CIRCULATORY CYTOKINES AND HEMATOLOGICAL PROFILES AS BIOMARKERS OF HIV AND AIDS PROGRESSION

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DECLARATION

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

Signature …….. Date ……

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SUPERVISORS' APPROVAL

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DEDICATION

To my family for their prayers, support and encouragement especially Teresiah, Kathrine, Angela and Eunice, and to those who encouraged me, particularly Patrick and Veronica.

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DEFINITION OF TERMS

Anemia - Less than the normal quantity of hemoglobin in the blood or a decrease in number of red blood cells (RBCs).

Biomarker - Any measurable biological characteristic encompassing the detection of physiologic and pathologic processes.

Cachexia - Loss of weight, fatigue, significant loss of appetite in someone who is not actively trying to lose weight, muscle atrophy, wasting syndrome and weakness.

Cytopenia- A shortage in numbers of any of the cellular elements of blood.

Epitope - The fraction of an antigen recognizable by the immune systems specially by the antibodies, B cells, or T cells also known as the antigenic determinant.

Leukopenia- A state of abnormally low number of circulating white blood cells in the blood.

Neoplasm - An abnormal growth of tissue caused by the rapid division of cells that have undergone some form of mutation.

Neutropenia - An abnormally low level of neutrophils in the blood.

Pleiotropic cytokines – Cytokines which affect activities of several types of cells.

Tat - protein encoded for by the tat gene in HIV-1.

Thrombocytopenia – A disorder caused by unusually low platelet numbers.

Thrombocytosis – A disorder in which there is an abnormally high amount of platelets.

ABREVIATION AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome
APCs	Antigen presenting cells
ART	Anti-retroviral therapy
BD	Beckton Dickinson
CAF	Cell antiviral factor
cART	Combination anti-retroviral therapy
CCC	Center for Comprehensive Care
CD	Cluster of differentiation
CDC	Centre for Disease Control and Prevention
CR	Chemokine receptor
CXCR	CXC Chemokine Receptor
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
FACS	Fluorescence Activated Cell Sorter
Gp	Gycoprotein
Gran#	The absolute content of granulocytes
HAART	Highly active antiretroviral therapy
HAD	Human immunodeficiency virus related dementia
HCT	Hematocrit

HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IFN-γ	Inter feron gamma
IgG	Immunoglobulin G
IL	Interleukin
INSTI	Integrase nuclear strand transfer inhibitors
LTNP	Long-term nonprogressors
Lymph#	Absolute content of lymphocytes
Lymph %	Relative content of lymphocytes
MCH	Mean cellular hemoglobin
MCHC	Mean cellular hemoglobin concentration
MCV	Mean cellular volume
Mid#	Absolute content of the mixture of monocytes, basophils and eosinophils
(Mid%)	Relative content of the mixture of monocytes, basophils and eosinophils
MPV	Mean platelet volume
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NtRTI	Nucleotide reverse transcriptase inhibitors
PCT	Platelet crit
PDW	Relative width of the distribution of platelets

PGH	Provincial general hospital
PI	Protease Inhibitor
PLT	Platelets
RBC	Red blood cells
RDW-CV	Relative distribution width of red blood cells by volume, coefficient of variation
RDW-SD	Relative distribution width of red blood cells by volume, standard deviation
RNA	Ribonucleic acid
Tat	Trans activator of transcription
T_C	Cytotoxic T cell
TCR	T-cell Co- Receptor
Th-1	T-helper subset 1
Th-2	T helper subset 2
TNF-α	Tumor necrosis factor alpha
TNF-β	Tumor necrosis factor beta
TNF-γ	Tumor necrosis factor gamma
VCT	Voluntary Counseling and Testing
WBC	White blood cells
WHO	World Health Organization

ABSTRACT

Cytokines are polypeptides that have a fundamental role in communication within the immune system and in allowing the immune system and host tissue cells to exchange information. They play a central role in the pathogenesis of many diseases including Acquired Immunodeficiency Deficiency Syndrome (AIDS). Cytokines reflect the local or systemic inflammatory *milieu,* and could serve as predictive biomarkers in Human Immunodeficiency Virus (HIV) infection. Hematological abnormalities are among the most common complications caused by HIV infection and seem to be dependent on the level of virus replication. The aim of this study was to identify circulatory cytokine biomarkers and hematological profiles measureable in blood that could predict the progression of HIV disease in the course of infection. This study was done at the Nakuru Provincial General Hospital (PGH). Eighty individuals were recruited for this study that comprised of forty individuals newly diagnosed with HIV-1 (treatment naïve), twenty HIV negative individuals and twenty HIV positive individuals that were on treatment with highly active antiretroviral therapy (HAART). Hematological parameters were analyzed using hematology auto analyzer system; cytokine types and concentrations were determined by flowcytometry using multiplex cytokine immunoassay and CD4 T cell counts were measured by flowcytometry using Becton and Dickinson Fluorescence Activated Cell Sorter (BD FACS) count. Statistical analysis were done using SPSS version 17. Descriptive statistics were applied; analyses of variance was used to determine the differences in mean circulatory cytokine levels, mean hematological profiles and mean CD4 T cell counts between the subgroups forming the study population. The associations between immunological and hematological profiles were determined. The study found significant differences in the mean Interleukin12p70 ($p<0.001$), Tumor Necrosis Factor ($p<0.05$), Interleukin 10 ($p<0.05$), Interleukin 6 ($p<0.005$) and Interleukin 1-β ($p<0.05$) between treatment naïve HIV patients, HIV patients on HAART and HIV negative patients. No significant differences were found between the mean White Blood Cell counts and Red Blood Cell counts ($p>0.05$) between the treatment naïve HIV patients, HIV negative patients and HIV patients on HAART but there were significant differences between the mean hematocrit (HCT) ($p<0.05$); hemoglobin (HGB) ($p<0.05$) and platelets (PLT) ($p<0.05$). This study also found significant differences in the mean volume of erythrocytes (MCV) ($p<0.05$), mean content of hemoglobin (MCH) ($p<0.05$), mean concentration of hemoglobin (MCHC) ($p<0.05$), mean platelet volume (MPV) ($p<0.001$), relative width of the distribution of platelets (PDW) ($p<0.001$) and the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#) ($p<0.05$). Significant differences in the mean CD4 T cell counts ($p<0.001$) were observed between the treatments naïve HIV patients, HIV negative patients and HIV patients on HAART. The study found several identical associations between immunological and hematological profiles among the study groups. The study showed that early period of HIV infection is characterized by varying circulatory cytokine levels and could be useful biomarkers and indicators of early immune activation of HIV disease. The results from this study also show that acute HIV infection affects hematological profiles, involving all the blood parameters, some of which may act as indicators of HIV disease progression. The study recommends the use of low cost hematologic tests as biomarkers of disease progression in resource limited settings and also a holistic treatment of HIV infected patients that include supplements to monitor and improve blood parameters and indices in addition to antiretroviral therapy.

CHAPTER ONE: INTRODUCTION

The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) and has become one of the world's most serious health and development challenges. The first cases of HIV were reported in 1981 and by 2017 approximately 77.3 million people had become infected with HIV since the start of the epidemic (UNAIDS, 2018). In addition, 35.4 million people have died from AIDS-related illnesses worldwide since the start of the epidemic. It was estimated that in 2017 about 36.9 million people globally were living with HIV while about 940,000 people died from AIDS- related illnesses (UNAIDS, 2018). Majority of people living with HIV are found in middle and low income countries. About 25.5 million HIV infected persons are said to be living in sub Saharan Africa with 19.4 million among them living in Eastern and Southern Africa. This is estimated as 43% of people living with HIV globally whilst women and girls accounts for more than half (59%) of them. Additionally, in 2017, an estimated 420,000 persons are said to have died of AIDS related illnesses in Eastern and Southern Africa (AIDSinfo, 2018).

New HIV cases have been reported from all regions of the world. An estimated 1.8 million people became newly infected with HIV in 2017 worldwide (UNAIDS, 2018). Approximately 790,000 new HIV infections were reported in Eastern and Southern Africa which accounted for about 44% of the global new HIV infections (UNAIDS, 2018). While there is still no cure for HIV, the majority of those that live with the virus or are at risk for HIV infection are not able to gain access to prevention, treatment and care (WHO, 2013). Various reports have indicated that in order to improve HIV testing and knowledge of HIV among young adults and

adolescents, a lot more needs to be done. About 59% of new HIV infections occur in persons aged between 15 - 24 years. This indicates that the virus mainly affects those in their most productive years (UNAIDS, 2014). In sub-Saharan Africa, three in four new infections occur among adolescents aged 15-19 are girls. Young women aged 15-24 years are twice more likely to be living with HIV than men. Every week around 7,000 young women aged 15-24 years become infected with HIV (UNAIDS, 2018). Whereas the immune systems of healthy individuals would normally clear a variety of infections, persons infected with HIV become more susceptible since the HIV weakens ones defense system and surveillance against infections (WHO, 2013).

Kenya's HIV epidemic is geographically diverse ranging from a prevalence of 21.0% in Siaya county to about 0.1% in Wajir county. Five counties with the highest adult HIV prevalence in 2017 in descending order included: Siaya (21.0%), Homa Bay (20.7%), Kisumu (16.3%), Migori (13.3%) and Busia (7.7%). Eight counties contributed to more than 50% of the people living with HIV out of the estimated adults (aged 15+ years) living with HIV in 2017 in Kenya. These are: Nairobi (182,856), Homa Bay (128,199, Kisumu (112,862), Siaya (113,605), Migori (79,146), Kiambu (56,622), Kakamega (48,752) and Mombasa (38,548); (MOH, 2018). Nonetheless Kenya has a generalized HIV epidemic in which all sections of the population are affected including men, women, young people and children (MOH, 2018) However, concentrated epidemics among certain groups have been identified as particularly vulnerable to HIV transmission in a number of studies in recent years. The Kenya AIDS Strategic Framework 2014/2015 – 2018/2019 which is the current HIV/AIDS strategy is in agreement with this when

describing concentrations of very high prevalence among key populations combined with deeply rooted HIV epidemic among the general population (KNACC, 2014).

Kenya's first case of HIV was detected in 1984 and by mid 1990s HIV became one of the main causes of death in the country placing huge demands on the economy, including the healthcare systems. In 1996, HIV prevalence peaked at 10.5 percent (MOH, 2014), and due to a combination of increased awareness, scaling up of prevention and treatment programs, there has been a decline in HIV prevalence in Kenya. In 2017, adult (15-49 years) prevalence declined to 4.9%. (Kenya HIV estimates, 2018). The total number of people living with HIV in Kenya was estimated at approximately 1,500,000 in 2017. This included 105,200 children less than 15 years old and 1,388,169 adults aged 15 years and above. Children under the 15 years of age accounted for 7% of all people living with HIV. Of the total number of people living with the virus in 2017, 184,718 (12%) were among youth 15-24 years of age. During the same year, there were approximately 52,800 new HIV infections across all ages; 44,800 among adults aged 15 years and above and 8,000 among children aged less than 14 years (MOH, 2018). A decline in AIDS related deaths has been reported that has been directly attributed to the wider access to antiretroviral therapy among other factors. However, in 2017, approximately 28,200 people died of AIDS-related causes that included 4,300 children aged less that 15 years and 23,900 adults (15 years and above) (MOH, 2018).

Most of the symptoms associated with HIV infection are due to effects on the effecter mechanisms of the host's immune system especially the CD4+ T helper cells. The human immunodeficiency virus infects a T cell which has a CD4+ receptor on its surface; CD4+ T helper cells participates in fundamental roles in the immune system through cytokines secretions that are involved in regulating the immune responses. Circulating cytokines are critical in fighting off an infection and they are also essential in other immune responses (Dinarello, 2000). They regulate a number of physiological and pathological functions including innate immunity, acquired immunity and several inflammatory responses (www.sinobiological.com/role of cytokines.html. 2019). The secretion of circulatory cytokines is in response to non-specific and specific stimuli while their production is by an array of cell types and they have been found as being significantly involved in inflammation, hematopoiesis and immunity (Korn *et al.*, 2009). Pro-inflammatory cytokines are produced predominantly by activated macrophages and are important in cell signaling and promote systemic inflammation. They are involved in the upregulation of inflammatory reactions, in contrast to anti-inflammatory cytokines, which promote healing and reduce inflammation; pro-inflammatory cytokines may act to make a disease worse in certain situations (Zhang and An, 2007).

Circulatory cytokines detectable in persons infected with HIV can be useful indicators of individuals' immune responses to HIV. The first weeks of HIV infection is characterized by circulatory cytokine production in the blood plasma (Stacey *et al.*, 2009). An increased production of pro-inflammatory cytokines is a part of an immune response during HIV infection (Bebell *et al.*, 2008; Stacey *et al.*, 2009). These cytokines augment the replication of HIV and

the loss of CD4 T cells through activation-induced apoptosis of by stander T cells, in activating and recruiting CD4 T cell targets for HIV infection and also by directly promoting proviral transcription (Osborne *et al.,* 1989; Lin *et al.,* 1997; Swingler *et al.,* 1999). As cytokines may suggest systemic inflammatory or a local milieu, they might function as biomarkers during the prognosis of early stages in HIV infection.

There are several hematological abnormalities that have been identified to be manifested during the course of HIV infection that include anemia, neutropenia, thrombocytopenia and coagulation abnormalities (Attili *et al.,* 2008). The most important of them are cytopenias. Anemia and neutropenia are generally caused by inadequate production because of suppression of the bone marrow by the HIV infection through abnormal cytokine expression and alteration of bone marrow microenvironment (Coyle, 1997).

HIV infection may lead to anemia in many ways: the important causes include; defective iron metabolism and reutilization (Fuchs *et al.,* 1993; Bain, 1999), opportunistic infections (Claster, 2002), administration of chemotherapeutic agents (Volberding *et al.,* 1990) and Vitamin B12 deficiency (Levine *et al.,* 2001). Neutropenia is common and the incidence rises from 13% to 44% with disease progression from HIV to AIDS (Kaslow *et al.,* 1987; Henry, 1998). In different study settings, the prevalence of anemia in persons with AIDS has been estimated at 63% to 95% (Hillman *et al.,* 1998), making it more common than thrombocytopenia or leukopenia in patients with AIDS. This high prevalence of anemia may be due to the high incidence of anemia, a long duration of anemia, or a combination of both. The incidence and severity of cytopenia are

generally correlated with the stage of the disease. Other causes of cytopenia in these patients include treatment-related adverse events or secondary to the opportunistic infections, malignancies, other preexisting or co-existing medical problems. Optimal management of the underlying HIV infection is essential, and mild cytopenia in asymptomatic patients may need no specific management (Coyle, 1997; Salond, 2005; Cosby, 2007).

Association of HIV infection with thrombocytopenia was long ago recognized (Sullivan *et al.*, 1997). Incidence of thrombocytopenia is around 40% of HIV infected persons, and in approximately 10% of the patients, it may be the first sign of AIDS (Pechere *et al.*, 1993). Thrombocytopenia is caused by immune-mediated destruction of the platelets, in addition to inadequate platelet production. Thrombocytopenia is caused by immune-mediated destruction of the platelets, in addition to inadequate platelet production. The incidence and severity of cytopenia are generally correlated to the stage of the disease. Other causes of cytopenia in these patients include treatment-related adverse events or secondary to the opportunistic infections or malignancies, or other preexisting or co-existing medical problems (Attili *et al.*, 2008). This study aimed at identifying cytokines measureable in plasma and hematologic profiles that could be used as biomarkers during acute HIV-1 infection that can predict HIV disease progression.

1.2 Problem Statement

Among the recently diagnosed human immunodeficiency virus patients, the progression rate of the infection can vary greatly whereby a number of patients may progress quickly to occurrence of opportunistic infection, while others might remain entirely well and maintain normal CD4 T cell counts, for months or even years following infection while not taking antiretroviral therapy. Although long-term nonprogressors may represent a small number of persons infected with the human immunodeficiency virus, this inconsistency may suggest the necessity of recognizing certain factors which might reveal the progression rate of the infection. Types and levels of the host immune products and their interactions throughout the early stage of the human immunodeficiency virus disease might predict some risks of the disease progression.

Profiling of the circulatory cytokines is a very pertinent parameter of immune responses. Diverse circulating cytokines have the capability of controlling other cytokines production and they may also have functions that are overlapping biologically (Vishwanath *et al.*, 2011). However, there are few studies on the relationship between circulatory cytokines which are formed during the early stages in HIV infection and disease prognosis. Analysis of production and function of cytokines expressed in recently diagnosed HIV infected patients may be valuable in predicting disease progression, which might be useful in disease management strategies. Relationships between cytokine profiles and HIV infection may form part of the immunological responses that are likely to occur, leading to more insight of HIV pathogenesis in the early stages of infection. This insight could be useful in advocating for reduction of HIV transmissions.

A variety of hematological profiles have been reported at every stage of HIV and AIDS and they frequently create a huge challenge in management of the disease. These expressions could damage the quality of life among the HIV patients and bring about life threatening symptoms; they might also be a sign of their body's immune status. Disorders that occur in the hematopoietic system might be caused by the direct effect of HIV disease among the infected individuals, side effects of treatment or neoplasm (Kusfa *et al.,* 2017). There are few studies on hematological profiles in HIV and very few have correlated results with disease progression. Hematological profiles in relation to HIV were analyzed and compared with circulatory cytokine profiles.

1.3 Justification of the Study

The relationships between the human immunodeficiency virus and an individual's immune responses are complicated. The CD4+T cell count is the laboratory test generally accepted as the best marker of the immediate state of immunological competency of the patient with HIV infection (Rübsamen-Waigmann *et al.,* 2003). An important measure of the health of immune systems in the course of HIV infection is the CD4 T cell count. When the CD4 T cell counts are lower than the normal range, it is an indication of a great harm done by the human immunodeficiency virus. The normal CD4 T cell counts range is 500 cells/ µL to 1600 cells/ µL (USDHHS, 2018). Several factors have been identified which can predict risks that are associated with the progression of HIV infection in terms of measuring the decline rate of CD4 T cell counts, progressing to opportunistic infections or loss of life, and even the danger of CD4 T cell counts declining to less than 200/µL. One indicator of the risks for progressing HIV

infection is the degree of human immunodeficiency virus replication which is usually indicated by levels of the plasma human immunodeficiency virus – ribonucleic acid (HIV RNA) (Fry *et al.,* 2001). There is need to identify more indicators that could be useful predictors of the progression in HIV infection, particularly in the early stages of the disease.

Quantifying circulatory cytokines produced during an immune response is an important tool in immunological assays for determining several processes of a disease. Following HIV infection, there might be an alteration within the production of hosts chemokines and cytokines that may favor the virus' success and survival within the hosts, thereby escalating the hosts' vulnerability to opportunistic diseases. Sometimes the cytokines might exert effects that are contrasting on the immune response whereas nearly all of them use pleiotropic effects (Peter *et al.,* 2011). The levels of circulatory cytokines in human blood are able to provide important information on *in vivo* immune position. Measuring and determining the levels of circulatory cytokines might also be useful in monitoring HIV disease progression including rates of inflammation. This approach can be used in relating systemic inflammation or immune status to disease outcome. Even though the stage of acute infection with HIV is said to be linked to increased productions of pro-inflammatory cytokines, the relationships of circulatory cytokine levels and HIV disease progression is not known. There is need for carrying out more studies on cytokines produced during the various stages of HIV disease; concentrations and types of cytokines may indicate their role during early stages of HIV infection.

Within a few days or weeks after HIV infection, the amount of HIV in the blood gets very high. HIV infection has several stages; the first one being primary HIV infection or the acute HIV infection, a stage where individuals infected may ignore the symptoms which include fever, fatigue, rash, headache, among others. It is important to check the hematological profiles at this stage to be able to determine the HIV disease progression. As HIV disease advances, there might be increases of hematological abnormalities that form part of the complications that are commonly seen in HIV disease. While the mechanism that underlies the abnormalities is unclear, an explicit diagnosis of the causes and mechanisms is necessary since a particular remedy might be required to correct these abnormalities. However, before HIV disease advances, analysis of hematological parameters and indices may be useful in assessing disease progression.

When individuals from resource limited settings are diagnosed with HIV disease, they are mostly referred to a more advanced facility with equipment that may determine their CD4+ T cell counts along with probably their viral load. Some individuals who are infected with HIV do not notice anything hence failing to see the signs during this early stage of HIV infection. At this point, some immune damage may occur although a number of individuals may assume that there's little harm and hence find no need to seek further treatment in a more advanced health facility.

The most common laboratory test done in almost all of the health facilities is the total blood counts that give information with reference to numbers of cells in the blood and their kinds particularly the platelets (PLT), red blood cells (RBC) and white blood cells (WBC). These parameters are considered very useful in diagnosing conditions, infections and disorders and

could be used to determine the progression of HIV to a certain extent, at the local set up. Blood count analysis often has other parameters that allow exploration of the situation in more detail, such as mean cellular hemoglobin (MCH), the mean cellular hemoglobin concentration (MCHC) and the mean cellular volume (MCV). These in addition to other blood parameters and indices are rarely used during a routine complete hemogram test and could present helpful clues for determining HIV disease progression, particularly during early stages before much damage is done to the immune system and also in reducing risks of infecting other persons. The determination of most applicable blood parameters and indices that would serve to assess early HIV disease progression is of great importance. Although there have been studies in HIV/AIDS, there are scant data available from Kenya on hematological and cytokine profiles of HIV/AIDS patients.

1.4 Research Questions

i) What are the cytokine profiles in HIV patients on highly active antiretroviral therapy (HAART), those who are not on highly active antiretroviral therapy (HAART/treatment naïve) and those that are HIV negative?

ii) What are the hematological profiles in HIV patients on highly active antiretroviral therapy (HAART), those who are not on highly active antiretroviral therapy (HAART/treatment naïve) and those that are HIV negative?

iii) What is the association between hematological profiles and CD4 profiles in HIV patients on highly active antiretroviral therapy (HAART), those that are not on highly active antiretroviral therapy (HAART/treatment naïve) and those who are HIV negative?

iv) What is the association between cytokines and CD4 profiles in HIV patients on highly active antiretroviral therapy (HAART), those that are not on highly active antiretroviral therapy (HAART/treatment naïve) and those who are HIV negative?

1.5 Null Hypotheses

i). There is no association between cytokines and hematological profiles in HIV patients on highly active antiretroviral therapy (HAART), those who are not on highly active antiretroviral therapy (HAART/treatment naïve) and those who are HIV negative.

ii) There is no association between cytokines and CD4 profiles in HIV patients on highly active antiretroviral therapy (HAART), those who are not on highly active antiretroviral therapy (HAART/treatment naïve) and those who are HIV negative.

iii) There is no association between hematological and CD4 profiles in HIV on highly active antiretroviral therapy (HAART), those who are not on highly active antiretroviral therapy (HAART/treatment naïve) and those who are HIV negative.

1.6 Objectives

1.6.1 General Objective

To assess the use of circulatory cytokines and hematological profile as biomarkers of HIV progression.

1.6.2 Specific Objectives

i) To determine circulatory cytokine profiles in patients infected with the human immunodeficiency virus that are on highly active antiretroviral therapy (HAART), those that are treatment naïve and those that are HIV negative.

ii) To determine hematological profiles in patients infected with HIV using highly active antiretroviral therapy (HAART), those that are treatment naïve and those who are HIV negative.

iii) To determine the association between hematological and CD4 profiles of patients infected with HIV and are on highly active antiretroviral therapy (HAART), those who are treatment naïve and those that are HIV negative.

iv) To determine the association between cytokine and CD4 profiles of HIV patients on highly active antiretroviral therapy (HAART), those who are treatment naïve and those that are HIV negative.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Human Immunodeficiency Virus

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is still among the top 10 causes of death globally (aidsinfonet.org). According to global HIV and AIDS statistics, 36.9 million adults and children were said to be living with HIV infection while 1.8 million became newly infected in 2017 (UNAIDS, 2018). Adults and children living with HIV in Kenya were estimated at 1,493,382 while new HIV infection were estimated at 52,767 in 2017, according to Kenya HIV estimates (MOH, 2018). Many countries have seen a considerable decrease in HIV/AIDS, though the decline rate differs across countries and regions. The reduction in new HIV infections has been strongest in the region most affected by HIV, Eastern and Southern Africa, where new HIV infections have been reduced by 30% since 2010. However, new HIV infections are rising in around 50 countries. In eastern Europe and central Asia the annual number of new HIV infections has doubled, and new HIV infections have increased by more than a quarter in the Middle East and North Africa over the past 20 years (UNAIDS, 2018).

