# INTERFERON-GAMMA AND INTERLUKIN-10 DERANGEMENTS IN HIV-1 ANTIRETROVIRAL NON-ADHERENT PATIENTS ATTENDING SIAYA COUNTY REFERRAL HOSPITAL, KENYA

**Fauzia Musa**

**A Thesis Submitted to the School of Public Health, Biomedical Science and Technology in Partial Fulfillment of the Requirements of the Award of the Degree of Master of Science in Medical Laboratory Sciences (Immunology Option) of Masinde Muliro University of Science and Technology**

**August, 2024**

## DECLARATION

This proposal is my original work prepared with no other than indicated sources and support and has not been presented elsewhere for a degree or any other award.

**Signature…… Date……...……**

Fauzia Musa,

Reg. No: HML/G/01/2016

## CERTIFICATION

We, the undersigned certify that we have read and hereby recommend for acceptance by the School of Public Health, Biomedical Sciences and Technology Masinde Muliro University of Science and Technology thesis titled ***"Interferon-gamma and interlukin-10 derangements in HIV-1 antiretroviral non-adherent patients attending Siaya County Referral Hospital, Siaya County Kenya"***

**Signature…… Date……...……**

Nathan M. Shaviya, Ph.D.

Department of Medical Laboratory Sciences

Masinde Muliro University of Science and Technology

**Signature…… Date……...……**

George A. Sowayi, MSc

Department of medical laboratory sciences

Masinde Muliro University of Science and Technology

## COPYRIGHT

This thesis is copyright materials protected under the Berne Convention, the Copyright Act 1999 and other international and national enactments in that behalf, on intellectual property. It may not be reproduced by any means in full or in part except for short extracts in fair dealing so for research or private study, critical scholarly review or discourse with acknowledgment, with the written permission of the Dean School of Graduate Studies on behalf of both the author and Masinde Muliro University of Science and Technology.

## DEDICATION

This thesis is dedicated to my family, whose unwavering support and encouragement have been my greatest sources of strength. To my parents, for their endless love and belief in my potential. To my friends, who provided both intellectual and emotional support throughout this journey. And to my mentors, whose guidance and wisdom have shaped my academic pursuits.

This work is a reflection of all the love, patience, and dedication you have shown me. Thank you for being my pillars of support.

## ACKNOWLEDGMENTS

Many individuals have provided unwavering support, guidance and encouragement which made this thesis possible. I am immensely grateful to all of them and take this chance to express my utmost gratitude.

My utmost gratitude goes to Dr. Nathan Shaviya and Mr. George Sowayi for their unwavering assistance, patience, and enlightened direction during my research journey. Their vast knowledge and motivation have been of immense significance in helping me surmount the project's hurdles.

I express my gratitude to Dr. Mustafa Barasa for providing me with constructive feedback and valuable suggestions, which have significantly improved the quality of this research. I am grateful to have had the opportunity to collaborate with my colleagues and peers, namely Erick Barasa, Elizabeth Sisianoi, Eliphalet Otunga, and Kevin Soita. Their companionship has not only made our research more enjoyable but also their contributions through constructive discussions have added immense value in enhancing my exploration. I cherish the privilege of working alongside such a creative group of personalities whose inspiration continues to be instrumental in my growth as an academic researcher! During this taxing time, I express my profound gratitude to my family and friends for their unwavering encouragement and comprehension. To Mr. And Mrs. Musa- My parents, thank you so much for being there with your unfailing support, love, belief in me all along the way!

In conclusion, I would like to convey my sincerest appreciation to Maulana Mweha for their steadfast assistance and endurance during the finalization of this thesis. Their comprehension and motivation have proven to be priceless assets throughout my journey. Thanks to all of you, this accomplishment has been made possible.

## ABSTRACT

Despite significant decline in HIV-1 infection rates from up-scaling of Anti-Retroviral Therapy (ART) accessibility, Kenya is among leading African countries in prevalence. Siaya County has the second-highest prevalence of HIV/AIDS in Kenya. During immune responses to infections, crucial cytokines such as Interferon-gamma (IFN-γ) and interleukin 10 (IL-10) are expressed by CD4+ T-cells. However, their production is dysregulated during HIV infection of these cells. Antiretroviral treatment (ART) aims to reduce viral load and restore balance within the immune system through normalizing IFN-γ levels alongside IL-10 levels while enabling stable CD4+ cell counts. Non-adherence inhibits this process impairing viral suppression that undermines restoration or maintenance of immunity equilibrium. Despite its importance, few studies have analyzed how ART non-adherence affects circulating cytokine levels like IFN-γ and IL-10. Therefore, we conducted a cross-sectional study involving 163 individuals who visited Siaya County Referral Hospital inclusive of ART-naive patients with or without HIV-infection living inside Western Province between October–December 2017. Our research aimed to determine whether there was any effect exerted on clinical therapy outcomes together with measuring changes made among participants' blood samples outlining differing adherence groups: those adherent wholly – 'HIV1+, ART-adherent' vs inadequately treated people - "HIV1 + , Art-nonadherent." HIV-1 status was determined by automated Abbott m2000 System, Viral load was measured using the COBAS® AmpliPrep/COBAS® TaqMan®, while CD4 count and cytokines measurement were done using BD PRESTO and Sandwiched ELISA respectively. The serum IFN-ɤ and IL-10 levels for HIV-1 -ve, HIV-1 [+] ART-naive, HIV-1 [+] ART-adherent, HIV-1 [+] ART-Non-adherent were: (5.73 vs. 5.89; 0.74 vs. 32.26; 2.55 vs. 14.9 and 0.98 vs. 26.73) pg/MmL. The CD4 T cell count was significantly lower in the HIV [+] ART-Naïve group (median, 395.9 IQR 349 copies per microliter of blood) as compared to HIV-1 negative (median 1407.4 IQR 1303 copies per microliter of blood), HIV [+] ART-Adherents, median, 575.4 IQR 374 copies per microliter of blood) and HIV [+] ART-Non-Adherents, median, 423.6, IQR, 252 copies per microliter of blood); $P$ <0.001. Conversely, the viral load was higher in HIV [+] ART-Non-Adherents, median, 4.6, IQR 1.1 copies per microliter of blood), than HIV [+] ART-Adherents, median, 3.4, IQR 1.9 copies per microliter of blood) and HIV [+] ART-Naïve, median, 4.5, IQR, 1.6 copies per microliter of blood); $P$ <0.001. Interleukin 10 (IL-10) levels correlated positively with viral load (ρ= 0.272; $P$=0.004) and inversely with CD4 T cell count (ρ= -0.627; $P$<0.0001) as well as BMI (ρ= -0.376; $P$<0.0001). Nevertheless, there was a negative correlation observed between IFN-γ and both viral load (ρ= -0.326; $P$<0.0001) and BMI (ρ= -0.342; $P$<0.0001). Conversely, a positive correlation was found between IFN-γ and CD4 T cell count (ρ= 0.619; $P$<0.0001). The current study has uncovered that the deficiency in compliance with highly active antiretroviral therapy (HAART) among HIV-1 infected patients is having a substantial impact on the quantities of pro-inflammatory and anti-inflammatory cytokines present within their blood. This discrepancy in cytokine balance could potentially disturb the sensitive equilibrium between these two conflicting factors. It is suggested that monitoring serum levels of IFN-γ and IL-10 may be an effective technique for tracking advancements made regarding HIV-1 infection management.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| APC | Antigen-presenting Cells |
| ART | Anti-Retroviral Therapy |
| ARV's | Anti-Retrovirals |
| BD | Becton, Dickinson |
| CCL14 | C-C motif chemokine ligand 14 |
| CCR5 | C-C chemokine receptor 5 |
| CD4+ | A cluster of Differentiation 4 |
| CHS | Centre for Health Solutions |
| CPE | cytopathic effects |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| HAART | Highly active antiretroviral therapy |
| DNA | Deoxyribonucleic Acid |
| HIV | Human Immunodeficiency Virus |
| IFN-γ | Interferon-gamma |
| IL | Interleukin |
| iNKR | Inhibitory natural killer cell receptors |
| KEMRI | Kenya Medical Research Institute |
| LN | Lymph nodes |
| MDM | Monocyte-derived macrophages |
| MHC | Major Histo-Compatibility |
| NASCOP | National AIDS and STIs Control |
| NK | Natural Killer |
| pDC | plasmacytoid dendritic cells |
| PLHIV | People Living with Human Immunodeficiency Virus |
| RNA | Ribonucleic Acid |
| SCRH | Siaya County Referral Hospital |
| STI | Sexually Transmitted Infections |
| TGF | Tumour Growth Factors |

| | |
|---|---|
| Th | T-Helper |
| TNF-α | Tumor Necrotic Factor |
| UN | United Nations |
| UNAIDS | United Nations Programme on HIV |

## OPERATIONALIZATION DEFINITION OF KEY TERMS

**HIV-1 naive -** HIV-1 positive participants who had not started using ARVs yet

**HIV-1 positive- ARTs adherent-**These are participants who were initiated on ART, they take their medicines every day and on time as prescribed, and keep all medical appointments

**HIV-1 positive- ARTs non-adherent -**Participants who had not taken their ARVs 7 days before the data collection period were considered as non-adherent.

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background to the study

Due to HIV's ability to weaken the immune system, individuals are at risk of developing life-threatening opportunistic infections (Bhatti et al., 2016). As a result of its high prevalence worldwide, particularly in Sub-Saharan Africa where countries like Kenya and Siaya County reside, effective antiretroviral therapy (ART) is critical for controlling transmission and reducing viral replication. This is necessary not only for preventing further spread but also minimizing the impact on victims' immune systems. Considering that two-thirds of people living with HIV/AIDS globally live in sub-Saharan Africa while this region accounts for over 70% of total global burden associated with infection rates; efforts towards implementing ART programs need greater prioritization across these regions than ever before or else consequences could be detrimental. Currently, around twenty million adults aged between fifteen and forty-nine have been diagnosed with HIV within sub-Saharan African alone per Dwyer-Lindgren et al.'s research from 2019 data analysis findings now widely supported by numerous other researchers globally throughout recent years too

According to the Kenya AIDS indicator survey (KAIS, 2017), sub-Saharan Africa ranks Kenya as having the third-highest number of HIV-positive individuals. The National AIDS Control Program's data for 2017 estimates an adult prevalence rate of 4.9%, where females showed a higher prevalence rate at 5.2% compared to males with only 4.5%. Geographically speaking, HIV epidemic in Kenya is dispersed across various locations

with wide-ranging rates - from highest in Siaya County at around21 .3 % and lowest estimated approximately being0 .1 %in Wajir county location-wise

According to NASCOP (2018), the virus that impacts all demographic groups is mainly transmitted through sexual contact. The effective control of infection transmission measures in the population may be compromised by the high prevalence of non-adherence of infected individuals to prescribed ART regimens, which may be the cause of the high HIV prevalence in Siaya County (Essien-Baidoo et al., 2019; Iacob et al., 2017). The cumulative rate of non-adherence to the HIV-1 antiretroviral therapy regimen in Africa is 23%, while the national rate in Kenya is 13% (CHS, 2017b). Siaya County's rate is 22%, which is significantly higher than the national rate (CHS, 2017b).

Numerous studies have demonstrated the effectiveness of antiretroviral therapy (ART) in reducing the incidence of HIV-associated illnesses and mortality rates. Therefore, it is recommended to routinely monitor patients who test positive for HIV to assess their infection progression and response to ART, as stated by several studies including Ahmed et al., 2018; Shoko & Chikobvu, 2019; Tsibris & Hirsch, 2010. This monitoring involves evaluating plasma viral load (VL) levels and assessing CD4-positive T-lymphocyte cell counts. Failure to adhere strictly with prescribed ART regimens has important implications not only on individual healthcare but also on communities at large that are affected by those living with or having AIDS/HIV-related sicknesses

The possibility of HIV developing resistance to currently available antiretroviral medications (ARVs) is a potential outcome that could necessitate more complex and expensive second or third-line ART regimens. In severe cases, it may even result in death

(Chalker et al., 2008). Individuals who are infected with HIV have been observed to experience chronic immunological activation, altered cytokine levels, and constant declines in their CD4 T-cell count (Shebl et al., 2012). It should be noted that the efficacy of these drugs in lessening the impact on the immune system can be jeopardized if ARV treatment discontinues - which must not go overlooked as critically important information.

