year to 60 years old

Patients who tested positive for visceral leishmaniasis

Patients who have not been put on treatment for visceral leishmaniasis

3.3.2 Exclusion Criteria

Patients who declined to give consent or assent to take part in the study

Patients who were screened and found to have no visceral leishmaniasis

Patients who had already started medication for VL as this may interfere with cell morphology

3.4 Sample Size Determination

The appropriate samples size for the study was calculated using Bernard Roster formula;

n= Z^2 p (1-p)/e^2 (Bernard Roster: Fundamentals of Biostatistics, Sixth Edition, pages 416-420)

Where *Z* is the value (1.96) corresponding to 95% confidence level, assuming *p* is the estimated proportion (50%) of confirmed VL patients by microscopy who tested positive with the splenic aspirate/bone marrow, and *e* is the acceptable margin of error (5%). Therefore the sample size n=384.

Considering that the number of VL positive patients by microscopy seen at Kimalel (N~91) in a year is less than 10 times the calculated sample size of 384 which is 3,840, a corrected sample size to be attained has been calculated using the finite

population correction (FPC) and the new sample size n' given by the formula n'=n/ (1+ (n/N) is 74VL positive.

Calculation:

- samples size was calculated using Bernard Roster formula; $n= Z^2 p (1-p)/e^2$
- Where n is the sample size, z = confidence interval at 95% (1.96) e = margin of error at 5% (0.05) ,p=anticipated prevalence used 50%(0.5)

$$n= Z^2 p (1-p)/e^2 \qquad \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} = \frac{3.8416 \times 0.25}{0.0025} \qquad n= 384$$

Considering number of VL positive patients seen by microscopy at Kimalel (N~91) in a year is less than 10 times the calculated sample size of 384 which is 3,840 a corrected sample size to be attained was calculated using the finite population correction (FPC) the new sample size n' given by the

formula n'=n/ (1+ (n/N) N=91

$$\frac{384}{1+\frac{383}{91}}$$

$$1+ 4.219 \quad \frac{384}{5.219} = 74 \text{ samples}$$

3.5 Sampling Method

The sampling method was convenience sampling. Patients came to the medical facility seeking for treatment. The clinician examined and talked to them about the study once he found them with signs and symptoms of VL. Those who met the

inclusion criteria were enrolled into the study after signing the informed consent or assent forms.

Laboratory request forms for complete blood count and splenic aspirates for examination were done. Samples were collected and sent to the Kimalel hospital laboratory for analysis and those that became positive microscopically for VL were enrolled to the study. This procedure was followed until the numbers of patients required for the study were reached.

3.6 Recruitment and Consenting

Patients who were referred or walk-ins were first seen at the outpatient clinic. For purposes of this study, all patients had clinical signs and symptoms of VL. Patients were then referred to the clinician's room for consultations. Those Patients suspected of VL had their blood taken for CBC and prothrombin time index (PTI). After the clinician had reviewed, the patient was referred to the observation room where he/she undergo pre-admission observation performed by the nurse. The in-patient files were opened and then the patient was taken to the ward. Patient suspected of VL underwent full informed consent or assent process. Once the patient had signed the informed consent/assent he/she receives a study participant number. The patients consented were entered into the screening log. The patients then received a study file and underwent a spleen aspirate performed by qualified clinician for parasitological examination and inclusion/exclusion procedures as defined in the study protocol. Blood samples were collected for full complete blood count and peripheral blood film from qualified patients. The guardians/parents of the eligible children were reffered to the study nurse who described the study to them. Written informed consent/assent was obtained from parents/guardians on behalf of children willing to participate by the clinician following detailed explanation of the study to them. For an illiterate parent/guardian, a literate witness signed who do not have connection to the study team.

3.7 Laboratory Procedures

3.7.1 Sample Collection

2mls of blood was collected in ethylene diamine tetra acetic acid (EDTA) tubes for Complete blood count (CBC) and peripheral blood film (PBF) from patients who had signs and symptoms of visceral leishmaniasis (VL)

Splenic aspirates were obtained from patients suspected to have VL.

3.7.2 Sample Analysis

3.7.2.1 Splenic Aspirate Smear Preparations, Staining and Examination for VL Detection

Aspirates were done on participants at bed site and the materials obtained were put on clean frost end microscope slides and smears made immediately before they dry up. The smears were labeled using a lead pencil with participant's initials and study number. The smears were then transported to the laboratory and let to air dry and fixed in absolute methanol for 2 seconds. The fixed smears were allowed to dry and stained with 10% Giemsa stain for 10 minutes. Smears were then washed gently in running tap water and let to air dry and microscopically examined for VL bodies using X100 oil immersion objective. Results were recorded in appropriate Laboratory report forms and study record book.

3.7.2.2 Complete Blood Count and Peripheral Blood Films for Differential Count

2mls of whole blood was gently mixed on the blood mixer and entered into the AcT10 Coulter Counter hematology machine which was available and in use in Kimalel sub-county hospital laboratory. Complete blood count was done and includes white blood cells, lymphocyte count, platelets, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Two copies of results were printed after each

run. One copy was sent to the clinician for participants' management and the other copy was kept for the study records.

3.7.2.3 Thin Blood Films Preparation for Differential Count

A small drop of blood (about 2-3mm in diameter) mixed in Ethylene Diamine Tetra acetic Acid (EDTA) anticoagulant was placed on a clean slide and a thin film made. The films were labeled with the participants' initials and study numbers. Films air dried and was fixed with absolute methanol. The dry films were stained with 10% Giemsa stain for 10minutes in the staining rack. The stain was gently rinsed using tap water and let to air dry. A drop of oil immersion was placed at the body of the film and examined using x100 objective. Each type of white blood cell was counted in every field to 100 white cells and recorded as percentage. Platelets and Red Blood Cells (RBC) morphology was examined. The morphology of all cells e.g. Red Blood Cells, White blood Cells:-Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Platelets were examined under the microscope and findings recorded. The differential counts were recorded in the laboratory report form and study record book.

3.8 Quality Control

The following controls were used to setup and monitor the Coulter AcT analyzer performance.

AcT Cell Control Plus (commercial cell controls*)* –Abnormal Low, Normal and Abnormal High levels of cell controls. These cell controls were analyzed prior to running study samples.

All reagents were used according to the manufacturer's recommendations and within their expiration date.Two slide smears of splenic aspirates and Peripheral Blood Film (PBF) for differential counts were prepared and stained with 10% Giemsa stain.

Freshly prepared 10% Giemsa stain was used daily and checked with known smears for quality control purposes.Internal quality control sample source: The internal quality control samples were obtained from Technomed Kenya Limited.

3.9 Data Management

The following data were collected from each subject: Gender, age, CBC and PBF, and Spleen aspirate. All collected data were double entered into computer in Microsoft excel (Ms-Excel) computer application. To avoid loss or tampering, back up of the data was done in compact discs, external hard disk and printing of the same data in hard copies. The printed hard copies were filed separately in labeled box files which were kept in a secure lockable drawer away from physical and mechanical hazards and also to maintain privacy and confidentiality of information of the participants. Cleaning and validation of the data was going on during data collection and entry in readiness for exportation of the data to SPSS (Statistical Package for Social Sciences) version 20 for data analysis. Any additional information collected and observed during data collection was recorded on hard cover laboratory study book and some kept in a lockable drawer for privacy, confidentiality, avoid loss and tampering of the information.

3.10 Ethical Considerations

Approval was sought from KNH/UoN ERC ethical committee.

Kimalel Hospital: permitted the use of their facilities and personnel in carrying out the study.

Written consent or assent was obtained from eligible study participants. Participation in this study was voluntary and participants' could withdraw at any point. Participant's confidentiality was upheld at all times. Data generated was kept under key and lock.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of the Study Population Analysis

Blood from 74 positive cases of visceral leishmaniasis (VL) was analyzed for various blood cellular parameter changes. The study targeted age 1 to 60 years old of all gender. Majority of the patients in this cohort were males at 58.1% (n = 43) and the females at 41.9% (n = 31). The largest age group was 1-17 year accounting for 55.4% (n = 41) with males accounting for 28.4 % (n = 21) of this age group and 27 % (n = 20) for the females. This was closely followed by patients aged 18 years and above at 44.6% (n=33) and 29.7% (n=22) of the population were males above 18 years old while females were 14.9% (n=11) of the same age (Table 1).

4.2 Blood Cellular Characteristic Features Observed on Different Techniques

4.2.1. Red Blood Cells Count

Out of 74 samples, analyzed 49 samples had hypochromic and normocytic anemia. This is a condition where red blood cells have fewer colours than normal cells and is due to a disproportionate reduction of red cell hemoglobin in proportion to the volume of cells. Clinically the colour of the cells can be evaluated by the Mean Cell Hemoglobin Concentration (MCHC).Hypochromic and normocytic cells have normal same size but the red colour is reduced. The findings showed that the Mean Cell Volume (MCV) that measures the average size of red blood cells in hypochromic and normocytic anaemia was within normal (80 – 99.9fl).While the Red Blood Cells (RBCs) and Hemoglobin (Hgb), mean Cell Hemoglobin (MCH), Hematocrit (HCT) and MCHC were reduced.

The findings of 23 samples analyzed were hypochromic and microcytic. This is anemia where RBCs are smaller than normal and often hypochromic RBCs are usually characterized by a low MCV (mean cell volume) less than 80fl. The RBC, Hb, MCV, MCH, HCT and MCHC in this, findings were reduced below normal and iron deficiency is the most common cause of microcytic anaemia. Two (2) samples

out of the 74 samples analyzed were found with hypochromic and macrocytic RBCs a kind of anemia where RBCs are larger than normal. The cells are folate, and vitamin B12 deficiency Here MCV is greater than 99.9fl. The HCT (hematocrit) counts were less than normal in all the samples analyzed.

4.2.2 Microscopic Examinations for WBCs Showed the Following Features

4.2.2.1 Neutrophil

Sixty (60) samples showed normal distribution of neutrophils (40-75%). All samples 74 had low WBC count (leucopenia) than normal ranges which are 4.5 – $10.5x10^3$/ul).There was no high neutrophil counts found in the examination. Fourteen (14) samples had neutropenia which involves having lower than normal levels of neutrophil in the circulating blood.

4.2.2.2 Lymphocytes

During the microscopic examinations the findings showed that out of 74 patients examined,37(50%) patients had normal lymphocyte counts and 37(50%) patients had higher than normal lymphocyte counts (lymphocytosis) (Normal values of lymphocytes counts ranges between 25 – 45% and high lymphocyte counts ranges above 45% which is termed lymphocytosis).