Human immunodeficiency virus infection has become a manageable chronic illness for people who can be tested and treated. Interventions such as antiretroviral therapies (ART), prophylaxis, and preventing transmission of mother-to-child have been successful (aidsinfonet.org, 2019). Due to the impact of antiretroviral therapy roll-out, the number of AIDS-related deaths is the lowest this century (940, 000), having dropped below 1 million for the first time in 2016. Yet,

the current pace of decline is not fast enough to reach the 2020 target of fewer than 500, 000 AIDS-related deaths (UNAIDS, 2018).

Human immunodeficiency virus (HIV) is a retrovirus that primarily infects components of the human immune system such as $CD4^+$ T cells, macrophages and dendritic cells. Consequently, it directly and indirectly destroys $CD4^+$ T cells (Alimonti *et al.*, 2003). HIV is a member of the genus *Lentivirus*, part of the family *Retroviridae* (International Committee on Taxonomy of Viruses, 2002). Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA virus. Upon entry into the target cell, the viral ribonucleic acid (RNA) genome is converted (reverse transcribed) into double-stranded deoxyribonucleic acid (DNA) (Figure 2.1) by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors (Smith and Daniel, 2006). Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system (Martínez, 2010). Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew (Fuster *et al.*, 2014)

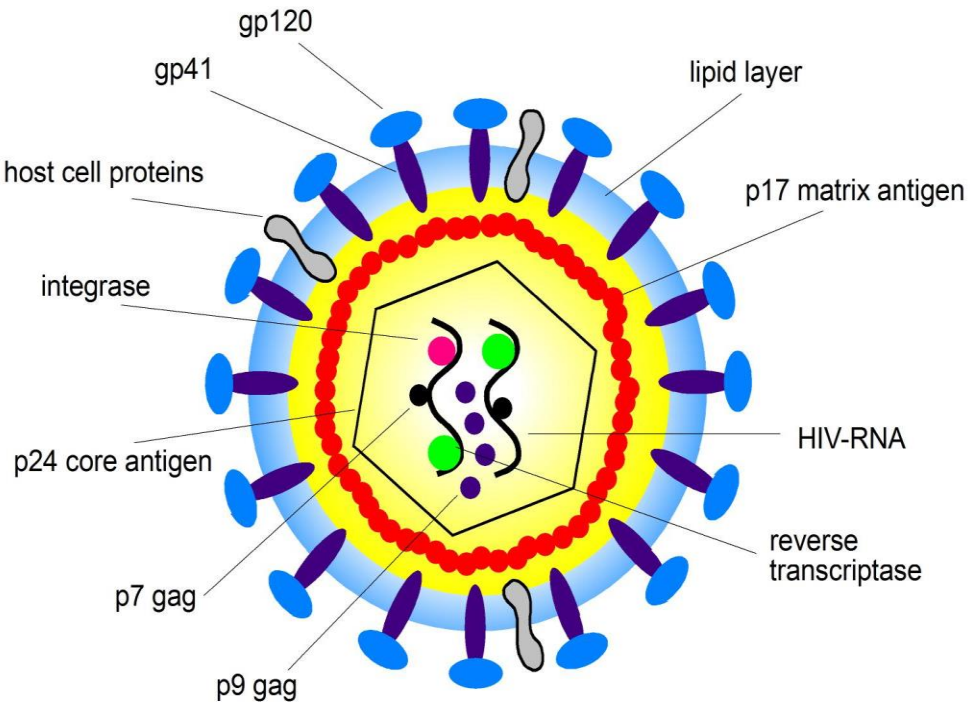


Figure 2.1: The human immunodeficiency virus (HIV) structure (source: www.discovery magazine.com).

When HIV is outside a cell it is known as a virion and is surrounded by a protective envelope that surrounds a number of viral proteins and some genetic material necessary to make new viruses. The main cells that HIV infects are those carrying a molecule called CD4 on their surface. CD4 is found on immune cells, most particularly on T-helper cells and on macrophages.HIV gets inside these cells by binding to the CD4 receptor using a molecule on the surface of the virus called gp120 (Figure 2.2). Once HIV has bound to CD4, it activates other proteins on the surface of the human cell known as CCR5 and CXCR4 in order to complete its fusion with the cell (Michael, 2014).

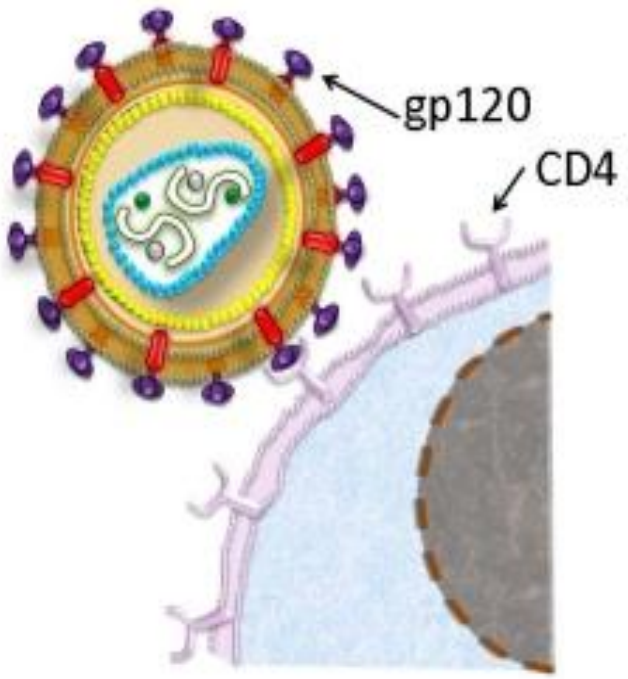


Figure 2.2: Binding of the virus on CD4 T cell (source: www.discovery magazine.com)

Once fusion has occurred, the inside of the virus (the RNA and some important enzymes) is absorbed into the human cell. A viral enzyme called reverse transcriptase performs the process required to translate HIV's genetic material (RNA) into DNA. The newly formed viral DNA is then integrated with the DNA of the human host cell using a viral enzyme called integrase. This allows HIV to reprogramme the human cell to make more HIV. In this stage, the two strands of

DNA divide and form a new strand of viral RNA, sometimes called messenger RNA (Michael, 2014) (Figure 2.3).

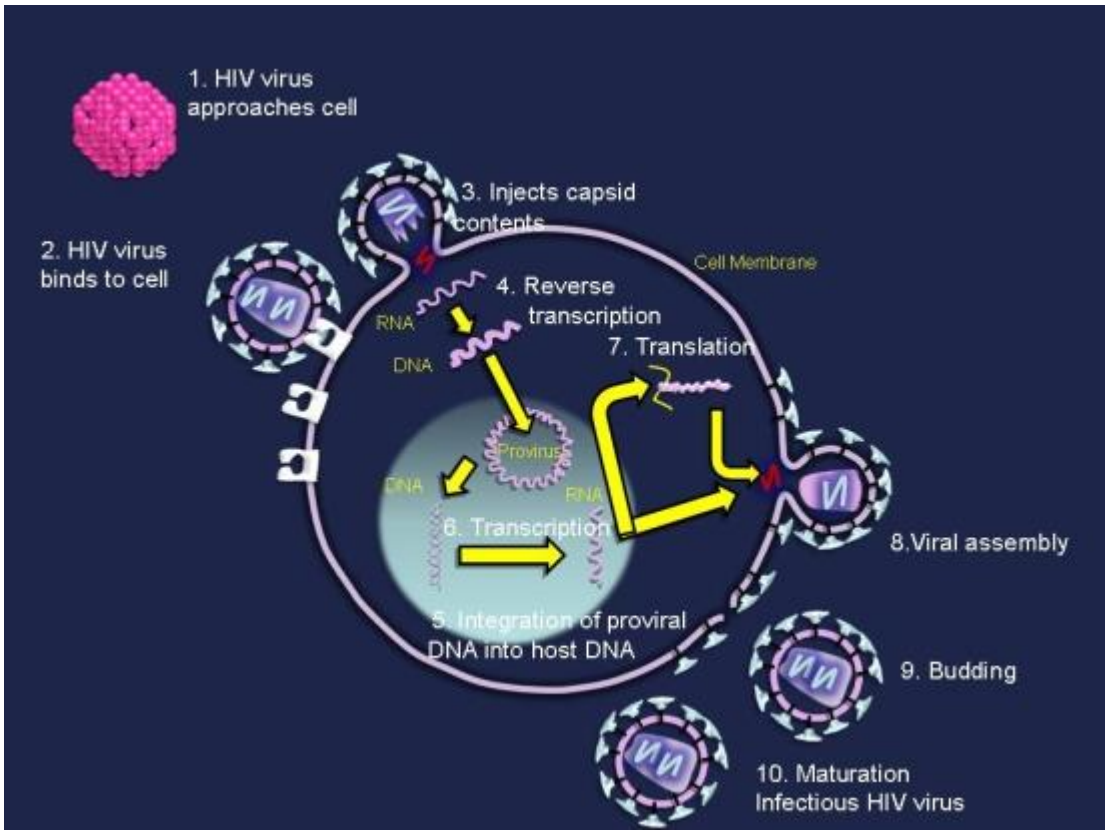


Figure 2.3: Human immunodeficiency Virus replication cycle (Source:www.discoverymagazine.com).

Next the protein building blocks, which will go on to form the new HIV particle, are assembled within the human cell. These blocks are laid out in turn through the translation of the information contained in the messenger RNA. The protein building blocks are then cut into smaller pieces by a viral enzyme called protease. These pieces form the structure of the new HIV particle, including each of the enzymes and proteins needed to repeat the reproductive process. Once this assembly has occurred, the new viral particle buds off the human cell, floats off into the bloodstream and is able to infect other cells. It is estimated that about 10.3 billion new HIV virions are produced every day in people who are not on HIV treatment (Michael, 2014).

Currently, there are two types of HIV that have been characterized: HIV-1 and HIV-2. The human immunodeficiency virus 1 (HIV-1) is the virus that was initially discovered, and is said to be more virulent, more infective, (Gilbert, 2003) and is the cause of the majority of HIV infections globally. The human immunodeficiency virus 2 (HIV-2) is said to have a lower infectivity compared with HIV-1 which implies that fewer people exposed to HIV-2 are infected per exposure. HIV-2 has is known to have a relatively poor capacity for transmission and is largely confined to West Africa and some European and South American countries (Reeves and Doms, 2002).

2.2 How the HIV infection is acquired

Human Immunodeficiency Virus (HIV) is transmitted by three major routes: sexual contact, significant exposure to infected body fluids or tissues, and from mother to child during pregnancy, delivery, or breastfeeding, known as vertical transmission (Markowitz, 2007). There is no risk of acquiring HIV if exposed to feces, nasal secretions, saliva, sputum, sweat, tears, urine, or vomit unless these are contaminated with blood (Kripke, 2007). It is likely to be co-infected by more than one strain of HIV - a condition known as HIV super infection (van der Kuyl and Cornelissen, 2007).

The most common mode of transmission of HIV is through sexual contact with an infected person; majority of all transmissions worldwide occur through heterosexual contacts (Markowitz, (2007). On the other hand, the pattern of transmission varies significantly among countries (http://www.cdc.gov/hiv/group/msm/index.html, 2019). The risk of transmission increases in the occurrence of many sexually transmitted infections (Ng *et al.,* 2011) and genital ulcers. Genital ulcers seem to increase the risk about fivefold (Boily *et al.,* 2009). Other sexually transmitted infections, such as gonorrhea, chlamydia, trichomoniasis, and bacterial vaginosis, are coupled with somewhat lesser increases in risk of transmission (Dosekun and Fox (2010).

The viral load of an infected person is a significant risk factor in both sexual and mother-to-child transmission (Anderson, 2012). A person's infectiousness is twelve times higher during the first 2.5 months of an HIV infection due to this high viral load (Dosekun and Fox, 2010). When the

person is in the late stages of HIV infection, rates of transmission are approximately eightfold greater (Boily *et al.,* 2009).

The second most common mode of HIV transmission is via blood and blood products (Markowitz, 2007). Blood-borne transmission can occur by needle-sharing during intravenous drug use, needle stick injury, transfusion of contaminated blood or blood product, or medical injections by unsterilised equipment. The risk from sharing a needle during drug injection is between 0.63 and 2.4% per act, with an average of 0.8% (Baggaley *et al.,* 2006). The risk of acquiring HIV from a needle stick from an HIV-infected person is estimated as 0.3% (about 1 in 333) per act and the risk following mucous membrane exposure to infected blood as 0.09% (about 1 in 1000) per act (Kripke, 2007).

HIV is transmitted by use of infected blood in about 93% of blood transfusions (Baggaley *et al.,* 2006). The risk of acquiring HIV from a blood transfusion is extremely low in developed countries(less than one in half a million) where improved donor selection and HIV screening is performed. Estimates have shown that up to 15% of HIV infections in low income countries happen when infected blood and blood products are being transfused, representing between 5% and 10% of global infections (Markowitz, 2007). Studies have shown that it is possible to acquire HIV from organ and tissue transplantation although rare because of screening (Simonds, 1993).

Unsafe medical injections have been shown to play a significant role in HIV spread in sub Saharan Africa. In 2007, between 12 and 17% of infections in this region were attributed to medical syringe use (Reid, 2009). According to the World Health Organization, the risk of HIV transmission as a result of a medical injection in Africa is estimated to be at 1.2% (Reid, 2009). Substantial risks are also associated with invasive procedures, assisted delivery, and dental care in this area of the world (Reid, 2009). It has been suggested that people giving or receiving tattoos, piercings, and scarification are theoretically at risk of infection but no confirmed cases have been documented (CDC, 2012). Studies have shown that it is not possible for mosquitoes or other insects to transmit HIV (Crans, 2010).

HIV can also be transmitted from mother to child during pregnancy, delivery, or through breast milk resulting to infection in the baby (Coutsoudis *et al.*, 2010; USDHHS, 2011). This is the third most common mode of HIV transmission globally (Markowitz, 2007). When no treatment is taken, the risk of transmission before or during birth is around 20% while in those who breastfeed, the risk of transmission is 35% (Coutsoudis *et al.,* 2010). If appropriate treatment can be given, the risk of mother-to-child infection can be reduced to about 1% (Coutsoudis *et al.,* 2010). Treatment that can be useful in prevention of HIV transmission to the child involves the mother taking antiretroviral during pregnancy and delivery – that includes two types of drugs from the nucleotide reverse transcriptase inhibitors (NRTIs) for example Zidovudine and Lamivudine; one drug from the non-nucleoside reverse transcriptase inhibitors (NNRTIs) for example Nevirapine plus two or three drugs from other groups (entry inhibitors, protease inhibitors and integrase inhibitors; USDHH,2015). Other methods are an elective caesarean section, avoiding breastfeeding, and administering antiretroviral drugs to the newborn (Thorne

and Newell, 2007). When the antiretroviral are taken by either the mother or the infant, they decrease the risk of transmission in those who do breastfeed (White, *et al*., 2014). Another factor that may pose a risk of transmission of HIV is if blood contaminates food during pre-chewing (CDC, 2018).

HIV is believed to spread between CD4+ T cells by two parallel routes: cell-free spread and cell-to-cell spread; it is said to employ hybrid spreading mechanisms (Zhang *et al.*, 2015). In the cell-free spread, the virus particles are said to bud from an infected T cell, enter the blood/extracellular fluid and then infect another T cell following a chance encounter (Zhang *et al.*, 2015) HIV can also spread by direct transmission from one cell to another by a process of cell-to-cell spread (Jolly *et al.*, 2004; Sattentau, 2008). It has been suggested that the hybrid spreading mechanisms of HIV contribute to the virus's ongoing replication against antiretroviral therapies (Sigal *et al.*, 2011; Zhang *et al.*, 2015).

2.3 Symptoms and stages in HIV infection

Following initial infection, a person may not notice any symptoms or may experience a short period of influenza-like illness (WHO, 2015) .Usually this is followed by a prolonged period with no symptoms (CDC, 2015). As the infection progresses, it interferes further with the immune system increasing the risk of common infections as well as other opportunistic infections and tumors that seldom affect people who have working immune systems (WHO, 2015).

The period known as acute HIV infection can be referred to by different names such as primary HIV infection, acute retroviral syndrome and acute HIV syndrome. All these names describe this brief stage immediately following HIV infection (AIDS Clinical Trial Unit, 2016). Acute HIV infection and primary HIV infection both describe the period immediately after infection when the patient is viremic and has detectable p24 antigen and/or HIV RNA without diagnostic HIV antibodies. Recent infection is generally used to describe the 6-month period after infection occurs. Early infection refers to both acute and recent infection, after which infection is defined as chronic (USDHHS, 2018).

There are three main stages of HIV infection: acute infection, clinical latency and AIDS (USDHHS, 2018). The initial period following the contraction of HIV is called acute HIV, primary HIV or acute retroviral syndrome (WHO, 2007; Mandell *et al.,* 2010). Many individuals develop an influenza-like illness or a mononucleosis-like illness 2–4 weeks post exposure while others have no notable symptoms (Marshall, 2008; Mandell *et al.,* 2010). Symptoms occur in 40–90% of cases and most usually include fever, large tender lymph nodes, throat inflammation, a rash, headache, and/or sores of the mouth and genitals (WHO, 2007; Mandell *et al.,* 2010). The rash, which occurs in 20–50% of cases, presents itself on the trunk and is typically maculopapular (Vogel *et al.,* 2010). Some people also acquire opportunistic infections at this stage (WHO, 2007). Gastrointestinal symptoms such as nausea, vomiting or diarrhea may arise as may neurological symptoms of peripheral neuropathy or Guillain-Barre syndrome (Mandell *et al.,* 2010). The time of the symptoms varies, but is usually one or two weeks. Owing to their nonspecific character, these symptoms are not often recognized as signs of HIV infection.

Therefore, it is recommended that HIV be considered in people presenting an unexplained fever who may have risk factors for the infection (Mandell *et al.,* 2010). The risk of sexual transmission of HIV during acute or recent infection is significantly higher than during chronic infection (Pilcher *et al*., 2004; Pinkerton, 2008; Hollingsworth *et al*., 2008; Hollingsworth *et al*., 2015).

The early symptoms are followed by a stage called clinical latency, asymptomatic HIV, or chronic HIV (USDHHS, 2018). With no treatment, this second stage of the natural history of HIV infection can last from about three years (Evian, 2006) to over 20 years (Charles and Hicks, 2001), on average, about eight years (Elliot, 2012). Whereas usually there are few or no symptoms at first, by the end of this stage many people experience fever, weight loss, gastrointestinal problems and muscle pains (USDHHS, 2018). Between 50 and 70% of people additionally develop persistent generalized lymphadenopathy, characterized by unexplained, non-painful swelling of more than one group of lymph nodes (other than in the groin) for over three to six months (Mandell and Dolan, 2010).

Though the majority of HIV-1 infected persons have a detectable viral load and in the absence of treatment will ultimately progress to AIDS, a small proportion (about 5%) retain high levels of CD4+ T cells (T helper cells) without antiretroviral therapy for more than 5 years (Mandell *et al.,* 2010; Blankson, 2010). These individuals are classified as HIV controllers or long-term nonprogressors (LTNP) (Blankson, 2010). An additional group consists of those who sustain a

low or undetectable viral load without anti-retroviral treatment, known as "elite controllers" or "elite suppressors". They represent about 1 in 300 infected persons (Walker, 2007).

Acquired immunodeficiency syndrome (AIDS) can be defined in terms of either a CD4+ T cell count below 200 cells per μL or the occurrence of specific diseases in connection with an HIV infection (Mandell and Dolan, 2010). With lack of proper treatment, around half of people infected with HIV develop AIDS within ten years (Mandell and Dolan, 2010). The most frequent initial conditions that signal to the presence of AIDS are pneumocystis pneumonia (40%), cachexia in the form of HIV wasting syndrome (20%), and esophageal candidiasis. Additional common signs include recurring respiratory tract infections (Mandell and Dolan, 2010).

There are various organisms that may cause opportunistic infections including bacteria, viruses, fungi, and parasites that are usually controlled by the immune system (Holmes *et al.,* 2003). Different types of infections may occur depending partly on what organisms are common in the person's environment (Mandell and Dolan, 2010). The infections might affect almost every organ system (Chu and Selwyn, 2011). Studies have indicated that people with AIDS are at a bigger risk of developing various viral-induced cancers, including Kaposi's sarcoma, Burkitt's lymphoma, primary central nervous system lymphoma, and cervical cancer (Vogel *et al.,* 2010). Kaposi's sarcoma is the most common cancer that occurs in 10 to 20% of people with HIV (Mandell and Dolan, 2010). The second most common cancer is lymphoma that causes death to nearly 16% of people with AIDS and also it is the initial sign of AIDS in 3 to 4% (Mandell and

Dolan, 2010).These two types of cancers are linked with human herpesvirus 8 (Mandell and Dolan, 2010). Cervical cancer occurs more commonly in those with AIDS because of its association with human papillomavirus (HPV) (Mandell and Dolan, 2010). Additionally, conjunctival cancer (of the layer that lines the inner part of eyelids and the white part of the eye) is more common in those with HIV (Mittal *et al.,* 2013).

In addition people with AIDS commonly have systemic symptoms such as extended fevers, sweats (particularly at night), swollen lymph nodes, chills, weakness, and unintentional weight loss (https://www.nlm.nih.gov/medlineplus/ency/article/000594.htm.2018). Diarrhea is also a common symptom, present in about 90% of people with AIDS (Sestak, 2005). They can in addition be affected by diverse psychiatric and neurological symptoms independent of opportunistic infections and cancers (Murray *et al.,* 2012).

For surveillance purposes two main clinical staging systems are used to classify HIV and HIV-related disease: the World Health Organization (WHO) disease staging system for HIV infection and disease (Walker, 2007) and the Centre for Disease Control (CDC) classification system for HIV infection (Schneider *et al.,* 2008). The WHO system uses the following categories: Primary HIV infection: May be either asymptomatic or associated with acute retroviral syndrome; Stage I: HIV infection is asymptomatic with a $CD4^+$ T cell count (also known as CD4 count) greater than 500 per microlitre (µl or cubic mm) of blood. May include generalized lymph node enlargement; Stage II: Mild symptoms which may include minor mucocutaneous manifestations and recurrent upper respiratory tract infections. A CD4

count of less than 500/µl; Stage III: Advanced symptoms which may include unexplained chronic diarrhea for longer than a month, severe bacterial infections including tuberculosis of the lung, and a CD4 count of less than 350/µl and stage IV or AIDS: severe symptoms include toxoplasmosis of the brain, candidiasis of the esophagus, trachea, bronchi or lungs and Kaposi's sarcoma and a CD4 count of less than 200/µl (Walker, 2007).

The Centre for Disease Control and Prevention's (CDC) system classifies HIV infections based on CD4 count and clinical symptoms and describes the infection in three stages: Stage 1: CD4 count ≥ 500 cells/µl and no AIDS defining conditions; Stage 2: CD4 count 200 to 500 cells/µl and no AIDS defining conditions; Stage 3: CD4 count ≤ 200 cells/µl or AIDS defining conditions and Unknown: if insufficient information is available to make any of the above classifications (Schneider *et al.,* 2008).

2.4 HIV diagnostic procedures

Diagnosing HIV infection during the acute phase of disease is particularly important; persons with acute HIV infection are highly infectious, because HIV concentrations are extremely high in plasma and genital secretions following initial infection (Pilcher *et al*., 2004; Wawer *et al*., 2005; Hollingsworth *et al*., 2008; Miller *et al*., 2010). Nevertheless tests for HIV antibodies are often negative during this phase of infection, causing persons to mistakenly believe they are uninfected and unknowingly continue to engage in behaviors associated with HIV transmission. Among individuals with acute HIV infection, 50%–90% are symptomatic, many of whom might seek medical care (Schacker *et al*., 1996; Weintrob *et al*., 2003).

HIV infection can be diagnosed by serologic tests that detect antibodies against HIV-1 and HIV-2 and by virologic tests that detect HIV antigens or ribonucleic acid (RNA). Testing begins with a sensitive screening test, usually an antigen/antibody combination or antibody immunoassay. Available serologic tests are both highly sensitive and specific and can detect all known subtypes of HIV-1. Most can also detect HIV-2 and uncommon variants of HIV-1, such as group O and group N (CDC, 2015). Rapid HIV tests enable clinicians to make a preliminary diagnosis of HIV infection within 30 minutes. However, most rapid antibody assays become reactive later than conventional laboratory-based antibody or combination antigen/antibody serologic assays, and thus can produce negative results in recently infected persons (CDC, 2018).