The nutritional status of adults with HIV/AIDS is a crucial diagnostic and prognostic indicator. Low-income countries' current guidelines recommend monitoring BMI regularly to assess patients' response to ART treatment. Malnutrition in adults can result from several factors such as increased metabolic requirements, malabsorption of essential nutrients, infections, diarrhea, nausea/vomiting and reduced appetite due the effects of the virus on their bodies (Bantie et al., 2024). Compared to healthy individuals who require less energy consumption; asymptomatic adult HIV/AIDS-positive patients need at least an extra 10%, symptomatic ones necessitate between 20-30% more while those recuperating may require up-to-the-minute multiplied intake by about 30%. Sub-Saharan African nations report undernourishment rates for nearly one-fourth (23.7%) residing with the disease condition there (Seid et al.,2023).

Pro-inflammatory and anti-inflammatory cytokines are the two primary categories into which cytokines can be broadly classified (Zhao et al., 2021). CD4+T-lymphocytes (Th cells) and monocytes/macrophages (mØs) are the primary cellular sources of cytokines (Breen, 2002). The immune response against foreign entities, such as HIV infection, is stimulated and inflammatory reactions are enhanced by the production of pro-inflammatory cytokines (Maharaj et al., 2017). Tumour necrosis factor-alpha (TNF-α),

interferon-gamma (IFN-γ), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-8 (IL-8), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), and interleukin-17 (IL-17) are the primary pro-inflammatory cytokines (Enayati et al., 2015).

In contrast, the anti-inflammatory cytokines are a group of immunoregulatory molecules that counteract the effects of pro-inflammatory cytokines, thereby limiting the current level of inflammation (Al-Qahtani et al., 2024). Enayati et al. (2015) have identified IL-6, IL-10, IL-13, and TGF-β as the primary anti-inflammatory cytokines.

Prior research has suggested that individuals infected with HIV have compromised immune systems, as evidenced by the impaired proliferation of CD4+ T-cells. (Noble et al., 2001) observed that this phenomenon is caused by reduced levels of IL12. The subsequent decrease in IL-2 and IFN-γ as a consequence of the reduction in IL-12 leads to immunosuppression.

The development of opportunistic infections is a defining characteristic of HIV progression towards AIDS, facilitated by the immunosuppressive state it creates. Both innate and adaptive immunity are largely regulated by proinflammatory cytokines like IFN-γ and IL-12, which have been shown to impact anti-HIV treatment efficacy in vitro studies when their activity is altered. Evidence suggests that HIV infection leads to a shift from predominantly Th1 immune responses (featuring production of IFN-γ and IL-2) to Th2 responses (marked by increased secretion of IL-4 &IL-10), with increased production of proinflammatory cytokines such as TNFα, typically observed alongside illness (Nittayananta et al.,2014).

Interferon-gamma (IFN-γ) plays multiple roles in the development of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS). IFN-γ production can be detected from the early stage to the end phase of HIV-1 infection in patients. It was initially created to eradicate the initial infection; however, it, along with other inflammatory cytokines, contributes to the development of persistent immune activation, which exacerbates the clinical symptoms of AIDS. In vivo investigations of IFN-γ therapy in patients infected with the virus have demonstrated that the cytokine does not possess any direct antiretroviral effects against HIV-1 in primary cultures, as per a study conducted by Roff et al. (2014). Studies conducted both in vitro and in vivo have demonstrated the ability of IFN-γ to increase HIV-1 proliferation, thereby increasing the risk for related diseases. However, it has also been found that IFN-γ can boost the functionality of natural killer cells and cytotoxic T-lymphocytes when confronted with HIV-infected cells. These activities are crucial for halting viral replication within an individual (Januškevica et al., 2016).

It has been shown that IL-10 plays a critical role in the preservation of immune function equilibrium during a variety of disorders. For example, research conducted on animal models has shown that the deletion of the IL-10 gene can enhance the body's capacity to combat pathogens and significantly accelerate the elimination of viral infections. Nevertheless, in certain infections, these interventions result in a more severe manifestation of the disease. This is due to the immune system's inability to effectively regulate immune-mediated harm and limit the pathogen burden (Kwon et al., 2019). IL-10's function is achieved through its interaction with a variety of cell types, which results in a substantial reduction in the synthesis of nearly all pro-inflammatory cytokines and chemokines.

Furthermore, the expression of genes that encode natural antagonists for these molecules is concurrently stimulated by IL-10. Furthermore, IL-10 has been shown to regulate the expression of certain co-stimulatory molecules, such as CD80 and CD86, as well as MHC class II, thereby significantly influencing the ability of antigen-presenting cells (APC) to activate T cells (Levings et al., 2002).

Critical immune system cells, such as CD4+ T-cells, blood monocytes, dendritic cells and tissue macrophages can be infiltrated by HIV-1. Even without the presence of CD4s, virus-monocyte interaction may stimulate IL-10 production at mRNA and protein levels in CD14+ monocytes. The current discovery suggests that HIV induces the synthesis of the immunosuppressive interleukin-10 (IL-10) in monocytes, irrespective of the presence of CD4 molecules. As per Ji et al. (2005), it is feasible that the interference with HIV entrance through CD4 molecules may not effectively mitigate the impact of IL-10 in the context of HIV infection. Therefore, the analysis of IL-10 and INF-γ can offer valuable information regarding the disease's progression, the extent of the immune response, and the level of immunological activation (Williams et al., 2013). Assessing cytokine levels alongside viral load and CD4+ T-cell counts is an effective tool for evaluating HIV-infected individuals' adherence to prescribed ART regimens.

### 1.2.Problem statement

Since the initial identification of HIV in 1981, the virus has emerged as a significant global health crisis, infecting approximately 77 million individuals worldwide. Currently, around 36.9 million people live with HIV, and the epidemic has resulted in tens of millions of deaths due to AIDS-related complications. HIV/AIDS continues to be a significant health concern in Kenya, causing approximately 29% of adult deaths, 20% of maternal fatalities

and resulting in the demise of around 15% children under five years old. Siaya County has the most substantial burden relating to HIV with an occurrence rate at an alarming high level-21.3%, which is noticeably greater than Kenya's national average figure standing at almost one-fifth (4.9 %).

A major challenge in managing HIV in Siaya County is the high rate of non-adherence to antiretroviral therapy (ART). The county's non-adherence rate is 22%, compared to the national average of 13% and the African average of 23% (CHS, 2017). This high rate of non-adherence undermines efforts to control the spread of HIV. Factors contributing to non-adherence include late detection, stigma, discrimination, difficulty accessing healthcare facilities, adverse effects of medications, comorbidities, psychosocial factors, economic constraints, and the availability of ART facilities(Basti et al., 2017).

Adherence to ART is crucial for its effectiveness. Studies in sub-Saharan Africa show that about 25% of patients discontinue ART within one year, and this rises to 40% after two years. Optimal adherence is defined as taking at least 95% of prescribed doses, while suboptimal adherence is taking 85-94%. Factors influencing adherence include stigma, poor service delivery, medication burden, and drug misuse. Strict adherence to ART is necessary to achieve optimal therapeutic outcomes (MoH, 2018), as non-adherence can lead to viral rebound, acute retroviral syndrome, increased risk of HIV transmission, decreased CD4 counts, accelerated disease progression, and the development of drug resistance.

Monitoring adherence is essential, but current methods largely rely on self-reporting, which can be unreliable. Plasma viral load and CD4 count are used to monitor HIV progression.

Viral load measurement is a more direct indicator of ART effectiveness than CD4 count, which can sometimes be misleading. However, many healthcare facilities in Kenya face significant challenges in performing these tests due to financial constraints, technical limitations, and the labor-intensive nature of the processes. Viral load testing is currently available at only a few facilities, such as Kenya Medical Research Institute (KEMRI) - Kisian in Kisumu County and KEMRI-Alupe in Busia County.

To improve monitoring, it is crucial to develop additional biomarkers that are cost-effective and easy to assay. Cytokines, which play a significant role in regulating HIV replication and the immune response, could be potential biomarkers. Assessing levels of cytokines like interleukin-10 and IFN-γ can provide valuable information about the immune response and viral replication rate. These biomarkers could offer a more accessible and cost-effective method for monitoring ART adherence and HIV progression.

The relationship between cytokine levels and ART adherence is significant. For instance, changes in interleukin-10 and IFN-γ levels can reflect the patient's adherence to ART and the progression of HIV. These biomarkers are cost-effective and simple to test, making them practical for widespread use in resource-limited settings. Evaluating the efficacy of cytokines as biomarkers could enhance the objectivity and reliability of adherence monitoring.

In summary, HIV/AIDS continues to be a major global health issue, with significant impacts in Kenya, particularly in Siaya County. High rates of non-adherence to ART pose challenges to controlling the epidemic. Effective monitoring of adherence and HIV progression is crucial for managing the disease. Developing cost-effective biomarkers like

cytokines could improve the reliability of adherence monitoring and help in better managing HIV/AIDS in resource-limited settings.

### 1.3. Objective of the study

#### 1.3.1. Broad objective

The objective of this study is to assess how non-compliance with ART regimens impacts the levels of INF-γ and IL-10, as well as clinical progression biomarkers for HIV disease in individuals who are not adhering to their treatment. The research will focus on patients living with HIV who attend Siaya County Referral Hospital.

#### 1.3.2. Specific objectives

i. To assess the plasma concentrations of INF-γ and IL-10 in HIV-1 patients who are ART-naive, ART-non-adherent or ART-adherent during their attendance at Siaya County Referral Hospital.

ii. To investigate The CD4+ T-cell count, BMI and HIV-1 viral load of ART-naive, non-adherent and adherent patients with HIV-1 attending Siaya County Referral Hospital will be evaluated to determine their health status.

iii. To establish a correlation between the levels of INF-γ and IL-10 in serum with BMI, CD4+ T-cell count, and HIV-1 viral load among HIV-positive patients who are ART-naive or undergoing non-adherent and adherent ART regimens while receiving medical attention at Siaya County Referral Hospital.

### 1.4. Research questions

i. What are the levels of INF-γ and IL-10 in the plasma of HIV-1 patients who have not adhered to ART treatment at Siaya County Referral Hospital?

ii. What is the difference in CD4+ T-cell counts and viral load (VL) levels between HIV patients who adhere to their ART regimen versus those who do not, at Siaya County Referral Hospital?

**HYPOTHESES**

i. **Ho:** The association of plasma levels of INF-γ and IL-10 with BMI, CD4+ T-cell counts, and viral load does not correspond to ART-naive or non-adherent HIV patients attending Siaya County Referral Hospital.

### 1.5. Justification for the study

Kenya ranks fourth worldwide in terms of high HIV prevalence. The region of Siaya has a consistently high prevalence rate of HIV, with a quarter of the population being afflicted by the virus. The introduction of antiretroviral therapy (ART) causes a gradual reduction in the amount of HIV RNA, which in turn improves the body's immune response and lowers the occurrence of illness and mortality related to HIV/AIDS (Mayer & Venkatesh, 2010). In addition, this phenomenon leads to a reduction in immunological activity associated with HIV, as evidenced by the presence of proinflammatory cytokines and T-cell activation, ultimately causing harm to several organs (Klatt et al., 2013). Nevertheless, our comprehension of cytokine presentation in persons who initiated antiretroviral therapy (ART) but later stopped treatment is still restricted. At present, the assessment of HIV

clinical disease progression heavily relies on two indicators - viral load and CD4 count. While CD4 cell count is a more cost-effective marker than viral load, it does not consistently correspond with either viral load or disease advancement. Therefore, there is a need for alternative indicators that are both dependable and cost-effective to assess the effectiveness of treatment, the advancement of the disease, and the extent to which patients adhere to prescribed antiretroviral medication plans. Although there have been many studies that have thoroughly investigated the connections between IFN-γ and IL-10 in regard to HIV infection in both HIV-1 ART-naive and -experienced patients, a complete knowledge of these interactions has not yet been attained This study sought to investigate how non-adherence to antiretroviral therapy (ART) relates to the levels of interferon-gamma and interleukin-10 in the bloodstream. Specifically, it aimed at gauging whether these biomarkers can effectively differentiate between those who adhere strictly versus those who don't stick with their prescribed ART regimen.

### 1.6. Significance of the Study

The results from this research offer noteworthy data that could assist in identifying further cost-effective markers for predicting the prognosis of HIV illness, in addition to developing a marker for detecting non-adherence to antiretroviral therapy (ART).

This investigation has yielded data that help enhance comprehension of the dynamics and interplay between IFN-γ and IL-10 throughout the progression of HIV infection and its therapy. Study's scope.