4.2.2.3 Monocytes

Microscopic examination showed thirty eight, 38(52.4%) patients had normal ranges of monocytes while, 36 (48.6%) patients had low monocyte counts.

4.2.2.4 Eosinophils

Eosinophils were not found during the microscopic examination in all the samples an indication that VL is among the infections that depletes the eosinophils from the circulation system.

4.2.2.5 Platelets

Examination Findings of Blood Cell count revealed that 65(87.8%) platelet count had thrombocytopenia while 9(12.2%) samples had normal distribution.

Table 4.1: Examination Findings of Blood Cellular Parameters

PARAMETERS	NORMAL COUNTS	LOW COUNTS	HIGH COUNTS	WHO REFERENCE RANGES
WBCs x 10^3/ul	0	74	0	4.5 - 10.5
RBCs x 10^6/ul	0	74	0	4.0 - 6.0
HgB g/dl	0	74	0	11 - 18
HCT %	2	72	0	35 - 60
MCV Fl	49	23	2	80 - 99.9
MCH pg	25	48	1	27 - 31
MCHC g/dl	3	70	1	33 - 37
PLATELETS X 10^3/ul	9	65	0	150 - 450
NEUTROPHILS %	60	14	0	45 - 75
LYMPHOCYTES %	37	0	37	25 - 45
MONOCYTES %	38	36	0	2 - 8
EOSINOPHILS %	0	0	0	0 - 6
BASOPHILS %	0	0	0	0 - 1

4.2.3 Microscopic Examinations for RBCs Showed the Following Features

Microscopic examination showed 49(66.2%) hypochromic and normocytic anemia cases, which is associated with a reduction of RBCs and low hemoglobin in circulation and 23(31.1%) hypochromic microcytic anemia cases associated with iron deficiency anemia, which is also a reduction of RBCs size and hemoglobin content of the cells.

During microscopic examinations 14(18.9%) Neutropenia, 37(50%) Lymphocytosis, 36(48.6%) monocytopenia and 65(87.8%) thrombocytopenia were identified

After microscopic examinations of WBCs, platelets and RBCs there were no specific blood cellular changes that can provide unique characteristics of identifying visceral leishmania infection. Also no unique cellular characteristic were found that can aid in early and accurate diagnosis of visceral leishmania

The study findings showed that VL affected most of the blood cells where Eosinophils were more affected cellular parameters, as they were not found during microscopic examinations.

(See appendices the blood cell smears for the results)

Table 4.2: Abnormalities of Blood Cellular Smears in VL Patients

Blood Smear Findings	**Cases (n-74)**	
	Number	**Percentage (%)**
Red blood cells		
Hypochromic and normocytic	49	66.2
Hypochromic and microcytic	23	31.1
Hypochromic and macrocytic	2	2.7
White blood cells		
Decreased Neutrophils	14	18.9
Increased lymphocytes	37	50.0
Decreased monocytes	36	48.6
Decreased platelets	65	87.8

4.2.4 Data Analysis and Presentations

Data analysis was conducted using SPSS^R statistical software version 20, 2011 once entered into MS-EXCEL. The number and percent distribution was calculated and the Student t-test was used to test significance at $p<0.05$. Descriptive means, frequencies and percentages were used to describe and summarize data. Tables were used to present result.

4.2.5 The T-Test tor Red Blood Cells, White Blood Cells and Platelets

The hematological red cell analytical parameters including the ranges, mean and the SD for both categories of patients are generally low as indicated in the table except for the MCV mean of 83.43. The p-values for both gender ranged from 0.63 to 0.96 which is statiscally insignificant since their p-value are above 0.05 (Table 4.3).

The mean total WBC count was 1.8 and 2.0 while the platelet counts were 74.6 and 100.3 for females and males respectively. The means for the differential white blood cells were: neutrophils (50, 53), lymphocytes (49, 45), monocytes (1.47, 1.97) for males and females respectively. The p-values were 0.0001 and 0.1107 for WBC and the platelets respectively. The p-value for the differentials ranged from 0.0971 to 0.3094 this is above the p-value 0.05 and therefore it is statistically insignificant. (Table 4.4).

Table 4.3: The T-Test for Red Blood Cells and Red Blood Cell Indices

	GENDER OF STUDIED POPULATION								
HEMATOLOGICAL	**MALE(N=43)**				**FEMALE(N=31)**				
PARAMETERS	**Range**	**WHO Range**	**Mean**	**SD**	**Range**	**WHO range**	**Mean**	**SD**	***P-Value***
RBC'S (x10^3/µl)	1.19-3.85	4.5 – 6.0	2.60	0.58	1.13-3.83	4.5 – 6.0	2.54	0.54	0.63
HB in(g /dl)	3.8-9.8	14 – 18	6.32	1.28	3.6-10.2	12 – 16	6.29	1.28	0.91
HCT (%)	12-33.5	35 – 47	20.38	4.49	12.3-33.5	35 – 60	20.32	4.20	0.96
MCV (fl)	56.2-103.2	80 -99.9	83.43	9.73	56.2-103.2	80 – 99.9	83.38	8.86	0.94
MCH(Pg)	16.2-32.1	27 – 31	25.56	3.15	16.2-34	27 – 31	25.50	3.03	0.94
MCHC(g/dl)	25.6-42.9	33 – 37	30.59	2.67	25.6-42.9	33 – 37	30.53	2.20	0.92

Table 4.4: The T-Test for White Blood Cells and Platelet Changes in Leishmaniasis

PARAMETER		GENDER OF THE STUDIED POPULATION						
		MALE(N=43)			FEMALE(N=31)			
	WHO range	Range	Mean	S. D	range	mean	S.D	p-value
PLT(10^3/µl)	150-450	21-225	74.55	41.62	19 -409	100.29	81.30	0.1107
WBC(x10^3/µl)	4.5-10.5	0.6-3.3	1.8	0.66	0.6 - 3	2.0	0.61	0.0001
NEUT	45-75	29-71	50	10.49	32 - 73	53.0	11.05	0.3094
LYMPH	25-45	26-70	49	10.8	24 - 66	45.0	11.53	0.2453
MONO	2-10	0-5	1.47	1.28	0-5	1.97	1.25	0.0971

Key: PLT-Platelets, WBC-White blood cells, Neut-Neutrophils, Lymph-Lymphocytes, Mono-Monocytes

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDATION

5.1 Discussion

Visceral leishmaniasis is an endemic parasitic disease transmitted by the bite of the female Phlebotomus sand-fly. VL manifestations include fever, hepatomegaly, splenomegaly, lymphadenopathy and pancytopenia. Challenges of the disease in Kenya are that mapping has not been done along endemic counties. Counties rely mostly on national government support in the management of visceral leishmaniasis. Undertaking the leishmania control activities has been hindered by inadequate budget provisions. Number of trained personnel is limited in case management and diagnosis leading to crippled surveillance systems and late detection of cases which lead to outbreak conditions.

The study targeted all gender aged between 1 to 60 years old. Blood from 74 positive cases of visceral leishmaniosis (VL) was analyzed for various blood cellular parameters. Majority of the patients in this cohort were males at 58.1% (n = 43) and the females at 41.9% (n = 31). The largest age group was between 1-17 years accounting for 55.4% (n = 41) with males accounting for 28.4 % (n = 21) of this age group and 27 % (n = 20) for the females. This was closely followed by patients aged 18 years and above at 44.6% (n=33) and 29.7% (n=22) of the population were males above 18 years old while females were 14.9% (n=11) of the same age.

Out of 74 samples, analyzed 49 samples had hypochromic and normocytic anemia. This is a condition where red blood cells have fewer colours than normal cells and is due to a disproportionate reduction of red cell hemoglobin in proportion to the volume of cells. Clinically the colour of the cells can be evaluated by the Mean Cell Hemoglobin Concentration (MCHC).Hypochromic and normocytic cells have normal same size but the red colour is reduced. The findings showed that the Mean Cell Volume (MCV) that measures the average size of red blood cells in hypochromic and normocytic anaemia was within normal (80 – 99.9fl).While the

Red Blood Cells (RBCs) and Hemoglobin (Hgb), mean Cell Hemoglobin (MCH), Hematocrit (HCT) and MCHC were reduced.

The analyzes of 23 samples were found to be hypochromic and microcytic. This is anemia in which RBCs are smaller than normal and often hypochromic. RBCs are usually characterized by a low MCV (mean cell volume) less than 80fl. The RBC, Hb, MCV, MCH, HCT and MCHC in this, findings were reduced below normal and iron deficiency is the most common cause of microcytic anaemia. Out of 74 samples analyzed two (2) were found with hypochromic and macrocytic RBCs a kind of anemia where RBCs are larger than normal. The cells are folate, and vitamin B12 deficiency Here MCV is greater than 99.9fl. The HCT (hematocrit) counts were less than normal in all the samples analyzed.

Sixty (60) samples showed normal distribution of neutrophils (40-75%). All 74 samples had low WBC count (leucopenia) than normal ranges which are 4.5 – $10.5x10^3$/ul) according to the WHO standards. There was no high neutrophil counts found in the examination. Fourteen (14) samples had neutropenia which involves having lower than normal levels of neutrophil in the circulating blood.

The lymphocyte examination showed that 37(50%) of the patients had normal counts while 37(50%) had lymphocytosis. (High counts than normal. Normal counts ranges between 25-42%).

Thirty eight 38(52.4%) of the patients showed normal range of monocytes while, 36(48.6%) had low monocyte counts.

Eosinophils were not found during microscopic examinations this could be probably due to chronic VL infection. Platelet count revealed that 65(87.8%) of the patients had thrombocytopenia and 9(12.2%) had normal counts.

The fever was the striking clinical features of VL which was present in all cases of our study. This was observed in other studies, (Zijlstra and El-Hassan, (2001). Splenomegaly and Hepatomegaly were common in our study patients which are in agreement with other studies (Meinecke et al., 1999), Thabet et al., 1999). All the

patients had anemia of varying degrees. This could be due to iron deficiency caused by bone marrow stores depletion and megaloblastic erythropoiesis due to folate deficiency caused by dietary deficiency. The pathogenesis of anemia in VL is hypersplenism ineffective erythropoiesis and hemolysis (Meinecke et al., 1999).The significant decrease of RBCs count which shows Hematological changes is due to the destruction of RBCs in the reticuloendothelial system caused by macrophage due to abnormal RBCs such as hypochromic and anisocytosis. Our study showed significant decline in Hb concentration in all VL infected age groups. This is in tandem with other studies (Al-Muhammadi et al., 2004).There was decrease of WBCs count in our study patients compared to WHO normal values. This is in tandem with other studies (Meinecke et al., 1999), Singh et al., 1999).The WBCs count decrease is due to spleen sequestration and massive parasitization of the reticuloendothelial system. The platelet counts showed significant decrease in both cases of our study. These findings corroborates the study by (Kager, 1988), Meinecke et al., 1999), Singh et al., 1999) who found that platelets count was significantly reduced. The reduction of platelets count would have been due to the peripheral destruction other than failure in production, (Meinecke et al., 1999).The study noted low neutrophil percentage decrease in all cases. This is similar with other studies (Kager, 1988) and Meinecke et al., 1999) the study showed significant lymphocytosis in all study patients which is in agreement with other studies (Singh et al., 1999).