The recommended diagnostic algorithm by Centre for Disease Control and Prevention (CDC) for HIV infection consists of a laboratory-based immunoassay, which if repeatedly reactive is followed by a supplemental test that includes an HIV-1/HIV-2 antibody differentiation assay, Western blot, or indirect immunofluorescence assay. However, available HIV laboratory antigen/antibody immunoassays detect HIV infection earlier than these supplemental tests. Therefore, during very early stages of HIV infection, discordant HIV test results have been erroneously interpreted as negative (Masciotra *et al.,* 2011). This problem is minimized by use of a combination HIV-1/HIV-2 antigen-antibody (Ag/Ab) immunoassay, which if reactive is followed by an HIV-1/HIV-2 antibody differentiation assay (CDC and Association of Public Health Laboratories, 2014). This algorithm confers an additional advantage, as it can detect HIV-2 antibodies after the initial immunoassay. Although HIV-2 is uncommon, accurate identification is important because monitoring and therapy for HIV-2 differs from that for HIV-1 (CDC, 2018).

2.5. Prevention and treatment strategies for HIV

Some methods of HIV prevention include safe sex, needle exchange programmes, treating those who are infected, and male circumcision (WHO, 2015). HIV infection in a baby can often be prevented by giving both the mother and child antiretroviral medication (WHO, 2015). Currently, there is no cure or vaccine; however, antiretroviral treatment can slow the pattern of the disease and may lead to a near-normal life expectancy (UNAIDS, 2012; CDC, 2015). HIV treatment is recommended to commence as soon as the diagnosis is made (WHO, 2015), because studies have shown that without treatment, the average survival time after infection is 11 years (UNAIDS, 2018).

Antiretroviral therapy (ART) is the use of HIV medicines to treat HIV infection. There are several classes of antiretroviral agents that act on different stages of the HIV life-cycle. The use of multiple drugs that act on different viral targets is known as highly active antiretroviral therapy (HAART). Highly active antiretroviral therapy decreases the patient's total burden of HIV, maintains function of the immune system, and prevents opportunistic infections (More and Chaissom, 1999). There are five classes of drugs, which are usually used in combination, to treat HIV infection. Use of these drugs in combination can be termed anti-retroviral therapy (ART), combination anti-retroviral therapy (cART) or highly active anti-retroviral therapy (HAART). Anti-retroviral (ARV) drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits (USDHHS, 2018).

Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two currently available agents in this class. Maraviroc works by targeting CCR5, a co-receptor located on human helper T- cells (Liebeman-Blum *et al.,* 2008). To prevent fusion of the virus with the host membrane, enfuvirtide can be used (Bai *et al.,* 2013). Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription. Examples of currently used NRTIs include Zidovudine, Abacavir, Lamivudine, Emtricitabine, and Tenofovir (Das and Arnold, 2013). Non-Nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme; NNRTIs act as non-competitive inhibitors of reverse transcriptase. NNRTIs affect the handling of substrate (nucleotides) by reverse transcriptase by binding near the active site. NNRTIs can be further classified into 1st generation and 2nd generation NNRTIs. 1st generation NNRTIs include Nevirapine and Efavirenz. 2nd generation NNRTIs are Etravirine and Rilpivirine (Das and Arnold, 2013).

Protease inhibitors block the viral protease enzyme necessary to produce mature virions upon budding from the host membrane (Wensing *et al.,* 2010). Virus particles produced in the presence of protease inhibitors are defective and mostly non-infectious. Examples of HIV protease inhibitors are Lopinavir, Indinavir, Nelfinavir, Amprenavir and Ritonavir. Darunavir and Atazanavir are currently recommended as first line therapy choices (USDHHS, 2015). Resistance to some protease inhibitors is high (Wensing *et al.,* 2010). Integrase inhibitors (also known as integrase nuclear strand transfer inhibitors or INSTIs) inhibit the viral enzyme

integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. There are several integrase inhibitors; Raltegravir became the first integrase inhibitor to receive approval. Two other clinically approved integrase inhibitors are Elvitegravir and Dolutegravir (Metifiot. *et al.*, 2013).

Antiretroviral therapy (ART) combines HIV medicines from at least two different HIV drug classes, making it very effective at preventing HIV from multiplying. Having less HIV in the body protects the immune system and prevents HIV from advancing to AIDS. Although ART can't cure HIV, the HIV medicines help people with HIV live longer, healthier lives; HIV medicines also reduce the risk of HIV transmission (AIDSInfo, 2016). Typical combinations include two Nucleoside reverse transcriptase inhibitors (NRTIs) as a "backbone" along with one Non-Nucleoside reverse transcriptase inhibitors (NNRTI), Protease inhibitor (PI) or integrase nuclear strand transfer inhibitor (INSTI) as a "base" (USDHHS, 2019).

Several studies have shown that without antiretroviral therapy (ART), most HIV-infected individuals eventually develop progressive immunodeficiency marked by CD4 T cell depletion, leading to AIDS-defining illnesses and premature death. The primary goal of ART is to prevent HIV-associated morbidity and mortality, a goal that is best accomplished by using effective ART to maximally inhibit HIV replication to sustain plasma HIV-1 RNA (viral load) below limits of quantification by commercially available assays. Durable viral suppression improves immune function and overall quality of life, lowers the risk of both AIDS-defining and non-AIDS-

defining complications, and prolongs life (Clinical Guidelines Portal, 2016). In addition high plasma HIV-1 RNA is a major risk factor for HIV transmission, and effective ART can reduce viremia and transmission of HIV to sexual partners by more than 96% (Tindall and Cooper, 1991; Niu *et al.*, 1993). Studies have suggested that expanded use of ART may lower incidence and, eventually, prevalence of HIV on a community or population level (Kinloch-de Loes *et al.*, 1993). Consequently, a secondary goal of ART is to reduce the risk of HIV transmission.

There are several evidences that support a decision to begin HIV treatment at the time of diagnosis (Lundgren and Babiker, 2015). Initiation of antiretroviral therapy (ART) during acute infection may have several beneficial clinical outcomes, including improved preservation of immunologic function, reduced time to viral suppression, and reduction of the viral reservoir, that could be important for cure strategies (Pires *et al*., 2004; Streek *et al.*, 2006; Koegl *et al.*, 2009; Le *et al.*, 2013; Pilcher *et al*., 2015). Recognizing and diagnosing acute infection is important to linking patients to care early and presents an important opportunity for prevention. Factors that may contribute to the increased risk for transmission during acute infection include: hyperinfectivity associated with both noticeably increased HIV-RNA levels and increased infectiousness of the virus (Ma *et al*., 2009; Quinn *et al*., 2000) missed HIV diagnosis (Chin *et al*., 2013) because the nonspecific influenza or mononucleosis-like symptoms during acute illness are often unrecognized; a diagnosis would prompt health care providers to recommend treatment and risk-reduction counseling that could reduce both viral load levels and high-risk behavior (Colfax *et al*., 2002; Steward *et al*., 2009; Fonner *et al*., 2012).

Detection of acute HIV infection can be a very important link in the chain of prevention for many reasons. Evidence demonstrates that patients with a recent diagnosis of HIV are more likely to reduce risk behaviors if they are given counseling at the time of testing (Steward *et al*., 2009; Fonner *et al*., 2012) and are linked to primary HIV care (Metsch *et al*., 2008). Besides, for those who choose to initiate ART, their risk of transmission is reduced significantly (Cohen *et a*l., 2011).According to the guidelines for the use of antiretroviral agents in HIV-1-Infected adults and adolescents, ART is recommended for all HIV-infected individuals, regardless of CD4 T cell count, to reduce the morbidity and mortality associated with HIV infection. ART is also recommended for HIV-infected individuals to prevent HIV transmission. When initiating ART, it is important to educate patients about the benefits of ART, and to address barriers to adherence and recommend strategies to optimize adherence (Clinical Guidelines Portal, 2016).

The decision to initiate ART should always include consideration of a patient's comorbid conditions and his or her willingness and readiness to initiate therapy. Thus, on a case-by-case basis, ART may be deferred because of clinical and/or psychosocial factors; however, therapy should be initiated as soon as possible Patients should also understand that currently available ART does not cure HIV. To improve and maintain immunologic function and maintain viral suppression, ART should be continued indefinitely (Clinical Guidelines Portal, 2016).

2.6 Immunology of HIV

Once a person is infected with HIV, the virus begins to attack and destroy the CD4 cells of the immune system (AIDSInfo, 2016), although every arm of the immune response maybe affected by HIV infection. Progressive reduction in numbers of circulating CD4+ T cells occurs in almost all cases of untreated HIV infection. The number of circulating CD4+ T cells is extensively used as a gauge of global immune competence and provides a predictor of the immediate risk for opportunistic illnesses (Masur *et al.,* 1989). Many HIV-infected persons have a syndrome of generalized lymphadenopathy characterized by accumulation of lymphocytes within inflammatory lymph nodes and upregulation of adhesion molecule expression earlier in the course of infection. Early on in the course of HIV infection, there is selective depletion of memory CD4+ T cells from circulation; as disease advances, CD4+ T cells of both the naive and memory phenotype are lost from circulation (Roederer *et al.,* 1995). All CD4 T cell populations are depleted from circulation and from lymphoid tissue sites in advanced HIV disease (Stevenson, 2003).

During early HIV infection, the CD8+ T-cell numbers tend to increase, reflecting expansion of memory CD8+ T cells, mainly HIV-reactive cells. CD8+ T cell expansions continue until far advanced stages of HIV disease, when all T-cell numbers tend to fall (Margolick *et al.,* 1995). Proportions of naive CD8 cells tend to fall in early infection, in contrast to memory CD8 cell expansions, but absolute numbers of these cells do not fall until HIV disease progresses (Roederer *et al.,* 1995). For example, in earlier disease CD8+ T cells that identify

Cytomegalovirus are present in huge numbers, but in advanced disease the cytolytic function of CD8+ T cells directed against opportunistic pathogens is evidently impaired (Rook *et al.*, 1985).

HIV infects immune cells of the macrophage and T-cell lineage. In order for the virus to enter into these cells, it requires CD4 as a receptor in addition to a co-receptor which most frequently is either chemokine receptor CCR5 or CXCR4 (Gorry and Ancuta, 2011: Figure 2.4). Two HIV envelope glycoproteins gp120 and gp41 are required for binding and entry into human cells. Gp41 possesses a transmembrane domain and is associated with the viral envelope while Gp120 is present in association with Gp41 but does not insert into or contact the viral membrane (Tagliamonte *et al.*, 2011). These two viral glycoproteins are present in HIV as tetramers. Therefore, three Gp41 molecules associate within the viral membrane, while three molecules of Gp120 associate with Gp41 (Tagliamonte *et al*., 2011). To facilitate HIV-1 entry into human cells, Gp120 binds to human cellular CD4 with high affinity. Binding causes a conformational change in Gp120 that reveals a co-receptor binding site. Binding to one of the chemokine receptors is then facilitated which in turn induces a conformational change in theglycoprotein gp41 N-terminus (Tagliamonte *et al*., 2011). A fusion peptide portion of gp41inserts into the host cell membrane and lowers energy that is required for fusion of the host and viral membranes (Tagliamonte *et al*., 2011). The viral core is then translocated into the cytoplasm of the host cell.

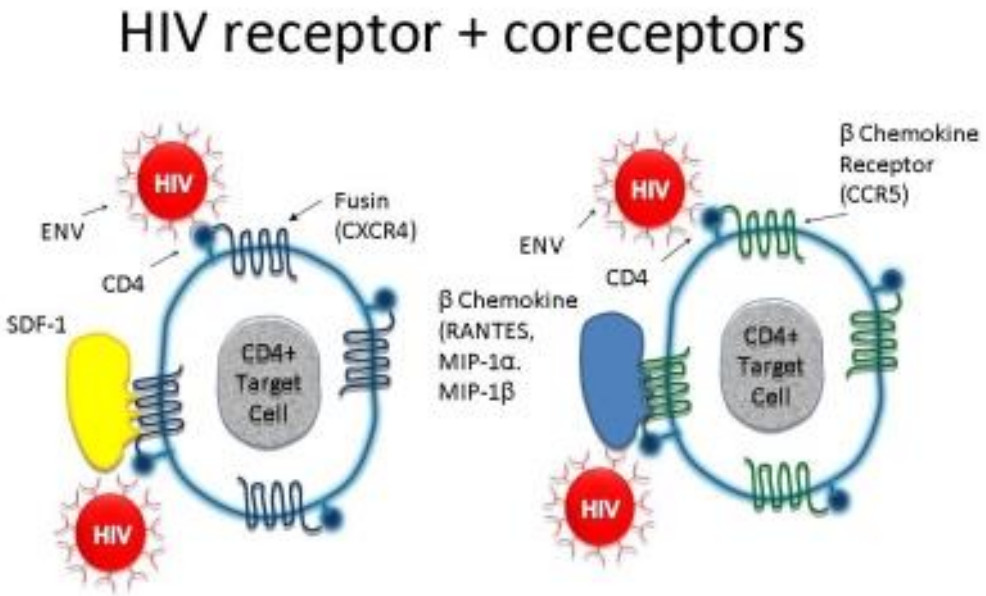


Figure 2.4: Human immunodeficiency virus entry into the body (Source: www.discovery magazine.com)

During untreated HIV infection, lymph nodes show inflammation with increased expression of cytokines such as interferon-gamma, IL-1, IL-2, and IL-12 (Emilie *et al.,* 1990; Boyle *et al.,* 1993; Galanaud, 1994; Trumpfheller *et al.,* 1998).The inflammatory state of the lymphoid tissues probably is a consequence of high-level HIV replication at these sites. These inflammatory lymph nodes are also characterized by increased expression of molecules such as intercellular adhesion molecules and vascular cell adhesion molecules (Bucy *et al.,* 1999). The ensuing condition likely results in sequestration of circulating lymphocytes in these sites. As disease

advances, there is progressive destruction of lymphoid architecture and eventually lymphoid tissues are, in the same way as the circulation, depleted of lymphocytes (Schacker *et al.,* 2002).

Under normal conditions, the immune system utilizes a Th1 subset response to viral infections. Activated antigen presenting cells (APC) secrete interleukin-12 (IL-12) which causes the cell differentiation into the Th1 subset of cells (Clerici *et al.,* 1993). These Th1 cells then secrete a characteristic Th1 profile of cytokines consisting of interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor-beta (TNF-β). IL-2 induces proliferation of naïve Th cells, amplifying the Th response. IFN-γ induces further IL-12 production in activated APCs, amplifying the Th1 response, and suppressing any Th2 response. IFN-γ also plays an important role in the activation of cytotoxic (T_C) cells which destroy virally infected cells.

The normal Th1 response to viral infection is shifted to a Th2 response in individuals infected with HIV (Klein *et al.,* 1997; Osakwe *et al.,* 2010). An increase in Th2 cytokines as well as a decrease in Th1 cytokines has been revealed in serum cytokine levels of HIV infected patients (Klein *et al.*, 1997; Osakwe *et al.,* 2010). Various assays have shown elevated serum IL-4 levels in HIV infected individuals (Clerici *et al.,* 1993): IL-4, in the presence of proliferating T cells leads to their differentiation into the Th2 subset. The Th2 cells promote B-cell proliferation, classswitching, and eosinophil activation (Clerici *et al.,* 1993). This Th2 response is not appropriate for control of intracellular pathogens such as HIV, and so allows it to persist and spread in CD4+ T-cells.

Plasma levels of TNF-alpha, IL-1, and IL-6 are said to be elevated in the course of HIV infection, and both TNF and IL-6 levels also are said to be directly correlated with plasma HIV RNA levels (Dezube *et al.,* 1997).In lymphoid tissue, the primary site of HIV replication, levels of TNF-alpha are not usually increased, even though expression of IL-1, IL-2, IL-6, IL-12, and interferon-gamma may be elevated (Andersson *et al.,* 1998). During administration of antiretroviral therapies, these indices of immune activation are likely to fall, demonstrating that HIV replication induces the state of high-level activation (Evans *et al.,* 1998; Bisset *et al.,* 1998; Burgisser *et al.,* 1999; Tilling *et al.,* 2002; Brazille *et al.,* 2003).

2.7 Hematological profiles associated with HIV infection

There are several hematologic abnormalities that are clinically significant common in patients with HIV infection and often pose a great challenge in the comprehensive management. They may cause symptoms that are life-threatening and impair the quality of life of these patients (Volberding *et al.,* 2003). Impaired hematopoiesis, immune-mediated cytopenias, and altered coagulation mechanisms have all been described in HIV-infected individuals. These abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and associated conditions (http://hivinsite.ucsf.edu/insite?page=kb-04-01-09).

Anemia has been reported as a very common finding in patients with HIV infection, particularly in individuals with more advanced HIV disease (Zon and Groopman, 1988). Some of the possible etiologies of anemia in patients with HIV infection are infections including B19

parvovirus, *Mycobacterium tuberculosis* and *Pneumocystis carinii* among others (http://hivinsite.ucsf.edu/InSite?page=kb-04-01-09). *Mycobacterium avium* complex is diagnosed in up to 18% of patients with advanced HIV infection during the course of their illness (Hawkins *et al.,* 1986).It causes high-grade bacteremia and widely disseminated infection that usually involves the bone marrow. Anemia tends to occur out of proportion to other cytopenias in such patients (Bogner *et al.,* 1990). There are other conditions associated with HIV infection which can cause anemia as a result of direct involvement of the bone marrow (Northfelt *et al.,* 1991). It has also been noted that HIV infection alone, without other complicating illness, may produce anemia in some patients (Spivak *et al.,* 1989).

Thrombocytopenia is commonly associated with HIV infection. Likely causes of thrombocytopenia in patients with HIV infection include: immune-mediated destruction, thrombotic thrombocytopenic purpura, impaired hematopoiesis, and toxic effects of medications. (Kaslow *et al.,* 1987). In many instances, however, thrombocytopenia is a relatively isolated hematologic abnormality associated with a normal or increased number of megakaryocytes in the bone marrow and elevated levels of platelet-associated immunoglobulin. Patients presenting with such manifestations have the clinical syndrome commonly referred to as immune thrombocytopenic purpura (ITP) (Murphy *et al.,* 1987). Patients with thrombocytopenia have true HIV – immune thrombocytopenic purpura (HIV-ITP) if there is no other condition or treatment that could cause thrombocytopenia, although most such patients are otherwise well. HIV-ITP is most often an early manifestation of HIV infection, occurring before the development of any CDC AIDS-defining condition (Jost *et al.,* 1988). HIV-Immune

thrombocytopenic purpura is commonly included among those conditions characterizing the middle-stage HIV disease (Abrams, 1988). HIV-infected patients are also susceptible to developing thrombocytopenia for reasons unrelated to their HIV infection, such as alcohol use, splenomegaly and liver disease, or drug effects (http://hivinsite.ucsf.edu/InSite?page=kb-04-01-09).

Granulocytopenia is a problem that is commonly encountered in patients with HIV infection. Although low granulocyte counts usually reflect the toxicity of therapies for HIV infection or associated conditions, studies of untreated patients have also shown a high incidence of granulocytopenia, particularly in patients with more profound immunodeficiency (Kaslow *et al.,* 1987).Pathogenesis of granulocytopenia in HIV infected patients is multifactorial. An autoimmune mechanism that involves antigranulocyte antibodies (Murphy *et al.,* 1987; Van der Lelie *et al,.* 1987) and impaired granulopoiesis (Stella *et al.,* 1987; Folks *et al.,* 1988) has been said to account for granulocytopenia in some patients. Any infiltrative process involving the bone marrow (infection, malignancy) may also produce granulocytopenia. Clinically, drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV infection (Jacobson *et al.,* 1997).

2.8 HIV and AIDS Vaccine

Vaccines stimulate the body's immune system to provide protection against infection or disease. Vaccines against HIV are being developed, and they are in various stages of clinical trial but at

present none have proven effective (who.int/hiv/topics/vaccines/Vaccines/en/). Vaccines are the most powerful public health tools available and an HIV vaccine would play a powerful role in ensuring the end to the AIDS epidemic (Global Advocacy for HIV Prevention, 2019). An HIV vaccine may have the purpose of protecting individuals who do not have HIV from being infected with the virus (a preventive vaccine), or treating an HIV-infected person (a therapeutic vaccine; Wikipedia.html) .A preventive HIV vaccine is given to people who do not have HIV, with the goal of preventing HIV infection in the future. The vaccine teaches the person's immune system to recognize and effectively fight HIV in case the person is ever exposed to HIV (AIDSInfo, 2019). A therapeutic HIV vaccine is given to people who already have HIV. The goal of a therapeutic HIV vaccine is to strengthen a person's immune response to the HIV that is already in the person's body. Researchers are developing and testing therapeutic HIV vaccines to slow down the progression of HIV to AIDS, and treating people with these vaccines would ideally keep HIV at undetectable levels without the need for regular antiretroviral therapy (ART). A therapeutic HIV vaccine may also make it less likely that a person could transmit HIV to others (AIDSInfo, 2019)

AIDS vaccine development is complex. First attempts to develop a vaccine against HIV in the late 1980s were based on eliciting an antibody response, which is how most vaccines are thought to work. However, because HIV mutates rapidly, and its outer spike protein conceals itself from the immune system, creating the appropriate viral antigens to use in a vaccine proved remarkably difficult, and the approach was abandoned. More recently, many scientists believe that an AIDS vaccine candidate will provide robust protection against HIV infection only if it engages both

arms of the adaptive immune system, i.e. cell-mediated and antibody-based immune responses. HIV vaccine development is complicated by the incredible variability of the virus, and in particular its envelope protein at both the individual and population level. Thus, the evolving number of virus subtypes and recombination renders vaccine development targeting the viral envelope constituents very difficult. Consequently, vaccines may have to be carefully adapted to the virus forms in circulation in the precise location where their use is intended (WHO, 2019).

Theoretically, any possible HIV vaccine must inhibit or stop the HIV virion replication cycle. (Collier *et al.,* 1998) The targets of a vaccine could be the following stages of the HIV virion cycle: free state, attachment, penetration, uncoating, replication, assembling and releasing (Wikipedia.html). It is important to conduct research to find an effective vaccine because: the availability of a safe, highly effective and accessible preventive HIV vaccine would be an important complement to other preventive interventions, that would considerably contribute to the interruption of the sequence of HIV transmission; if the HIV immunization strategies are well received, they may possibly reach populations where other interventions are not satisfactorily successful and the research on preventive HIV vaccines is providing new information on the possible use of vaccines as therapeutic interventions, to be used in association with antitretroviral therapies, which could lead to a lowering in the cost of the treatments and to an increase on their long-term efficacy (WHO, 2019). Some of the areas of interest being studied in clinical trials include: the safety of preventive vaccines - whether a preventive vaccine protects against HIV infection and whether a preventive vaccine controls HIV if a person gets HIV while

enrolled in a study. Included also are the immune responses that occur in people who receive a preventive vaccine and different ways of giving preventive vaccines (AIDSINfo, 2019).

Encouragingly, data from the first HIV vaccine trial to show a positive protective signal were released in 2009. The trial, termed RV 144 was performed in Thailand. It used a combination of two vaccines in a heterologous prime-boost paradigm, i.e. one vaccine given in four doses was then "boosted" by two doses containing both vaccines. Analysis of the trial showed that the group receiving the vaccine had an infection rate 31.2% lower than the group that received the placebo. Although this result is not enough to qualify the vaccine for licensure, RV144 has provided very useful pointers for a way forward. Several trials are planned incorporating lessons from RV 144 (WHO, 2019). In Kenya, Scientists have made great development strides in the search for an HIV cure. There are plans to start a trial of a new vaccine that stops the virus from infecting cells. This is work is being done by a group of researchers at the Kenya AIDS Vaccine Initiative, which is the site of the planned clinical trial (mobile.nation.co.ke/blogs/editorial/HIV-vaccine-trial-good-news/3112610-515906-gmaha9/index.html, 2019).

CHAPTER THREE: MATERIALS AND METHOD

3.1 Study Site

This study was done at Nakuru Provincial General Hospital (PGH), also known as Nakuru Level 5 Hospital. The hospital serves patients particularly those referred from other district hospitals and health centers in Nakuru County. Convenient sampling technique was used to identify Nakuru Provincial General Hospital as a study site because being a referral hospital made it an ideal site for this study because sampling of the study group would include individuals from a wider region. The hospital is situated in the northern part of Nakuru town and is about 2 kilometers from the town centre (Figure 3.1).

3.2 Study Population

At the beginning of the study the researcher, with the guidance and assistance from the staff of the Center for Comprehensive Care (CCC) sought consent from the study population. Those who consented were enrolled, and in accordance with the Helsinki Declaration (WHO, 2013), they were asked to complete the informed permission forms. Permission to carry out the study was sought from the hospital's administration. The study involved 80 patients; subdivided into three subgroups comprising of 40 patients that were newly diagnosed with HIV and not yet on therapy, 20 patients who were on antiretroviral therapy (HAART) and 20 HIV negative individuals. Blood samples for laboratory investigation from all the study groups were collected as they visited the VCT and labeled appropriately. Hematological profiles, CD4 counts, and cytokine assays of the study population were analyzed at the laboratories of Nakuru Provincial General Hospital.