The study aimed to track individuals who had stopped their HIV-1 antiretroviral therapy (ART) treatment and those who had continued, both of whom were HIV-positive. The

investigation aimed to evaluate the INF-γ and IL-10 cytokine levels of individuals who agreed to be part of this study. Additionally, CD4 count and HIV viral load were analyzed as markers for monitoring the progression of HIV AIDS in participants aged 18 or older attending Siaya County Referral Hospital. The group included both defaulters and non-defaulters receiving HIV-1 ART regimen treatment.

This study is expected to make a significant contribution towards the attainment of sustainable development objective number 3, which focusses on promoting good health. The utilisation of cytokine levels as objective indicators for monitoring ART adherence leads to improved accuracy in adherence monitoring, hence boosting the effectiveness of HIV ART. By doing this, the quality of life for persons living with HIV will significantly enhance, leading to increased productivity.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Origin and Structure Human Immunodeficiency Virus Human

The high rates of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) worldwide are a major issue, as evidenced by the tremendous number of people impacted. Over 70 million individuals have been affected by HIV up to this point, leading to 35 million fatalities. Presently, approximately 36.7 million individuals are living with the disease, and alarmingly, there are approximately 14,000 new infections reported daily (Fajardo-Ortiz *et al*., 2017). According to estimates, the African continent is home to over 70% of those living with HIV, while Asia accounts for approximately 20% of the affected population. The classification of the HIV places it in the Lentivirus genus, which belongs to the Retroviridae family and the Ortho-retrovirinae subfamily (Lopez *et al*., 2010). HIV is categorized into two subtypes, HIV-1 and HIV-2. These types share genetic characteristics but have unique viral antigen variants. Simian immunodeficiency virus (SIV) belongs to the Lentivirus genus as well and causes an immune deficiency in non-human primates. Research on epidemiology and phylogeny indicates that between 1920 to 1940, humans were first exposed to HIV based on the available evidence so far retrieved from investigations conducted thus far. HIV-1 is believed to have originated from immunodeficiency viruses found in non-human primates, specifically Central African chimpanzees (SIVcpz), while HIV-2 is thought to have evolved from similar viruses found in West African sooty mangabeys (Ling *et al*., 2003).

The HIV-1 virion exhibits a spherical morphology, characterized by a diameter of around 100 nm, which is equivalent to one-tenth of a micron. In accordance with Figure 2.1. The virion is enclosed by an external layer known as the viral envelope, which serves to safeguard the internal constituents of the virus. Additionally, the viral envelope contains specific proteins that facilitate the virus in identifying and binding to novel host cells. The virion contains viral structural proteins which undergo assembly to form a shell, commonly referred to as a capsid. This capsid serves the purpose of enclosing and arranging the viral DNA. The genome of HIV-1 is comprised of two single-stranded RNA molecules, which are positive-sense in nature. Each of these RNA strands contains a comprehensive collection of viral genes. The primary role of the virion is to facilitate the transfer of viral RNA from an infected cell, known as the "producer cell," to a recipient host cell. In the case of HIV-1, the virion can assume two unique morphological states, as described by Pornillos and Ganser-Pornillos in 2008 (Ganser-Pornillos *et al*., 2008). The process of virion formation involves the budding of an immature form from a producer cell. This immature form consists of a spherical capsid that is primarily formed of a precursor structural protein known as Gag (alternatively referred to as p55 or Pr55). The molecular weight of Gag is around 55 kDa. A morphological change from the immature to the mature virion is required for the acquisition of infectivity. Initiation of the phenomena known as "HIV-1 Maturation" occurs when the viral protease processes or cleaves the precursor Gag protein at a specific location ((Briggs & Kräusslich, 2011).

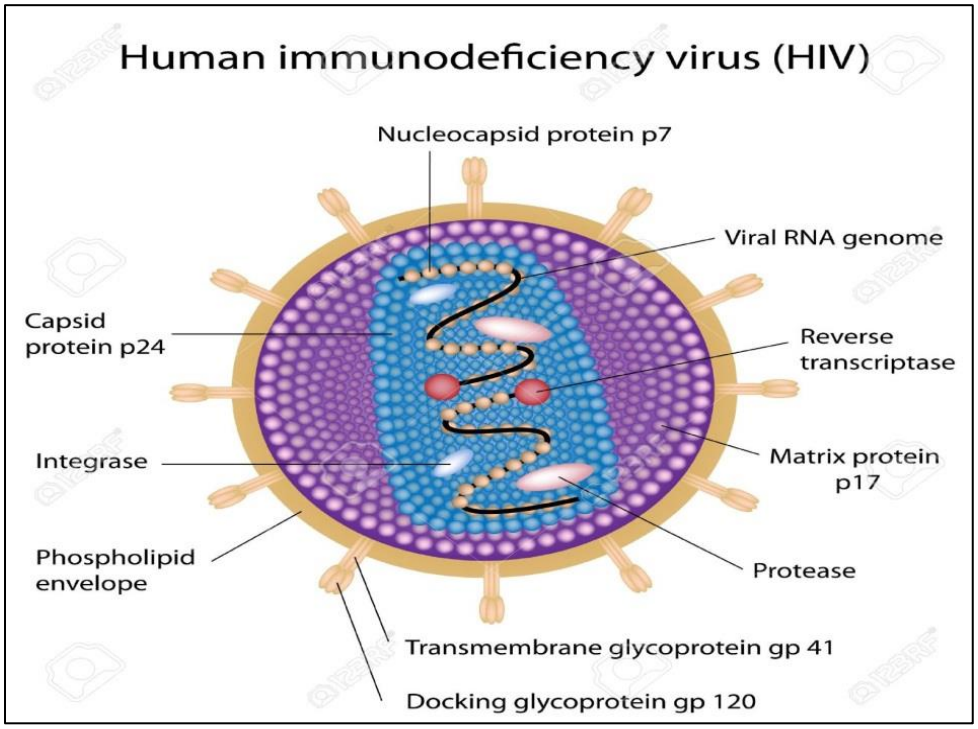



Figure 2: HIV structure

Duane (2009) stated that the structure of HIV includes an enveloped virus with two conspicuous envelope glycoproteins, namely gp120 and gp41. Furthermore, it is made up of two segments of genomic RNA along with numerous enzymes and other crucial viral proteins.

### 2.2 Pathogenesis of HIV

HIV-1 is transferred by sexual contact across mucosal surfaces, as well as through maternal-infant exposure and percutaneous inoculation. The preferred transmission of CCR5-tropic viruses (referred to as R5 viruses) through all pathways remains incompletely understood. The process of transmission is thereafter accompanied by a systematic manifestation of viral and host indicators of infection within the blood plasma (Shaw & Hunter, 2012). The systematic evaluation of the sequential manifestation of laboratory markers in new HIV-1 infection was conducted by Fiebig et al. (2003), who developed a

laboratory staging system for acute and early infection (Fiebig *et al*., 2003). The phase immediately following cellular infection and preceding the initial detection of the virus in the bloodstream is referred to as the eclipse phase. The anticipated duration of this interval ranges from 7 to 21 days, as determined by clinical histories of occurrences involving high-risk exposure (Grazinoli Garrido *et al*., 2020). Activation of natural killer (NK) cells and production of type I interferon (IFN-/) by plasmacytoid dendritic cells (pDC) are two of the most important aspects of innate antiviral responses (Barber, 2001). Significant implications for pathogenesis and the design of an efficient vaccine-induced response may result from HIV-1 infection's effect on both processes. The generation of immunostimulatory substances that aid in the formation of antigen-specific adaptive immune responses is facilitated in part by natural killer (NK) cells, which also play a critical role in the elimination of infected cells (Vivier *et al*., 2008). There is a fine balance between positive and negative signals provided by ligand-receptor systems that controls NK cell activity. Inhibitory natural killer cell receptors (iNKR) have been shown by Sivori *et al*. (2019) to prevent NK cell-mediated cytolysis by recognizing major histocompatibility complex (MHC) class I molecules on target cells (Sivori *et al*., 2019).

CD4+ T cells' ability to specialize into various T helper (Th) cell subsets is pivotal in regulating immunological responses. These subsets of Th cells are essential for immune system function because they recruit and activate other immune cells such B cells, CD8+ T cells, macrophages, and effector cells. CD4+ T lymphocyte functions are mostly determined by the cytokines they produce and where in the body they are located. In a study published in 1986, Mosmann *et al*. divided T cell clones into two groups, $Th_1$ and $Th_2$ (Mosmann *et al*., 1986). IFN, IL-4, and IL-13 are cytokines referred to as "signature

cytokines" which have been observed to be produced by the aforementioned cell subtypes. Th1 cells significantly enhance the cytotoxic effector functions of NK cells, CD8+ T cells, and macrophages. This is crucial in eliminating intracellular pathogens such as viruses residing within host's cellular structures or bacterium that inhabits a similar environment along with protozoan parasites. While these types of T-cells can often be found in peripheral blood samples collected from individuals they tend to occur more frequently throughout lymph nodes (LNs), gastrointestinal tracts (GI) this includes other related tissues typically targeted for detection purposes via various medical diagnostic methods. Memory cells expressing the CCR5 receptor make up a sizable population of CD4+ T lymphocytes in the GI tract, as reported by Poles *et al*. (2001)(Poles *et al*., 2001). It is thought that viruses can easily infect these cells. There is no question that these cells are very receptive to HIV infections in culture.

Direct cytopathic effects (CPE) of the virus are principally responsible for the distinctive decrease in CD4+ T cells that is seen in HIV infection. The main factors to this phenomena have been identified as syncytia formation (viral-induced cell fusion) and premature cell death (Costin, 2007). Measurements of the dynamics of cell growth and lysis indicate that the cytopathic effect is linked mostly to viral generation by infected cells; this includes the development of gigantic syncytial cells. Leonard et al. (1988) report that HIV infection is transient in the vast majority of T4 cells (Leonard *et al*., 1988). However, only a minority (10-30%) of these infected cells express the virus and die after being activated by phytohemagglutinin. This points to the importance of immune activation and cell differentiation in both viral generation and eventual cell death.

### 2.3. The burden of HIV-1 ART Regimen Non-Adherence

ART adherence refers to the extent to which patients follow a specified treatment plan, including the regular and timely consumption of medications and adherence to dietary and medication restrictions ( Stricker et al., 2014; Addo et al., 2022;). The effectiveness of ART hinges on consistent and sustainable adherence to prescribed regimens (Mills et al., 2006). However, a significant minority of HIV-infected individuals fail to achieve optimal adherence, leading to severe public health consequences.

A study in the UK found a non-adherence rate of 58% among HIV patients, especially high in those co-infected with TB (Mukui et al., 2016). In the US, 21% of AIDS patients missed a dose within 24 hours, and 34% skipped a dose within three days, due to factors like dietary restrictions, social stigma, and lifestyle adjustments (Aye et al., 2017). In Cuba, a study of 847 HIV patients showed a non-adherence rate of 29.4% (Aragonés-López et al., 2012).

A meta-analysis by (Mills et al., 2006) reported a global non-adherence rate of 36%, with North America at 45% and Africa at 23%. Despite higher non-adherence in developed nations, adherence rates are slightly better in developing countries. In Africa, a study by (Chime, 2019) found a 23% non-adherence rate. Optimal adherence remains challenging due to the complexity of ART regimens, side effects, stigma, psycho-social factors, clinical environments, and cultural influences (Freeman et al., 2021).

In Sub-Saharan Africa, adherence rates vary: 63% in South Africa (Schatz, 2019), 68% in Uganda (Nabukeera-Barungi et al., 2015), 54% in Nigeria (Anyaike et al., 2019), 24% in Southwest Ethiopia (Tesfaye et al., 2019), and 13% in Cameroon (Fonsah et al., 2017). In

Cameroon, higher adherence was linked to consistent treatment, counseling services, and social and economic support (Fonsah et al., 2017). According to Fonsah et al. (2017), achieving virological suppression of HIV necessitates a minimum adherence level of 80%. Instances when adherence rates fall below this threshold are indicative of suboptimal adherence among patients.

In Kenya, non-adherence rates are inconsistent. The Kenya AIDS Indicator Survey (KAIS) 2012 Report found that 58% of HIV-positive individuals aged 15-64 were eligible for ART. A 2014 study by (Maina et al., 2014), showed that 63% were receiving ART, with 78% achieving viral suppression. Non-adherence rates varied: 36% in Mombasa (Mahmood, 2020), 48% in Nairobi's Kibra slums (Wekesa, 2019), 44.2% in Eldoret, and 18% in Nairobi (Kioko & Pertet, 2017); Mahmood et al., 2020; (Wekesa, 2019). A 2017 CHS study in Siaya found a 22% non-adherence rate, with barriers including non-disclosure of HIV status, alcohol use, low literacy, medication side effects, social stigma, and distance from healthcare facilities (Adeniyi et al., 2018); (Ahmed et al., 2018); (Oluoch et al., 2019).