The visceral leishmaniasis (VL) is associated with the following hematological changes: general reduction in white blood cells (WBC), Red blood cells (RBC) and platelets in infected patients. Among the WBC differentials, reductions have been noted in neutrophils and eosinophils, as well as the platelets and an increase in lymphocytes. These findings are in tandem with studies undertaken by (Singh et al., 1999) with a contradiction in previous reports by (Agrawal et al., 2013) that is potentially due to variations in the study age groups involved. Agrawal and colleagues study cohort was centered on patients below 30 years while this study focused on a wide range of 1-60 years however, the lowest age enrolled in the study was a 6 year old. (Agrawal et al., 2013) study had predominantly showed normocytic normochromic in RBCs and thrombocytosis. These were not similar to our study.

Our study on peripheral blood cells were dominated with microcytic hypochromic anemia and thrombocytopenia. Due to the endemicity of the disease in this region, exposure is enhanced by the nature of activities performed by various age groups. Patients within the age group of 1-17 years old were more affected while pasturing animals in the surrounding bushes compared to the older generation who spend more time around their homesteads. While women fetching firewood were expected to have a rise in exposure, this was not demonstrable from the data obtained for both sexes above 17 years.

Based on the nature and anatomical sites affected by the disease, pancytopenia is a common complication. This was noted in all the age groups regardless of the duration of exposure. While this study could not be able to establish the exact time of exposure and infection, all patients enrolled manifested with mild to moderate pancytopenia. Majority of the patients had a microcytic hypochromic anemia 46 (62%) with some exhibiting a normochromic picture 28 (38%). The anemia findings may be attributed to poor nutrition. Both sexes were equally affected reinforcing the dietary challenges afflicting the community. (Aikat et al., 1979). All patients enrolled into the study had a positive splenic aspirate for leishmaniasis as part of their inclusion criteria. This was purposely done to ensure that the hematological changes found in this cohort could be extrapolated and associated with the disease. Based on these findings, the total leukocytes (white blood cells, WBCs) were significantly reduced compared to World held organization WHO normal values given though studies by (Al-Muhammad et al., 2004) found significantly low levels of total leucocytes.

Platelets were equally found to be lower than normal, a finding that has always been attributed to bone marrow involved as well as the pooling effects of the spleen. All the patients enrolled in the study had splenomegaly and is thought to have contributed significantly to the pancytopenia (Kasili, 1980).

The utility of hematological findings to aid diagnosis of leishmaniasis has been contentious for long due to the lack of sensitivity and specificity to the disease. This study could not demonstrate its utility either but reinforces the hematological

changes associated with the disease. Such findings in an endemic area may be a key pointer to the presence of the disease and may warrant some therapeutic intervention. The hematological red cell analytical parameters including the ranges, mean and the SD for both categories of patients are generally low except for the MCV mean of 83.43. The p-values for both gender were statistically not significant as they are >0.05. The RBCs and its indices were affected except the MCV that remained normal in the study. The mean total WBC count showed marked leukopenia while the platelet counts exhibited marked thrombocytopenia for females and males respectively. The Neutrophils, Monocytes and platelets were lower than the WHO normal values given while the lymphocytes were increased above normal values of those given by WHO. Complete blood count was performed in the laboratory that included WBCs-lymphocytes, Neutrophils, Monocytes Basophils and Eosinophils and platelets. RBCs- Hb, PVC, MCV, MCH, MCHC. The films examination showed there were no specific characteristics that differentiate between VL infected cells and the cells of other infected patients. The VL infected cells give similar features to other infected cells by other infections. Therefore, the appearance of cell morphology alone in the study would not give a diagnosis for VL Eosinophils were not identified in all the smears which indicated that the disease destroy the eosinophils from blood circulation at the time cells are mounting defense against VL this could also happen from other diseases. Though most cells were affected by VL Eosinophils were the most affected cellular parameters.

5.2 Conclusion

Laboratory findings of VL patients in the study revealed significant decrease in Hb, RBCs and RBC indices (except MCV, which remained within the normal ranges in all cases compared to the WHO normal ranges. There was also considerable decrease in white blood cells and Platelets counts. The differential counts and microscopic examinations showed a complete absence of eosinophils that rules presence of visceral leishmania.

The study did not find the specific blood cellular changes that can provide unique characteristics for diagnosing and identifying the visceral leishmania infection

neither unique cellular characteristic were found that can aid in early and accurate diagnosis of visceral leishmania.

All cellular parameters were affected but, eosinophils were mostly affected in the study.

5.3 Recommendation

There is need to further investigate other diseases for differential diagnosis of visceral leishmaniasis co-morbidities.

More research should be conducted on various test methodologies to ascertain the most appropriate laboratory diagnosis for VL. These need to target simple and affordable techniques that can even be afforded by the resource poor and under developing countries.

REFERENCES

Adams, E.R., Jacquet, D., Schoone, G., Gidwani, K., Boelaert, M. & Cunningham, J., 2012. Leishmaniasis direct agglutination test: using pictorials as training materials to reduce inter-reader variability and improve accuracy. *PLoS neglected tropical diseases, 6*(12), .e194

Agrawal, Y., Sinha, A.K., Upadhyaya, P., Kafle, S.U., Rijal, S. & Khanal, B., (2013). Hematological profile in visceral leishmaniasis. *International Journal of Infection and Microbiology, 2*(2), 39-44.

Aikat, B.K., (1979). Haematological investigations in kala-azar patients in Bihar. *Indian Journal of Medical Research, 70,* 571-582

Al-Jurayyan, N.A., Al-Nasser, M.N., Al-Fawaz, I.M., Al Ayed, I.H., Al Herbish, A.S., M-Mazrou, A.M. & Al Sohaibani, M.O., (1995). The haematological manifestations of visceral leishmaniasis in infancy and childhood. *Journal of tropical pediatrics, 41*(3), 143-148.

Al-Muhammadi, M.O., Al-Shujiri, G.S.H. & Noor, M.H., (2004). Hematological changes in children suffering from visceral leishmaniasis (Kala azar). *Medical Journal of Babylon, 1*(3-4)

Alvar, J., den Boer, M. & Dagne, D.A., (2021). Towards the elimination of visceral leishmaniasis as a public health problem in east Africa: reflections on an enhanced control strategy and a call for action. *The Lancet Global Health, 9*(12), e1763-e1769

Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., Boer, M.D. & WHO Leishmaniasis Control Team, (2012). Leishmaniasis worldwide and global estimates of its incidence. *PloS one, 7*(5), e35671.

Araújo, V.E.M.D., Pinheiro, L.C., Almeida, M.C.D.M., Menezes, F.C.D., Morais, M.H.F., Reis, I.A., Assunção, R.M. & Carneiro, M., (2013). Relative risk

of visceral leishmaniasis in Brazil: a spatial analysis in urban area. *PLoS Neglected Tropical Diseases, 7*(11), e2540.

Arce, A., Estirado, A., Ordobas, M., Sevilla, S., García, N., Moratilla, L., De La Fuente, S., …& Iriso, A., (2013). Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. *Eurosurveillance, 18*(30).

Aronson, N., Herwaldt, B.L., Libman, M., Pearson, R., Lopez-Velez, R., Weina, P., Carvalho, E.M., …& Magill, A., (2016). Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clinical infectious diseases, 63*(12), e202-e264.

Azizi, M.W., Belhadi, S., Yallese, M.A., Mabrouki, T. & Rigal, J.F., (2012). Surface roughness and cutting forces modeling for optimization of machining condition in finish hard turning of AISI 52100 steel. *Journal of mechanical science and technology, 26,* 4105-4114.

Babiker, A., Ravagnan, S., Cassini, R., Salviato, A., Drago, A., Natale, A., Hassan, M.M., … & MMukhtar, M., (2012). Genetic diversity of Leishmania from Sudan and possible correlation to clinical signs: preliminary results. *Facoltà Di Medicina Veterinaria, .65.*

Bajaj, P., Gosavi, S. & Puneet, K., 2013. Haematological Parameters In Visceral Leishmaniasis. *International Journal of Pharmaceutical Science Invention, 2*(8), 06-08.

Barrett, M.P. & Croft, S.L., 2012. Management of trypanosomiasis and leishmaniasis. *British medical bulletin, 104*(1), 175-196.

Berman, J.D., (1997). Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clinical infectious diseases, 24*(4), 684-703.

Bern, C., Hightower, A.W., Chowdhury, R., Ali, M., Amann, J., Wagatsuma, Y., Haque, R., … & Akter, T., 2005. Risk factors for kala-azar in Bangladesh. *Emerging infectious diseases*, *11*(5), 655.

Bern, C., Maguire, J.H. & Alvar, J., (2008). Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS neglected tropical diseases*, *2*(10), e313.\

Berriatua, E., Maia, C., Conceição, C., Özbel, Y., Töz, S., Baneth, G., Pérez-Cutillas, P., … & Pereira, A., (2021). Leishmaniases in the European Union and neighboring countries. *Emerging Infectious Diseases*, *27*(6), 1723.

Boelaert, M., Bhattacharya, S., Chappuis, F., El Safi, S.H., Hailu, A., Mondal, D., Rijal, S., … & Peeling, R.W., (2007). Evaluation of rapid diagnostic tests: visceral leishmaniasis. *Nature Reviews Microbiology*, *5*(11), S31-S39.

Boelaert, M., Criel, B., Leeuwenburg, J., Van Damme, W., Le Ray, D. & Van Der Stuyft, P., (2000). Visceral leishmaniasis control: a public health perspective. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *94*(5), 465-471.

Boelaert, M., El-Safi, S., Hailu, A., Mukhtar, M., Rijal, S., Sundar, S., Wasunna, M., … & Desjeux, P., (2008). Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in East Africa and the Indian subcontinent. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(1), 32-40.