Figure 3.1 LOCATION OF PROVINCIAL GENERAL HOSPITAL IN NAKURU TOWN (Source, Google Map)

3.3 Design of the Study

A prospective cross sectional study design was used which involved the selection of males and females who gave their consent, and who were attending the Voluntary Counseling and Testing (VCT) centre at the hospital. The individuals who attended the Centre for Comprehensive Care for the first visit were advised to have HIV test done at the VCT. Recruitment of the study group was done at the VCT and at the CCC.

3.4 Sampling and sample size determination

Simple random sampling using random numbers was done to get sample size for the study after consecutive sampling technique yielded larger sample than was required from individuals attending the Comprehensive Care Centre. The determination of a representative sample size was done based on Yamane's (1967) as follows:

$$n_o = \frac{z^2 p(1-p)\, N}{z^2 p\,(1-p) + Ne^2}$$

Where:

n_o = sample size

z = confidence interval corresponding to a level of confidence

p = population proportion

N = population size

e = precision or error limit

The Yamane formula assumes a normal distribution and the individuals seeking treatment at the CCC were assumed to be normally distributed in terms of the parameters that were needed for investigation. According to the VCT records, the monthly average attendance was about 100 individuals. A 95% confidence level was deemed acceptable and thus statistically z=2. The proportion of consenting male and female that would have been relevant to the study was p. If p = 0.5, a new formula was derived:

$$n = \frac{N}{1 + Ne^2}$$

Where:

n = sample size

N = population size

e = level of precision or sampling error (Yamane, 1967).

Using the formula at a 95% confidence level and a sampling error of ±5% resulted in:

$$n = \frac{100}{1 + 100\ (0.05)^2}$$

$$= 80$$

A sample size of 80 patients was used.

Three subgroups were sampled from the study sample size, using simple random sampling as recommended by Sudman (1976), who suggested that in each subgroup, a sample of 20 to 50

individuals can be used. The subgroups comprised 40 recently diagnosed HIV patients that had not started HAART, 20 HIV patients that were on HAART and 20 HIV negative patients; their age and gender were determined.

3.5 Ethical considerations

The individuals that accepted to be enrolled signed a consent form (Appendix 1). Those under 18 years had their parents and guardians sign on their behalf. Ethical approval for this research was obtained from the PGH Ethical and Research Board (Appendix 11).

3.6 Inclusion and exclusion criteria

Those who were recruited for the study included consenting males and females, recently diagnosed with HIV infection and had not started highly antiretroviral therapy (HAART), those that with HIV infection and were on therapy in addition to HIV negative persons. All individuals who were HIV positive and negative but had no co-morbidities and not using any medication were included in the study. Those having other co-morbidities or those who were on other medication were excluded from the study.

3.7 Collection of Blood Samples

About 10 ml of whole blood was collected from cubital vein into EDTA treated tubes and inverted carefully, 6-10 times after draw and stored at room temperature. Part of the blood was

collected for hematological analyses and part for CD4 T cell enumeration. The blood samples were them centrifuged at room temperature and the supernatant (plasma) was aspirated and aliquoted into plasma vials that were labeled with the information of individuals in the study population. The plasma was stored at -24^{0}C until when the entire cohort had been collected in order to determine cytokines of the samples simultaneously

3.8 Determination of hematological profiles

Blood parameters were determined using Quintus 5 – part hematology analyzer according to the manufacturer's protocol (Buole Meidal, AB, Sweden; Plate 3.1.). Quintus is a complete hematology system instrument which is accompanied with 3 reagents, controls, calibrator and cleaners. The system is designed to measure up to 24 parameters using whole blood that includes leukocyte, erythrocyte and thrombocyte parameters. Quintus diluents is used for both diluting the sample and focusing the stream of white blood cells in the flow cell, the lyse is used for hemolyzing red blood cells releasing hemoglobin and enabling the 5 – part white blood cells differential while the stopper is for stopping the white blood cells lysing process. The general measuring principle include: the optical laser method for 5 – part white blood cells (WBC) differential parameters, the impedance method for the total white blood cells (WBC), red blood cells (RBC), Hematocrit (HCT) plus platelets (PLT) and the spectrophotometric method for hemoglobin (HGB). The result screen displays all 24 parameters' scatter and histograms.

Plate 3.1: Quintus 5 – part hematology analyzer (Buole Meidal, AB, Sweden).

About 100 µl of whole blood was used for hematological analysis. Each blood sample was fed into a single tube inlet of the auto blood analyzer that automatically detected and analyzed the sample. The result screen displayed 24 analyzed blood parameters that were acquired through a computer printout.

3.9 Enumeration of CD4 T cells

CD4T cell counts were carried out using Beckton Dickson (BD) Fluorescence Activated Cell Sorter Count (FACSCount) system (BD Biosciences, USA; Plate 3.2) according to the protocol by the manufacturer (David *et al.,* 2004). Beckton Dickson FACSCount is a complete system incorporating instrument, reagents, controls and software. It utilizes a direct two-colour immunoflourence method for enumerating absolute counts of CD3 lymphocytes, CD4 lymphocytes and CD8 lymphocytes. In addition the system generated a ratio of CD4 and CD8. The BD FACSCount reagent kit consisted of paired reagent sets containing a mixture of monoclonal antibody reagents conjugated to two fluorochromes and a known number of fluorochrome – intergrated polystyrene beads. The first tube in each pair contained CD4 and CD3 antibodies while the second contained CD8 and CD3. The kit also contained formaldehyde fixative.

All the 80 labeled blood samples were thawed before the flow cytometry for the enumeration of CD4 cells and refrigerated after. Enumeration of CD4 cells was performed as follows: TruCount tubes (BD TruCOUNT tubes) were labeled with identification number. 20uL of CD3/CD8/CD45/CD4 monoclonal antibody reagent (BD MultiTEST), for determining percentages and absolute counts of human helper/inducer and suppressor cytotoxic T lymphocytes in erythrocyte-lysed whole blood was put into the bottom of each appropriately labeled tube. 50uL of anticoagulated whole blood was put into the bottom of the tubes. The tubes were vortex gently to mix, and then incubated for 15 minutes in the dark at room temperature (20-25C). 450uL of FACS lysing solution was added to the tubes, vortexed gently

to mix and incubated for 15 minutes in the dark at room temperature (20-25C). The samples were then processed and analyzed in the FACSCalibur flow cytometer using BD Cell Quest software. The results were reported as the percentage of positive cells per lymphocyte population and as the number of positive cells per microliter of blood (absolute count). The results were acquired through a computer printout.

3.10 Determination of Circulatory Cytokines

All the 80 labeled blood samples of blood plasma were thawed before flow cytometry for cytokine detection. The types and quantities of cytokines were detected by flow cytometry using a multiplex assay system that included Becton and Dickinson Cytometric Bead Array (BD CBA) Human Inflammatory Cytokine kit and Becton and Dickinson Fluorescence Activated Cell Sorter (FACSCalibur) flow cytometer ((FACSCount system; Plate3.2, BD Biosciences, U.S.A). The workflow consisted of the following steps according to the recommended procedure BD Biosciences):

3.10.1 Preparation of Human Inflammatory Cytokines Standards

A vial of lyophilized Human Inflammatory Cytokine Standard was opened and the standard spheres transferred to a 1 ml polypropylene tube. The tube was labeled “Top Standard” (Tube 10). The standards were reconstituted with 2 ml of assay diluent and allowed to equilibrate for 15 minutes at room temperature. The reconstituted protein was gently mixed by pipette.

Plate 3.2: FACSCount system (BD Biosciences, USA).

Eight 12x75 mm tubes were labeled and arranged in the following order: 1:2 (Tube 9; 2,500 pg/mL), 1:4 (Tube 8; 1,250 pg/mL), 1:8 (Tube 7; 625 pg/mL), 1:16 (Tube 6; 312.5 pg/mL), 1:32 (Tube 5; 156 pg/mL), 1:64 (Tube 4;80 pg/mL), 1:128 (Tube 3; 40 pg/mL) and 1:256 (Tube 2; 20 pg/mL). 300 µl of assay diluent was pipetted in each of the tubes. Serial dilution was performed by transferring 300µl from the Top Standard to the 1:2 dilution tube and mixed thoroughly by pipette, and continued by transferring 300µl from 1:2 tube to the 1:4 tube until to the 1:256 tube.

One tube was prepared containing only assay diluent to serve as the 0 pg/mL negative control. This was labeled as Tube 1 and had no standard dilution. The standard curve for each protein covers a defined set of concentrations from 20 to 5,000 pg/mL (BD Biosciences).

3.10.2 Mixing Human Inflammatory Cytokine Capture Beads

Six Capture Beads that had been bottled individually were pooled before using them in the assay. Each capture bead suspension was vortexed before mixing. 10 µl aliquot of each capture bead, for each assay tube to be analyzed, was added into a single tube labeled "Mixed Capture Beads". Eighty assay tubes were to be analyzed and so the mixture comprised of the following capture beads: 800 µl (10 x 80) of interleukin 8 (IL-8), 800 µl of interleukin 1β (IL-1β), 800 µl of interleukin 6 (IL-6), 800 µl of interleukin 10 (IL-10), 800 µl of tumor necrosis factor (TNF) and 800 µl of interleukin 12p-70 (IL-12p70). The mixture of capture beads was vortexed thoroughly and was ready for transfer to the assay tubes.

3.10.3 Performance of the Human Inflammatory Cytokine Assay

After preparing the standards and mixing the capture beads, the next step was to perform the assay. The mixed capture beads were vortexed and 50 µl added to all assay tubes. 50 µl of the sample was added to eighty labeled tubes each containing about 12.5 µl of plasma that included forty from HIV positive patients, twenty from HIV negative and twenty from HIV positive patients on HAART. The assay tubes were incubated for 1.5 hours at room temperature. After the incubation period, 1 ml of wash buffer was added to each assay tube and centrifuge at 200g

for 5 minutes; the supernatant was aspirated and discarded. 50 µl of the human inflammatory cytokine phycoerythrin (PE) detection reagent was added to all assay tubes and incubated for 1.5 hours at room temperature. After this second incubation period, 1 ml of wash buffer was added to each assay tube and centrifuged at 200g for 5 minutes; the supernatant was aspirated and discarded from the assay tubes and finally 300 µl of wash buffer was added to each assay tube to re-suspend the bead pellet.

3.10.4 Sample Acquisition and analysis of data

The samples were acquired on the flow cytometer. The assay setup procedure was followed according to the protocol by the manufacturers (BD Biosciences) and the appropriate acquisition template was available. Each sample was vortexed for 3-5 seconds immediately before acquiring on the flow cytometer. Tube 1 (0 standard) was vortexed and ran in setup mode according to the protocol. Sample acquisition continued by running Tube 2 (20 pg/mL), followed by Tube 3 (40 pg/mL), and so on throughout Tube 10 (Top Standard). The test samples were run after the standards. Data for the detection of individual proteins was analyzed using BD CellQuest software, according to the recommended protocol (BD Biosciences).

3.11 Data analyses

The data obtained from the computer printouts were entered in a Microsoft Office 2007 Excel worksheet and SPSS version 17 was used for the statistical analysis. Descriptive statistics were applied for all the parameters that were measured and included the means, standard deviations

and variances of individual parameter in each of the sub group that formed the study population. Q-Q plot was used for testing normality. The data was not normally distributed and hence was Log transformed. Analyses of variance (ANOVA) were used to determine the differences in mean circulatory cytokines, mean hematological parameters and mean CD4 T cell counts of the study population while Tukey's post-hoc tests were used to compare means of the parameters between the subgroups. Associations between cytokines and hematological parameters; between cytokines and CD4 +T cells count and between CD4+ T cell counts and hematological profiles were determined using Chi-square. A p value <0.05 was considered statistically significant.

CHAPTER FOUR: RESULTS

4.1 Study Population Characteristics

A total of 80 individuals participated in the study: 40 individuals were sampled from those that were diagnosed with the human immunodeficiency virus (HIV) and had not started therapy, (treatment naïve HIV patients), 20 were sampled from HIV patients that started therapy using highly antiretroviral therapy (HAART) and 20 were sampled from HIV negative individuals. The mean age was 33.65 years (SD±12.59) for treatment naïve HIV patients; 30.55 years (SD± 12.94) for HIV positive patients on HAART and 26.60 (SD ±6.95) years for HIV negative patients. There were 16 males and 24 females of the treatment naïve HIV patients; 4 males and 16 females of the HIV positive patients on HAART and 9 males and 11 females of the HIV negative patients. The females were more than the males in any of the three groups (Table 1).

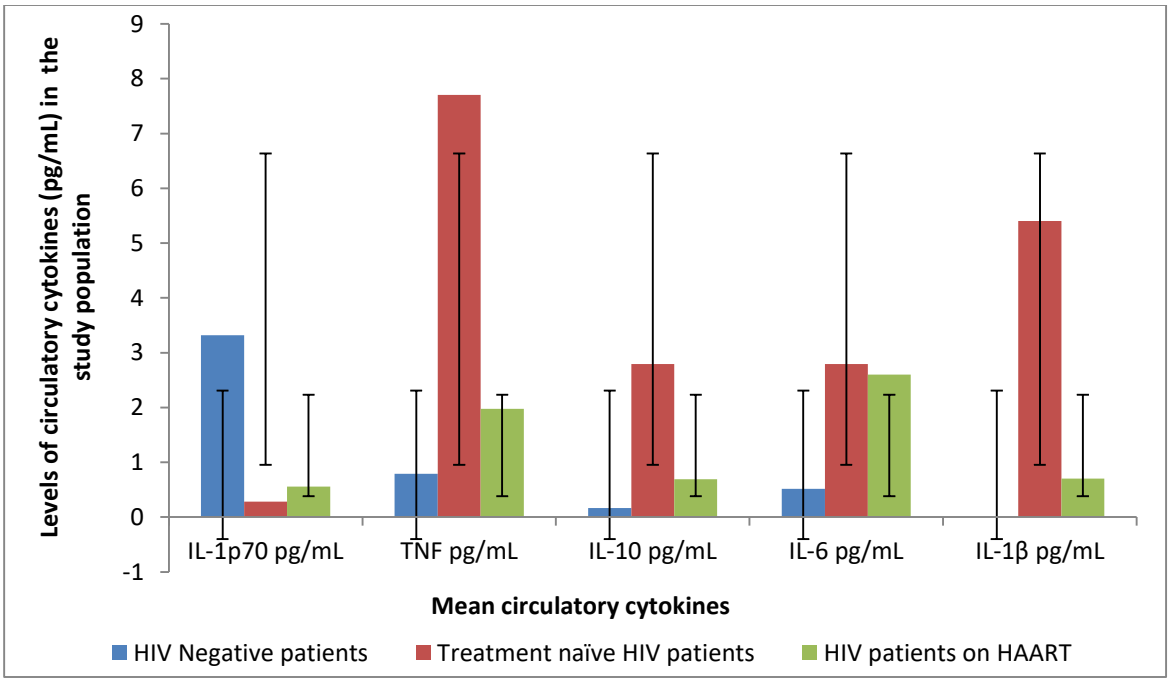
Analysis of variance (ANOVA) for comparison of mean age indicated that there was no significant age difference between the groups ($F = 2.51$; $p = 0.088$) and the means of age in the three groups in the study population was not significantly different.

Table 4.1: The study population - characteristics by HIV status, age and gender

Sub groups	HIV Negative patients (N=20)	Treatment naïve HIV positive patient (N=40)	HIV positive patients on HAART (N=20)
Mean Age	$\bar{x}$ =26.60 SD ± 6.95 Minimum = 19 years Maximum = 50 years	$\bar{x}$ =33.65 SD ± 12.59 Minimum =7 years Maximum=72 years	$\bar{x}$ =30.55 SD ± 12.9 Minimum = 2 years Maximum = 53 years
Gender	Male=9 (45.0%) Female=11 (55.0%)	Male = 16 (40.0%) Female =24 (60.0%)	Male =4 (20.0%) Female =16 (80.0%)

4.2 Circulatory Cytokine Profiles of the study population

The detectable cytokines in the study population were interleukin 1β pg/mL(IL1β), interleukin 6 pg/mL (IL-6), interleukin 10 pg/mL (IL-10), interleukin 12p70 pg/mL (IL-12p70) and tumor necrosis factor pg/mL(TNF). IL- IL-6 pg/mL, IL-10 pg/mL, 12p70 pg/mL and TNF pg/mL were detectable in HIV negative patients; in HIV positive patients on HAART and also in treatment naïve HIV patients. IL-1β was not detected in HIV negative patients; however, it was detectable in HIV positive patients on HAART and in treatment naïve HIV patients (Figure 4.1).



Key: IL-12p70 pg/mL= Interleukin 12p70 pg/mL; TNF pg/mL = Tumor necrosis factor pg/mL; IL-10 pg/mL = Interleukin 10 pg/mL; IL-6 pg/mL= Interleukin 6 pg/mL; IL-1β pg/mL= Interleukin 1 *beta* pg/mL.

Figure 4.1: Mean circulatory cytokines (± 1SD): IL-12p70, TNF, IL-10, IL-6 and IL-1β in HIV negative patients (N=20), treatment naïve HIV patients (N=40) and the HIV patients on HAART (N=20). Number over bars indicates sample sizes.

Analysis of variance (ANOVA) showed that there were significant differences in the mean IL-12p70 (F = 10.376; p=0.000), TNF (F = 4.883; p=0.010), IL-10 (F = 6.515; p=0.002), IL-6 (F = 7.231; p=0.001) and a significant difference in the mean IL-1β (F = 5.253; p=0.007) between HIV negative patients, HIV patients on HAART and treatment naïve HIV patients (Table 4.2).

Table 4.2: Analysis of variance for the mean circulatory cytokines in HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Asterisks mean significantly different.

	HIV negative patients (n=20)	Treatment naïve HIV patients (n=40)	HIV patients on HAART (n=20)		
Circulatory cytokines (pg/ml)	**Mean**	**Mean**	**Mean**	**ANOVA (F)**	**Difference (p value)**
IL-12p70	3.317 ±4.44	0.2815 ±0.514	0.5593±1.396	10.376	0.000*
TNF	0.7895 ±2.247	7.707 ±13.399	1.9750±4.980	4.883	0.010*
IL-10	0.1630 ±0.503	2.794 ±4.437	0.6918±1.628;	6.515	0.002*
IL-6	0.5135±1.206	2.794± 4.437	2.6020 ±4.620	7.231	0.001*
IL-1β	0.0000	5.401 ±10.136	0.7028 ±4.445	5.253	0.007*

Key: IL-12p70 = Interleukin 12p70 pg/mL; TNF = Tumor necrosis factor pg/mL; IL-10 = Interleukin 10 pg/mL; IL-6= Interleukin 6 pg/mL IL-1β= Interleukin 1 *beta* pg/mL.

*= Significant at $p<0.05$.

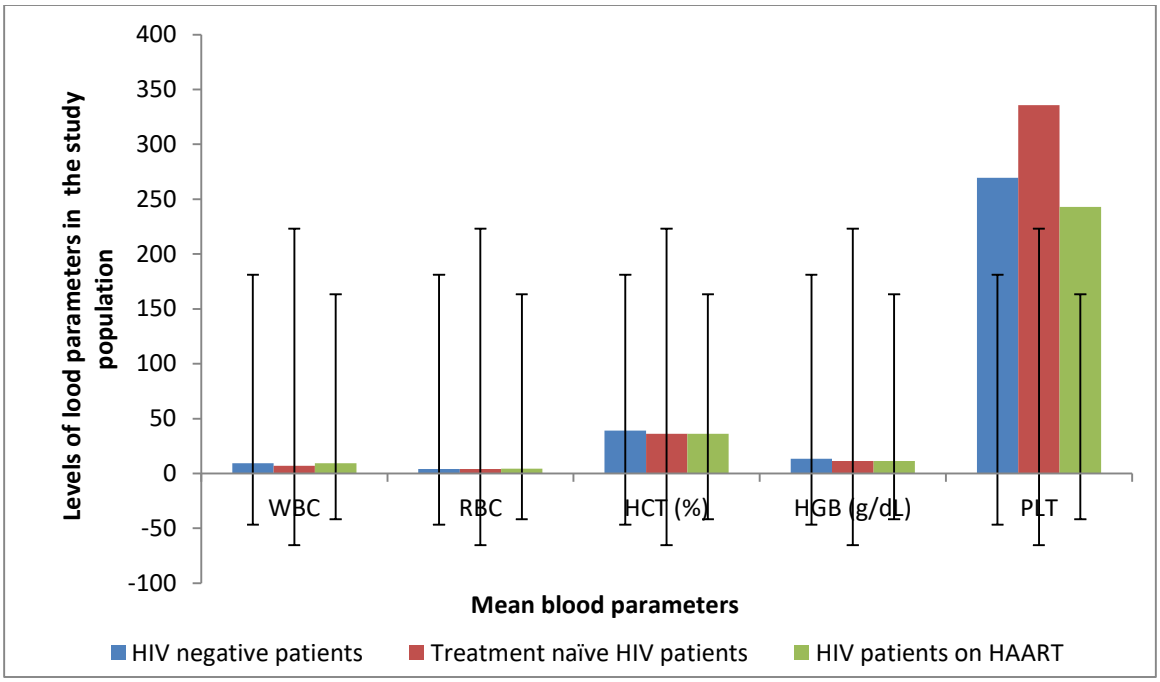
Tukey's post Hoc test showed that the mean Interleukin - 12p70 pg/mL (IL-12P70; $\bar{x}$ = 3.317, SD = 4.441) in the HIV negative patients was significantly higher (p = 0.000) when compared to the mean IL-12p70 pg/mL levels of treatment naïve HIV patient ($\bar{x}$ = 0.2815, SD 0.5145). It also differed significantly (p=0.001) from the mean IL-12p70 pg/mL of HIV patients on HAART ($\bar{x}$ = 0.5593, SD = 1.397). Among the treatment naïve HIV patients, the mean tumor necrosis factor pg/mL (TNF; $\bar{x}$ =7.707, SD = 13.40) was observed as being significantly higher (p=0.015) when compared to that of HIV negative patients ($\bar{x}$ = 0.7895, SD = 2.247) and also differed significantly (p = 0.025) from that of HIV patients on HAART ($\bar{x}$ = 1.9750, SD = 4.980). The mean interleukin - 6 pg/mL (IL-6) in treatment naïve HIV patients ($\bar{x}$ = 2.794, SD =

4.437) was seen as being significantly higher (p=0.001) when compared to that of HIV negative patients ($\bar{x}$ = 0.5135, SD 1.206) and also differed significantly (p = 0.013) from the mean IL-6 levels of HIV patients on HAART ($\bar{x}$ = 2.6020, SD 4.620).

The mean interleukin 1-β pg/mL (IL-1β) of treatment naïve HIV patients ($\bar{x}$ = 5.401, SD = 10.14) was significantly higher (p = 0.000) than the mean IL-1β of HIV negative patients ($\bar{x}$ = 0.0000) and also differed significantly (p = 0.014) from that of HIV patients on HAART ($\bar{x}$ = 0.7028, SD = 4.445). In HIV negative patients, there was no detectable IL-1β (Table 4.2). Among the treatment naïve HIV patients, the mean interleukin – 10 (IL-10; ($\bar{x}$ = 2.794, SD = 4.437) was observed as being significantly higher (p=0.004) when compared to the mean IL-10 of HIV negative patients ($\bar{x}$ = 0.1630, SD = 0.5032) and differed significantly (p = 0.008) from the mean IL-10 levels of HIV patients on HAART ($\bar{x}$ = 0.6918, SD = 1.628).

4.3 Blood parameters of the study population

The mean White Blood Cell counts (WBC 10^9/L), Red Blood Cell counts (RBC10^{12}/L), Hematocrit (HCT %), Hemoglobin (HGB g/dL) and platelets (PLT10 ^{9}/L) in treatment naïve HIV patients, HIV patients on HAART and in HIV negative patients were determined (Figure 4.2)



Key: RBC = Red Blood Cells (10^{12}/L); WBC = White Blood Cells (10^{9}/L; HCT = hematocrit (%); HGB = hemoglobin (g/dL); PLT = Platelets (10^{9}/L). (Normal blood parameters values according to the Nakuru Provincial General Hospital Laboratory: RBC (3.50-5.50 x 10^{12}/L); WBC (4.0-10.0 x 10^{9}/L); HCT (37.050.0%); HGB (11.0-16.0 g/dL); PLT (100-300 x 10^{9}/L)).