Non-compliance with ART is a complex issue that challenges long-term patient monitoring. A multidisciplinary approach is recommended to address this issue (Iacob et al., 2017). Besides reducing illness and death, achieving viral suppression is a secondary goal of HIV treatment. Poor adherence correlates with reduced efficacy in viral suppression, posing immediate health risks and increasing the likelihood of developing resistance to treatment (Bangsberg, 2006). This could impact treatment costs and the range of available therapeutic options.

The factors contributing to suboptimal adherence to ART are multifaceted and include the complexity of therapeutic regimens, such as the burden of pill consumption and frequency of dosing. Treatment-related side effects, inadequate health literacy, subpar patient-physician rapport, and restricted availability of ART due to formulary limitations or financial constraints also play significant roles (Schaecher, 2013). Stigma, both social and self-imposed, remains a considerable barrier to adherence, as individuals may fear discrimination or judgment from others. This stigma can deter patients from seeking treatment or adhering to their medication regimen.

Furthermore, psycho-social factors such as depression and lack of social support can negatively impact adherence. Clinical environments that are not patient-friendly or do not provide adequate counseling and support services can also hinder adherence. Cultural influences, including traditional beliefs and practices, may conflict with ART regimens and affect patients' willingness to adhere.

To improve ART adherence, interventions must address these barriers comprehensively. Strategies may include simplifying ART regimens, enhancing patient education about the importance of adherence, and providing robust support systems. Health care providers should focus on building strong, trustful relationships with patients and creating an environment that reduces stigma and encourages open communication.

Innovative approaches, such as the use of technology for reminders and support, peer support programs, and community-based interventions, have shown promise in improving adherence rates. Additionally, addressing socio-economic barriers by ensuring access to affordable medications and healthcare services is crucial.

In conclusion, while ART adherence is vital for the effectiveness of HIV treatment, achieving optimal adherence remains challenging due to various factors. Improving adherence rates and enhancing the health outcomes of individuals living with HIV requires a comprehensive strategy that encompasses patient education, support systems, reducing stigma, and improving healthcare access.

### 2.4. Human Immunodeficiency virus infection and cytokines

Cytokines are a class of signaling proteins that interact with specific receptors to modulate the process of cell differentiation, particularly within the immune system. These polypeptides exert their effects on signaling molecules and cells, inducing their activation and migration towards regions of inflammation, infections, and traumas. They also modulate main lymphocyte growth factors and other biological processes. The excessive activation or amplification of host defense mechanisms, as shown in certain cases of infective and sterile inflammation, can lead to tissue harm due to the actions of these cytokines (Holdsworth & Gan, 2015). Throughout the course of chronic HIV infection, CD4 and CD8 T cell immunological activation is impacted by both homeostatic mechanisms and inflammation resulting from HIV. The aforementioned disparities are evident in the manners by which these T cell populations react to the inflammatory and homeostatic milieu (Le Saout *et al*., 2012).

Based on the findings of structural research, it has been observed that a significant proportion of cytokines can be categorized into one of four distinct types. The hematopoietin family has a role in the process of hematopoiesis. For instance, IL-2, also known as interleukin-2, and the interferon family exhibit a particular role of impeding viral

replication within the cellular environment of a host organism. As an example, IFN-γ, the chemokine group (including CCL14), and the tumor necrosis factor cluster (with abilities to induce cell death and carry out diverse functions). For example, the study conducted by Reeves et al. (2018) investigated the role of TNF-α (Reeves *et al*., 2018).

HIV infection causes changes in both the cytokine profile inside the body (in vivo) and in test tubes (in vitro). Th1 cytokines like IL-2 and IFN-gamma are often suppressed during HIV-1 infection. Meanwhile, there is an increase in Th2 cytokines such as IL-4 and IL-10, while pro-inflammatory cytokines including TNF-alpha, IL-6,IL8,and Interleukins 1 decrease significantly. Impaired cell-mediated immunity, brought on by cytokine overproduction, contributes to the disease's origin (Kedzierska & Crowe, 2001). Multiple cytokines can alter CD4 T lymphocytes and macrophage lineage cells to regulate HIV-1 infection and replication *in vitro* (Künzli & Masopust, 2023). The category of "HIV-inducing cytokines" encompasses several molecules, such as TNF-alpha, TNF-beta, IL-1 and IL-6. Both T cells and monocyte-derived macrophages (MDM) are vulnerable to having their HIV-1 replication stimulated by these particular cytokines. Other cytokines like IL2, 7 or 15 have also been linked with the upregulation of HIV-1 in T cells while macrophage-colony stimulating factor alternatively named MCSF has been found effective in promoting MDM's proliferation of HIV-I virus. Inhibitors of HIV-1 replication in T cells and monocyte-derived macrophages (MDM) include the HIV-suppressive cytokines IFN-alpha, IFN-beta, IL-16, IL-10, and IL-13. Iketleng *et al*. (2016) showed that IFN-gamma, IL-4, and granulocyte-macrophage colony-stimulating factors are all bifunctional cytokines with inhibitory and stimulatory effects on HIV-1(Iketleng *et al*., 2016).

A shift from a predominantly T-helper type 1 (Th1) to T-helper type 2 (Th2) immune response has been associated to HIV infection, according to a number of studies. Interleukin (IL)-2 and interferon (IFN)-y production decreases during this phase, while IL-4 and IL-10 secretion increases (Clerici & Shearer, 1993). Additionally, there is an elevated production of proinflammatory cytokines such as IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α.

Interleukin-10 (IL-10), interferon-gamma (IFN-y), and interleukin-2 (IL-2) are three of the most important cytokines that have been associated to HIV infection and illness progression (Katsikis *et al*., 2011). Interleukin-2 promotes the rapid and selective expansion of effector T cell populations, such as CD4+ and CD8+ T cells, after they have been activated by antigen, as stated by (Boyman & Sprent, 2012). Significant effects on cellular metabolism and glycolysis, processes essential to the long-term health of T cells, have also been identified. Natural killer (NK) cells benefit from interleukin-2 (IL-2) because it promotes their expansion and survival. IL-2 is also important because it stimulates the production of cytokines including tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-), and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are produced by natural killer (NK) cells (Moro-García *et al*., 2018). Furthermore, it has been noted that IL-2 and IL-12 together exhibit a synergistic impact, leading to an increase in natural killer (NK) cell cytotoxicity (Maia *et al*., 2024).

TGF-β1, a cytokine with both anti-inflammatory and profibrotic effects, may be a contributing factor to the increased prevalence of non-AIDS-related ailments and extended immunosuppression. This cytokine is consistently elevated in both untreated and virally suppressed individuals infected with HIV, as indicated by Theron *et al*. (2017)(Theron *et*

*al*., 2017). T regulatory cells (Tregs) exert their suppressive roles by multiple ways, one of which involves the production of TGF-β1. Additionally, Tregs produce IL-10, which is a broadly active anti-inflammatory cytokine (Shalev *et al*., 2011). TGF-β1 is usually a protective cytokine that possesses anti-inflammatory properties. However, overproduction of the same can result in severe pathological consequences. Various research studies have revealed increased levels of TGF-β1 among HIV-1 infected individuals' bloodstream due to augmented presence of regulatory T cells (Tregs). These findings suggest that these cells are likely responsible for producing this cytokine primarily. According to Choi et al.'s observations (2021), patients with CD4 counts below 200 cells/μL exhibit exceptionally high amounts of TGF-β1 concentration indicative and negatively correlated with both circulating CD8 and CD4 cell counts but no significant relationship observed concerning their HIV viral load levels.

### 2.5 Effects of HIV ART Non-Adherence on Cytokines

Failure to adhere to antiretroviral therapy (ART) could potentially result in negative outcomes for individuals living with HIV and their immediate social circle. The reported outcomes include the development of resistance to present treatment regimens, as well as the hastening of disease progression. The cytokine profile experiences a shift due to HIV infection in both laboratory and within the body. Individuals infected with HIV-1 typically exhibit reduced levels of T-helper type 1 cytokines, such as IL-2 and INF-γ. Conversely, there is an increase observed in production of T-helper type 2 cytokines like IL-4 and IL-10 alongside pro-inflammatory ones like TNF-α,IL-6,IL8&and I L-. Irregular generation of these molecules hampers cellular immunity leading to disease progression. Past studies

have indicated that distinct types may regulate replication & transmission rates for CD4-T cells or macrophages affected by this condition (Kedzierska & Crowe 2001).

IL-10 is a cytokine with anti-inflammatory properties that has a crucial function in controlling immunological responses. Within the realm of HIV, T cells that produce IL-10, namely CD8+ T cells, have been observed to hinder the destructive activity of other T cells and restrain the generation of pro-inflammatory cytokines such as IL-2 (Elrefaei et al., 2007). This inhibition can contribute to immunological dysregulation observed in HIV infection, potentially resulting in a reduced response to antiretroviral therapy (ART) and increased non-adherence due to the perception of treatment inefficacy or adverse effects. According to studies, an association exists between higher quantities of T cells that produce IL-10 and the advancement of HIV disease. This indicates that IL-10 may assist in maintaining the virus' presence while evading immune system detection (Elrefaei et al., 2007; Mohammadi et al., 2023). Moreover, having these regulatory T cells could weaken the ability of one's immune system to appropriately respond to ART medication, potentially aggravating adherence challenges.

Alternatively, IFN-γ is a cytokine that supports inflammation and holds significant importance in shielding the body from viruses. By stimulating CD4+ and CD8+ T cells activation alongside an increase in major histocompatibility complex (MHC) molecules production, it enhances immune responses. These molecules are crucial for T cells to identify infected cells. Although IFN-γ signalling is crucial for controlling HIV replication, an excessive or unregulated IFN-γ response can cause immune activation. This immune

activation can potentially lead to non-adherence in patients, especially if they experience negative side effects from antiretroviral therapy (ART) or if they believe that their treatment is ineffective due to ongoing immune activation (Duggal et al., 2012).

The interaction between IL-10 and IFN-γ plays a crucial role in determining the immunological milieu in patients with HIV infection. Antiretroviral medicines such as tenofovir have been demonstrated to hinder the generation of IL-10, which may result in a shift towards a more pro-inflammatory condition (Hughes et al., 2020). This transition has the potential to cause a rise in immune activation, which, although advantageous in certain situations, might also lead to intensified adverse effects from antiretroviral therapy (ART) and, as a result, decreased compliance. Furthermore, the existence of IL-10 can mitigate the impact of IFN-γ, resulting in a less than ideal immune response against the virus. This implies that the equilibrium between these cytokines has a vital role in defining both the efficacy of ART and the compliance with treatment protocols.

Thus, both IL-10 and IFN-γ have a substantial impact on ARV non-adherence by modulating immune modulation and inflammation. The immunosuppressive qualities of IL-10 may decrease the effectiveness of ART, whereas the role of IFN-γ in boosting immunological activation can cause negative consequences that discourage adherence. Gaining insight into these processes is crucial for formulating methods to enhance treatment adherence among those who have HIV.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Study site

Patients were recruited from the Siaya County Referral Hospital. The hospital was established in 1976 and currently serving six sub-county hospitals, and 113 primary health care facilities as a referral center. The hospital serves mostly the communities living within the central Nyanza part of Kenya. It serves both the inpatient and outpatient including the patient support center for HIV patients on ART. Currently, more than 3,524 HIV patients are supported by the center. The hospital in-patient has a bed capacity of 220.

## 3.2 Study Design

Siaya County Referral Hospital was the location of a cross-sectional study that quantitatively examined HIV-infected patients who were not adhering to ART treatment.

## 3.3. Study Population

The research was conducted on adults with HIV who were adhering to ART, as well as those who were non-adhering and attended the HIV comprehensive care clinic at Siaya County Referral Hospital. Additionally, individuals from the same area but presumed healthy and HIV negative were included in the study as controls.

### 3.3.1 Inclusion Criteria

The study included adults aged 18 years and above who gave their consent, comprising ART-compliant HIV-positive patients, previously non-adherent individuals initiated on ART, as well as treatment-naive participants.

### 3.3.2 Exclusion criteria

Individuals who were HIV positive and under 18 years old, as well as those with co-infections such as HBV or malaria and those who did not provide consent, were excluded from the study on ART.