Boelaert, M., Rijal, S., Regmi, S., Singh, R., Karki, B., Jacquet, D., Chappuis, F., … & Koirala, S., (2004). A comparative study of the effectiveness of diagnostic tests for visceral leishmaniasis. *The American journal of tropical medicine and hygiene*, *70*(1), 72-77.

Bray, C., Bell, L.N., Liang, H., Haykal, R., Kaiksow, F., Mazza, J.J. & Yale, S.H., (2016). Erythrocyte sedimentation rate and C-reactive protein

measurements and their relevance in clinical medicine. *Wmj*, *115*(6), 317-21.

Braz, R.F., Nascimento, E.T., Martins, D.R., Wilson, M.E., Pearson, R.D., Reed, S.G. & Jeronimo, S.M., (2002). The sensitivity and specificity of Leishmania chagasi recombinant K39 antigen in the diagnosis of American visceral leishmaniasis and in differentiating active from subclinical infection. *The American journal of tropical medicine and hygiene, 67*(4), 344-348.

Cartwright, G.E., Chung, H.L. & Chang, A.N., (1948). Studies on the pancytopenia of kala-azar. *Blood*, *3*(3), 249-275.

Carvalho, A.M.R.S., Mendes, T.A.D.O., Coelho, E.A.F., Duarte, M.C. & Menezes-Souza, D., (2018). New antigens for the serological diagnosis of human visceral leishmaniasis identified by immunogenomic screening. *PLoS One*, *13*(12), .e0209599.

Chappuis, F., Rijal, S., Soto, A., Menten, J. & Boelaert, M., (2006). A meta-analysis of the diagnostic performance of the direct agglutination test and rK39 dipstick for visceral leishmaniasis. *Bmj*, *333*(7571), 723.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R.W., Alvar, J. & Boelaert, M., (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?. *Nature reviews microbiology*, *5*(11), 873-882.

Chowdhury, M.S., El Harith, A., Al Massum, A., Al Karim, E. & Al Rahman, A., 1993. Prevalence of agglutinating anti-Leishmania antibodies in two multi-thousand Bengoli communities. *Parasitology research, 79*(6), 444-450.

Cooper, C., (2018). Global, regional, and national Disability-Adjusted Life-Years (DALY) for 359 diseases and injuries and Health Life Expectancy (HALE) for 195 countries and territories, 1990-2017: A systematic

analysis for the global burden of disease study 2017. *The Lancet, 392*(10159), 1859-1922.

Cota, G.F., de Sousa, M.R., Fereguetti, T.O. & Rabello, A., (2013). Efficacy of anti-leishmania therapy in visceral leishmaniasis among HIV infected patients: a systematic review with indirect comparison. *PLoS neglected tropical diseases, 7*(5), .e2195.

Cotterell, S.E., Engwerda, C.R. & Kaye, P.M., (2000). Enhanced hematopoietic activity accompanies parasite expansion in the spleen and bone marrow of mice infected with Leishmania donovani. *Infection and immunity*, *68*(4), 1840-1848.

Cotterell, S.E., Engwerda, C.R. & Kaye, P.M., (2000). Leishmania donovani infection of bone marrow stromal macrophages selectively enhances myelopoiesis, by a mechanism involving GM-CSF and TNF-α. Blood, *The Journal of the American Society of Hematology*, *95*(5), 1642-1651.

Cunningham, J., Hasker, E., Das, P., El Safi, S., Goto, H., Mondal, D., Mbuchi, M., … & Sundar, S., (2012). A global comparative evaluation of commercial immunochromatographic rapid diagnostic tests for visceral leishmaniasis. *Clinical infectious diseases*, *55*(10), 1312-1319.

da Silva Solcà, M., Guedes, C.E.S., Nascimento, E.G., de Sá Oliveira, G.G., dos Santos, W.L.C., Fraga, D.B.M. & Veras, P.S.T., (2012). Qualitative and quantitative polymerase chain reaction (PCR) for detection of Leishmania in spleen samples from naturally infected dogs. *Veterinary parasitology, 184*(2-4), 133-140.

da Silva, S.M., Rabelo, P.F.B., de Figueiredo Gontijo, N., Ribeiro, R.R., Melo, M.N., Ribeiro, V.M. & Michalick, M.S.M., (2010). First report of infection of Lutzomyia longipalpis by Leishmania (Leishmania) infantum from a naturally infected cat of Brazil. *Veterinary parasitology, 174*(1-2), 150-154

Das, N.K., (2020). Post kala-azar dermal leishmaniasis. *Indian Journal of Dermatology, 65*(6), 450-451.

Dash, S.Garewal, G, Marwaha, N, Sarode, R, & gupta, R.K. (1991). Clinco-hematological characteristics in patients with kala-azar.*Trop Georg Med. 43*, 357-362

Datta, U., Rajwanshi, A., Rayat, C.S., Sakhuja, V. & Sehgal, S., (1984). *Kala-azar in Himachal* Pradesh: a new pocket.

Davies, C.R., Kaye, P., Croft, S.L. & Sundar, S., (2003). Leishmaniasis: new approaches to disease control. *Bmj, 326*(7385), 377-382.

de Almeida, A.S., de Andrade Medronho, R. & Werneck, G.L., (2011). Identification of risk areas for visceral leishmaniasis in Teresina, Piaui State, Brazil. *The American journal of tropical medicine and hygiene, 84*(5), 681.

de Godoy, N.S., da Costa Lima-Junior, M.S., Lindoso, J.A.L., Pereira-Chioccola, V.L., Okay, T.S. & Braz, L.M.A., (2020). A PCR and RFLP-based molecular diagnostic algorithm for visceral leishmaniasis. *Asian Pacific Journal of Tropical Medicine, 13*(2), 62.

de Paiva-Cavalcanti, M., de Morais, R.C.S., Pessoa-e-Silva, R., Trajano-Silva, L.A.M., Goncalves-de-Albuquerque, S.D.C., Tavares, D.D.H.C., Brelaz-de-Castro, M.C.A., Silva, R.D.F.E. & Pereira, V.R.A., (2015). Leishmaniases diagnosis: an update on the use of immunological and molecular tools. *Cell & bioscience, 5*, 1-10.

de Souza, D.K., Picado, A., Bessell, P.R., Liban, A., Wachira, D., Mwiti, D., Matendechero, S.H., … & Cruz, I., 2022. Strengthening visceral leishmaniasis diagnosis capacity to improve access to care in Kenya: the example of Marsabit county. *Frontiers in Tropical Diseases, 2*, 80975.

Dube, B., Arora, A., Singh, V.P., Kumar, K.A.I.L.A.S.H. & Sundar, S.H.Y.A.M., (1995). Platelet function studies in Indian kala-azar. *The Journal of Tropical Medicine and Hygiene, 98*(3), 166-168

Dujardin, J.C., Campino, L., Cañavate, C., Dedet, J.P., Gradoni, L., Soteriadou, K., Mazeris, A., Ozbel, Y. & Boelaert, M., (2008). Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. *Emerging infectious diseases, 14*(7), p.1013

Dutta, A., Sarkar, D., Gurib-Fakim, A., Mandal, C. & Chatterjee, M., (2008). In vitro and in vivo activity of Aloe vera leaf exudate in experimental visceral leishmaniasis. *Parasitology Research, 102*(6), 1235-1242.

El Hag, I.A., Hashim, F.A., El Toum, I.A., Homeida, M., El Kalifa, M. & El Hassan, A.M., (1994). Liver morphology and function in visceral leishmaniasis (Kala-azar). *Journal of Clinical Pathology, 47*(6), 547-551.

El Harith, A., Kolk, A.H., Leeuwenburg, J., Muigai, R., Huigen, E., Jelsma, T. & Kager, P.A., (1988). Improvement of a direct agglutination test for field studies of visceral leishmaniasis. *Journal of clinical microbiology, 26*(7), 1321-1325.

El-Shoura, S.M., (1994). Visceral leishmaniasis: III. Effect of parasitaemia level on the bone marrow ultrastructure. *Applied parasitology, 35*(1), 61-69.

Gavgani, A., Mohite, H., Edrissian, G.H., Mohebali, M. & Davies, C.R., (2002). Domestic dog ownership in Iran is a risk factor for human infection with Leishmania infantum. *The American journal of tropical medicine and hygiene, 67*(5), 511-515.

Guerin, P.J., Olliaro, P., Sundar, S., Boelaert, M., Croft, S.L., Desjeux, P., Wasunna, M.K. & Bryceson, A.D., 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *The Lancet infectious diseases, 2*(8), 494-501.

Gupta, S., Raychaudhury, B. & Datta, S.C., (2009). Host peroxisomal properties are not restored to normal after treatment of visceral leishmaniasis with sodium antimony gluconate. *Experimental parasitology*, *123*(2), 140-145.

Hailu, A., Mudawi Musa, A., Royce, C. & Wasunna, M., (2005). Visceral leishmaniasis: new health tools are needed. *PLoS medicine*, *2*(7), e211.

Hailu, A., van Baarle, D., Knol, G.J., Berhe, N., Miedema, F. & Kager, P.A., (2005). T cell subset and cytokine profiles in human visceral leishmaniasis during active and asymptomatic or sub-clinical infection with Leishmania donovani. *Clinical Immunology*, *117*(2), 182-191.

Harith, A.E., Kolk, A.H.J., Kager, P.A., Leeuwenburg, J., Muigai, R., Kiugu, S. & Laarman, J.J., (1986). A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Transactions of the royal society of tropical medicine and hygiene*, *80*(4), 583-586.

Hasker, E., Kansal, S., Malaviya, P., Gidwani, K., Picado, A., Singh, R.P., Chourasia, A., … & Wilson, M.E., (2013). Latent infection with Leishmania donovani in highly endemic villages in Bihar, India. *PLoS neglected tropical diseases*, *7*(2), e2053.

Herwaldt, B.L., (2001). Laboratory-acquired parasitic infections from accidental exposures. *Clinical microbiology reviews*, *14*(4), 659-688.

Houghton, R.L., Petrescu, M., Benson, D.R., Skeiky, Y.A., Scalone, A., Badaró, R., Reed, S.G. & Gradoni, L., (1998). A cloned antigen (recombinant K39) of Leishmania chagasi diagnostic for visceral leishmaniasis in human immunodeficiency virus type 1 patients and a prognostic indicator for monitoring patients undergoing drug therapy. *The Journal of infectious diseases*, *177*(5), 1339-1344.