Figure 4.2: Mean blood parameters (±SD): WBC (10^{9}/L)**, RBC** (10^{12}/L); **HCT**(%)**;, HGB**(g/dL) **and PLT**(10^{9}/L). **of HIV Negative n=20 patients, treatment naïve HIV patients n=40 and HIV patients on HAART, n= 20**

Analysis of variance showed no significant differences between the mean white blood cell count (WBC) (F = 2.615; p=0.080) and between the mean red blood cell count (RBC) (F = 1.153; p=0.321) in HIV negative patients, HIV patients on HAART and in treatment naïve HIV patients. There were significant differences between the mean hematocrit (HCT) (F = 3.998; p=0.022); hemoglobin (HGB) (F = 4.086; p=0.021) and platelets (PLT) (F = 3.555; p=0.033) between HIV negative patients, HIV patients on HAART and in treatment naïve HIV patients (Table 4.3).

Table 4.3: Analysis of variance for the means of blood parameters in HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Asterisks mean significantly different.

	HIV Negative patients (n=20)	Treatment naïve HIV patients (n=40)	HIV patients on HAART (n=20)		
Blood parameters	**Mean**	**Mean**	**Mean**	**ANOVA (F)**	**Difference (p value)**
WBC (10^9/L	9.41 ± 3.75	6.91± 1.89	9.30± 4.98	2.615	0.080
RBC (10^{12}/L)	4.15 ± 0.83	4.11± 0.74	4.45± 0.79	1.153	0.321
HCT (%)	39.08 ± 4.14	36.16± 4.41	36.13± 2.84	3.998	0.022*
HGB (g/dl)	13.37 ± 2.57	11.43± 2.74	11.49± 2.36	4.086	0.021*
PLT (10^9/L)	269 ± 63.00	335.70± 175.90	242.85± 93.24	3.555	0.033*

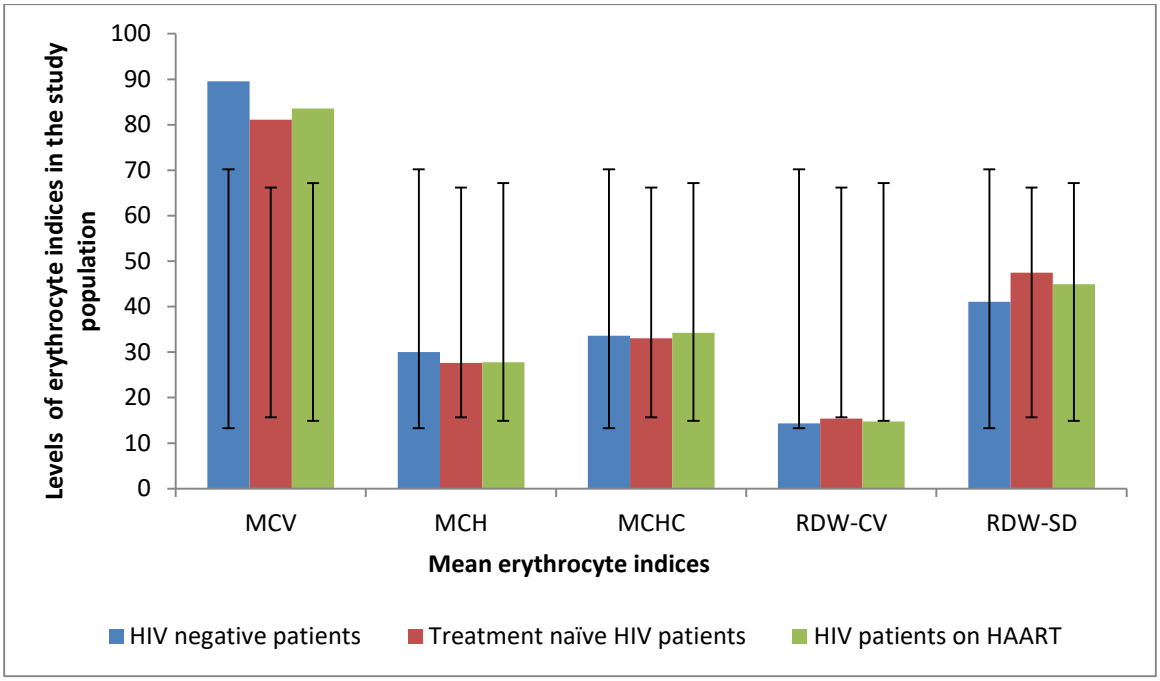
Key: RBC = red blood cells; WBC = white blood cells; HCT = hematocrit; HGB =hemoglobin; PLT = platelets.

*= Significant at $p<0.05$

Tukey's post Hoc test showed that the mean red blood cell counts (RBC) and of the white blood cell counts (WBC) between the subgroups were not significantly different. However, the mean hemoglobin (HGB) in treatment naïve HIV patients ($\bar{x}$ = 11.43, SD= 2.74) was found to be significantly lower (p=0.022) compared to that of HIV negative patients ($\bar{x}$ = 13.37, SD = 2.57) but not significant (p=0.996) when compared with that of HIV patients on HAART ($\bar{x}$ =11.49, SD = 2.36). The mean hematocrit (HCT, ($\bar{x}$ = 39.08, SD = 4.14) in HIV negative patients was found to be significantly higher (p = 0.026) compared to that of treatment naïve HIV patients ($\bar{x}$ =36.16, SD = 4.41) and of HIV patients on HAART ($\bar{x}$ = 36.13, SD = 2.84). The mean platelets (PLT, ($\bar{x}$ = 335.70, SD = 175.90) in treatment naïve HIV patients was significantly higher (p = 0.041) than that of HIV negative patients ($\bar{x}$ = 269.45, SD = 63.00) and of HIV patients on HAART ($\bar{x}$ = 242.85, SD = 93.24).

Erythrocyte indices

Figure 4.3 shows the mean volume of erythrocytes (MCV), the mean content of hemoglobin (MCH), the mean concentration of hemoglobin (MCHC)**,** the relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and the relative distribution width of red blood cells by volume, standard deviation (RDW-SD) in HIV negative patients, HIV patients on HAART and in treatment naïve HIV patients.



Key: MCV = mean volume of erythrocytes; MCH = mean content of hemoglobin; MCHC = mean concentration of hemoglobin; RDW-CV = relative distribution width of red blood cells by volume, coefficient of variation; RDW-SD = relative distribution width of red blood cells by volume, standard deviation. (Normal erythrocyte indices values according to the Nakuru Provincial General Hospital Laboratory: MCV (82-95fL); MCH (27-31pg); MCHC (32-36g/dL); RDW-CV (11.5-14.5%); RDW-SD(35-56); Lymph# (0.8-4.0); Mid# (0.1-1.2); Gran# (2-7); Lymph% (20-40); Mid% (3-14); Gran% (50-70)).

Figure 4.3: Mean erythrocyte indices (± SD): MCV, MCH, MCHC, RDW-CV and RDW-SD of HIV negative n=20 patients, treatment naïve HIV n=40 patients and HIV n=20 patients on HAART

Analysis of variance showed significant differences between the mean volume of erythrocytes (MCV; F = 4.108, p = 0.020; the mean content of hemoglobin (MCH; F = 3.359, p = 0.040) and the mean concentration of hemoglobin (MCHC; F = 4.988, p = 0.009) among the HIV negative patients, treatment naïve HIV patients and the HIV patients on HAART. There was no significant difference between the mean the relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and the relative distribution width of red blood cells by volume, standard deviation (RDW-SD) among the HIV negative patients, HIV patients on HAART and the treatment naïve HIV patients (Table 4.4).

Tukey's post Hoc tests showed that the mean volume of erythrocytes (MCV) ($\bar{x}$ = 89.53, SD = 5.68) of HIV negative patients was significantly higher (p = 0.020) when compared to that of treatment naïve HIV patients ($\bar{x}$ = 81.13, SD = 5.51) but not significantly different (p=0.068) when compared with the mean of HIV patients on HAART ($\bar{x}$ = 83.55, SD = 12.43). The mean content of hemoglobin (MCH) ($\bar{x}$ = 30.03, SD = 1.81) of HIV negative patients was significantly higher (p=0.041) compared to the mean of treatment naïve HIV patients ($\bar{x}$ = 27.74, SD = 2.09) but was not significantly different (p=0.106) when compared to the mean of HIV patients on HAART ($\bar{x}$ = 27.64, SD = 4.56).

The mean concentration of hemoglobin (MCHC) ($\bar{x}$ = 33.02, SD = 1.44) of treatment naïve HIV patients was significantly lower (p=0.007) than the mean of HIV patients on HAART ($\bar{x}$ = 34.27, SD = 1.55); there was no significant difference (p=0.302) between the mean MCHC of HIV negative patients ($\bar{x}$ =33.62, SD = 1.38) and of the mean of treatment naïve HIV patients.

Table 4.4: Analysis of variance for the means of blood indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Asterisks mean significant differences.

	HIV Negative patients (n=20)	**Treatment Naïve HIV Patients (n=40)**	**HIV Patients on HAART (n=20)**		
Blood indices	**Means**	**Means**	**Means**	**ANOVA (F)**	**Difference (p value**
Erythrocyte indices					
MCV	89.53±5.51	81.13±5.51	83.55±12.43	4.108	0.020*
MCH	30.03±1.81	27.64±4.56	27.74±2.09	3.359	0.040*
MCHC	33.62±1.55	33.02±1.44	34.27±1.55	4.988	0.009*
RDW-CV	14.32±1.96	15.36± 2.85	14.78±3.29	0.992	0.376
RDW-SD	41.08±4.37	47.49±14.65	44.95±7.54	2.423	0.095
Platelet indices					
MPV(fL)	9.03± 0.91	10.16±1.05	9.55±0.90	9.600	0.000*
PDW	14.62± 0.45	15.03±0.44	14.77±0.33	30.793	0.000*
PCT (%)	0.24±0.09	0.25±0.05	0.30±0.13	1.536	0.222
Leukocyte indices					
Lymph# (10^9/L	2.69±1.71	2.42±1.89	2.43±1.00	0.198	0.821
Mid# (10^9/L)	0.48±.19	0.69± 0.29	0.68±0.45	3.116	0.048*
Gran# (10^{10}/L)	6.19±4.73	3.87±1.63	6.05±2.41	2.908	0.061
Lymph%	35.76±13.99	28.91±9.73	29.67±16.21	1.492	0.231
Mid%	7.18±1.65	9.06±1.67	8.24± 2.03	3.641	0.031*
Gran%	55.72±13.09	64.04±10.11	62.09±17.49	1.776	0.176

Key: MCV = mean volume of erythrocytes; MCH = mean content of hemoglobin; MCHC = mean concentration of hemoglobin; RDW-CV = relative distribution width of red blood cells by volume, coefficient of variation; RDW-SD = relative distribution width of red blood cells by volume, standard deviation; MPV(f/L) = mean platelet volume; PDW = the relative width of the distribution of platelets; PCT (%) = platelet crit; LYMP# = the absolute content of lymphocytes; MID# = absolute content of the mixture of monocytes, basophils and eosinophils;

GRAN# = the absolute content of granulocytes; LYMPH% = the relative (%) content of lymphocytes; MID% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; GRAN% = the relative (%) content of granulocytes.

* = Significant at $p < 0.05$

There was no significant difference between the means of the relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and of the relative distribution width of red blood cells by volume, standard deviation of the study subgroups (RDW-SD), (Table 4.4).

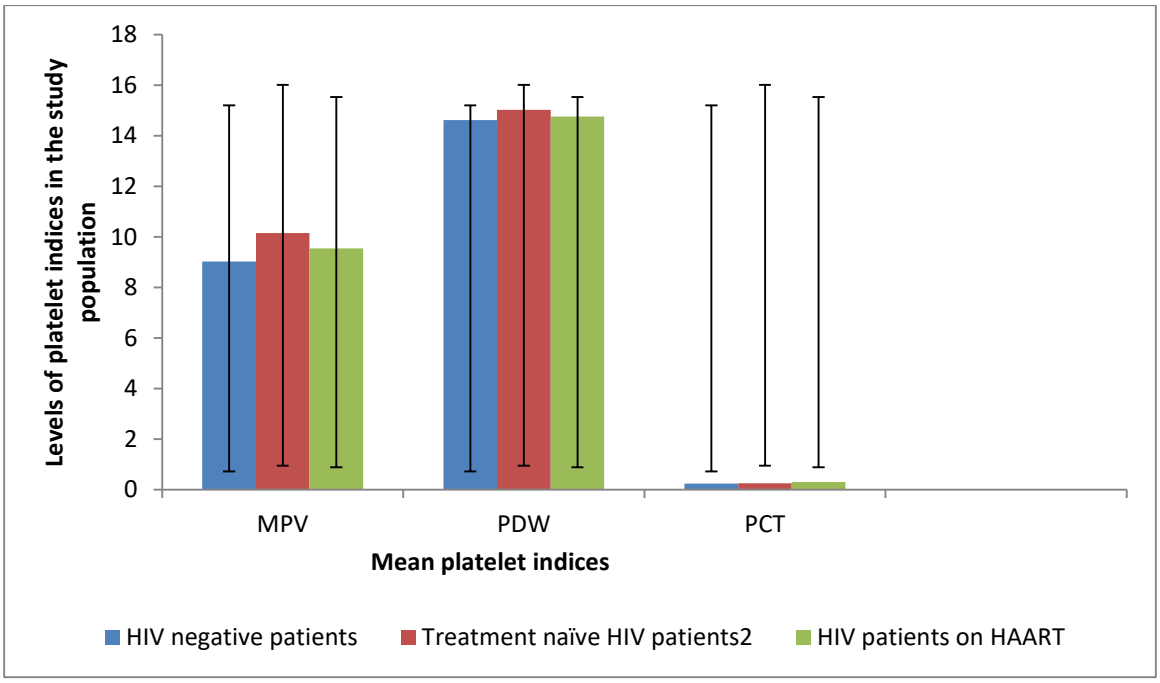
Platelet indices

Mean platelet volume (MPV), the relative width of the distribution of platelets (PDW) and platelet crit (PCT%) in HIV negative patients, in HIV patients on HAART and in treatment naïve HIV patients were analyzed (Figure 4.4).

Analysis of variance showed significant differences between the means of the mean platelet volume (MPV; $F = 9.600$, $p<0.001$) and the mean of the relative width of the distribution of platelets (PDW; $F = 30.793$, $p < 0.001$) of study population groups. The difference between the means of platelet crit (PCT) was not significant (Table 4.4).

Tukey's post Hoc tests showed that the treatment naïve HIV patients had significantly higher ($p=0.000$) means of mean platelet volume (MPV) ($\bar{x} = 10.16$, SD = 1.05) compared to those of HIV negative patients ($\bar{x} = 9.03$, SD = 0.91).There was no significant difference ($p=0.123$)

between the mean MPV of treatment naïve HIV patients compared to that of HIV patients on HAART ($\bar{x}$ = 9.55, SD = 0.90).



Key: MPV = mean platelet volume; PDW = relative width of the distribution of platelets; PCT = platelet crit. (Normal platelet indices values according to the Nakuru Provincial General Hospital Laboratory: MPV (7-11fL); PDW (15-17); PCT (0.108-0.282%).

Figure 4.4: Mean platelet indices (± SD): MPV, PDW and PCT of HIV negative n=20 patient, treatment naïve n=40 patients and HIV n=20 patients on HAART.

Tukey's post Hoc tests showed that the treatment naïve HIV patients had significantly higher (p=0.000) means of mean platelet volume (MPV) ($\bar{x}$ = 10.16, SD = 1.05) compared to those of HIV negative patients ($\bar{x}$ = 9.03, SD = 0.91).There was no significant difference (p=0.123) between the mean MPV of treatment naïve HIV patients compared to that of HIV The mean of the relative width of the distribution of platelets (PDW) ($\bar{x}$ = 15.03, SD = 0.44) of treatment

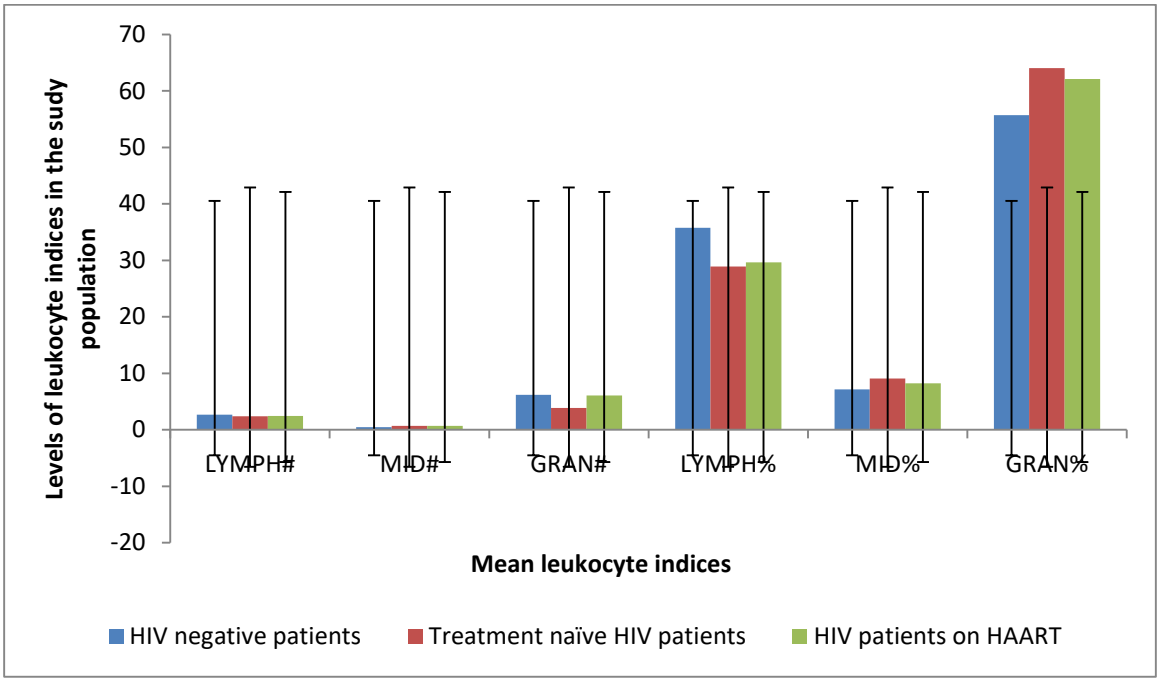
naïve HIV patients was significantly higher (p=0.000) compared to the mean of HIV negative patients ($\bar{x}$ = 14.62, SD = 0.45) and also differed significantly (p=0.000) compared to the mean PDW ($\bar{x}$ = 14.77, SD = 0.33) of HIV patients on HAART. There was no significant difference between the means of platelet crit (PCT) among the groups.

Leukocyte indices

Figure 4.5 shows the mean the absolute content of lymphocytes (Lymph#), the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#), the absolute content of granulocytes (Gran#), the relative (%) content of lymphocytes (Lymph %), the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%) and the relative (%) content of granulocytes (Gran%) in HIV negative patients, in HIV patients on HAART and in treatment naïve HIV patients.

There were significant differences between the mean absolute content of the mixture of monocytes, basophils and eosinophils (Mid#; F = 3.16, p = 0.050) and of the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%; F = 3.641, p = 0.031) while no significant differences were seen between the mean absolute content of lymphocytes (Lymph#), the absolute content of granulocytes (Gran#), the relative (%) content of lymphocytes (Lymph%) and the relative (%) content of granulocytes (Gran%) of the groups).The mean absolute content of the mixture of monocytes, basophils and eosinophils (Mid#, $\bar{x}$ = 0.48, SD = 0.19) of the HIV negative patients was significantly lower (p = 0.048) than the means of treatment naïve and HIV

patients. There were no significant differences in the means of the other leukocyte indices (Table 4.4)



Key: LYMP# = the absolute content of lymphocytes; MID# = absolute content of the mixture of monocytes, basophils and eosinophils; GRAN# = the absolute content of granulocytes; LYMPH% = the relative (%) content of lymphocytes; MID% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; GRAN% = the relative (%) content of granulocytes. (Normal leukocyte indices values according to the Nakuru Provincial General Hospital Laboratory: Lymph# (0.8-4.0); Mid# (0.1-1.2); Gran# (2-7); Lymph% (20-40); Mid% (3-14); Gran% (50-70)).

Figure 4.5: Mean leukocyte indices (± SD): Lymph#, Mid#, Gran#, Lymph%, Mid% and Gran% of HIV negative n=20 patients, treatment naïve n=40 patients and HIV n=20 patients on HAART.

After Tukey's post hoc tests, there were no significant differences between the mean absolute content of lymphocytes (Lymph#), of the absolute content of granulocytes (Gran#), of the relative (%) content of lymphocytes (Lymph%), of the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%) and the relative (%) content of granulocytes (Gran%) between treatment naïve HIV patients and HIV negatives, between treatment naïve HIV

patients and HIV patients or between HIV negative patients and HIV patients on HAART. However, there was a significant difference (p=0.048) between the mean absolute content of the mixture of monocytes, basophils and eosinophils (Mid#) of treatment naïve HIV patients and HIV negative patients.

4.4. CD4 T cell counts of the study population

Mean CD4 T cell counts of the study population were analyzed. The mean CD 4 T cell counts in treatment naïve HIV patients was 364.03 ±SD 284.064, the mean CD4 T cell counts of HIV patients on HAART was 485.85 ±SD 232.278 while the mean CD4 T cell counts of HIV negative patients was 1063.45 ±SD 971.705 (Figure 4.6)

There were statistically significant differences between the subgroup mean CD4 T cell counts as determined by analysis of variance ($F = 11.672$, $p=0.000$). Tukey's post hoc tests showed that the mean CD4 T cell counts of HIV negative patients was significantly higher (p=0.000) when compared to that of treatment naïve HIV patients and also significantly higher (p=0.003) compared to the mean CD4 T cell counts of HIV patients on HAART. There was no significant difference (p=0.688) in the mean CD4 T cell counts of treatment naïve HIV patients and of HIV patients on HAART.

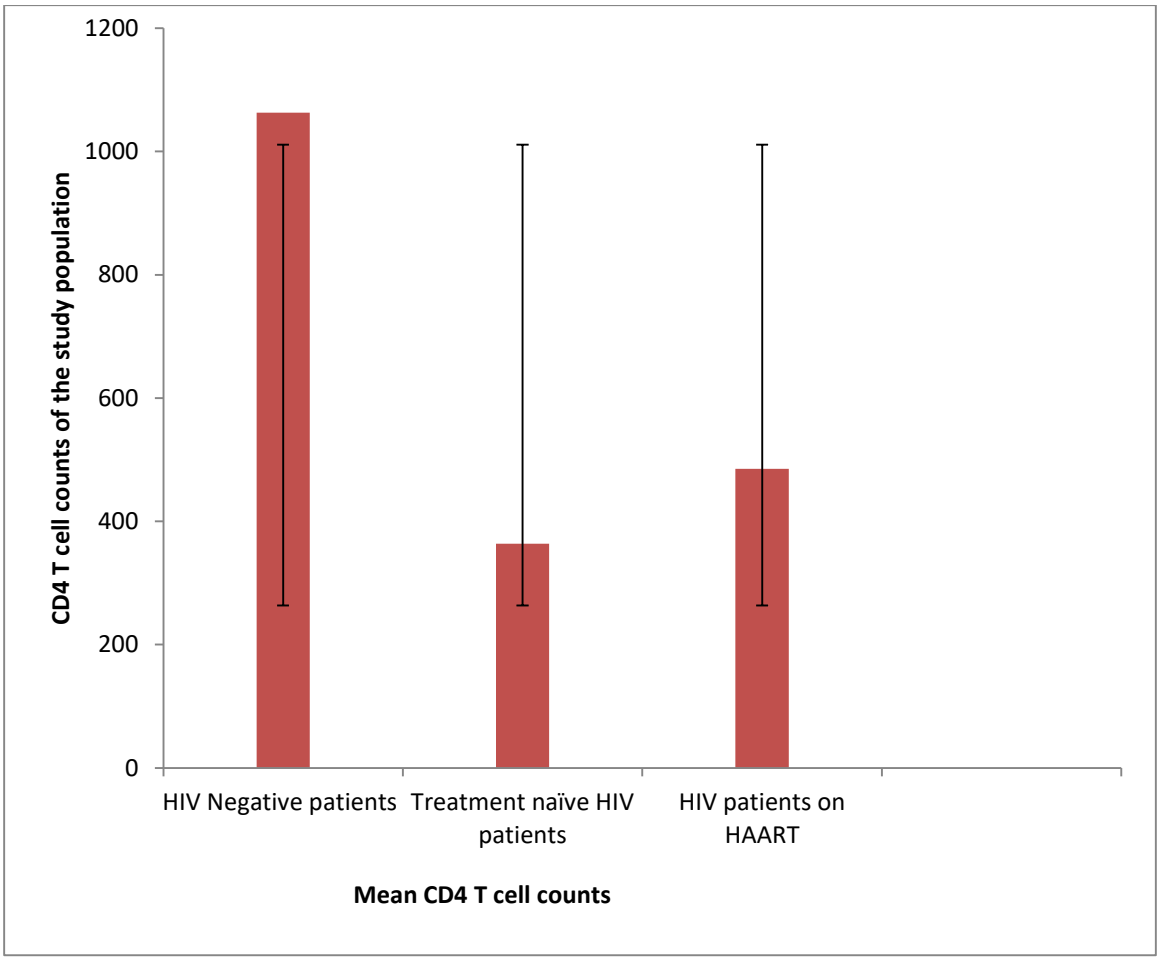


Figure 4.6: Mean CD4 T cell counts (± SD) of HIV negative patients, n=20, $\bar{x}$ = 1063, treatment naïve patients, n=40, $\bar{x}$ = 364 and HIV patients on HAART, n=20, $\bar{x}$ =485.