### 3.4 Sample size and Sampling

The (Charan & Biswas, 2013) sample size calculation method were used, this formula was found to be suitable for quantitative case-control studies

Sample size = $\frac{r+1}{r}\frac{SD^2\left(Z_\beta+Z_{\frac{\alpha}{2}}\right)^2}{d^2}$

Where

r = Ratio of control to cases, 1 for an equal number of case and control

d = Expected mean difference between case and control of which will be 0.15

Zβ = Standard normal variate for power = for 95% power value is 1.96.

Zα/2 = Standard normal variate for level of significance (0.05) which is 1.96

Sample size = $\frac{1+1}{1}\frac{1^2(0.15+1.96)^2}{0.15^2}$ =197.8

The samples used in the study 47 HIV positive patients on ART, 23 patients not on ART, 42 on ART but not adhering and 51 negatives as controls, total of 163 participants.

The respondents for this study were selected using a simple purposive sample method. Cases and controls were recruited from the HIV comprehensive care clinic. The Morisky Medication Adherence Scale was utilized to assess the adherence of patients to ART.

### 3.5. Sample Collection

The variables examined in this study encompassed the CD4+ T cell count, as well as the plasma levels of cytokines and viral load. Five (5) mls of blood samples were obtained from individuals who were either receiving ART or had not yet started medication treatment. The blood was collected in 4 ml tubes that contained ethylenediaminetetraacetic acid (EDTA). Following the collecting process, the samples were carefully packaged in a cool box and subsequently sent to the laboratory for the purpose of conducting CD4 T cell count analysis. The centrifugation process was employed to separate whole blood into its constituent components, resulting in the isolation of plasma. The obtained plasma was then carefully stored in accordance with the prescribed guidelines provided with the test kits. This was done in preparation for later laboratory analyses, which encompassed the examination of cytokine levels as well as the determination of viral load. The CD4 T cell count was determined by the utilization of flow cytometry, whereas the viral load and cytokines were assessed utilizing the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Roche Diagnostics, Mannheim, Germany) and ELISA procedures, respectively.

#### 3.5.1. Determination of CD4 count

The enumeration of cells positive for the cluster of Differentiation 4 marker was conducted utilizing the Becton Dickinson (BD) FACSPresto instrument (Software V01.00.00) (Becton-Dickinson™, Franklin Lakes, USA). The BD FACSPresto™ cartridge,

specifically the CD4/%CD4/Hb cartridge, comprises of desiccated antibody reagents that are coupled with fluorochromes. Upon the interaction between blood and the reagents, the antibodies present in the reagents exhibit affinity towards the surface antigens located on the lymphocytes and monocytes. Following the incubation time, the cells were subjected to analysis using the BD FACSPresto Near-Patient CD4 Counter (Becton-Dickinson™, Franklin Lakes, USA), which served as the instrumental platform for this study. According to Afolabi et al. (2017), the program is capable of identifying the specific cell populations that are of interest. Additionally, it is able to determine CD4 absolute counts, CD4 percentages of lymphocytes, and hemoglobin concentration.

A volume of approximately 25-30 µl of blood was introduced onto the cartridge by directly filling the cartridge intake. Throughout this process, the cartridge lid remained closed, and the cartridge was consistently held in a horizontal position. The cartridge was thereafter inserted into the door with the channel oriented in an upward direction. Upon insertion, the door promptly closed, triggering an automatic processing of the sample. The processed results were afterwards displayed on the screen. Following the end of the procedure, the door was opened, and the results were printed out.

### 3.5.2. Determination of the Viral Load

The concentration of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma was determined with a nucleic acid amplification test called the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Roche Diagnostics, Mannheim, Germany). Currently, the process of preparing specimens is automated by utilizing the COBAS® AmpliPrep Instrument. Also, either the COBAS® TaqMan® Analyzer or COBAS®

TaqMan ® 48 Analyzer automate amplification and detection tasks. Roche Molecular Systems (Clarke et al., 2014) state that this examination relies on three fundamental methods.

### 3.5.3. Specimen preparation

The COBAS® AmpliPrep Instrument was used for automated specimen preparation, utilizing a silica-based capture method. The experimental procedure involved processing 850 microliters (μL) of plasma and lysing HIV-1 virus particles by heating them in the presence of protease and chaotropic lysis/binding solution. This caused nucleic acids such as HIV-1 RNA to be released while also protecting against ribonucleases (RNases). Protease, along with predetermined amounts of HIV-1 QS Armored R molecules were injected into each sample alongside magnetic glass particles and the lysis reagent. Incubation then led to binding between the surface of these magnetic glass particles and both types of RNA present in specimens: HIV-1 QS RNA plus HIV - 1RNA . Having separated from unbound contaminants through particle washes which removed salts & proteins present throughout serum , this material containing freed RNAs could undergo amplification mixture addition prior introduction within either Roche Diagnostics' COBAS® TaqMan Analyzer or their COBAS® TaqMan®48 analyzer subsequent thereto being subjected another incubation period following forced amalgamation..

### 3.5.4. Reverse transcription of the target RNA

The production of complementary DNA was achieved by performing reverse transcription and PCR amplification using the thermostable recombinant enzyme Thermus species DNA polymerase (Roche, 2007). Under appropriate buffer conditions and in the presence of

manganese ions (Mn2+), Z05 demonstrates a dual capacity as both a reverse transcriptase and a DNA polymerase. The concurrent execution of reverse transcription and PCR amplification, along with the ability to identify the amplicon in real-time, was made possible by integration.

### 3.5.5. Simultaneous PCR amplification of target cDNA and detection of cleaved dual- labeled oligonucleotide probe specific to the target

Both the HIV-1 target RNA and the HIV-1 QS RNA were reverse-transcribed, and then the reaction mixture was thermal-cycled in a COBAS® TaqMan® Analyzer or a COBAS® TaqMan® 48 Analyzer (Roche Diagnostics, Mannheim, Germany). Denaturation of the RNA: cDNA hybrid and exposure of the desired primer target sequences were achieved by cycling the reaction mixture through an elevated temperature in this thermal cycler. Chilling caused the primers to anneal to their respective target DNA. The annealed primers on the target template were extended using DNA Polymerase from the thermostable Thermus species Z05, yielding a double-stranded DNA molecule known as an amplicon. The required number of cycles for the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (Roche Diagnostics, Mannheim, Germany) has been preprogrammed by the manufacturer.

Using a dual-labeled oligonucleotide probe that's specific to both the target and QS areas of HIV-1, we successfully identified the amplified DNA. With this probe, we were able to detect both the HIV-1 amplicon and HIV-1 QS amplicon separately. Real-time polymerase chain reaction (PCR) is at the core of Roche Diagnostics' COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test in Mannheim, Germany. This technique tracks PCR product

accumulation by measuring fluorescent reporter dye emission intensity throughout amplification processes.

### 3.5.6. Sandwich ELISA determination of plasma level of IL-10

The Invitrogen Hu IL-10 kit is a solid-phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA) from InvitrogenTM, Life Sciences based in California, USA. This examination operates on the following principle: Human interleukin-10 (Hu IL-10)-specific monoclonal antibodies are utilized to pre-coat the microtiter strips. The samples include known standards for Hu IL-10 concentration control specimens and unknowns which were pipetted into the wells. When bound together with immobilized antibody, human Interleukin 1 molecule has binding affinity forming complex due to it's collected Il - 19 being tracked continually through biotinylated monoclonal antibody recognizing only that of human nature.

Microtiter wells were pre-filled with 50 μL of standards, samples or controls. Next, incubation buffer was added to the wells containing standards and samples. A cover was then placed over the plate and left to incubate for two hours at room temperature. The next step involved washing the sample before applying a biotinylated anti-IL-10 (Biotin Conjugate) solution in each well - 100 μL per well. The test plate was sealed once again and allowed to incubate for another two hours under room temperature conditions while being washed four more times afterward. A working solution comprising Streptavidin-HRP (Thermo Fisher Scientific, Massachusetts, USA), which consists of about 100 uL volume per filled microtiter hole on plates formerly prepared plus an additional thirty minutes' leave out time interval after sealing up covered dishes followed by performing

washings resulted in refilling these holes back during this process period as required said that "four further washes were performed." Once completed with all those activities aforementioned above subsequently adding Stabilized Chromogen into every aperture within one's dish amongst final stages hasn't escape tight monitoring processes like any other scientific experiment you've heard so far because there are certain factors responsible such situations such make sure delicate testing instruments don't get interference causing them failures may end up skewing results leaving no value whatsoever also adds gravity tests already carried through stated researchers need take notice how they should have kept proper records documenting everything done towards achieving ideal outcomes. Afterward allowing dished stand idle durability reasons depending upon lab safety precautions stopper got poured bible reading using optical density readings taken from stopped sequences we determined IL-10 concentration utilizing standard curve(s). As Shebl et al.(2012) demonstrated convincingly enough – his work serves evidence-based proof backed-up rightly so empirical research findings showed accuracy concerning efficacy(commensurately applicable measures where called-for existence).

### 3.5.7. Sandwich ELISA determination of plasma IFN-γ levels

An example of a commercially available ELISA that employs a solid phase sandwich format is the Invitrogen IFN- kit (Invitrogen™, Life Sciences, California, USA). The oligoclonal approach used in this assay involves the use of several monoclonal antibodies (MAbs 1) specific for various IFN-γ epitopes. All reagents were allowed to reach room temperature before use. 50 µL of each sample, standard, and control was pipetted into their respective wells. Next, anti-IFN-γ HRP Conjugate (at a volume of 50 µL) was added to each well. The solution sat for two hours at room temperature on a horizontally positioned

shaker turning at 700 revolutions per minute while being incubated. Afterward the solution was aspirated and the wells rinsed four times with water before adding in quickly produced chromogen-substrate (a mixture of H2O2 and TMB) that filled every well within fifteen minutes following washing steps which amounted up accordingly depending upon how you prepared it beforehand Then this plate would be incubated another fifteen more-minute session under protection against direct sunlight using shield if necessary! Finally we introduced Stop Solution - consisting only one molar hydrochloric acid(HCl)-reaching fifty microliters for every single last instance where absorption readings were taken as all samples had measurable quantities determined via curve-building from Shebl et al.'s work done back during 2012.

### 3.6. Data Analysis

The data were analyzed using SPSS V23. Mean and standard deviation (SD) summarized continuous variables, whereas counts and proportions (%) summarized categorical variables. A simple linear regression model evaluated the relationship between CD4 count, viral load, and cytokine levels; adjusted for patient age, gender, education level and marital status. In addition to this analysis method used above the Kruskal Wallis H-test with Dunn's post-hoc test determined statistically significant differences in cytokines levels among HIV patients adhering ART as compared to those who didn't comply along with CD4 count and viral load. The statistical significance was set at $P \leq 0.05$.

### 3.7. Ethical Considerations

Ethical and review approval was sought from the respective committees of Siaya County Referral Hospital, Masinde Muliro University of Science and Technology, and the National Commission for Science, Technology, and Innovation (NACOSTI), ensuring compliance with ethical research standards.

The study implemented measures to safeguard the well-being of the participants and uphold their dignity. The participants were selected based on their eligibility as individuals who were either non-adhering to their HIV positive ART regimen or adhering to it. Detailed information regarding the study, such as the collection of anonymous samples, analysis of data, and the confidentiality of results, was provided to the participants. The study ensured that only codes representing the participants were used to maintain anonymity. Blood samples were exclusively obtained from patients who provided their consent, and this procedure was carried out by employees who had received proper training.

The study relied solely on voluntary participation, and participants were afforded the opportunity to withdraw from the study at any point if they so desired. The participants were adequately informed and assured about their involvement, enabling them to comprehend the consequences of participation and make an autonomous decision without any form of pressure.