Jiménez, M., González, E., Martín-Martín, I., Hernández, S. & Molina, R., (2014). Could wild rabbits (Oryctolagus cuniculus) be reservoirs for Leishmania infantum in the focus of Madrid, Spain? *Veterinary parasitology*, *202*(3-4), 296-300.

Kasili, E.G., (1980). Haematological abnormalities in visceral leishmaniasis. *East African Medical Journal*, *57*(9), 634-640.

Khatun, M., Alam, S.S., Khan, A.H., Hossain, M.A., Haq, J.A., Jilani, M.S.A., Rahman, M.T. & Karim, M.M., (2017). Novel PCR primers to diagnose visceral leishmaniasis using peripheral blood, spleen or bone marrow aspirates. *Asian Pacific Journal of Tropical Medicine*, *10*(8), 753-759.

Kumari, S., Kottarath, S.K., Srivastava, S.K., Bhat, M., Dinda, A.K., Thakur, C.P. & Sharma, S., (2019). Leishmania donovani reduces the levels of retinoic acid–synthesizing enzymes in infected macrophages and favoring its own survival. *Parasitology research*, *118*(1), 63-71..

Lindoso, J.A.L., Cunha, M.A., Queiroz, I.T. & Moreira, C.H.V., (2016). Leishmaniasis–HIV coinfection: current challenges. *HIV/AIDS-research and palliative care*, 147-156.

Makau-Barasa, L.K., Ochol, D., Yotebieng, K.A., Adera, C.B. & de Souza, D.K., (2022). Moving from control to elimination of Visceral Leishmaniasis in East Africa. *Frontiers in Tropical Diseases*, *3*, 965609

Mandal, A., Das, S., Roy, S., Ghosh, A.K., Sardar, A.H., Verma, S., Saini, S. … & Mandal, C., (2016). Deprivation of L-arginine induces oxidative stress mediated apoptosis in Leishmania donovani promastigotes: contribution of the polyamine pathway. *PLOS Neglected Tropical Diseases*, *10*(1), e00043.

Mandal, J., Khurana, S., Dubey, M.L., Bhatia, P., Varma, N. & Malla, N., (2008). Evaluation of direct agglutination test, rk39 Test, and ELISA for the

diagnosis of visceral leishmaniasis. *The American journal of tropical medicine and hygiene, 79*(1), 76-78.

Manson-Bahr, P.E.C. & Bell, D.R., (1987). Visceral leishmaniasis. *Manson's tropical diseases*, 19th ed. Bailliere Tindall, London, England, pp.87-113.

Martínez-López, M., Soto, M., Iborra, S. & Sancho, D., (2018). Leishmania hijacks myeloid cells for immune escape. *Frontiers in microbiology, 9*, 883.

Meinecke, C.K., Schottelius, J., Oskam, L. & Fleischer, B., (1999). Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. *Pediatrics, 104*(5), e65-e65.

Mendez, S., Traslavina, R., Hinchman, M., Huang, L., Green, P., Cynamon, M.H.& Welch, J.T., (2009). The antituberculosis drug pyrazinamide affects the course of cutaneous leishmaniasis in vivo and increases activation of macrophages and dendritic cells. *Antimicrobial agents and chemotherapy, 53*(12), .5114-5121.

Meredith, S., den Boer, M., Burza, S. & Croft, S.L., (2024). KalaCORE: A Programme to Tackle Visceral Leishmaniasis in South Asia and East Africa (2014–2019). *In Challenges and Solutions against Visceral Leishmaniasis (pp. 19-41).* Singapore: Springer Nature Singapore

Meredith, S.E., Kroon, N.C., Sondorp, E., Seaman, J., Goris, M.G., Van Ingen, C.W., Oosting, H., … & Oskam, L., (1995). Leish-KIT, a stable direct agglutination test based on freeze-dried antigen for serodiagnosis of visceral leishmaniasis. *Journal of Clinical Microbiology, 33*(7), 1742-1745.

Mondal, S, Pradyot, B, & Nahid, A. (2010). Current diagnosis and treatment of visceral leishmaniasis." *Expert review of anti-infective therapy 8*(8), 919-944.

Mondiale de la Santé, O. & World Health Organization, (2016). Leishmaniasis in high-burden countries: an epidemiological update based on data reported in 2014. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire, 91*(22), 286-296.

Mondiale de la Santé, O. & World Health Organization, (2018). Global leishmaniasis surveillance update, 1998–2016–Le point sur la situation Mondiale de la leishmaniose, 1998-2016. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire, 93*(40), 530-540.

Muhammad, A.G., (2004). *Khuluq al-Muslim*. Damascus: Dar al-Qalam.

Murray, H.W., (2002). Kala-azar—progress against a neglected disease. *New England Journal of Medicine, 347*(22), 1793-1794

Murray, H.W., Lu, C.M., Mauze, S., Freeman, S., Moreira, A.L., Kaplan, G. & Coffman, R.L., (2002). Interleukin-10 (IL-10) in experimental visceral leishmaniasis and IL-10 receptor blockade as immunotherapy. *Infection and immunity, 70*(11), 6284-6293.

Mutinga, M.J., Ngoka, J.M. & Odhiambo, T.R., (1984). Epidemiological, investigations of visceral leishmaniasis in the West Pokot District, Kenya. *International Journal of Tropical Insect Science, 5*(6), 521-525.

Neki, N. & Singh, J., (2017). Hematological changes in visceral leishmaniasis. *Int J Curr Res Med Sci, 3*(6), 36-40.

Oskam, L., Nieuwenhuijs, J.L. & Hailu, A., (1999). Evaluation of the direct agglutination test (DAT) using freeze-dried antigen for the detection of anti-Leishmania antibodies in stored sera from various patient groups in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 93*(3), 275-277.

Pacheco-Fernandez, T., Volpedo, G., Gannavaram, S., Bhattacharya, P., Dey, R., Satoskar, A., Matlashewski, G. & Nakhasi, H.L., (2021). Revival of

leishmanization and leishmanin. *Frontiers in Cellular and Infection Microbiology, 11,* 639801.

Pakzad, R., Dabbagh-Moghaddam, A., Mohebali, M., Safiri, S. & Barati, M., (2017). Spatio-temporal analysis of cutaneous leishmaniasis using geographic information system among Iranian Army Units and its comparison with the general population of Iran during 2005–2014. *Journal of parasitic diseases, 41*(4), 1114-1122.

Pippard, M.J., Moir, D., Weatherall, D.J. & Lenicker, H.M., (1986). Mechanism of anaemia in resistant visceral leishmaniasis. *Annals of Tropical Medicine & Parasitology, 80*(3), 317-323.

Rafati, S., Hassani, N., Taslimi, Y., Movassagh, H., Rochette, A. & Papadopoulou, B., (2006). Amastin peptide-binding antibodies as biomarkers of active human visceral leishmaniasis. *Clinical and Vaccine Immunology, 13*(10), 1104-1110.

Rajagopala, S., Dutta, U., Chandra, K.P., Bhatia, P., Varma, N. & Kochhar, R., (2008). Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis–case report and systematic review. *Journal of Infection, 56*(5), 381-388.

Reithinger, R. & Dujardin, J.C., (2007). Molecular diagnosis of leishmaniasis: current status and future applications. *Journal of clinical microbiology, 45*(1), 21-25.

Ruiz-Postigo, J.A., Jain, S., Mikhailov, A., Maia-Elkhoury, A.N., Valadas, S., Warusavithana, S., Osman, M., … & Gasimov, E., (2021). Global leishmaniasis surveillance: 2019-2020, a baseline for the 2030 roadmap/Surveillance mondiale de la leishmaniose: 2019-2020, une periode de reference pour la feuille de route a l'horizon 2030. *Weekly epidemiological record, 96*(35), 401-420.

Safavi, M., Eshaghi, H. & Hajihassani, Z., (2020). Visceral leishmaniasis: Kala-azar.

Salotra, P. & Singh, R., (2006). Challenges in the diagnosis of post kala-azar dermal leishmaniasis. *Indian Journal of Medical Research*, *123*(3), 295.

Sarkari, B., Chance, M. & Hommel, M., (2002). Antigenuria in visceral leishmaniasis: detection and partial characterisation of a carbohydrate antigen. *Acta tropica*, *82*(3), 339-348.

Scarpini, S., Dondi, A., Totaro, C., Biagi, C., Melchionda, F., Zama, D., Pierantoni, L., … & Lanari, M., (2022). Visceral leishmaniasis: Epidemiology, diagnosis, and treatment regimens in different geographical areas with a focus on pediatrics. *Microorganisms*, *10*(10), 1887.

Schallig, H.D.F.H., Schoone, G., Kroon, C., Hailu, A., Chappuis, F. & Veeken, H., (2001). Development and application of'simple'diagnostic tools for visceral leishmaniasis. *Medical Microbiology and Immunology*, *190*(1), 69-71.

Schwing, A., Pomares, C., Majoor, A., Boyer, L., Marty, P. & Michel, G., (2019). Leishmania infection: misdiagnosis as cancer and tumor-promoting potential. *Acta tropica*, *197*, 104855.

Shahbazi, F., Shahabi, S., Kazemi, B., Mohebali, M., Abadi, A.R. & Zare, Z., (2008). Evaluation of PCR assay in diagnosis and identification of cutaneous leishmaniasis: a comparison with the parasitological methods. *Parasitology research*, *103*, 1159-1162.

Silva, L.D.A., Romero, H.D., Prata, A., Costa, R.T., Nascimento, E., Carvalho, S.F.G. & Rodrigues, V., (2006). Immunologic tests in patients after clinical cure of visceral leishmaniasis. *Am J Trop Med Hyg*, *75*(4), 739-743.

Silvestre, R., Cordeiro-Da-Silva, A., Santarém, N., Vergnes, B., Sereno, D. & Ouaissi, A., (2007). SIR2-deficient Leishmania infantum induces a defined IFN-γ/IL-10 pattern that correlates with protection. *The journal of immunology*, *179*(5), 3161-3170.

Singh, O.P. & Sundar, S., (2015). Developments in diagnosis of visceral leishmaniasis in the elimination era. *Journal of parasitology research, 2015*(1), 239469

Singh, O.P., Hasker, E., Sacks, D., Boelaert, M. & Sundar, S., (2014). Asymptomatic Leishmania infection: a new challenge for Leishmania control. *Clinical Infectious Diseases, 58*(10), 1424-1429.

Skinner, J., Yankey, B. & Shelton, B.K., (2019). Hemophagocytic lymphohistiocytosis. *AACN advanced critical care*, *30*(2), 151-164.