4.5 Correlations of Immunological and Hematological parameters

The correlations of immunological and hematological parameters in the study population were determined by Pearson' product-moment correlation coefficient (Pearson's correlation).

4.5.1 Correlation of Circulatory Cytokine Profiles and Hematological Parameters

HIV negative patients

There was no significant correlation between cytokines and blood parameters; cytokines and erythrocyte indices; cytokines and platelet indices or between cytokines and leukocyte indices among the HIV negative patients.

Treatment Naïve HIV Patients

Significant negative correlations were observed between cytokines and some blood parameters among the treatment naïve HIV patients, for example, between interleukin -12p70 (IL-12p70) and platelets (PLT), ($r = -0.552$, $p < 0.05$); between Interleukin – 10 (IL-10) and platelets ($r = -0.501$, $p < 0.05$); plus also between Interleukin – 6 (IL-6) and platelets ($r = -0.596$, $p < 0.05$).

There were no significant correlations between cytokines and erythrocyte indices. Platelet crit (PCT) was the only platelet indices that had significant correlations with cytokines. There were significant negative correlations between interleukin – 12p70 (IL-12p70) and platelet crit (PCT), ($r=-0.541$, $p<0.005$); between interleukin – 10 (IL-10) and platelet crit ($r = -0.508$, $p < 0.05$); plus also between Interleukin -6 (IL-6) and platelet crit ($r=-0.604$, $p < 0.05$).Several significant correlations were seen between cytokines and leukocyte indices: significant positive correlations were observed between interleukin - 6 and the absolute content of lymphocytes (Lymph#), ($r = 0.0448$, $p < 0.05$) plus between Interleukin - 6 and the relative (%) content of lymphocytes (Lymph%), ($r=0.0517$, $p<0.05$).

Significant negative correlations were observed between Tumor Necrosis Factor (TNF) and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), ($r=-0.563$, $p<0.05$); between interleukin – 10 and absolute content of the mixture of monocytes, basophils and eosinophils (Mid#) ($r=-0.502$, $p<0.05$); between interleukin – 10 and the absolute content of granulocytes (Gran#), ($r=-0.490$, $p<0.05$); between IL-10 and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), ($r = -0.655$, $p < 0.05$); between Interleukin – 10 and the relative (%) content of granulocytes (Gran%), ($r = -0.0601$, $p < 0.05$); between interleukin – 6 and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), ($r = -0.462$, $p < 0.05$) and also between Interleukin – 6 and the relative (%) content of granulocytes (Gran%), ($r=-0.585$, $p<0.05$) (Table 4.5).

Table 4.5: Pearson' product-moment correlation coefficient of circulatory cytokines and hematological parameters in treatment naïve HIV patients. Only the significant correlations are shown.

	Circulatory cytokines				
	IL-12p70	**TNF**	**IL-10**	**IL-6**	**IL-1B**
Hematological parameters					
PLT	r = -0.552 p=0.012*	NS	r = -0.501 p = 0.024*	r = -0.596 p = 0.005*	NS
PCT	r = -0.541 p = 0.014*	NS	r = -0.508 p=0.022*	r = -0.604 p =0.005*	NS
Lymph#	NS	NS	NS	r =0.448 p =0.047*	NS

Mid#	NS	NS	r = -0.502 p = 0.024*	NS	NS
Gran#	NS	NS	r = -0.490 p= 0.028*	NS	NS
Lymph%	NS	NS	NS	r = -0.517 p = 0.020*	NS
Mid%	NS	r = -0.563 p = 0.010	r = -0.655 p = 0.002	r= -0.462 p = 0.040*	NS
Gran%	NS	NS	r = -0.601 p = 0.005**	r = -0.585 p = 0.007*	NS

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*. PLT = platelets; PCT = platelet crit; Lymph# = the absolute content of lymphocytes; Mid# = absolute content of the mixture of monocytes, basophils and eosinophils; Gran# = the absolute content of granulocytes; Lymph% = the relative (%) content of lymphocytes; Mid% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; Gran% = the relative (%) content of granulocytes.
NS – Not significant;

*=Significant at $p<0.05$;

HIV Patients on HAART

There were no significant correlations between cytokines and blood parameters and between cytokines and erythrocyte indices among the HIV patients on HAART. However, significant positive correlations were observed between cytokines and platelet indices that included the correlations between interleukin - 12p70 (IL-12p70) and mean platelet volume (MPV), $r=0.330$, $p<0.05$); between tumor necrosis factor (TNF) and mean platelet volume (MPV), ($r=0.393$, $p<0.05$) and between tumor necrosis factor (TNF) and relative width of the distribution of platelets (PDW), $r=0.334$, $p<0.05$).

Correlation between cytokines and platelet crit (PCT) were not significant. The significant correlations between cytokines and leukocyte indices included the correlations between interleukin – 6 (IL-6) and the relative (%) content of lymphocytes (Lymph%), r=0.318, p<0.05) and between interleukin - 6 (IL-6) and the relative (%) content of granulocytes (Gran%), (r=0.325, p<0.05). Other leukocyte indices were not significantly correlated with cytokines (Table 4.6).

Table 4.6: Pearson' product-moment correlation coefficients of circulatory cytokines and hematological parameters in HIV patients on HAART showing only the significant correlations.

	Circulatory cytokines				
	IL-12p70	TNF	IL-10	IL-6	IL-1B
Hematological parameters					
MPV	r=0.330 p=0.037*	r=0.393 p=0.012*	NS	NS	NS
PDW	NS	r=0.334 p=0.035*	NS	NS	NS
Lymph%	NS	NS	NS	r=0.318 p=0.045*	NS
Gran%	NS	NS	NS	r=0.325 p=0.041*	NS

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta.* MPV = mean platelet volume; PDW = relative width of the distribution of platelets; Lymph% = the relative (%) content of lymphocytes; Gran% = the relative (%) content of granulocytes.

NS – Not significant; *=Significant at p<0.05

4.5.2 Correlation of hematological parameters and CD4 T cell counts

There were correlations, though not significant, between blood parameters and CD4 T cell counts, between erythrocyte indices and CD4 T cell counts; between platelet indices and CD4 T cell counts and between leukocyte indices and CD4 T cell counts among the HIV negative patients and the HIV patients on HAART.

Among the treatment naïve HIV patients, there were no significant correlations between blood parameters and CD4 T cell counts; between erythrocyte indices and CD4 T cell counts and neither between leukocyte indices and CD4 T cell counts. There was a significant correlation between mean platelet volume (MPV) and CD4T cell counts, ($r = 0.326$, $n = 40$, $p<0.05$). Other platelet indices were not significantly correlated with CD4 T cell counts.

4.6 Associations between Immunological and Hematological parameters

A chi-square test was performed to determine the associations between cytokine profiles and blood parameters; between cytokine profiles and blood indices; and between cytokine profiles and CD4 T cell counts.

4.6.1 Associations between Circulatory Cytokines and Hematological parameters

Some similar associations were observed between cytokines and blood parameters among the HIV negative patients and HIV patients on HAART that were not seen in treatment naïve HIV

patients. In HIV negative patients, for example, the association between interleukin – 12p70 (IL-12p70) and white blood cells (WBC), χ^2 (192, N=20) = 200.0, p=0.331), between IL-12p70 and red blood cells (RBC), χ^2 (216, N=20) = 220.0, p=0.412), between IL-12p70 and hematocrit (HCT), χ^2 (216, N=20) = 220.0, p = 0.412), between IL-12p70 and hemoglobin (HGB), χ^2 (204, N=20) = 208.8, p=0.395), and between IL-12p70 and platelets (PLT), χ^2 (216, N=20) = 220.0, p=0.412) were similar to the associations between interleukin – 6 (IL-6) and white blood cells (WBC), χ^2 (192, N=20) = 200.0, p=0.331), between IL-6 and red blood cells (RBC), χ^2 (216, N=20) = 220.0, p=0.412), between IL-6 and hematocrit (HCT), χ^2 (216, N=20) = 220.0, p=0.4120), between IL-6 and hemoglobin (HGB), χ^2 (204, N=20) = 208.8, p=0.395) and between IL-6 and platelets (PLT), χ^2 (216, N=20) = 220.0, p=0.412). Several other similar associations were observed between cytokines and blood parameters among the HIV negative patients, for example, the association between tumor necrosis factor (TNF) and white blood cells (WBC), χ^2 (128, N=20) = 145.6, p=0.137) and the association between interleukin 10 (IL-10) and white blood cells (WBC) χ^2 (128, N=20) = 145.6, p= 0.137) were similar. The only similar associations observed among the treatment naïve HIV patients were those between interleukin - 12p70 (IL-12p70) and white blood cells (WBC), χ^2 (340, N=40) = 339.3, p=0.500) and between - IL-12p70 and red blood cells (RBC); χ^2 (340, N=40) = 339.3, p=0.500) (Table 4.7). These associations were not significant.

Among the treatment naïve HIV patients, significant associations ($p<0.05$) were observed between interleukin-12p70 (IL-12p70) and hemoglobin (HGB), χ^2 (310, N=40) = 358.67,

p=0.030); and between tumor necrosis factor (TNF) and hemoglobin (HGB), χ^2 (341, N=40) = 398.62, p=0.017). Other relationships between cytokines and blood parameters were not significant and neither were they similar to any other associations (Table 4.7).

Similar associations were observed among the HIV patients on HAART, for example, the association between tumor necrosis factor (TNF) and white blood cells(WBC), χ^2 (48, N=20) = 60.0, p=0.115), between tumor necrosis factor (TNF) and red blood cells (RBC), χ^2 (54, N=20) = 49.0, p=0.652), between tumor necrosis factor and hematocrit (HCT), χ^2 (54, N=20) = 60.0, p=0.267), between tumor necrosis factor (TNF) and hemoglobin (HGB), χ^2 (51, N=20) = 60.0 p=0.182) and between tumor necrosis factor and platelets (PLT), χ^2 (57, N=20) = 60.0, p=0.368) were similar to the associations between interleukin – 6 (IL-6) and white blood cells (WBC), χ^2 (48, N=20) = 60.0, p=0.115), between interleukin – 6 (IL-6) and red blood cells (RBC), χ^2 (54, N=20) = 49.0 p=0.652), between interleukin – 6 (IL-6) and hematocrit (HCT), χ^2 (54, N=20) = 60.0, p=0.267), between interleukin – 6 (IL-6) and hemoglobin (HGB), X^2(51, N=20) = 60.0, p=0.182), between interleukin – 6 (IL-6) and platelets (PLT), χ^2 (57, N=20) = 60.0, p=0.368) (Table 4.7). However, these associations though identical were not significant.

Examples of other similar associations, though not significant, among the HIV patients on HAART were the associations between interleukin - 12p70 (IL-12p70) and white blood cells (WBC), χ^2 (80, N=20) = 85.8, p=0.309) and between interleukin - 1β (IL-1β) and white blood

cells (WBC), χ^2 (80, N=20) = 85.8, p=0.309), between interleukin – 12p70 (IL-12p70) and red blood cells (RBC), χ^2 (90, N=20) = 89.3, p=0.500) and between interleukin - 1β (IL-1β) and red blood cells (RBC), χ^2 (90, N=20) = 89.3, p=0.500). Other similar associations were those between interleukin - 1β (IL-1β) and red blood cells (RBC), χ^2 (90, N=20) = 89.3, p=0.500), between interleukin 1β (IL-1β) and hematocrit (HCT), χ^2 (90, N=20) = 89.3, p=0.500) and between interleukin 1β (IL-1β) and platelets (PLT), χ^2 (90, N=20) = 89.3, p=0.500) (Table 4.7).

Table 4.7: Chi-square test (χ^2) for the associations between circulatory cytokines and blood parameters of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks and significant ones are shown by superscripts.

	Cytokines:	**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Blood parameters**	**Association of cytokines and blood parameters: p values**				
HIV Negative patients	WBC*	0.331*	0.137*	0.137*	0.331*	-
	RBC*	0.412*	0.367*	0.579*	0.412*	
	HCT*	0.412*	0.367*	0.579*	0.412*	-
	HGB*	0.395*	0.208	0.390	0.395*	-
	PLT*	0.412*	0.579	0.579*	0.412*	-
Treatment naïve Patients	WBC	0.500	0.414	0.754	0.460	0.221
	RBC	0.500	0.473	0.531	0.381	0.381
	HCT	0.806	0.908	0.500	0.992	0.960
	HGB	0.030[a]	0.017[a]	0.353	0.194	0.129
	PLT	0.352	0.346	0.359	0.330	0.426
HIV patients on HAART	WBC**	0.309**	0.115**	0.157	0.115**	0.309**
	RBC**	0.500**	0.652**	0.772	0.652**	0.500**

HCT**	0.221	0.267**	0.297	0.267**	0.500**
HGB**	0.127	0.182**	0.221	0.182**	0.353
PLT**	0.343	0.368**	0.381	0.368**	0.500**

Key: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*; RBC = red blood cells; WBC = white blood cells; HCT = hematocrit; HGB =hemoglobin; PLT = platelets.

* Similar associations in HIV negative patients; ** similar associations in HIV patients on HAART.

[a]=Significant at $p<0.05$

Erythrocyte indices

Among the HIV negative patients, similar associations were observed between tumor necrosis factor (TNF) and erythrocyte indices; and between interleukin – 6 (IL-6) and erythrocyte indices. The relationship between tumor necrosis factor (TNF) and mean volume of erythrocytes (MCV), χ^2 (54, N=20) = 60.0, p=0.267) was similar to the association between IL-6 and MCV, χ^2 (54, N=20) = 60.0, p=0.267); the association between TNF and mean content of hemoglobin (MCH), χ^2 (57, N=20) = 60.0, p=0.368) was identical to the association between IL-6 and mean MCH, χ^2 (57, N=20) = 60.0, p=0.368); the association between TNF and mean concentration of hemoglobin (MCHC), χ^2 (42, N=20) = 49.4, p=0.201) was similar to the association between IL-6 and MCHC, χ^2 (42, N=20) = 49.4, p=0.201); the association between TNF and relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV), χ^2 (45,

N=20) = 35.3, p= 0.850) was similar to the association between IL-6 and RDW-CV, χ^2 (45, N=20) = 35.3, p= 0.850) and the association between TNF and relative distribution width of red blood cells by volume, standard deviation (RDW-SD), χ^2 (39, N=20) = 38.8, p=0.478) was similar to the association between IL-6) and RDW-SD, χ^2 (39, N=20) = 38.8, p=0.478). These associations were not significant (Table 4.8).

Although such similar associations were not seen among the treatment naïve HIV patients, a significant association ($p<0.05$) between tumor necrosis factor (TNF) and relative distribution width of red blood cells by volume, standard deviation (RDW-SD), χ^2 (231, N=40) = 284.8, p=0.009) was observed among this group.

Similar associations between cytokines and erythrocyte indices were also observed among HIV patients on HAART. For example, the association between interleukin – 12p70 (IL-12p70) and mean volume of erythrocytes (MCV), χ^2 (216, N=20) = 228.8, p=0.263) was similar to the association between interleukin- 6 (IL-6) and MCV, χ^2 (216, N=20) = 228.8, p=0.263); the association between IL-12p70 and mean content of hemoglobin (MCH), χ^2 (216,N=20) = 220.0, p=0.412) was similar to the association between IL-6 and MCH, χ^2 (216,N=20) = 220.0, p=0.412); the association between IL-12p70 and mean concentration of hemoglobin (MCHC), χ^2 (216, N=20) = 240.0, p=0.126) was identical to the association between IL-6 and MCHC, χ^2 (216, N=20) = 240.0, p=0.126); while the associations between tumor necrosis factor (TNF) and mean volume of erythrocytes (MCV), χ^2 (144, N=20) = 149.2, p=0.367) and

between tumor necrosis factor (TNF) and mean content of hemoglobin (MCH), χ^2 (144, N=20) = 149.2, p=0.367) and those between of interleukin – 10 (IL-10) and mean volume of erythrocytes MCV, χ^2 (144, N=20) = 149.2, p=0.367) and between IL-10 and mean content of hemoglobin (MCH), χ^2 (144, N=20) = 149.2, p=0.367) were similar. Other identical associations are shown in Table 4.8. However, these associations though identical were not significant.

Table 4.8: Chi square test (χ^2) for the associations between circulatory cytokines and erythrocyte indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks and those significant are shown by superscript.

	Cytokines:	**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Erythrocyte indices**	**Association of cytokines and blood parameters: p values**				
HIV Negative patients	MCV	0.500	0.267*	0.297	0.267*	-
	MCH	0.343	0.368*	0.381	0.368*	-
	MCHC	0.224	0.201*	0.390	0.201*	-
	RDW-CV	0.803	0.850*	0.494	0.850*	-
	RDW-SD	0.496	0.478*	0.841	0.478*	-
Treatment naïve Patients	MCV	0.363	0.305	0.432	0.181	0.984
	MCH	0.463	0.668	0.209	0.303	0.258
	MCHC	0.544	0.145	0.224	0.292	0.105
	RDW-CV	0.355	0.245	0.494	0.381	0.946
	RDW-SD	0.237	0.009[a]	0.819	0.767	0.920
HIV patients on HAART	MCV	0.263**	0.367**	0.367**	0.263**	0.500
	MCH	0.412**	0.367**	0.367**	0.412**	0.221
	MCHC	0.126**	0.171**	0.171**	0.126**	0.221
	RDW-CV	0.460	0.208**	0.208**	0.210	0.224
	RDW-SD	0.283	0.813	0.405	0.543	0.247

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*; MCV = mean volume of erythrocytes; MCH = mean content of hemoglobin; MCHC = mean concentration of hemoglobin; RDW-CV = relative distribution width of red blood cells by volume, coefficient of variation; RDW-SD = relative distribution width of red blood cells by volume, standard deviation.

* Similar associations in HIV negative patients; ** Similar associations in HIV patients on HAART. [a] =Significant at $p<0.05$

Platelet indices

Among the HIV negative patients, similar associations, though not significant, were observed between tumor necrosis factor (TNF) and mean platelet volume (MPV), χ^2 (36, N=20) = 35.3, p=0.502) and between interleukin – 6 (IL6) and MPV, χ^2 (36, N=20) = 35.3, p=0.502); between TNF and relative width of the distribution of platelets (PDW), χ^2 (33, N=20) = 10.6, p=1.000) and between IL-6 and PDW, χ^2 (33, N=20) = 10.6, p=1.000); between TNF and platelet crit (PCT), χ^2 (54, N=20) = 60.0, p=0.267) and between IL-6 and PCT, χ^2 (54, N=20) = 60.0, p=0.267); (Table 4.9).

There were no similar associations that were observed among the treatment naïve HIV patients. However, significant associations were observed among this group that included: the relationships between Interleukin – 12p70 (IL-12p70) and mean platelet volume (MPV) ($p<0.5$); between tumor necrosis factor (TNF) and mean platelet volume(MPV), ($p<0.05$); between tumor necrosis factor (TNF) and relative width of the distribution of platelets (PDW), ($p<0.05$); between interleukin – 10 (IL-10) and relative width of the distribution of platelets (PDW), ($p<0.05$); between interleukin – 6 (IL-6) and mean platelet volume (MPV), ($p<0.05$);

between interleukin – 6 (IL-6) and relative width of the distribution of platelets (PDW), ($p<0.05$); between interleukin - 1β (IL-1β) and mean platelet volume (MPV), ($p<0.05$); and between interleukin 1- β and IL-1β and relative width of the distribution of platelets (PDW) ($p<0.05$); (Table 4.9).

Table 4.9: Chi-square test (χ^2) for the associations between circulatory cytokines and platelet indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks and significant associations are shown by superscripts.

Cytokines:		IL-12p70	TNF	IL-10	IL-6	IL-1β
Groups of patients	**Platelet indices**	**Association of cytokines and blood parameters: p values**				
HIV negative patients	MPV	0.090	0.502*	0.909	0.502*	-
	PDW	0.940	1.000*	0.999	1.000*	-
	PCT	0.221	0.267*	0.297	0.267*	-
Treatment naïve Patients	MPV	0.003[a]	0.000[a]	0.114	0.011[a] ($p<0.05$)	0.011[a]
	PDW	0.162	0.022[c]	0.001[a]	0.006[a]	0.001[a]
	PCT	0.232	0.454	0.268	0.552	0.297
HIV patients on HAART	MPV	0.285**	0.521**	0.521**	0.285**	0.097
	PDW	0.723**	0.585	0.978	0.723**	0.678
	PCT	0.246**	0.208	0.208	0.246**	0.353

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*; MPV = mean platelet volume; PDW = relative width of the distribution of platelets; PCT = platelet crit.

* Similar associations in HIV negative patients; ** Similar associations in HIV patients on HAART.

[a] = Significant at $p<0.05$

Among the HIV patients on HAART, similar associations were observed between interleukin – 12p70 (IL-12p70) and mean platelet volume (MPV), $\chi^2(168, N=20) = 177.9$, $p=0.285$) and

between interleukin - 6 (IL-6) and mean platelet volume (MPV), χ^2 (168, N=20) = 177.9, p=0.285); between IL-12p70 and relative width of the distribution of platelets (PDW), χ^2 (120, N=20) = 110.4, p=0.723) and between IL-6 and PDW, χ^2 (120, N=20) = 110.4, p=0.723); between IL-12p70 and platelet crit (PCT), χ^2 (204, N=20) = 217.5, p=0.246 and between IL-6 and PCT, χ^2 (204, N=20) = 217.5, p=0.246. Other similar associations in this group included the association between tumor necrosis factor (TNF) and mean platelet volume (MPV), χ^2 (112, N=20) = 110.6, p=0.521) and the association between interleukin – 10 (IL-10) and mean platelet volume (MPV), χ^2 (112, N=20) = 110.6, p=0.521); (Table 4.9). Although these associations were similar, they were not significant (Table 4.9).

Leukocyte indices

Among the HIV negative patients, significant associations were observed between IL-10 and the absolute content of lymphocytes (Lymph#) ($p<0.05$), and between IL-10 and absolute content of the mixture of monocytes, basophils and eosinophils (Mid#) ($p<0.05$). Identical associations were observed: between tumor necrosis factor (TNF) and the absolute content of lymphocytes (Lymph#), χ^2 (36, N=20) = 45.9, p=0.125) and between interleukin – 6 (IL-6) and Lymph#, χ^2 (36, N=20) = 45.9, p=0.125); between tumor necrosis factor (TNF) and the mixture of monocytes, basophils and eosinophils (Mid#), χ^2 (21, N=20) = 30.0, p=0.92) and between IL-6 and the mixture of monocytes, basophils and eosinophils (Mid#), χ^2 (21, N=20) = 30.0, p=0.92); between TNF and the absolute content of granulocytes (Gran#), χ^2 (51, N=20) = 60.0,

p=0.182) and between IL-6 and the absolute content of granulocytes (Gran#), χ^2 (51, N=20) = 60.0, p=0.182); between TNF and the relative (%) content of lymphocytes (Lymph%), χ^2 (57, N=20) = 60.0, p=0.368) and between IL-6 and the relative (%) content of lymphocytes (Lymph%), χ^2 (57, N=20) = 60.0, p=0.368); between TNF and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), χ^2 (48, N=20) = 60.0, p=0.115) and between IL-6 and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), χ^2 (48, N=20) = 60.0, p=0.115) and also between TNF and the relative (%) content of granulocytes (Gran%), χ^2 (57, N=20) = 60.0, p=0.368) and between IL-6 and the relative (%) content of granulocytes (Gran%), χ^2 (57, N=20) = 60.0, p=0.368; Table 4.10). However, though these associations were similar they were not significant.