# CHAPTER FOUR

## RESULTS

### 4.1. Demographic profiles of the study participants.

A total 163 adults, females, 90 (55.2%) and males, 73 (44.8%) were recruited into the study. The distribution of the gender across the groups was not significantly different, *P* = 0.108. Age differences was also not significantly different across the groups; HIV-1 negative 37.69 (6.0), HIV [+] ART-naive, 39.0 (7.0), HIV [+] ART-adherents, 37.5 (7.0), HIV [+] ART non-adherents, 37.86 (9.3); *P* = 0.838. Height was comparable across the study groups, even though it was not significantly across the group; HIV-1 negative 1.69 (0.1), HIV [+] ART-naive, 1.64 (0.1), HIV [+] ART-adherents, 1.67 (0.1), HIV [+] ART non-adherents, 1.67 (0.1); *P* = 0.465. However, weight was significantly different across the study groups; HIV-1 negative 67.82 (18.0), HIV [+] ART-Naïve, 56.94 (11.0), HIV [+] ART-adherents, 66.64 (14.7), HIV [+] ART non-adherents, 59.15 (11.8); *P*= <0.001. Similarly, BMI was also significantly different across the group with HIV [+] ART-naive recorded lowest, 20.37 (5.11) as compared to HIV-1 negative 24.47 (5.27), HIV [+] ART-adherents, 23.97 (5.49), HIV [+] ART Non-adherents, 21.07 (3.72); *P* = <0.001. Additionally, BMI was further categorized into either underweight ≤18.5 or overweight ≥18.5. Majority of HIV [+] ART-Non-Adherents, 9 (40.0) were underweight even though the distribution of underweight and overweight individual was not significantly different across the group; *P* = 0.101. Consistent with height, waist circumference was not significantly different across the clinical groups: HIV-1 negative 76.0 (10.0), HIV [+] ART-naive, 72.0 (10.0), HIV [+] ART-adherents, 72.0 (10.0), HIV [+] ART Non-adherents, 76 (9.3); *P* = 0.088. However, hips circumference was significantly different

across the study group; HIV-1 negative 90.0 (10.0), HIV [+] ART-naive, 90.0 (11.0), HIV [+] ART-adherents, 88.0 (5.0), HIV [+] ART non-adherents, 91.5 (7.0); *P* = 0.049. Similarly, MUAC was significantly differently across the groups; HIV-1 negative, 25.0 (3.0), HIV [+] ART-naive, 24.0 (4.0), HIV [+] ART-adherents, 24.0 (2.0), HIV [+] ART non-adherents, 25.0 (2.0); *P* = 0.015. In contrast to MUAC and hips circumference, BUST was not significantly different across the groups, HIV-1 negative, 84.0 (6.0), HIV [+] ART-naive, 83.0 (8.0), HIV [+] ART-adherents, 24.0 (2.0), HIV [+] ART non-adherents, 85.0 (4.0); *P* = 0.372 (Table 4.1.)

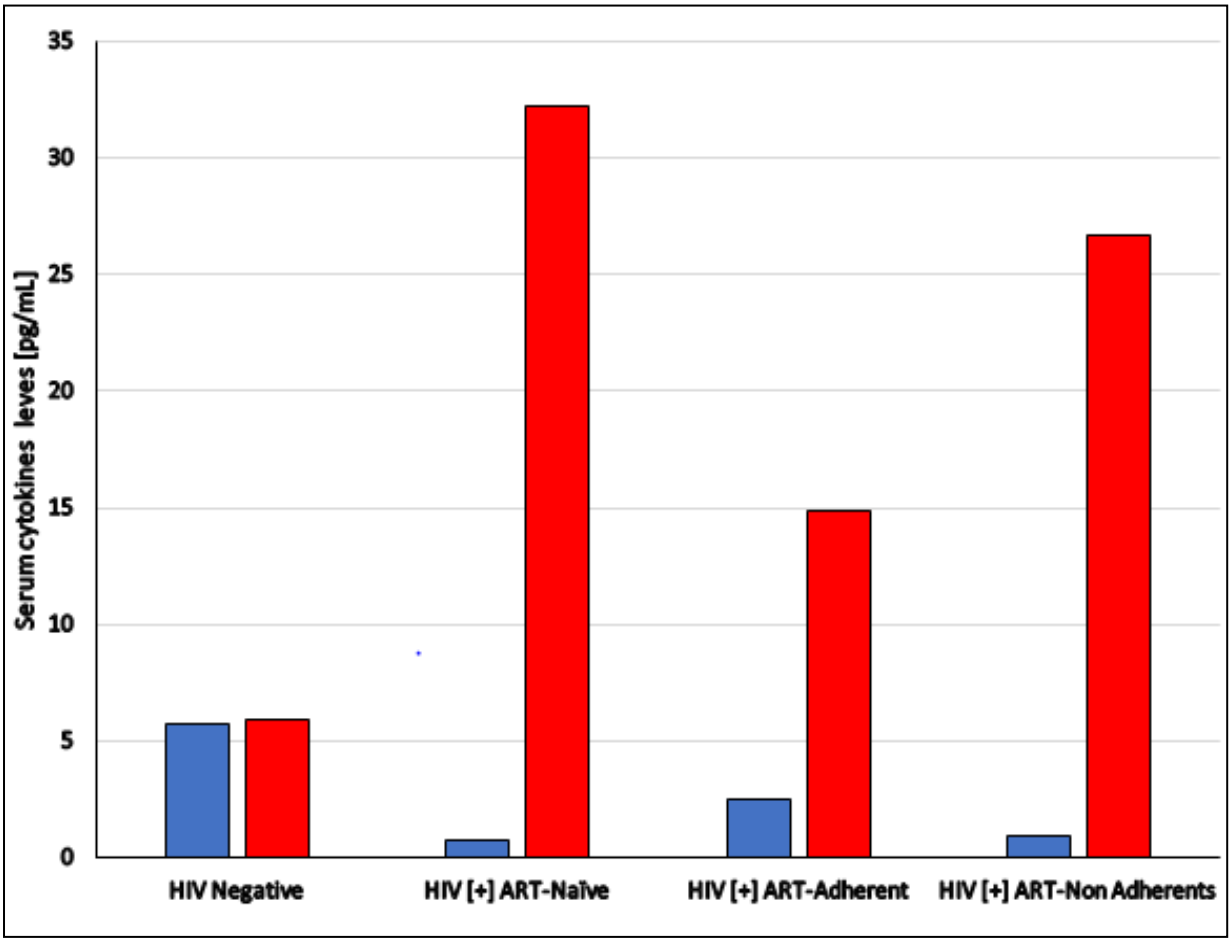
Table: 4. 1. Demographic characteristics of the study participants

| Characteristics | HIV Negative | HIV [+] ART-naive | HIV [+] ART-Adherents | HIV [+] ART-Non-Adherents | *P* |
|---|---|---|---|---|---|
| Gender n, % | | | | | |
| Female | 27 (30.0) | 1415.6) | 32 (35.6) | 17 (18.9) | 0.108 |
| Male | 24 (32.9) | 9 (12.3) | 15 (20.5) | 24 (34.2) | |
| Age | 37.69 (6.0) | 39.0 (7.0) | 37.5 (7.0) | 37.86 (9.3) | 0.838 |
| Height | 1.69 (0.1) | 1.64 (0.1) | 1.67 (0.1) | 1.67 (0.1) | 0.465 |
| Weight | 67.82 (18.0) | 56.94 (11.0) | 66.64 (14.7) | 59.15 (11.8) | **<0.001** |
| BMI | 24.47 (5.27) | 20.37 (5.11) | 23.97 (5.49) | 21.07 (3.72) | **<0.001** |
| BMI <18.5 | 4 (20.0) | 5 (25.0) | 3 (15.0) | 9 (40.0) | 0.101 |
| BMI >18.5 | 47 (32.9) | 119 (13.3) | 44 (30.8) | 33 (23.1) | 0.101 |
| Waist (cm) | 76.0 (10.0) | 72.0 (10.0) | 72.0 (10.0) | 76 (9.3) | 0.088 |
| Hips | 90.0 (10.0) | 90.0 (11.0) | 88.0 (5.0) | 91.5 (7.0) | 0.049 |
| MUAC | 25.0 (3.0) | 24.0 (4.0) | 24.0 (2.0) | 25.0 (2.0) | 0.015 |
| Bust | 84.0 (6.0) | 86.0 (10.0) | 83.0 (8.0) | 85.0 (4.0) | 0.372 |

The data is presented as medians (IQR, interquartile range). HIV-1[+], which stands for human immunodeficiency virus type-1 positive, ART represents anti-retroviral treatment. F/M refers to Female and Male while MUAC signifies mid-upper arm circumference. The Kruskal-Walis H test was used in the analysis of data across groups set at $P<0.01$ for continuous measures and Chi-Square tests were employed for gender distribution and BMI or body mass index. The significant values are indicated in bold font.

### 4.2. The Serum IFN-γ and IL-10 cytokines levels among the study participants

The serum IFN-γ and IL-10 in HIV-1 individual, HIV-1 [+] ART-naive, HIV-1 [+] ART-adherent, HIV-1 [+] ART-Non-adherent were: (5.73 vs. 5.89; 0.74 vs. 32.26; 2.55 vs. 14.9 and 0.98 vs. 26.73) pg/mmL. This are summarized in the bar graph *Figure 4.1*.




*KEY*
*Blue bars - IFN-γ*
*Red bars - IL-10*

**Figure: 4.1. Serum mean cytokines levels among the study participants**

The figure above is showing the mean cytokine levels (pg/ml), HIV [+]; human immunodeficiency virus type-1 positive; ART; anti-retroviral treatment. The blue bars represent interferon gamma and red bars represent interlekins-10.

### 4.3. CD4 T cell counts and viral load of the *HIV-negative, ART-naive and -experienced ART regimen non-adherent and -adherent patients*

The CD4 T cell counts CD4 T cell counts were significantly lower in HIV [+] ART-Naive 395.9 (349) group as compared to HIV-1 negative 1407.4 (1303), HIV [+] ART-Adherents, 575.4 (374) and HIV [+] ART Non-Adherents, 423.6 (252); *P* = <0.001. Additionally, majority of HIV [+] ART-Naïve had lower CD4 count CD4 <300, 10 (43.5) as compared to HIV [+] ART-Adherents 5 (10.6) and HIV [+] ART Non-Adherents 18 (42.9), *P* = <0. 001. Conversely, the viral load was higher in HIV [+] ART Non-Adherents, 4.6 (1.1), as compared to HIV [+] ART-Adherents, 3.4 (1.9) and HIV [+] ART-Naïve 4.5 (1.6); *P* <0.001. Majority of HIV [+] ART Non-Adherents individuals presented with >1000 copies per u/l of blood, 39 (92.9) as compared to HIV [+] ART-Naïve 9 (82.6), HIV-1 negative 0 (0.0), HIV [+] ART-Adherents, 27 (57.4), *P* = <0.001. In addition, few HIV [+] ART Non-Adherents individuals presented with <1000, HIV-1 viral copies 3 (7.1) as compared to HIV [+] ART-Naïve 4 (17.4) and HIV [+] ART-Adherents 20 (42.6), *P* = <0.001 (Table 2).

**Table: 4. 2. HIV-1 viral load and CD4 T cell count of the study participants.**

| Clinical Characteristics | HIV Negative | HIV [+] ART-naive | HIV [+] ART-adherent | HIV [+] ART-non-adherents | *P* |
|---|---|---|---|---|---|
| CD4 | 1407.4 (1303) | 395.9 (349) | 575.4 (374) | 423.6 (252) | <0.001 |
| CD4 <300 | 0 (0.0) | 10 (43.5) | 5 (10.6) | 18 (42.9) | <0.001 |
| CD4 300-499 | 3 (5.9) | 7 (30.4) | 13 (27.7) | 12 (28.6) | <0.001 |
| CD4 >500 | 48 (94.1) | 6 (26.1) | 29 (61.7) | 12 (28.6) | <0.001 |
| Viral load | | 4.5 (1.6) | 3.4 (1.9) | 4.6 (1.1) | <0.001 |
| VL <1000 | 51 (100.0) | 4 (17.4) | 27 (57.4) | 3 (7.1) | <0.001 |
| VL >1000 | 0 (0.0) | 9 (82.6) | 20 (42.6) | 39 (92.9) | <0.001 |

The data is presented as medians (IQR, interquartile range). HIV-1[+] represents human immunodeficiency virus type-1; ART [-] means anti-retroviral treatment was not administered while ART [+] implies that it was given. VL indicates viral load and CD4 T cell count refers to the cluster of differentiation. The analysis involved using Kruskal-Wallis H test across groups with post-hoc P<0.01 for continuous measures and Chi-Square test for categorical information which revealed significant differences between the groups studied. The bold values are significant.

### 4.4. Correlation of circulating cytokine levels with HIV-1 viral load, CD4 T cell count and BMI among HIV-1 ART-naive, and ART-experienced regimen -adherent and non-adherent patients

Interleukins -10 (IL-10) correlated positively with viral load (ρ= 0.272; *P*=0.004) and inversely with CD4 T cell count (ρ= -0.627; *P*<0.0001) as well as BMI (ρ= -0.376; *P*<0.0001). However, IFN-γ correlated inversely with viral load (ρ= -0.326; *P*<0.0001) and BMI (ρ= -0.342; *P*<0.0001), positively with CD4 T cell count (ρ= 0.619; *P*<0.0001) (Table 3).