Slutzky, G.M., Londner, M.V. & Greenblatt, C.L., (1985). Lipid and Lipopolysaccharide-Like Antigens of Leishmania Promastigotes 1. *The Journal of protozoology, 32*(2), 347-352.

Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G. & Baneth, G., (2011). LeishVet guidelines for the practical management of canine leishmaniosis. *Parasites & vectors, 4*, 1-16.

Srivastava, A., Sweat, J.M., Azizan, A., Vesely, B. & Kyle, D.E., (2013). Real-time PCR to quantify Leishmania donovani in hamsters. *The Journal of parasitology*, *99*(1), 145-150.

Srividya, G., Kulshrestha, A., Singh, R. & Salotra, P., (2012). Diagnosis of visceral leishmaniasis: developments over the last decade. *Parasitology research, 110*, 1065-1078

Sundar, S. & Rai, M., (2005). Treatment of visceral leishmaniasis. *Expert opinion on pharmacotherapy, 6*(16), 2821-2829.

Sundar, S., Jha, T.K., Thakur, C.P., Engel, J., Sindermann, H., Fischer, C., Junge, K., Bryceson, A. & Berman, J., (2002). Oral miltefosine for Indian visceral leishmaniasis. *New England journal of medicine, 347*(22), 1739-1746.

Thabet, F., Tabarki, B., Fehem, R., Yacoub, M., Selmi, H. & Essoussi, A.S., (1999). Syndrome of inappropriate macrophage activation associated with infantile visceral leishmaniasis. *La Tunisie Médicale, 77*(12), 648-650.

Thakur, C.P., Dedet, J.P., Narain, S. & Pratlong, F., (2001). Leishmania species, drug unresponsiveness and visceral leishmaniasis in Bihar, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 95*(2), 187-189.

Tlamcani, Z., (2016). Visceral leishmaniasis: an update of laboratory diagnosis. *Asian Pacific Journal of Tropical Disease, 6*(7), 505-508.

Tonui, W.K. & Titus, R.G., (2006). Leishmania major soluble exo-antigens (Lm SEAgs) protect neonatal BALB/c mice from a subsequent challenge with L. major and stimulate cytokine production by Leishmania-naïve human peripheral blood mononuclear cells. *Journal of Parasitology, 92*(5), 971-976.

Tripathi, P., Singh, V. & Naik, S., (2007). Immune response to leishmania: paradox rather than paradigm. *FEMS Immunology & Medical Microbiology*, *51*(2), 229-242.

Vallur, A.C., Tutterrow, Y.L., Mohamath, R., Pattabhi, S., Hailu, A., Abdoun, A.O., Ahmed, A.E., … & Almeida, R.P., (2015). Development and comparative evaluation of two antigen detection tests for Visceral Leishmaniasis. *BMC Infectious Diseases, 15*(1), 1-10.

van Griensven, J. & Diro, E., (2012). Visceral leishmaniasis. *Infectious Disease Clinics, 26*(2), 309-322.

van Griensven, J. & Diro, E., (2019). Visceral leishmaniasis: recent advances in diagnostics and treatment regimens. *Infectious Disease Clinics, 33*(1), 79-99.

Varani, S., Cagarelli, R., Melchionda, F., Attard, L., Salvadori, C., Finarelli, A.C., Gentilomi, G.A., … & Scalone, A., (2013). Ongoing outbreak of visceral

leishmaniasis in Bologna Province, Italy, November 2012 to May 2013. *Eurosurveillance, 18*(29), 20530.

Varani, S., Ortalli, M., Attard, L., Vanino, E., Gaibani, P., Vocale, C., Rossini, G., … & Mastroianni, A., (2017). Serological and molecular tools to diagnose visceral leishmaniasis: 2-years' experience of a single center in Northern Italy. *PloS one, 12*(8), e0183699.

Varma, N. & Naseem, S., (2010). Hematologic changes in visceral leishmaniasis/kala azar. *Indian Journal of Hematology and Blood Transfusion, 26*(3), 78-82.

World Health Organization, (2024). *Disease outbreak news: annual compendium 2022*. Rome: World Health Organization..

Zijlstra, E.E. & El-Hassan, A.M., (2001). Leishmaniasis in Sudan. 3. Visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 95*(Supplement_1), S27-S58.

Zijlstra, E.E., Musa, A.M., Khalil, E.A.G., El Hassan, I.M. & El-Hassan, A.M., (2003). Post-kala-azar dermal leishmaniasis. *The Lancet infectious diseases, 3*(2), 87-98.

APPENDICES

Appendix I: Informed Consent Forms

Study Title: Blood cellular hematological changes in patients with visceral leishmaniasis attending Kimalel sub-county hospital, Baringo County

Consent explanation: My name is Agura Lukas Seremani, P O Box 20778-00202 Nairobi, Kenya; Tel: 0722892207), a Masters student at the College of Health Sciences, School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology

P O Box 62000-00200 Nairobi, Kenya; Phone: +254(067) 52124, +254(067) 52028.

I am conducting a study to find out if there are specific unique characteristic blood cellular hematological changes in patients with VL. The information in this form will help you make an informed decision whether or not to participate in this study. Please read through carefully and feel free to ask any question(s) about the study. I will read it out and explain to you if you are not able to read.

Purpose: This study is interested in finding the specific blood cellular changes that can provide unique characteristics of identifying the visceral leishmania infection. If successful, the study can aid in early diagnosis of VL avoiding the invasive methods currently in use.

Benefits: By participating in this study, there is no direct benefit. However, you will receive the necessary drug(s) to cure you from the disease called Kala azar. All expenses for diagnosis and treatment are at no cost to you.

Risks: There is a possibility of mild discomfort at the needle entry when collecting blood. The amount of blood to be collected from you will be 2 milliliters (equivalent of 1tea spoonful). The risks associated with procedures for diagnosing Kala azar include:

i) Internal bleeding following splenic aspiration. This is uncommon and known to occur in 1 per 1000 patients

ii) ii) Discomfort during bone marrow aspiration. A clinician minimizes the risks by performing a blood test checking blood levels, bleeding tendency and using standardized technique for bone marrow aspirations. Local anesthesia is used to reduce the pain. In case any complications occur e.g. bleeding, the clinician will take care of you until you are fully recovered.

Confidentiality: The information that we collect from this study will be kept confidential. No one, except the study Principal investigator and the research team who are able to access the information about you that will be collected during the study. Any information related to you, e.g. clinical and laboratory results will have initials and a number on it instead of your name, which will be recognized only by the Principal investigator and the research team.

Procedure: If you accept to participate in this study, you will undergo face-to-face interviews with the study clinician using a language you are comfortable with to answer a few questions concerning your health as well as collect sample from you

Voluntarism: Participation in this study will be voluntary without any coercion

Subject's rights: Since participation in this study is voluntary, you have the right to withdraw or discontinue participating at any time without penalty. If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact–KNH/UoN ERC (Chairperson of the Scientific Steering Committee, PO Box 20732 Nairobi, Kenya; Phone: 02-7263000 Ext 44102.

I have read this form or had it read to me in a language that I understand. My questions have been answered satisfactorily. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time without penalty or harm. In case of withdrawal, I understand that the Clinicians will however, take care of me like any other patient.

Participant's Name: _____

Participant's Signature: _____

Date: _____

Name of Clinician: _____

Clinician signature: _____

Date: _____

Appendix II: Parental/Guardian Ascent Form for Minors Aged 1 – 17 Years

Study Title: Blood cellular hematological changes in patients with visceral leishmaniasis attending Kimalel sub-county hospital, Baringo County

Consent explanation: My name is Agura Lukas Seremani, P O Box 20778-00202 Nairobi, Kenya; Tel: 0722607476), a Masters student at the College of Health Sciences, School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology

P O Box 62000-00200 Nairobi, Kenya; Phone: +254(067) 52124, +254(067) 52028.

I am conducting a study to find out if there are specific unique characteristic blood cellular hematological changes in patients with VL. The information in this form will help you make an informed decision whether or not to participate in this study. Please read through carefully and feel free to ask any question(s) about the study. I will read it out and explain to you if you are not able to read.

Purpose: This study is interested in finding the specific blood cellular changes that can provide unique characteristics of identifying the visceral leishmania infection. If successful, the study can aid in early diagnosis of VL avoiding the invasive methods currently in use.

Benefits: By participating in this study, there is no direct benefit. However, your child will receive the necessary drug(s) to cure him/her from the disease called Kala azar. All expenses for diagnosis and treatment are at no cost to you.

Risks: There is a possibility of mild discomfort at the needle entry when collecting blood. The amount of blood to be collected from your child will be 2 milliliters (equivalent of 1tea spoonful). The risks associated with procedures for diagnosing Kala azar include:

i) Internal bleeding following splenic aspiration. This is uncommon and known to occur in 1 per 1000 patients

ii) ii) Discomfort during bone marrow aspiration. A clinician minimizes the risks by performing a blood test checking blood levels, bleeding tendency and using standardized technique for bone marrow aspirations. Local anesthesia is used to reduce the pain. In case any complications occur e.g. bleeding, the clinician will take care of your child until he/she is fully recovered.

Confidentiality: The information that we collect from this study will be kept confidential. No one, except the study Principal investigator and the research team who are able to access the information about you that will be collected during the study. Any information related to your child, e.g. clinical and laboratory results will have initials and a number on it instead of your name, which will be recognized only by the Principal investigator and the research team.

Procedure: If you accept your child to participate in this study, you will undergo face-to-face interviews with the study clinician using a language you are comfortable with to answer a few questions concerning your child's health as well as collect sample from him/her.

Voluntarism: Participation in this study will be voluntary without any coercion

Subject's rights: Since participation in this study is voluntary, your child has the right to withdraw or discontinue participating at any time without penalty. If you have questions about your child's rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact – KNH/UoN ERC (Chairperson of the Scientific Steering Committee, PO Box 20732 Nairobi, Kenya; Phone: 02-7263000 Ext 44102.

I have read this form or had it read to me in a language that I understand. My questions have been answered satisfactorily. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time without penalty or harm. In case of withdrawal, I understand that the Clinicians will however, take care of me like any other patient.