Among the treatment naïve HIV patients, significant associations ($p<0.05$) were observed between IL-12p70 and the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%), χ^2 (300, N=40) = 351.8, p=0.021); between IL-10 and the absolute content of granulocytes (Gran#), χ^2 (306, N=40) = 366.0, p=0.018; between IL-6 and Gran#, χ^2 (476, N=40) = 532.3, p=0.038); between IL-1β and the absolute content of lymphocytes (Lymph#), χ^2 (22, N=40) = 40.0, p=0.011) and between IL-1β and absolute content of the mixture of monocytes, basophils and eosinophils (Mid#), χ^2 (10, N=40) = 19.5, p=0.034). There were no identical association between any cytokines and leukocyte indices among these patients. Among the HIV patients on HAART, identical associations were observed that included associations between IL-12p70 and Gran#, χ^2 (180, N=20) = 168.8, p=0.716) and between IL-6 and Gran#,

χ^2 (180, N=20) = 168.8, p=0.716); the associations between IL-12p70 and Lymph%, χ^2 (216, N=20) = 228.8, p=0.263) and between IL-6 and Lymph%, χ^2 (216, N=20) = 228.8, p=0.263); the associations between IL-12p70 and Mid%, χ^2 (204, N=20) = 220.0, p=0.210) and between IL-6 and Mid%, χ^2 (204, N=20) = 220.0, p=0.210); the associations between IL-12p70 and Gran%, χ^2 (228, N=20) = 240.0, p=0.280) and between IL-6 and Gran%, χ^2 (228, N=20) = 240.0, p=0.280). Identical associations were also observed between TNF and Gran#, χ^2 (120, N=20) = 101.4, p=0.890) and between IL-10 and Gran#, χ^2 (120, N=20) =101.4, p=0.890) and also in the associations between TNF and Gran%, χ^2 (152, N=20) = 160.0, p=0.312) and between IL-10 and Gran%, χ^2 (152, N=20) = 160.0 p=0.312). These associations were not significant (Table 4.10).

Table 4.10: Chi-square test (χ^2) for the associations between circulatory cytokines and leukocyte indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks those that are significant shown by superscripts.

	Cytokines:	**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Leukocyte indices**	**Association of cytokines and blood parameters: p values**				
HIV negative patients	LYMPH#	0.324	0.125*	0.021a	0.125*	-
	MID#	0.308	0.092*	0.044a	0.092*	-
	GRAN#	0.127	0.182*	0.221	0.182*	-
	LYMPH%	0.343	0.368*	0.381	0.368*	-
	MID%	0.223	0.115*	0.157	0.115*	-
	GRAN%	0.343	0.368*	0.381	0.368*	
Treatment naïve patients	LYMPH#	0.130	0.330	0.942	0.564	0.011a
	MID#	0.391	0.175	0.881	0.077	0.034a

	GRAN#	0.014	0.053	0.018a	0.038a	0.221
	LYMPH%	0.352	0.346	0.359	0.330	0.426
	MID%	0.021a	0.197	0.116	0.637	0.105
	GRAN%	0.230	0.220	0.241	0.405	0.381
HIV patients on HAART	LYMPH#	0.229	0.159	0.485	0.295	0.334
	MID#	0.978	0.912	0.967	0.994	0.850
	GRAN#	0.716**	0.890**	0.890**	0.716**	0.859
	LYMPH%	0.263**	0.367	0.171	0.263**	0.221
	MID%	0.210**	0.390	0.208	0.210**	0.127
	GRAN%	0.280**	0.312**	0.312**	0.28**0	0.343

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*; LYMP# = the absolute content of lymphocytes; MID# = absolute content of the mixture of monocytes, basophils and eosinophils; GRAN# = the absolute content of granulocytes; LYMPH% = the relative (%) content of lymphocytes; MID% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; GRAN% **=** the relative (%) content of granulocytes.

* Similar associations in HIV negative patients; ** Similar associations in HIV patients on HAART. [a] = Significant at $p<0.05$

4.6.2 Associations between CD4 T cell counts and Circulatory Cytokines

There were no significant associations between CD4 T cell counts and circulatory cytokines observed in either HIV negative patients, treatment naïve HIV patients or HIV patients on HAART. However, similar associations were observed between tumor necrosis factor (TNF) and CD4 T cell counts, χ^2 (57, N=20) = 60.0, p=0.368) and between interleukin – 6 (IL-6) and CD4

T cell counts, χ^2 (57, N=20) = 60.0, p= 0.368) among the HIV negative patients. Among the HIV patients on HAART, similar associations were observed between interleukin – 12p70 (IL-12p70) and CD4 T cell counts, χ^2 (228, N=20) =240.0, p=0.280) and between interleukin – 6 (IL-6) and CD4 T cell counts, χ^2 (228, N=20) = 240.0, p=0.280) while similar associations were also observed between TNF and CD4 T cell counts, χ^2 (152, N=20) = 160.0, p=0.312) and between interleukin – 10 (IL-10) and CD4 T cell counts, χ^2 (152, N=20) = 160.0, p=0.312). Such identical associations were not observed among the treatment naïve HIV patients (Table 4.11).

Table 4.11: Chi-square test (χ^2) for the associations between CD4 T cell counts and circulatory cytokines of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks.

Cytokines:		**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients		**Association of CD4 T cells and plasma cytokines: p values**				
HIV negative patients	CD4 T cells	0.343	0.368*	0.381	0.368*	-
Treatment naïve HIV patients	CD4 T cells	0.762	0.729	0.670	0.405	0.381
HIV patients on HAART	CD4 T cells	0.280*	0.312*	0.312*	0.280*	0.343

KEY: IL-12p70 = Interleukin 12p70; TNF = Tumor necrosis factor; IL-10 = Interleukin 10; IL-6= Interleukin 6; IL-1β= Interleukin 1 *beta*.

* Similar associations between CD4 T cell counts and some circulatory cytokines.

4.6.3 Associations between CD4 T cell counts and Blood parameters

There were no significant associations between CD4 T cell counts and blood parameters. However, some unique associations were observed: the association between CD4 T cell counts and white blood cells (WBC), χ^2 (304, N=20) = 320.0, p=0.253); between CD4 T cell counts and red blood cells (RBC), χ^2 (342, N=20) = 360.0 p=0.241); between CD4 T cell counts and hematocrit (HCT), χ^2 (342, N=20) = 360.0, p=0.241) and between CD4 T cell counts and hemoglobin (HGB), χ^2 (323, N=20) = 340.0, p=0.247) among the HIV negative patients were identical to the association between CD4 T cell counts and the same blood parameters among the HIV patients on HAART. The associations between CD4 T cell counts and platelets (PLT) differed in both these groups (Table 4.12).

It was also noticed that the association between red blood cells (RBC) and CD4 T cells counts and also the association between hematocrit (HCT) and CD4 T cells counts were similar in HIV negative patients and HIV patients on HAART, while the association between platelets (PLT) and CD4 T cells counts among HIV patients on HAART were identical to the associations between RBC and CD4 T cells counts; and HCT and CD4 T cells counts in HIV patients on HAART. The association between platelets (PLT) and CD4 T cell counts, χ^2 (1482, N=40) = 1520.0, p=0.241) among the treatment naïve HIV patients was the only similar association with the association between platelets (PLT) and CD4 T cell counts of HIV patients on HAART (Table 4.12).

Table 4.12: Chi-square test (χ^2) for the associations between CD4 T cell counts and blood parameters in HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated with asterisks.

Blood parameters:		WBC	RBC	HCT	HGB	PLT
Groups of patients		**Association of cytokines and blood parameters: p values**				
HIV Negative patients	CD4 T cells	0.253**	0.241**	0.241**	0.247**	0.236
Treatment naïve HIV patients	CD4 T cells	0.288	0.195	0.310	0.321	0.241
HIV patients on HAART	CD4 T cells	0.253**	0.241**	0.241**	0.247**	0.241

KEY; RBC = red blood cells; WBC = white blood cells; HCT = hematocrit; HGB =hemoglobin; PLT = platelets.

KEY: ** Similarities in association

4.6.4 Associations between CD4 T cell counts and erythrocyte indices

There were no significant associations between CD4 T cell counts and erythrocyte indices, among the HIV negative patients, treatment naïve HIV patients or in HIV patients on HAART. However, the association between CD4 T cell counts and mean volume of erythrocytes (MCV), χ^2 (342, N=20) =360.0, p=0.241) among the HIV negative patients were identical to the association between CD4 T cell counts and mean volume of erythrocytes (MCV), χ^2 (342, N=20) =360.0, p=0.241), between CD4 T cell counts and mean content of hemoglobin (MCH), χ^2 (342, N=20) =360.0, p=0.241) and between CD4 T cell counts and mean concentration of hemoglobin (MCHC), χ^2 (342, N=20) =360.0, p=0.241) of the HIV patients on HAART. An

identical association was observed between CD4 T cell counts and MCHC, χ^2 (266, N=20) =280.0, p=0.266) in HIV negative patients and between CD4 T cell counts and relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV), χ^2 (266, N=20) =280.0, p=0.266) in HIV patients on HAART. There were no identical associations between CD4 T cell counts and erythrocyte indices among the treatment naïve HIV patients (Table 4.13).

Table 4.13: Chi-square test (χ^2) for the associations between CD4 T cell counts and erythrocyte indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated by asterisks.

Erythrocyte indices:		**MCV**	**MCH**	**MCHC**	**RDW-CV**	**RDW-SD**
Groups of patients		**Association of cytokines and erythrocyte indices: p values**				
HIV negative patients	CD4 T cells	0.241**	0.236	0.266	0.259	0.273
Treatment naïve HIV patients	CD4 T cells	0.278	0.199	0.200	0.321	0.247
HIV patients on HAART	CD4 T cells	0.241**	0.241**	0.241**	0.266	0.287

KEY: MCV = mean volume of erythrocytes; MCH = mean content of hemoglobin; MCHC = mean concentration of hemoglobin; RDW-CV = relative distribution width of red blood cells by volume, coefficient of variation; RDW-SD = relative distribution width of red blood cells by volume, standard deviation.
KEY: ** Similar association

4.6.5 Association between CD4 T cell counts and platelet indices

There were no significant associations between CD4 T cell counts and platelet indices in either of the groups nor were their any identical associations observed (Table 4. 14).

Table 4.14: Chi-square test (χ^2) for the associations between CD4 T cell counts and platelet indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART.

Platelet indices:		MPV	PDW	PCT
Groups of patients		**Association of CD4 T cells and platelet indices: p values**		
HIV Negative patients	CD4 T cells	0.280	0.287	0.241
Treatment naïve HIV Patients	CD4 T cells	0.331	0.285	0.268
HIV patients on HAART	CD4 T cells	0.266	0.295	0.247

KEY: MPV = mean platelet volume; PDW = relative width of the distribution of platelets; PCT = platelet crit.

4.6.6 Associations between CD4 T cell counts and leukocyte indices

There were no significant associations between CD4 T cell counts and leukocyte indices in any of the groups. However, among the HIV negative patients, identical associations were observed between CD4 T cell counts and the relative (%) content of lymphocytes (Lymph%), χ^2 (361, N=20) =380.0, p=0.236) and between CD4 T cell counts and the relative (%) content of

granulocytes (Gran%), χ^2 (361, N=20) =380.0, p=0.236); and also between CD4 T cell counts and the relative (%) content of granulocytes (Gran%), χ^2 (361, N=20) =380.0, p=0.236) among the HIV patients on HAART. Identical associations were also observed between CD4 T cell counts and the relative (%) content of lymphocytes (Lymph%), χ^2 (1482, N=40) =1520.0, p=0.241) in treatment naïve HIV patients and between CD4 T cell counts and the relative (%) content of lymphocytes (Lymph%), χ^2 (1482, N=40) =1520.0, p=0.241) among the HIV patients on HAART (Table 4.15).

Table 4.15: Chi-square test (χ^2) for the associations between CD4 T cell counts and leukocyte indices of HIV negative patients, treatment naïve HIV patients and HIV patients on HAART. Similar associations are indicated by asterisks.

Leukocyte indices:		**LYMPH#**	**MID#**	**GRAN#**	**LYMPH%**	**MID%**	**GRAN%**
Groups of patients		**Association of cytokines and blood parameters: p values**					
HIV negative	CD 4 T cells	0.280	0.322	0.247	0.236**	0.253	0.236**
Treatment naïve Patients	CD 4 T cells	0.331	0.230	0.288	0.241**	0.333	0.249
HIV patients on HAART	CD 4 T cells	0.273	0.312	0.259	0.241**	0.247	0.236**

KEY: LYMP# = the absolute content of lymphocytes; MID# = absolute content of the mixture of monocytes, basophils and eosinophils; GRAN# = the absolute content of granulocytes; LYMPH% = the relative (%) content of lymphocytes; MID% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; GRAN% = the relative (%) content of granulocytes.

** Similar associations.

CHAPTER FIVE: DISCUSSION

5.1 Circulatory Cytokine Profiles

Levels of circulatory cytokines of persons infected with human immunodeficiency virus (HIV) are altered when compared to cytokine profiles found in those without the infection. Infection with the HIV along with the viral proteins is capable of disturbing cytokine production as well as disrupting their normal interactions, which results in normal immune function being disrupted (Vishwanath *et al.*, 2011). The altered levels of circulatory cytokines in individuals infected with HIV are likely to have a direct impact on the course of HIV disease by enhancing or suppressing HIV replication, and can affect the function of the immune system (Breen, 2002). This study sought differences in circulatory cytokine levels by measuring plasma cytokines in those infected with HIV without treatment (HAART naïve) and on treatment(on HAART), and comparing the cytokine levels with those found in individuals without HIV. The HIV patients who were on HAART had just been diagnosed with HIV infection since policy dictates that all individuals diagnosed as HIV positive are put on antiretroviral therapy. According to this study, statistically significant differences were observed in the levels of the pro-inflammatory circulatory cytokines, which stimulates the immune system, and the anti-inflammatory cytokines which suppresses the immune system among those infected and not infected with HIV.

In this study, Interleukin 1β (IL-β), Interleukin 6 (IL-6), Interleukin 12p70 (IL-12p70), Interleukin 10 (IL-10) and Tumor Necrosis Factor (TNF) were detectable and showed significant differences among the various groups in the study population. Higher concentration levels of four circulatory cytokines (1β, IL-10, IL-6 and TNF) were observed in treatment naïve HIV

patients while in HIV negative patients, higher IL-12p70 concentration levels were detected despite having no detectable IL-1β. The higher levels of IL-12p70 among the HIV negative patients in this study would be an indication of other infections apart from HIV since IL-12 is a Th1 cell cytokine: the Th1 lymphocytes are critical in the cellular immune responses as well as playing crucial functions in hosts' defense systems for intracellular microbial agents and viruses (www.sinobiology.com/T cell cytokines.html). Furthermore, the higher levels of IL-12p70 in these patients may be an indication of the immune system's ability to restore T cell numbers following T cell depletion or expansion and to maintain normal T cell counts. Studies have suggested that IL-12 plays a key role in T cell homeostasis (www.sinobiology/.com /T cell cytokines.html, 2019).

In this study the HIV patients on HAART and the treatment naïve HIV patients had lower levels of detectable IL-12 p70 when compared with the HIV negative patients, an observation that agrees with a study conducted by Tudela *et al.,* (2014) that established lower concentration levels of IL-12 in patients positive for HIV although it differs from another finding by Lindi *et al.,* (2010) which indicated that during acute HIV infection, there are elevated levels of IL-12p70. The lower plasma IL-12 p70 observed in this study, however, agrees with another study in which very low concentrations of IL – 12 during acute HIV infection were reported (Norris *et al.,* 2006). Studies conducted earlier had revealed that HIV-infected individuals have a weaker immune system; this is because the CD4+Tcells are not able to proliferate due to the decrease in the levels of IL-12 (Noble *et al.,* 2001). During an infection, dendritic cells and phagocytes produce IL-12 which is a pro-inflammatory cytokine (Trincheri, 2003); IL-12 is said to

contribute to cellular inflammation and functions in the differentiation, proliferation and promotion of Th1 and cytotoxity (www.sinobiological.com/Inflammatory cytokines.html, 2019).

It has been shown that the gradual deterioration of cellular effector response is one of the characteristics of HIV infection (Vishwanath *et al.,* 2011). Decreased levels of IL-12 is said to lead to impaired Th1 responses and increased susceptibility to intracellular pathogens (www.sinobiological.com/role of cytokines.html); consequently, the probability for opportunistic infections increases when IL-12 concentrations decreases (Vishwanath *et al.,* 2011).The decreased levels of IL-12p70 observed among the treatment naïve HIV patients in this study could be an indicator of HIV disease progression and is in line with Louis *et al.,* (2010), who stated that as HIV progresses, there is decreased CD4+ and CD8+ cells responses that is caused by decreased IL-12. In addition, it was noted that normal levels of CD4 + cells could not be restored when IL-12 therapy was administered in the late stages of HIV (Villinger and Ansari, 2010).

This study showed that Interleukin - 10 (IL-10) levels in treatment naïve HIV patients were significantly higher when compared to that of HIV patients on therapy and to that of HIV negative patients. Although some discrepant results concerning IL-10 levels during HIV infection had been reported in previous studies (Clerici and Shearer, 1994), the levels in this study agrees with a report by Kaur *et al.,* (2016) which indicated that the plasma levels of this cytokine showed a significant increase in individuals infected with HIV. The high IL-10 levels observed in treatment naïve HIV patients in this study agrees with a study by Tudela *et al.,*

(2014) and with a study by Lindi *et al.,* (2010). In another study by Stylianou *et al.*, (1999), significantly higher circulating IL-10 levels in HIV infected patients were demonstrated. However, during the administration of HAART, a significant fall in the levels of IL-10 were established in the same study that was also able to observe that antiretroviral therapy has effects on the levels of IL-10; one observation that agrees with this study. Higher concentration levels of IL-10 were observed in HIV patients on HAART compared to HIV negative patients in this study, hence agreeing with Haissman *et al.,* (2009) whose study found higher levels of IL-10 in individuals on HAART and lower levels of cytokines in HIV negative persons.

Being an anti inflammatory cytokine, Interleukin – 10 (IL 10) has been recognized as a mediator of the chronic inflammatory processes by participating in both humoral and cellular inflammation (Trincheri, 2007). This cytokine is said to be involved in immune suppression, decreased antigen presentation and MHC class 11 expressions of dendritic cells and down regulates pathogenic Th1, Th2 and Th17 responses (www.sinobiological.com/cytokine functions review html, 2019). The ability of IL-10 to inhibit pro-inflammatory cytokines such as IL-2, IL-6, IL-12, TNF-α and IFN-γ is the most important function of this cytokine (Trincheri, 2007). Both Th1 and Th2 cells are said to secrete IL-10 cytokine according to some studies (Brockman *et al.*, 2009); Hedrich and Bream, (2010) stated that IL-10 cytokine is also produced by the cells from the myeloid lineage that includes dendritic cells and macrophages. Decreased IL-10 is associated with immune pathology due to uncontrolled inflammation while increased levels are said to inhibit sterile immunity to some pathogens (www.sinobiological.com/cytokine functions review html).

The role of Interleukin 10 (IL-10) in HIV pathogenesis has been discussed in several studies. According to reports by Ji *et al.,* (2005), after infection with HIV, the main cells producing interleukin 10 cytokine in peripheral blood mononuclear cells are CD 14+ monocytes through interactions independent of CD4+ molecules; consequently it was concluded that the production of interleukin 10 is dependent on the presence of CD 14+ monocytes. Yeh *et al.,* (2000) observed that the frequency of IL-10 producing cells increases significantly as the patient progresses to advanced stages of HIV disease (Yeh *et al.,* 2000). The role played by IL-10 in HIV infected individuals is said to be governed by the different stages of the HIV disease. For example, IL-10 might promote viral replication by inhibiting effector immune responses from adaptive and innate immunity in acute HIV 1 infected individuals (Naicker *et al.,* 2009). Additionally, there were proposals that the increase in production of IL-10 levels lowers plasma viral loads and increased CD4+ cell counts and IL-10 resembled a protective role by reducing immune activation, inhibiting virus replication in macrophages in a chronic phase of infection (Naicker *et al.,* 2009).

Tumor necrosis factor (TNF) is a stimulus for IL-10 release from various cells in itself, whereas IL-10 is an effective down regulator of TNF production. There have been suggestions that the balance between TNF and IL-10 is of great importance in HIV disease pathogenesis (Stylianou *et al.,* 1999). Increased IL-10 may be viewed as beneficial in slowing HIV disease progression in light of its ability to suppress the production of pro-inflammatory cytokines and, under some conditions, suppress HIV replication (Breen, 2002).

Significantly higher concentration of tumor necrosis factor (TNF) was observed in treatment naïve HIV patients compared with HIV negative patients in this study, an observation that agrees with Haissman *et al.,* (2009) who observed high concentration levels of TNF in individuals infected with HIV and also in agreement with Lindi *et al.,* (2010) whose study found higher concentration levels of TNF in recently HIV infected persons compared with HIV negative individuals. Being a pro-inflammatory cytokine, TNF is said to play key roles in the origins and progressions of diseases including HIV-1 (Bahia and Silakari, 2010). The immune regulatory responses of the hosts are said to influence the pathogeneses of HIV 1 disease, triggering the production of TNF by natural killer cells, monocytes and macrophages (Alfano and Poli, 2005). Studies have revealed an increased TNF-α production by macrophages that is induced by HIV infection (Vishwanath *et al.,* 2011). Tumor necrosis factor is a said to be one of the pro-inflammatory cytokines that plays key roles in mediating acute inflammatory reactions; they produce fever, inflammation, tissue destruction, and in some cases, shock and death when administered to humans (www.sinobiological.com/ Inflammatory cytokines.html, 2019).

Significantly high levels of TNF were also observed in treatment naïve HIV patients compared with the HIV patients on therapy, which also agrees with a study by Haissman *et al.,* (2009) which revealed that upon administration of antiretroviral therapy in HIV patients there were decreases in TNF levels. Studies have shown that it is during the early phase of acute inflammatory diseases that TNF is secreted. Among its roles in the pathogenesis of HIV disease is stimulation of T lymphocytes' apoptosis and activation of nuclear factor kB (nf-kB). There have been suggestions that in order to prevent TNF progression to the chronic stage, it must be targeted at an appropriate time during production. Monitoring the cytokine development is vital

since its local effect may be beneficial to the host (Fernandez-Ortega *et al.,* 2004). The control of production and secretion of TNF is highly important because studies have demonstrated that in excess it may cause severe inflammatory damage and toxicity. Tumor necrosis factor would serve as a possible method for HIV-1 therapy and other diseases when its release is regulated. Other inflammatory cytokines such as IL-6 and IL-8 which aid in the up regulation of viral replication have also been found to be induced by TNF (Fernandez-Ortega *et al.,* 2004). The ability of TNF to stimulate production of anti-inflammatory cytokine IL-10, consequently preventing further inflammation by causing the inhibition of TNF has also been indicated in other studies (Leghmari *et al.,* 2008).

In this study, the treatment naïve HIV patients were found to have significantly higher levels of interleukin 6 (IL-6) compared with HIV negative patients and HIV patients on HAART, a finding that agrees with a study by Tudela *et al.,* (2014). Studies have shown that high plasma interleukin-6 levels are associated with morbidity and mortality in HIV patients (Duprez *et al.,* 2012; Kuller *et al.,* 2008; Fuster *et al.,* 2014). Plasma levels of IL-6 were also observed to be higher in HIV infected individuals in a study by Martyn *et al.,* (2015). The HIV negative patients in this study were found to have significantly low concentration levels of IL-6 compared with HIV patients on HAART. This observation agrees with Tudela *et al.,* (2014) who found significant increase of IL-6 in participants positive for HIV infection and another one by Haissman *et al.,* (2009) who also observed significantly higher levels of IL-6 in HIV infected individuals compared with HIV-uninfected. Haissman *et al.,* (2009) also observed that IL-6 decreased after administration of antiretroviral therapy.

IL-6 has pro- as well as anti-inflammatory properties and is a major player in hematopoiesis, as well as in acute phase and immune responses. It is commonly produced at local tissue sites and released into circulation in almost all situations of homeostatic perturbation (Heinrich *et al.,* 2003). In addition to its importance in the generation of immunity against chronic intracellular infections, circulating IL-6, together with TNF- and IL-1, is said to be required for the induction of acute phase reactions that includes fever, corticosterone release, and hepatic production of acute phase proteins many of which are protease inhibitors (Xing *et al.,* 1998).

A certain study showed that the induction by IL-6 by live HIV preparations occurred in the absence of T cells and could be neutralized by human anti-HIV serum indicating that HIV was responsible for this IL-6 inducing activity. Other studies have demonstrated that IL-6 can be produced by a variety of cells upon various kinds of stimulation: for example, monocyte/macrophages, one of the target cells of HIV, produced IL-6 upon stimulation with both live and inactivated HIV (Nakajima *et al.,* 1989). Several other studies have shown the increase of IL-6 expression within HIV infected cells, for example, a study by Miles *et al.,* (1990) found that IL-6 might serve as a growth factor for the HIV virus, thus encouraging HIV replication and proliferation.