**Table: 4. 3. Correlation of serum IL-10 and IFN-γ cytokine levels with HIV infection diagnostic BMI, Viral Load and CD4+ T-cell count**

| *Cytokine* | Log10 HIV-1 RNA copies | | CD4+ T cells | | BMI | |
|---|---|---|---|---|---|---|
| | ρ | *P* | ρ | *P* | P | *P* |
| *IL-10* | 0.272 | 0.004 | -0.627 | <0.0001 | -0.376 | <0.0001 |
| *IFN-γ* | -0.326 | <0.0001 | 0.619 | <0.0001 | 0.342 | <0.0001 |

The data presented is Spearman's rank correlation coefficient (rho, ρ) with the obtained values. The correlation between IL-10 (Interleukin-10) and IFN-γ (interferon-gamma) was determined through a Spearman's rank correlation analysis. Significant P-values are indicated by bolded values.

# CHAPTER FIVE

# DISCUSSION

## 5.1. Demographic profiles of the study participants

An analysis of demographic and anthropometric data indicates that individuals who are infected with the human immunodeficiency virus (HIV) but have not yet started antiretroviral treatment, exhibit significantly lower weight and body mass index (BMI), when compared to those individuals also infected with HIV but receiving anti-retroviral therapy. Furthermore, the majority of individuals who did not adhere to antiretroviral therapy (ART) for HIV were underweight. This was followed by those who were both HIV-positive and had not yet started ART. This discovery suggests that the use of anti-retroviral treatment may be a contributing factor to low weight, body mass index, and underweight in individuals who are HIV positive and not receiving anti-retroviral treatment, as well as those who are HIV positive but not adhering to their treatment, when compared to individuals who are HIV negative and adhering to their treatment. Furthermore, prior research has demonstrated a connection between the utilisation of ART and measures indicating decreased body weight, low BMI, and being underweight at the beginning stage of ART adoption (Pantazis et al., 2022; Hirigo et al., 2023). The metabolic complication of antiretroviral therapy (ART) has been associated with impaired absorption of nutrients, resulting in muscular atrophy and overall reduction in body weight (Duggal et al., 2012; (Thet & Siritientong, 2020)). The discovery supports the fact that advanced HIV infection and failure to follow treatment have been associated with HIV enteropathy, unresolved chronic diarrhoea, malabsorption, increased fever accompanied by weight loss

due to increased energy usage, and muscle wasting ((Elfstrand & Florén, 2010); (Siddiqui et al., 2022). Previous studies have shown an increase in body mass index (BMI) after starting antiretroviral therapy (ART) (Alebel et al., 2022; Mavarani et al., 2023). This finding contradicts the current discovery. An institutional retrospective analysis revealed an increase in weight and body mass index (BMI) within the first two years of initiating second-line antiretroviral therapy (ART) (Baraki et al., 2019). However, in our specific situation, not following medical instructions may play a vital role in worsening the increase of the amount of virus in the body, therefore causing harmful effects on the human body. In the setting of HIV-1 infection, individuals frequently suffer from underweight due to metabolic problems. This can result in deficits of important micronutrients as selenium, zinc, vitamins A, B12, and vitamin D. The aforementioned investigations undertaken by (Deshwal and Arora, 2019; Adhikari et al., 2022; Phiri et al., 2022) have demonstrated a clear correlation between these deficiencies and undernourishment, leading to future underweight.

### 5.2. The Serum IFN-γ and IL-10 cytokines levels of the study participants

The present study evaluated the levels of pro- and anti-inflammatory cytokines, body mass index (BMI), CD4+ T lymphocyte counts, and viral load in HIV-1-infected individuals who demonstrated noncompliance with highly active antiretroviral therapy (HAART). Afterwards, the indicated results were compared with the serum levels seen in patients who followed HAART treatment, as well as individuals who had not yet started HAART and were used as healthy controls. The study examined various cytokines, such as IFN-γ and IL-10, which have both pro-inflammatory and anti-inflammatory properties, respectively. Individuals infected with HIV-1 exhibited elevated levels of IFN-γ, which were

subsequently reduced with the consistent and prolonged use of HAART. Individuals who consistently followed regular HAART treatment showed a steady decrease in the levels of IL-10. Nevertheless, it was demonstrated that there was a rise in IL-10 levels among individuals who failed to adhere to the prescribed treatment plan. Conversely, it was noted that individuals infected with HIV-1 exhibited decreased blood levels of IFN-γ. Consistent and long-term use of HAART seemed to lead to higher levels of interferon-gamma (IFN-γ) in the blood, while not following the prescribed treatment schedule appeared to be linked to lower levels of IFN-γ in the blood. Contrary to these findings, other studies have demonstrated that there are no substantial changes in blood IL-10 levels detected in HIV patients who are using HAART (Musa et al., 2021; Osuji et al., 2018). Recent studies have shown that IL-10 levels in HIV patients who are on HAART medication tend to increase at the same time as the CD4 T cell count rises (Liu et al., 2024; Younas et al., 2016). Multiple longitudinal investigations have yielded comparable results to the current analysis, demonstrating a decrease in IL-10 levels with adherence to HAART (Dale et al., 2014; Osuji et al., 2018). Moreover, there is a lack of agreement on the effect of HAART on serum interferon-gamma (IFN-γ) levels in individuals with HIV. Multiple studies have observed a consistent increase in the levels of interferon-gamma (IFN-γ) in the bloodstream following frequent use of highly active antiretroviral therapy (HAART) (Roff et al., 2014; Watanabe et al., 2019). Conversely, some studies have documented a decrease in FN-γ levels in individuals with HIV who are undergoing HAART treatment (Hernández-Walias et al., 2020; Osuji et al., 2018; Watanabe et al., 2019). The current study reveals that patients who comply with the HAART regimen see a rise in serum levels of interferon-gamma (IFN-γ). IFN-γ, categorised as a Th1 cytokine, usually triggers a shift from Th1 to

Th2 during HIV infection (Reuter et al., 2012; Williams et al., 2013). with ART. It is anticipated that IFN-γ levels in the bloodstream will decline during infection without antiretroviral therapy (ART), while highly active antiretroviral therapy (HAART) may boost immune cell synthesis of IFN-γ. These pro-inflammatory cytokines have vital functions within HIV-1 infections. Our research outcomes propose a potential reversal of blood cytokine levels due to non-adherence to ART.

### 5.3 Correlation of serum IL-10 and IFN-γ cytokine levels with HIV infection diagnostic BMI, Viral Load and CD4+ T-cell count

Furthermore, a direct association was found between the levels of IFN-γ and both the counts of CD4 T cells and BMI. In contrast, a negative connection was seen between IL-10 levels and both CD4 T cell counts and BMI. Previous studies have found higher levels of IL-10 in persons with HIV who have a high viral load (Musa et al., 2021; Sun et al., 2023). During HIV-1 infection, the decrease in IFN-γ, which is a type 1 helper T cell cytokine, offers a favourable environment for the reproduction of the virus and the reduction of CD4 T cells (Cromarty et al., 2019; Ward et al., 2021). The results of this investigation are consistent with the hierarchical analysis, which showed that IFN-γ had a significant effect on both viral load and CD4 T cell counts.

In the current study, the anti-inflammatory properties of the cytokine IL-10 were examined. HIV infection appears to cause elevated levels of cytokines in the bloodstream. Observations revealed that the continued adherence to HAART over a prolonged period led to a reduction in the amounts of anti-inflammatory cytokines circulating in the body. However, it was demonstrated that HIV patients who did not follow to their treatment regimen had increased amounts of these cytokines in their bloodstream. Studies conducted

by Freeman et al. (2021) and Hokello et al. (2024) have shown that HIV infection leads to a shift from Th1 to Th2 cytokines, such as interleukin-10 (IL-10) (Freeman et al., 2021; Hokello et al., 2024). Consequently, it is expected that these cytokines will show elevated levels in the bloodstream after an infection. Consistent and ongoing use of HAART can result in a notable decrease in blood IL-10 levels. HAART works by restoring the equilibrium of cytokines (Pillay et al., 2020). In a recent study conducted by Jianu et al. (2021), the researchers examined the levels of serum IL-10 in HIV patients who were either treatment-naive or had prior experience with highly active antiretroviral therapy (HAART). The study found that there were similar outcomes in both groups, however there was a notable decrease in IL-10 levels among individuals who had undergone HAART treatment (Jianu et al., 2021). The results of this study indicate that strict adherence to HAART (Highly Active Antiretroviral Therapy) can result in a decrease in the levels of anti-inflammatory cytokines in the blood. This could potentially lead to better outcomes for patients with HIV-1. Nevertheless, it was noted that failure to comply with the prescribed treatment led to a rise in anti-inflammatory cytokines, so negating the benefits of HAART. Additionally, a direct relationship was found between IL-10 and viral load, while IL-10 showed an opposite connection with CD4 T cell counts and BMI. The data indicate that not following the prescribed HAART treatment has detrimental consequences, as it results in higher levels of anti-inflammatory cytokines. A correlation can be noted between higher levels of interleukin-10 (IL-10) and factors such as increased viral load, reduced CD4 T cell count, and body mass index (BMI).

Our work has shown that not following HAART treatment results in higher levels of serum interleukin-10 (IL-10), while simultaneously decreasing interferon-gamma (IFN-γ) levels.

The findings of this study suggest that there may be a possibility to reverse the effects linked to following HAART treatment and controlling the transition of Th1/Th2 cytokines. As a result, this ultimately leads to harmful outcomes and a reduced outlook for individuals affected by HIV-1. Furthermore, IFN-γ has a vital function in predicting disease progression indicators in HIV-1 patients who do not adhere to treatment.

# CHAPTER SIX

# CONCLUSIONS AND RECOMENDATIONS

## 6.1. Conclusions

i. The adherence to antiretroviral therapy (ART) has a considerable impact on the immunological function and viral load of patients with HIV-1. Individuals who adhere to antiretroviral therapy (ART) have higher levels of CD4+ T-cells and lower levels of viral load compared to patients who are not on ART or who are not adherent to ART. More precisely, patients who were adhering to antiretroviral therapy (ART) had a CD4+ T-cell count of 575.4 cells/µL and a viral load of 3.4 log copies/mL. In contrast, patients who were not receiving ART or were not adherent to it had lower CD4+ T-cell counts (395.9 and 423.6 cells/µL, respectively) and higher viral loads (4.5 and 4.6 log copies/mL, respectively).

ii. The blood concentrations of IFN-γ and IL-10 exhibit unique patterns and associations with illness indicators in individuals with HIV-1. The levels of IFN-γ show an inverse correlation with viral load ($\rho = -0.326$; $P<0.0001$) and a positive correlation with CD4+ T-cell count ($\rho = 0.619$; $P<0.0001$). This suggests that greater levels of IFN-γ are associated with improved immune function and reduced viral replication. On the other hand, there is a positive relationship between IL-10 levels and viral load ($\rho = 0.272$; $P=0.004$), and a negative relationship between IL-10 levels and CD4+ T-cell count ($\rho = -0.627$; $P<0.0001$). This suggests that higher IL-10 levels are linked to greater viral replication and a weaker immune system.

iii. Body Mass Index (BMI) shows a negative correlation with IL-10 levels ($\rho$= -0.376; P<0.0001) and a positive correlation with IFN-$\gamma$ levels ($\rho$= -0.342; P<0.0001), indicating that a lower BMI is linked to greater IL-10 levels and lower IFN-$\gamma$ levels. This suggests a potential correlation between the nutritional condition and the immunological response in individuals with HIV-1. A decreased BMI may signal a decline in general health and immune system functioning.

## 6.2 Recommendation

### 6.2.1 Recommendations for action

i. Enhance adherence support initiatives for HIV-1 patients to enable consistent and appropriate use of antiretroviral treatment (ART). This may encompass routine counselling, reminders to stick to treatment, and tackling obstacles to adherence, such as social stigma, adverse effects, and pharmaceutical accessibility. Enhanced compliance will result in enhanced immune function, increased CD4+ T-cell counts, and reduced viral loads, ultimately enhancing patient outcomes.

ii. Incorporate regular monitoring of cytokine levels, specifically IFN-$\gamma$ and IL-10, into the clinical care of HIV-1 patients. Quantifying these cytokines can offer vital information on the patient's immunological condition and the advancement of the disease, enabling the implementation of more tailored treatment approaches. For example, elevated IL-10 levels may suggest the necessity for more vigilant monitoring and possible therapies to combat heightened viral replication and immune suppression.

iii. Establish and execute nutritional assistance initiatives for individuals infected with HIV-1, specifically targeting those with a low body mass index (BMI). Optimizing

patients' nutrition can boost their general well-being, bolster immunological function, and potentially enhance their response to antiretroviral therapy (ART). Providing nutritional counselling and offering supplements or dietary help are crucial elements of complete HIV care.