Parent/Guardian's: Name: _____

Parent/Guardian's: Signature: _____

Date: _____

Name of Clinician: _____

Clinician signature: _____

Date: _____

Appendix III: Raw Data

Date of test	Pts Intls	Study No. 1-74	Age 1-60yrs	Gender M/F	WBC $x10^3$ul	RBC $x10^3$ul	HB g/dl	HCT %	MCV Fl	MCH pg	MCHC g/gl	Plt $x10^3$ul	N %	L %	M %	E %	B %	SA +
24-07-2015	LL	1	13yrs	M	2.6	2.42	6.2	21.2	87.6	25.9	28.2	44.7	60	39	1	0	0	+
24-07-2015	JM	2	16yrs	F	1.1	2.1	5	17.5	83.3	23.9	28.7	51	52	47	2	0	0	+
24-07-2015	MR	3	47yrs	F	1.1	2.33	5.6	18.5	78.3	24.9	28.7	19	46	53	1	0	0	+
27-07-2915	ML	4	17yrs	M	0.9	2.35	6.2	18.6	81.3	24.6	38.2	49	49	49	2	0	0	+
27-07-2015	LL	5	17yrs	M	1.4	2.31	6.4	22.4	88.1	28.6	29.5	53	60	40	0	0	0	+
28-07-2015	JK	6	35yrs	M	1.8	2.25	6.2	18.6	80.9	23.8	28.1	39	64	36	0	0	0	+
29-07-2015	RB	7	12yrs	F	2	2.85	7.9	23.6	87.9	27.1	31	59	50	50	0	0	0	+
30-07-2015	JL	8	14yrs	M	1.9	3.21	5.9	17.7	81.4	24.5	29	60	57	43	0	0	0	+
30-07-2015	LC	9	13yrs	M	2.9	3.56	6.9	20.7	78.4	22.5	27.2	95	55	45	0	0	0	+
30-07-2015	SE	10	22yrs	M	2.1	3.13	5.3	15.9	84.4	26.5	39.2	63	63	37	0	0	0	+
31-07-2015	KK	11	14yrs	F	1.4	2.33	6.4	19.2	94.2	26.9	28.6	43	59	40	1	0	0	+
31-07-2025	SK	12	24yrs	M	1.9	2.92	6.7	20.1	86.3	26.9	25.6	38	56	44	0	0	0	+
17-08-2015	LK	13	17yrs	M	2.6	2.66	7.4	23.6	88.9	28	31.5	81	40	59	1	0	0	+

17-08-2015	DY	14	23yrs	M	2.6	2.51	7	22.4	89.4	27.8	31.1	75	41	59	0	0	0	+
17-08-2015	LY	15	26yrs	M	1.8	1.88	5.3	16.6	88.6	28	31.6	53	38	62	0	0	0	+
17-08-2015	HK	16	38yrs	M	1.9	2.92	6.7	20.1	86.3	26.9	25.6	38	54	44	2	0	0	+
17-08-2015	JK	17	14yrs	F	2.5	2.63	7.1	23.4	88.9	27.2	30.6	83	37	62	1	0	0	+
18-08-2015	LY	18	7yrs	F	1.8	3.43	9.7	32.6	95	28.4	29.9	51	60	38	2	0	0	+
18-08-2015	CM	19	8yrs	F	2.4	2.77	7.6	26.6	95.7	27.5	28.7	69	36	63	1	0	0	+
18-08-2015	LY	20	8yrs	M	1.9	3.51	9.7	33.5	95.5	27.7	29	54	61	36	3	0	0	+
26-08-2015	JK	21	11yrs	F	1.7	2.66	5.8	18.2	68.5	21.6	31.6	149	41	57	2	0	0	+
27-08-2015	TL	22	15yrs	M	2	3.27	7.1	24.5	78.6	24.7	32.6	39	39	60	1	0	0	+
02-09-2015	KK	23	4yrs	M	2.7	2.91	4.5	15.2	56.2	16.2	29.6	144	29	70	1	0	0	+
02-09-2015	LN	24	10yrs	M	1.6	2.55	5.1	16.7	65.4	19.8	30.5	116	48	50	2	0	0	+

10-09-2015	KH	25	15yrs	M	1.5	2.2	5.2	16.7	75.5	23.6	31.2	74	50	49	0	0	0	+
10-09-2015	KK	26	23yrs	M	1.6	2.94	6.5	21.3	72.6	22.3	30.7	62	39	61	0	0	0	+
10-09-2015	JP	27	61yrs	M	1	2.81	7.5	21.3	96.6	30.4	31.5	40	57	40	0	0	0	+
17-09-2015	JK	28	35yrs	M	1.1	2.69	7.7	26.7	86.9	28.1	42.9	63	48	50	0	0	0	+

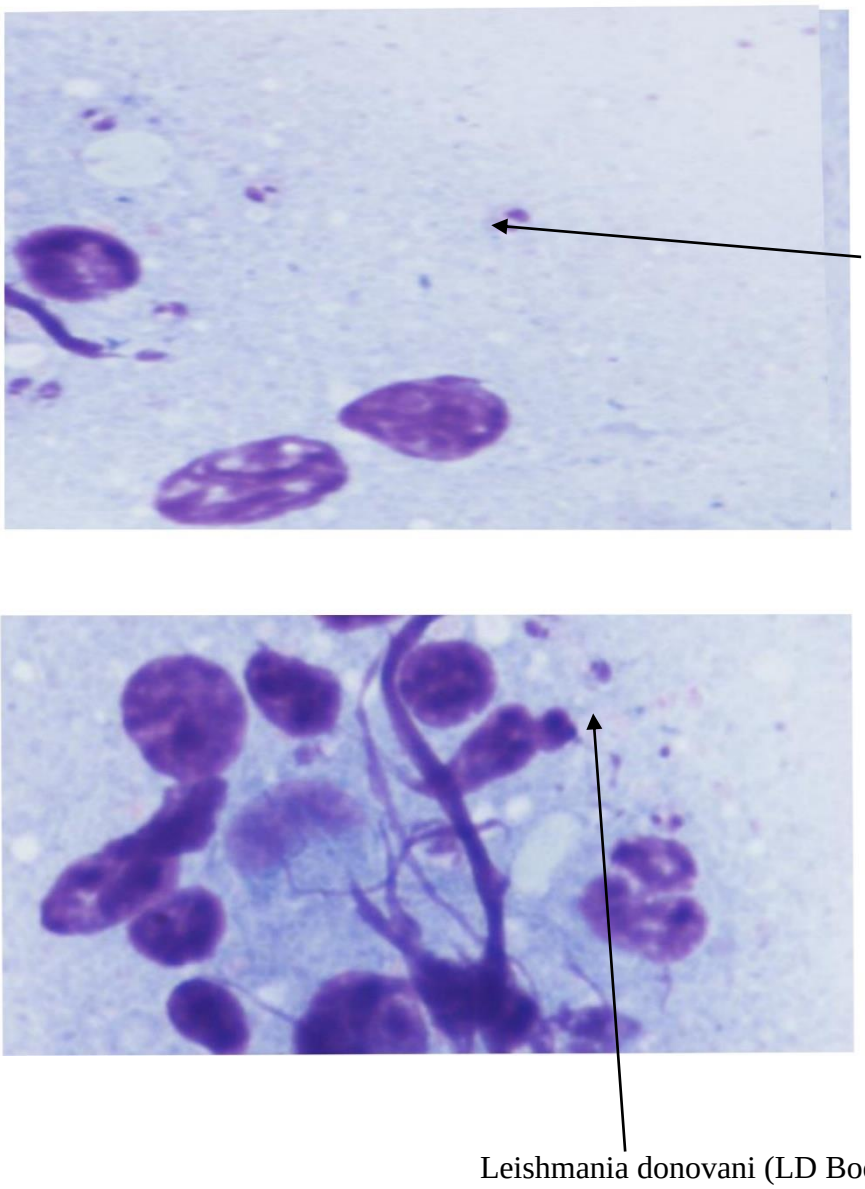
17-09-2015	IK	29	27yrs	M	1.3	3.07	8.6	23.5	87.4	28.6	32.7	63	49	50	1	0	0	+
25-09-2015	CR	30	25yrs	M	1.7	2.93	6	19.7	81.4	24.8	30.5	40	59	39	2	0	0	+
25-09-2015	SC	31	28yrs	M	1.2	2.54	5.2	19.1	74.9	20.6	27.5	31	52	45	2	0	0	+
25-09-2915	YO	32	23yrs	M	0.9	3.03	7	23.2	76.4	23	30.1	53	46	54	0	0	0	+
29-09-2015	JC	33	25yrs	M	0.7	2.31	6.6	22.5	79.6	25.3	29.6	34	41	59	0	0	0	+
29-09-2015	JJ	34	6yrs	F	1.4	2.25	5.7	17.5	70.6	24.7	30.9	43	40	60	0	0	0	+
30-09-2015	ML	35	40yrs	F	2.8	2.85	6.7	22.4	91.5	27.5	30	106	63	35	2	0	0	+
30-09-2015	RL	36	16yrs	M	1.8	2.79	6.4	24.6	98.4	24.3	31.3	58	39	60	1	0	0	+
30-09-2015	LC	37	9yrs	F	1.9	2.22	5.9	19	85.5	26.7	31.2	42	63	33	4	0	0	+
30-09-2015	EL	38	7yrs	F	2.1	1.55	4.3	15.1	96.8	27.4	28.3	54	37	62	1	0	0	+
06-10-2015	KM	39	50yrs	M	2.2	2.16	5.5	19.3	95.2	24.7	28.8	78	57	41	2	0	0	+
07-10-2025	JM	40	22yrs	F	1.5	1.13	3.6	12	99.1	29.5	31.6	35	59	38	3	0	0	+
16-10-2015	JA	41	9yrs	F	2.6	1.18	5.1	15.9	95.3	27.3	32	78	60	37	3	0	0	+
19-10-2015	JL	42	12yrs	F	1.3	2.83	5.4	17.2	60.1	19	31.3	111	42	56	2	0	0	+
19-10-2015	DK	43	10yrs	M	0.6	1.79	5	15	83.5	27.9	33.4	52	31	68	1	0	0	+
19-10-2015	EL	44	9yrs	M	2	2.47	7.2	22.1	89.3	29.1	32.6	84	59	38	3	0	0	+
19-10-2015	LL	45	15yrs	M	1.5	2.58	5.5	18	69.9	21.4	30.7	126	53	46	1	0	0	+
23-10-2015	LN	46	18yrs	M	1.1	2.18	5.5	17.3	79.4	25.4	32	70	44	54	2	0	0	+