In this study, the detectable IL-1β levels in treatment naïve HIV patients was significantly higher than in the HIV negative patients and in HIV patients on HAART. The HIV patients on HAART had higher IL-1β levels compared with HIV negative patients. These findings agree with Lindi *et*

al., (2010) who found increased levels of IL-1β during acute HIV infection compared with pre-infection samples but differ with an observation by Martyn *et al.,* 2015) who found lower levels of IL-β in HIV infected individuals compared with controls. IL-1β plays a crucial role in triggering the immune response during various diseases. It has been shown that IL-1β is an important mediator during systemic (Okusawa *et al.,* 1988; Ohlsson *et al.,* 1990) as well as local inflammation (Dayer and Fenner, 1992), and it may also be involved in the development of autoimmune diseases (Brennan and Feldmann,1992). This cytokine is thought to have an inducing effect on Kaposi's sarcoma in association with AIDS (Louie *et al.,* 1995) and possibly contributes to several other secondary complications during the course of HIV disease, such as neurological (Weiss and Sundar, 1992) and haematological disorders (Maury and Labdevirta, 1990). Additionally, it has been shown that IL-1 can induce HIV viral replication (Folks *et al.,* 1987; Granowitz *et al.,* 1991).

Interleukin 1 (IL-1) is a pro-inflammatory cytokine that plays an important role in host defense by inducing acute and chronic inflammation through activation of both arms of the innate and acquired immune systems (Nambu and Nakae, 2010). IL-1 is produced by activation of mononuclear phagocytes as well as microglia in the brain in response to normal immune stimuli (Burchett *et al.,* 1998). It has been reported that IL-1 will be produced by either the binding of gp120 to the CD4 molecules on mononuclear phagocytes or infection with HIV (Merrill *et al.,* 1989; Cheung *et al.,* 2008).

Increased levels of IL-1 have been demonstrated in some patients with HIV-1 (Lepe-Zuniga *et al.,* 1987). High levels of IL-1 (Lepe-Zuniga *et al.,* 1987; Weiss *et al.,* 1989; Molina *et al.,* 1989; Emilie *et al.,* 1990) are produced in the supernatant of cultured peripheral blood monocyte early in the onset of HIV disease (Lepe-Zuniga *et al.,* 1987).HIV virus has been shown to be present in mononuclear phagocytes and in the blood and brain of AIDS patients. Production of IL-1 from mononuclear phagocytes after stimulation with HIV-1 has been suggested to contribute to some of the symptoms of AIDS such as fever, cachexia and aseptic meningitis (Merill *et al.,* 1989).

It has been suggested that chronic infection and viral latency are typical of HIV-1 infection and that stimulation with IL-1β can stimulate viral replication in chronically infected cells (Devadas *et al.,* 2004). Monocytes are said to be major reservoirs for HIV-1 in infected tissue and vectors for virus transmission to target cells, as well as sources of effective cytokines that can affect cell function and virus replication (Devadas *et al.,* 2004). It is well established that the clinical manifestations of AIDS include both immunologic and neurologic disorders. It has been shown that in the brain, IL-1 induces activation and proliferation of astrocytes (Merrill *et al.,* 1989).

Studies have implicated IL-1 in the pathogenesis of HIV associated dementia (HAD) (Kaul *et al.,* 2001). While IL-1β is said to be highly expressed in the central nervous system of individuals with HAD, it is also said to correlate with neuronal injury and are implicated in the pathogenesis of HAD (Epstein and Gendelman, 1993); Brabers and Nottet, 2006). Microglia and macrophages in the brain are said to release IL-1β after stimulation with HIV-1 envelop protein gp120 (Wahl *et al.,* 1989; Merrill *et al.,* 1992), which increases in the brain during HIV (Tyor *et al.,* 1992) and

has been shown to be elevated in cerebral spinal fluid during HIV infection (Gallo *et al.,* 1989). It has been observed that about 25% of subjects with HIV will develop dementia, particularly HIV encephalitis, (HIVE) which can occur in spite of the use of HAART (Levy, 2007).

Macrophage inflammatory products that include IL-1β have been demonstrated in HIV related encephalitis in mouse and human brain tissue (Persidshy *et al.,* 1997). It has been suggested that the release of neurotoxins, including L-cysteine, from macrophages in the brain is mediated by IL-1 following stimulation of the macrophages by the HIV membrane protein gp120. L-cysteine is said to be released from human monocyte derived macrophages stimulated by either gp120 (Lipton, 1998); or by IL-1 (Yeh *et al.,* 2000). There have been suggestions that cytokines including IL-1 may mediate the neurotoxic actions of gp120. Consequently, immune activation of macrophages in the brain without direct HIV infection may lead to neural damage (Yeh *et al.,* 2000). Evaluation of brain tissue from HIV encephalitis cases showed increased IL1β in the frontal white matter (Maek-A-Nantawat *et al.,* 2007). In addition, IL-1β expression was detected in HIVp24-positive cells in the HIV encephalitis patients, which indicated that IL-1β was induced by HIV- 1 infection. It was concluded that a macrophage/microglia lineage is the main cell type to release cytokines in HIV encephalitis and that IL-1β expressed by HIV-1 infected cells may be one of the important factors for induction of HIV encephalitis (Maek-A-Nantawat *et al.,* 2007).

This study found higher levels of pro inflammation cytokines, tumor necrosis factor (TNF) and interleukin - 1β (IL-1β) among the treatment naïve HIV patients compared with HIV negative

patients and HIV patients on HAART. Inflammatory cytokines play a central role in the pathogenesis of anemia (Parinthi and Kulkarni, 2012). The inflammatory state seen during HIV infection may have several possible causes. Tumor necrosis factor, IL-1 and interferon gamma have all been shown to inhibit erythropoiesis in vitro (Henry and Hoxie, 2005). TNF levels have been shown to be consistently elevated in HIV infection and correlate with viral load (Coyle, 1997). The elevated TNF and IL-1β plasma level is probably determined by a synergistically harmful interaction of HIV-related and HIV-unrelated morbidity (Borges *et al.,* 2014). TNF levels have been shown to be consistently elevated in HIV infection (Coyle, 1997).Blood monocytes and resident macrophages have been found to be important in *vivo* cell targets for HIV infection and their role AIDS pathogenesis are well recognized. These cells of innate immune defences usually survive HIV infection, serve as a major virus reservoir, and function as immunoregulatory cells through secretion of several pro-inflammatory cytokines in response to HIV infection, thereby recruiting and activating new target cells for the virus, including CD4 T cells (Vishnawath *et al.,* 2011).

5.2 Hematological Profiles

Recognition of hematological features during the various stages of human immunodeficiency virus (HIV) infection, particularly in recently diagnosed HIV patients, is very important with the continuing challenges of monitoring HIV disease progression. Disorders of hematopoietic system are common but often overlooked complications of HIV infection which manifest at any stage of the disease (Sujata *et al.,* 2013).

The treatment naïve HIV patients in this study had significantly lower hemoglobin (HGB (g/dl;) levels compared with HIV patients on HAART and HIV negative patients which could be an indication of anemia. Low hemoglobin has also been reported by Friel and Scadden (2011) among the HIV positive patients, and observed that the yearly incidence of developing anemia increases with disease progression affecting patients with asymptomatic HIV infection. The findings of this study are also in agreement with another study by Daniel and Evelyn, (2011) that found low mean values of white blood cells (WBC (10^9/L;) and hemoglobin (HGB (g/dl;) in patients that were positive for HIV compared with patients that were HIV negative. Parinitha and Kulkarni (2012) also reported low mean values of hemoglobin in patients that were HIV positive. Findings from this study also confirms a study by Dangana *et al.,* (2010) who found low hemoglobin levels in HIV positive patients. In some patients, without other complicating sickness HIV infection alone might produce anemia. Serum immunoreactive erythropoietin in patients infected with HIV was studied in various stages of the disease. The study showed that the hormone levels failed to rise with increased anemia which suggested that one cause of anemia might be insufficient amounts of erythropoietin (Spivak *et al.,* 1989). There has been other suggestion that hematopoiesis may be inhibited by soluble factors in the serum of HIV infected persons or that direct infection with HIV of marrow progenitor cells might play a role in anemia production in addition to other hematological abnormalities linked to infection with HIV (Stella *et al.,* 1987; Folks *et al.,* 1988).

The HIV negative patients in this study had significantly higher mean hematocrit (HCT (%) and hemoglobin (HGB (g/dl) compared with the means in HIV patients on HAART and treatment naïve HIV patients which agrees with a study by Ositadimma *et al.,* (2016). The low hematocrit

of HIV patients on HAART and treatment naïve HIV patients in this study may be indicative of anemia. Richman *et al.,* (1987) also reported reduction in hemoglobin levels in HIV patients receiving therapy. Studies have shown that marrow erythroid hypoplasia, aplasia, and megaloblastic maturation have developed as a result of antiretroviral therapy (Walker *et al.*, 1988). Other studies have demonstrated that anemia is less common in patients with less advanced HIV disease (Volberding *et al.,* 1990; Collier *et al.,* 1990). Because the purpose of red blood cells is to transfer oxygen from the lungs to body tissues, a blood sample's hematocrit can become a point of reference of its capability of delivering oxygen. Additionally, hematocrit levels may expose possible diseases in individuals.

The treatment naïve patients in this study had lower means of white blood cells (WBC 10^9/L) suggestive of leucopenia, and also lower means of red blood cells (RBC 10^{12}/L), indicative of anemia, compared with HIV patients on HAART and HIV negative patients. These findings agrees with a study by Ositadimma *et al.,* (2016), that found low white blood cell counts (WBC 10^9/L) in HIV patients on HAART and in patients that were HIV positive compared to patients that were negative for HIV. Higher prevalence of leucopenia was also observed in treatment naïve HIV patients in a study by Sujata *et al.,* (2013). Findings of this study support a previous study by Anyaechie *et al.,* (2005) which showed a distinct pattern of leucopenia in individuals infected with HIV, thereafter concluding that a reduction in leucocyte profiles may be a further indicator of infection with HIV. An ineffective erythropoiesis or a decreased red blood cells production might be the cause of the low red blood cells (RBC). The source of anemia in HIV has been said to be multifactorial (Parinthi and Kulkarni, 2012): the pathophysiology of HIV-associated anemia may involve decreased red blood cells production, increased red blood cells

destruction and ineffective red blood cells production (Paul *et al.*, 2004). Anemia has been shown to be a statistically significant predictor of progression to AIDS and is independently associated with increased risk of death in patients with HIV (Daniel and Evelyn, 2011).

This study found that the mean platelets (PLT (10^9/L) were significantly higher in treatment naïve HIV patients, an indication of thrombocytosis, when compared to HIV patients on HAART and HIV negative patients. Higher platelets in treatment naïve HIV patients were confirmed from earlier works (Attili *et al.,* 2008; Dikshit *et al.,* 2009); Daniel and Evelyn, (2011) also found higher means of platelets in HIV positive patients thus agreeing with this study. However, these findings differs with studies done by Kaslow *et al.,* (1987); Murphy *et al.,* (1987) and Jost *et al.,* (1988) who associated thrombocytopenia with HIV infection. High platelet counts may occur as symptoms of another disease or existing condition (secondary or reactive thrombocytosis) such as anemia and inflammatory conditions.

In this study, significantly low mean volume of erythrocytes (MCV), significantly low mean content of hemoglobin (MCH) and significantly low mean concentration of hemoglobin (MCHC) of treatment naïve HIV patients were observed compared with the means of HIV negative patients and HIV patients on HAART; the treatment naïve HIV patients had higher mean relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and relative distribution width of red blood cells by volume, standard deviation (RDW-SD) compared to those of HIV negative patients and of HIV patients on HAART. Parinthi and Kulkarni (2012) reported higher mean volume of erythrocyte (MCV) but Tripathi *et al.,* 2005)

reported lower MCV. However, the observation in this study is in agreement with a study by Wankah *et al.,* (2014), and with another study by Enawgaw *et al.,* (2014) who found lower mean content of hemoglobin (MCH); lower mean concentration of hemoglobin (MCHC) and also lower mean volume of erythrocytes (MCV); and also found higher mean relative distribution width of red blood cells by volume (RDW) in treatment naïve HIV patients compared to HIV patients on HAART. Another study by Tripathi *et al.,* (2005) reported a high mean relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV), an indication of mild anisocytosis; they also reported low mean content of hemoglobin (MCH) and mean concentration of hemoglobin (MCHC) in treatment naïve HIV patients which is in agreement with this study. The low mean in mean volume of erythrocytes (MCV) and mean content of hemoglobin (MCH) in treatment naïve HIV patients compared to HIV negative patients may indicate that HIV positive patients experience microcytic hypochromic anemia conditions (Daniel and Evelyn, 2011).

This study showed significant differences between the means of the mean platelet volume (MPV) and the mean of the relative width of the distribution of platelets (PDW) between the study groups, while no significance difference of the means of platelet crit (PCT) was observed. There was significantly higher means of mean platelet volume (MPV) and of relative width of the distribution of platelets PDW in treatment naïve HIV patients compared to the HIV negative patients and HIV patients on HAART. This observation agrees with Enawgaw *et al.,* (2014) who found higher mean platelet volume (MPV) in treatment naïve HIV patients compared to HIV negatives and HIV patients on HAART.

5.3 Associations between immunological and hematological parameters

This study found that the mean CD4 T cell counts of treatment naïve patients was significantly lower compared to those of the HIV patients on HAART and of the HIV negative patients, an observation similar to a study by Tudela *et al.,* (2014). Studies have shown that from the moment HIV first enters the body, it triggers immune activation (Liz, 2010). One of the earliest immunological abnormalities of HIV infection is the reduction in absolute number of CD4 T cells and is said to be the most significant predictive indicator for risk of opportunistic infections development (Henry and Hoxie, 2005). Weak correlations between CD4 T cell counts and the blood parameters and blood indices were observed in this study. These findings agrees with those of Nwabuko *et al.,* (2013) that found weak correlations between the CD4 T cells count and white blood cells; between CD4 T cells count and platelets and between CD4 T cells count and hematocrit in the HIV negative and in HIV positive patients.

When comparing the associations between the CD4 T cell counts and the levels of the circulatory cytokines in the study population, associations were observed, in which several were identical. For example, the p-value of association between IL-12p70 and white blood cell counts was p=0.331 and the p-value of association between IL-6 and white blood cell counts was p=0.331. These observations agree with a study by Borges *et al.,* (2015) and another one by Bastard *et al.,* (2012). Given the nature of CD4 T cell counts as important biomarkers for HIV, these findings showed that the CD4 T cell counts were significantly lower in the treatment naïve HIV patients compared to the HIV negative patients. This may contribute to the varied associations observed in this study.

This study found several identical associations between circulatory cytokines and CD4 T cell counts; and between circulatory cytokines and hematological parameters. Several studies have found some associations of circulatory cytokines and CD4 T cells; and between cytokines and blood parameters during HIV infection, and is in agreement with this study. For instance Borges *et al.* (2015), found higher IL-6 when the CD4 T cell counts were low, Attili *et al.,* (2008) found associations between CD4 T cells count and several blood parameters and indices while Haissman *et al.,* (2009) found that IL-10 correlates with CD4 T cells count. Lowered CD4 T cell counts exposes patients infected with HIV to an increased risk of opportunistic infections. HIV disease is typically accompanied by changes in the synthesis and secretion of several immunologically significant cytokines, even though in humans the HIV is the etiological agent of AIDS (Iannello *et al.,* 2010). The disturbances of these cytokines play important roles in the progression of the human immunodeficiency virus disease to acquired immunodeficiency syndrome (Soufian *et al.,* 2012). Findings of this study might imply that there are many determinants that can compel different patterns of associations between immunological and hematological parameters which could be useful biomarkers for HIV pathogenesis.

This study found that the early stages of HIV infections (treatment naïve) are characterized by varying levels of different types of circulatory cytokines, both pro-inflammatory and anti-inflammatory, although the former appear more elevated than the latter; the Th2 cytokines seems to dominate this stage of infection. The inflammatory responses may be as a result of a complex interplay of many cell types using chemical messengers to communicate among themselves, hence forming heightened chains of command and feedback loops. The concentrations of circulatory cytokines during HIV infection before the start of antiretroviral therapy (HAART

naïve) could be informative biomarkers in HIV disease whereas the pro - inflammatory cytokines: Interleukin 1β (IL-1β) and Tumor Necrosis Factor (TNF) could be more informative as biomarkers of the disease. The administration of antiretroviral therapy to HIV patients alters the concentration levels of the secreted circulatory cytokines.

Blood parameters that include the white blood cells (WBC 10^9/L), hematocrit (HCT), hemoglobin (HGB g/dl) and platelets (PLT 10^9/L) may be more predictive biomarkers during early or acute HIV infection. The following parameters may also act as indicators of disease progress during this period: the erythrocyte indices that includes the mean content of hemoglobin (MCH), the mean concentration of hemoglobin (MCHC) and the mean volume of erythrocytes (MCV); the platelet indices including the relative width of the distribution of platelets (PDW) and the mean platelet volume (MPV) and the leukocyte indices that includes the relative content of the mixture of monocytes, basophils and eosinophils (Mid%) and the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#).

There are many immunological responses and altered processes such as the depletion of CD4 T cells, inflammation with increased expression of circulatory cytokines and the shifting of normal Th1 response to viral infection to Th2 response going on in the bodies of newly diagnosed HIV patients due to the virus, which might be the cause of unnoticed damages that could eventually have great impacts on individual's wellness and survival, despite the person appearing to be in good health. The effects of the virus on the immune system may not be the only attribute to the conditions seen during HIV infection but might also be due to the immune system's responses to

the virus. Since the immune system uses hundreds of signaling chemicals, some of which that could be traced in the bloodstream, they can be measured as biomarkers to provide clues about immune system activities.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

1. There were differences in means of circulatory cytokine levels among the groups that formed the study population. The higher levels of Tumor Necrosis Factor (TNF) and Interleukin 1-β (IL-1β) among the treatment naïve patients compared to HIV negative and HIV patients on HAART might be an indication of immunological responses to HIV infection being manifested in this group of patients. Tumor Necrosis Factor and Interleukin 1-β could be useful as possible biomarkers of HIV disease progression

2. Differences in means of blood parameters and blood indices were observed among the study groups. The higher mean platelets (PLT), mean relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and the relative width of the distribution of platelets (PDW) including lower mean white blood cells and the absolute content of granulocytes (Gran#) among the treatment naïve HIV patients compared to HIV negative patients and HIV patients on HAART might be an indication of some hematologic abnormalities due to the effect on HIV. These could be useful as possible biomarkers of HIV disease progression.

3. There were several similar associations between circulatory cytokines and blood parameters; between circulatory cytokines and erythrocyte indices; between circulatory cytokines and platelet indices and between circulatory cytokines and leukocyte indices among the HIV negative patients and HIV patients on HAART that were not seen among the treatment naïve HIV patients. The lack of similar associations between the circulatory cytokines and hematological

profiles among the treatment naïve HIV patients might be an indicator of the extent of the virus infection In addition; significant association between cytokines and platelet indices among the treatment naïve patients could also an indicator of certain conditions and a possible biomarker of HIV disease progression.

4. Similar associations between circulatory cytokines and CD4 T cell counts and between circulatory cytokines and hematological profiles were seen among the HIV negative patients and among the HIV patients on HAART that were not seen among the treatment naïve HIV patients. These could be indicators of the presence of the virus in these individuals and could act as possible indicators of HIV disease progression.

6.2 Recommendations

i) This study recommends the use of Interleukin 1 – β (IL-1β), Tumor Necrosis Factor (TNF), Interleukin 12p70 (IL-12p70), Interleukin 6 (IL-6) and Interleukin 10 (IL-10) as biomarkers of HIV disease progression.

ii) This study also recommends the use of platelets and platelet indices as well as erythrocyte indices as biomarkers of HIV disease progression.

iii) The associations between cytokines and hematological profiles are useful indicators of HIV diseases progression. Those that could be useful as biomarkers for HIV progression would include: the associations between interleukin-12p70 and hemoglobin (HGB); and between tumor necrosis factor (TNF) and hemoglobin. Also to include as biomarkers are associations of

cytokines and platelet indices that include the associations between Interleukin – 12p70 (IL-12p70) and mean platelet volume (MPV); between tumor necrosis factor (TNF) and mean platelet volume (MPV); between tumor necrosis factor (TNF) and relative width of the distribution of platelets (PDW); between interleukin – 10 (IL-10) and relative width of the distribution of platelets (PDW); between interleukin – 6 (IL-6) and mean platelet volume (MPV), between interleukin – 6 (IL-6) and relative width of the distribution of platelets (PDW); between interleukin - 1β (IL-1β) and mean platelet volume (MPV) and between interleukin 1- β and IL-1β and relative width of the distribution of platelets (PDW).

iv) The associations between cytokines and CD4 T cell counts and between hematological profiles and CD4 T cell counts could be useful biomarkers of HIV disease progress.

6.3 Suggestions for further research work

i) The correlations of immunological and hematological parameters; and the associations between the same parameters could be studied for a longer period of time, depending on the levels of immunity, using an enrolled group for a longitudinal study that can be monitored at regular basis. It is necessary to find out at which points the associations become similar or dissimilar and to find out the determinants of these scenarios.

ii) It would also be important to match the study population for age groups and /or gender.

iii) Viral loads could be included among the immunological parameters to find out their associations with cytokines, CD4 T cells and hematological parameters. Viral loads of HIV infected patients were not routinely determined at the site during this study.

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APPENDIX 1

PATIENT'S CONFIDNTIAL QUESTIONAIRE

INSTRUCTIONS:

1. DO NOT WRITE YOUR NAME ON THIS QUESTIONAIRE
2. ALL INFORMATION YOU GIVE WILL BE TREATED AS CONFIDENTIAL
3. WHERE THERE ARE BRACKETS, ( ), PUT A TICK, ( ), WHERE APPROPRIATE.
4. GIVE SHORT ANSWERS WHERE NECESSARY

N. B. Your contribution will be highly appreciated

1. Name of your village/estate _____

Division _____ District _____

2. Gender: Male ( ), Female ( ).

3. How old are you?

4. Marital status: Single ( ), Married ( ), Widow ( )

Widower ( ), Separated ( ).

5. What is your occupation? _____

6. Are you willing to undergo a HIV test? Yes ( ), No ( )

7. Why have you decided to have a HIV test?

- Ongoing campaigns on free HIV testing ( )
- Questioning my recent behavior ( )
- Questioning my partner's recent behavior ( )
- Feeling unwell in past few days/weeks ( )

- Others

_____

_____

8. Are you psychologically prepared for whichever outcome of the results? Yes ( ), No ( ).

PATIENTS ACCEPTANCE COMMITMENT

I accept to participate in this study and sign as follows:
The research team has described to me what is going to be done. I do understand that my decision to participate in this study will not alter my normal healthcare. In the use of information generated from this study, my identity will remain anonymous. I have been informed about the research study in which I an agreeing to participate.

Signature_____ Age_____

Date_____

Candidate's name _____

Witnessed by _____

Date_____

Signature of principal investigator (or authorized representative) _____

APPENDIX 11

APPROVAL TO UNDERTAKE RESEARCH

MINISTRY OF MEDICAL SERVICES

Telegrams: "PROVMED", NAKURU
Telephone: Nakuru 051-2215580-90
When replying please quote
FAX 051 2216497

PROVINCIAL GENERAL HOSPITAL
RIFT VALLEY PROVINCE
P.O. Box 71
NAKURU.

RII/VOL.I/08

Date 29/08/2013

To: Jane N. Mugwe
P.O. Box 1966
Nakuru

Dear Jane Mugwe

RE: APPROVAL TO UNDERTAKE RESEARCH AT THE RIFT VALLEY PROVINCIAL GENERAL HOSPITAL

Reference is made to your letter dated 8th August 2013 seeking approval to conduct a research on "Blood Cytokine and Hematological Profiles as Predictive Biomarkers of HIV and AIDS Progression in Nakuru Provincial General Hospital, Nakuru county."

Permission has been granted/Not granted for the research. It is hoped that you will adhere to the ethics and standards that relate to research at our institution.

Thank you.

Yours sincerely,

MEDICAL SUPERINTENDENT **CHAIRPERSON RESEARCH AND ETHICS COMMITTEE**

MEDICAL SUPERINTENDANT PROVINCIAL GENERAL HOSPITAL-NAKURU
04 SEP 2013
P. O. BOX 71, NAKURU