26-10-2015	HC	47	12yrs	F	2.2	2.97	7.1	23.4	78.4	24.1	30.5	91	56	41	3	0	0	+
30-10-2015	ESK	48	10yrs	M	0.9	1.19	3.9	12.3	103.2	32.1	33	52	30	68	2	0	0	+
10-11-2015	KN	49	19yrs	M	2.3	3.63	9.8	31.3	81.4	25.6	31.4	112	71	26	3	0	0	+
11-11-2015	CJ	50	20yrs	F	1.3	2.99	5.6	19.3	64.8	18.6	28.7	278	72	27	1	0	0	+
13-11-2015	JK	51	44yrs	M	1.3	2.28	5.7	18.1	79.6	25.1	31.5	183	55	44	1	0	0	+
13-11-2015	TC	52	63yrs	F	2.9	2.71	7.5	24	84.5	27.6	31.2	125	63	33	4	0	0	+

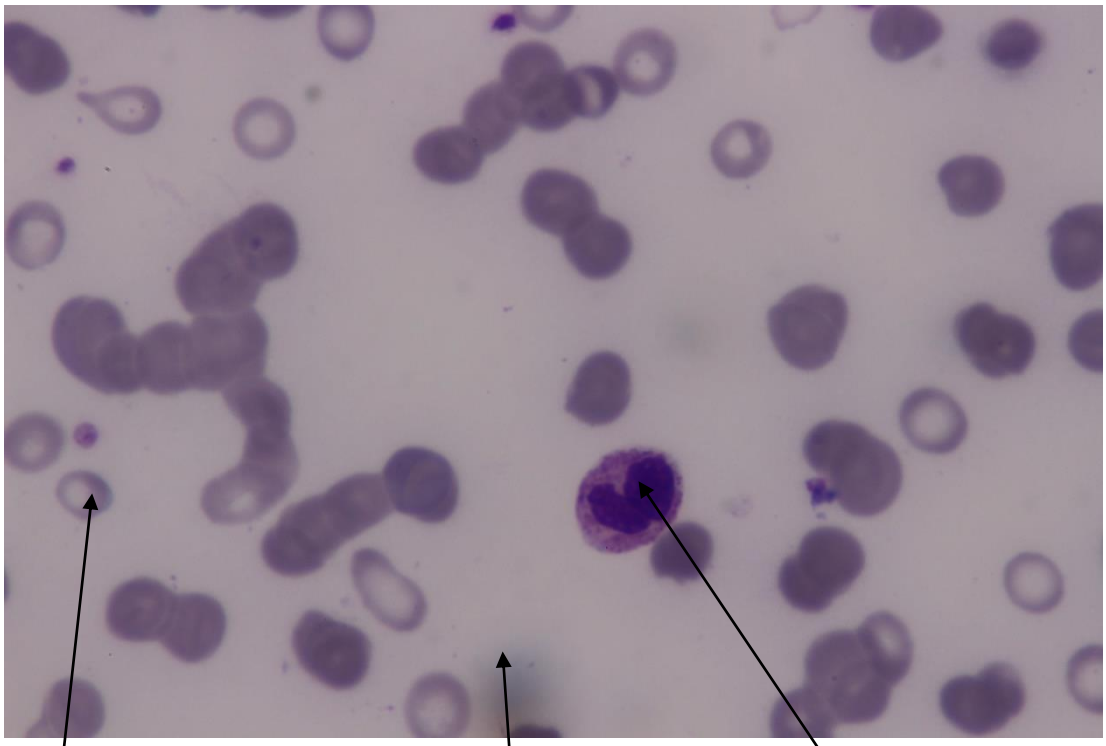
16-11-2015	CR	53	16yrs	F	1.5	1.34	4.1	13.4	96.7	29.6	32.6	22	57	41	2	0	0	+
17-11-2915	KA	54	10yrs	F	0.6	1.56	3.8	12.1	77.6	24.6	31.7	49	61	38	1	0	0	+
19-11-2015	LL	55	15yrs	M	1.4	2.12	5.9	19.1	89.4	29.6	31.1	49	50	47	3	0	0	+
19-11-2015	KL	56	14yrs	F	1.9	1.99	5	16.4	82	25.3	30.9	50	54	44	2	0	0	+
24-11-2015	JB	57	36yrs	M	2.3	2.83	6.9	22.2	78.4	24	30.9	49	59	36	5	0	0	+
27-11-2015	TC	58	50yrs	F	2	2.83	7.7	24.9	81.8	21.7	31.1	83	58	40	2	0	0	+
27-11-2015	JK	59	25yrs	M	1	1.32	3.8	12.9	97.8	28.8	29.5	41	46	50	4	0	0	+
12-01-2016	JK	60	12yrs	F	1.8	2.34	5.6	16.9	72.3	23.7	32.8	169	32	66	2	0	0	+
12-01-2016	SK	61	17yrs	M	1	2.3	5.6	17.8	77.3	24.4	31.6	156	49	50	1	0	0	+

15-01-2026	FL	62	30yrs	F	2.4	2.62	6.6	21.3	81.1	25	30.8	166	51	46	3	0	0	+
28-01-2016	FS	63	39yrs	F	2.2	2.47	6.2	20	80.8	25.2	31.1	160	44	55	1	0	0	+
29-01-2016	JK	64	19yrs	F	2.4	3.85	8.2	25.9	67.4	21.3	31.7	115	67	32	1	0	0	+
29-01-2016	JK	65	12yrs	F	3	2.84	6	21	73.9	21.2	28.7	409	41	55	4	0	0	+
10-02-2016	NI	66	6yrs	M	2.6	3.05	8	24.9	81.8	26.3	32.2	80	32	66	2	0	0	+
16-02-2016	CK	67	16yrs	F	2.6	2.15	7.3	23.1	101.4	34	31.7	101	59	36	5	0	0	+
22-02-2016	AK	68	22yrs	F	1.5	2.57	6.2	19.6	76.3	24.3	31.9	178	55	43	2	0	0	+
17-03-2016	EK	69	14yrs	M	3.3	2.31	5.6	19.3	83.8	24.5	29.2	225	60	40	0	0	0	+
18-03-2016	NA	70	22yrs	M	1.4	2.95	7.5	23.7	80.3	21.9	28.4	97	56	40	4	0	0	+
31-03-2016	CI	71	28yrs	M	2.2	2.17	5.9	19.1	87.8	27.1	30.9	88	66	31	3	0	0	+
14-04-2016	GB	72	35yrs	F	1.9	3.39	10.2	31	91.4	30.2	33	20	73	24	3	0	0	+
22-04-2016	KA	73	8yrs	M	3	2.08	5.3	17.6	80.4	25.5	31.4	104	35	64	1	0	0	+
22-04-2016	LK	74	7yrs	F	3	1.54	4.4	16.1	97.4	28.5	29.3	100	42	58	0	0	0	+

Appendix IV: Giemsa Stained Splenic Aspirate Smear Showing Leishmania Donovani Amastigotes (LD Bodies)



Appendix V: Giemsa Stained Blood Smear Showing Hypochromic and Normocytic Cells, a Neutrophil and Platelets

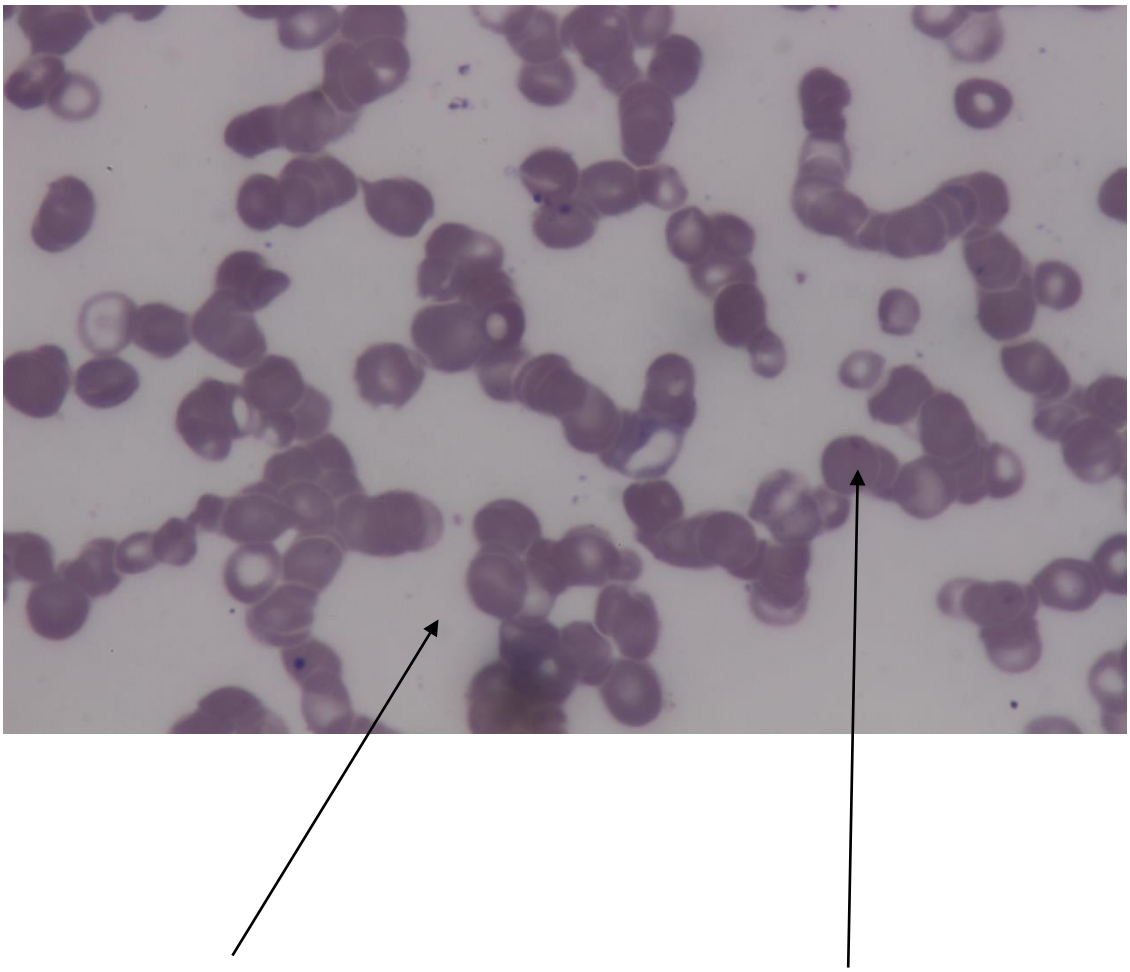


Hypochromic and normocytic cells

Platelet

Neutrophil cell

Appendix VI: Giemsa Stained Blood Smears Showing Hypochromic and Microcytic Cells With platelets



Platelets

Hypochromic and Michrocytic cells

Appendix VII: Giemsa Stained Blood Smear Showing Hypochromic and Machrocytic Cells with Platelets