### 6.2.2 Recommendation for further study

i. Perform longitudinal research to examine the prolonged impact of adhering to antiretroviral therapy (ART) on the immunological function and viral load in individuals with HIV-1. The primary objective of these research should be to investigate the temporal dynamics of CD4+ T-cell counts, virus loads, and cytokine levels (namely IFN-γ and IL-10) over prolonged durations. This research can offer profound insights into the correlation between consistent adherence to antiretroviral therapy (ART) and the course of diseases as well as the results experienced by patients.

ii. Examine the impact of targeted dietary interventions on immunological function and viral suppression in individuals with HIV-1 infection. Subsequent research should investigate the effects of various diets, supplements, or nutritional support programs on BMI, cytokine levels, CD4+ T-cell counts, and viral load. This research aims to determine optimal nutritional methods for promoting the health and managing the treatment of individuals with HIV-1.

iii. Perform mechanistic investigations to elucidate the underlying biological mechanisms by which IFN-γ and IL-10 impact the course of HIV-1 illness and the immune response. The objective of these investigations should be to clarify the pathways and interactions that result in the reported correlations between cytokine

levels, viral load, and CD4+ T-cell numbers. Gaining a comprehensive understanding of these pathways can facilitate the development of precise treatments that regulate cytokine levels in order to enhance patient outcomes.

## REFERENCES


Addo, M. K., Aboagye, R. G., & Tarkang, E. E. (2022). Factors influencing adherence to antiretroviral therapy among HIV/AIDS patients in the Ga West Municipality, Ghana. *IJID Regions, 3*, 218. https://doi.org/10.1016/j.ijregi.2022.04.009

Adeniyi, O. V., Ajayi, A. I., Ter Goon, D., Owolabi, E. O., Eboh, A., & Lambert, J. (2018). Factors affecting adherence to antiretroviral therapy among pregnant women in the Eastern Cape, South Africa. *BMC Infectious Diseases*, *18*(1), 175. https://doi.org/10.1186/s12879-018-3087-8

Adhikari, N., Bhattarai, R. B., Basnet, R., Joshi, L. R., Tinkari, B. S., Thapa, A., & Joshi, B. (2022). Prevalence and associated risk factors for tuberculosis among people living with HIV in Nepal. *PLoS ONE*, *17*(1), e0262720. https://doi.org/10.1371/journal.pone.0262720

Ahmed, S., Autrey, J., Katz, I. T., Fox, M. P., Rosen, S., Onoya, D., Bärnighausen, T., Mayer, K. H., & Bor, J. (2018). Why do people living with HIV not initiate treatment? A systematic review of qualitative evidence from low- and middle-income countries. *Social Science & Medicine (1982), 213*, 72–84. https://doi.org/10.1016/j.socscimed.2018.05.048

Alebel, A., Demant, D., Petrucka, P., & Sibbritt, D. (2022). Effects of undernutrition on opportunistic infections among adults living with HIV on ART in Northwest Ethiopia: Using inverse-probability weighting. *PLoS ONE*, *17*(3), e0264843. https://doi.org/10.1371/journal.pone.0264843

Al-Qahtani, A. A., Alhamlan, F. S., & Al-Qahtani, A. A. (2024). Pro-Inflammatory and Anti-Inflammatory Interleukins in Infectious Diseases: A Comprehensive Review.

*Tropical Medicine and Infectious Disease*, *9*(1), Article 1. https://doi.org/10.3390/tropicalmed9010013

Annunziato, F., Cosmi, L., Liotta, F., Maggi, E., & Romagnani, S. (2015). Human T helper type 1 dichotomy: Origin, phenotype and biological activities. *Immunology*, *144*(3), 343–351. https://doi.org/10.1111/imm.12399

Anyaike, C., Atoyebi, O. A., Musa, O. I., Bolarinwa, O. A., Durowade, K. A., Ogundiran, A., & Babatunde, O. A. (2019). Adherence to combined Antiretroviral therapy (cART) among people living with HIV/AIDS in a Tertiary Hospital in Ilorin, Nigeria. *The Pan African Medical Journal*, *32*, 10. https://doi.org/10.11604/pamj.2019.32.10.7508

Aragonés, C., Sánchez, L., Campos, J. R., & Pérez, J. (2011). Antiretroviral therapy adherence in persons with HIV/AIDS in Cuba. *MEDICC Review*, *13*(2), 17–23. https://doi.org/10.37757/MR2011V13.N2.6

Aye, W. L., Puckpinyo, A., & Peltzer, K. (2017). Non-adherence to anti-retroviral therapy among HIV infected adults in Mon State of Myanmar. *BMC Public Health*, *17*(1), 391. https://doi.org/10.1186/s12889-017-4309-5

Bangsberg, D. R. (2006). Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *43*(7), 939–941. https://doi.org/10.1086/507526

Bantie, B., Gebeyehu, N. A., Adella, G. A., Kassie, G. A., Mengstie, M. A., Abebe, E. C., Seid, M. A., Gesese, M. M., Tegegne, K. D., Anley, D. T., Zemene, M. A., Dessie, A. M., Feleke, S. F., Dejenie, T. A., Kebede, Y. S., Chanie, E. S., Kerebeh, G.,

Bayih, W. A., & Moges, N. (2024). Trends of Body Mass Index changes among adults on antiretroviral therapy in Northwest Ethiopia: A longitudinal data analysis. *Scientific Reports*, *14*(1), 5265. https://doi.org/10.1038/s41598-024-53701-0

Baraki, A. G., Gezie, L. D., Zeleke, E. G., Awoke, T., & Tsegaye, A. T. (2019). Body mass index variation over time and associated factors among HIV-positive adults on second-line ART in north-west Ethiopia: A retrospective follow-up study. *BMJ Open*, *9*(9), e033393. https://doi.org/10.1136/bmjopen-2019-033393

Barber, G. N. (2001). Host defense, viruses and apoptosis. *Cell Death and Differentiation*, *8*(2), 113–126. https://doi.org/10.1038/sj.cdd.4400823

Basti, B. D., Mahesh, V., Bant, D. D., & Bathija, G. V. (2017). Factors affecting antiretroviral treatment adherence among people living with human immunodeficiency virus/acquired immunodeficiency syndrome: A prospective study. *Journal of Family Medicine and Primary Care*, *6*(3), 482–486. https://doi.org/10.4103/2249-4863.222014

Bhatti, A. B., Usman, M., & Kandi, V. (2016). Current Scenario of HIV/AIDS, Treatment Options, and Major Challenges with Compliance to Antiretroviral Therapy. *Cureus*, *8*(3), 1–12. https://doi.org/10.7759/cureus.515

Boyman, O., & Sprent, J. (2012). The role of interleukin-2 during homeostasis and activation of the immune system. *Nature Reviews. Immunology*, *12*(3), 180–190. https://doi.org/10.1038/nri3156

Breen, E. C. (2002). Pro- and anti-inflammatory cytokines in human immunodeficiency virus infection and acquired immunodeficiency syndrome. *Pharmacology & Therapeutics*, *95*(3), 295–304. https://doi.org/10.1016/S0163-7258(02)00263-2

Briggs, J. A. G., & Kräusslich, H.-G. (2011). The molecular architecture of HIV. *Journal of Molecular Biology*, *410*(4), 491–500. https://doi.org/10.1016/j.jmb.2011.04.021

Chalker, J., Andualem, T., Minzi, O., Ntaganira, J., Ojoo, A., Waako, P., & Ross-Degnan, D. (2008). Monitoring adherence and defaulting for antiretroviral therapy in 5 East african countries: An urgent need for standards. *Journal of the International Association of Physicians in AIDS Care (Chicago, Ill.: 2002)*, *7*(4), 193–199. https://doi.org/10.1177/1545109708320687

Charan, J., & Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*, *35*(2), 121. https://doi.org/10.4103/0253-7176.116232

Chime, O. H., Ndibuagu, E. O., & Orji, C. J. (2019). Rates and predictors of adherence and retention for antiretroviral therapy among HIV-positive adults in Enugu, Nigeria. *Malawi Medical Journal*, *31*(3), 202–211. https://doi.org/10.4314/mmj.v31i3.7

Choi, S. M., Park, H. J., Choi, E. A., Jung, K. C., & Lee, J. I. (2021). Cellular heterogeneity of circulating CD4+CD8+ double-positive T cells characterized by single-cell RNA sequencing. *Scientific Reports*, *11*(1), 23607. https://doi.org/10.1038/s41598-021-03013-4

CHS. (2017a). Annual Reports. *CHS: Centre for Health Solutions - Kenya*. https://chskenya.org/annual-reports/

CHS, K. (2017b). Center for Health Solutions 2017 ANNUAL REPORT. *Chs*, *24*(11).

Clerici, M., & Shearer, G. M. (1993). A TH1—>TH2 switch is a critical step in the etiology of HIV infection. *Immunology Today*, *14*(3), 107–111. https://doi.org/10.1016/0167-5699(93)90208-3

Costin, J. M. (2007). Cytopathic Mechanisms of HIV-1. *Virology Journal*, *4*(1), 100. https://doi.org/10.1186/1743-422X-4-100

Cromarty, R., Sigal, A., Liebenberg, L. J. P., McKinnon, L. R., Abdool Karim, S. S., Passmore, J.-A. S., & Archary, D. (2019). Diminished HIV Infection of Target CD4+ T Cells in a Toll-Like Receptor 4 Stimulated in vitro Model. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01705

Dale, S., Cohen, M., Weber, K., Cruise, R., Kelso, G., & Brody, L. (2014). Abuse and Resilience in Relation to HAART Medication Adherence and HIV Viral Load Among Women with HIV in the United States. *AIDS Patient Care and STDs*, *28*(3), 136–143. https://doi.org/10.1089/apc.2013.0329

Deshwal, R., & Arora, S. (2019). High Prevalence of Vitamin D Deficiency in HIV Infected on Antiretroviral Therapy in a Cohort of Indian Patients. *The Journal of the Association of Physicians of India*, *67*(3), 42–45.

Duggal, S., Chugh, T. D., & Duggal, A. K. (2012). HIV and Malnutrition: Effects on Immune System. *Clinical and Developmental Immunology*, *2012*, 784740. https://doi.org/10.1155/2012/784740

Dwyer-Lindgren, L., Cork, M. A., Sligar, A., Steuben, K. M., Wilson, K. F., Provost, N. R., Mayala, B. K., VanderHeide, J. D., Collison, M. L., Hall, J. B., Biehl, M. H., Carter, A., Frank, T., Douwes-Schultz, D., Burstein, R., Casey, D. C., Deshpande, A., Earl, L., El Bcheraoui, C., … Hay, S. I. (2019). Mapping HIV prevalence in sub-Saharan Africa between 2000 and 2017. *Nature*, *570*(7760), 189–193. https://doi.org/10.1038/s41586-019-1200-9

Elfstrand, L., & Florén, C.-H. (2010). Management of chronic diarrhea in HIV-infected patients: Current treatment options, challenges and future directions. *HIV/AIDS (Auckland, N.Z.)*, *2*, 219. https://doi.org/10.2147/HIV.S13191

Elrefaei, M., Ventura, F. L., Baker, C. A. R., Clark, R., Bangsberg, D. R., & Cao, H. (2007). HIV-Specific IL-10-Positive CD8+ T Cells Suppress Cytolysis and IL-2 Production by CD8+ T Cells1. *The Journal of Immunology*, *178*(5), 3265–3271. https://doi.org/10.4049/jimmunol.178.5.3265

Enayati, S., Seifirad, S., Amiri, P., Abolhalaj, M., & Mohammad -Amoli, M. (2015). Interleukin-1 beta, interferon-gamma, and tumor necrosis factor-alpha gene expression in peripheral blood mononuclear cells of patients with coronary artery disease. *ARYA Atherosclerosis*, *11*(5), 267–274.

Essien-Baidoo, S., Obiri-Yeboah, D., Opoku, Y. K., Ayamga, E., Hodi Zie, K., Attoh, D., Obboh, E., Hayfron Benjamin, A., & Afrifa, J. (2019). ART Regimen and Other Sociodemographics Do Not Affect Cytokine Expression in HIV Patients in Ghana. *The Canadian Journal of Infectious Diseases & Medical Microbiology = Journal Canadien Des Maladies Infectieuses et de La Microbiologie Médicale*, *2019*, 2730370. https://doi.org/10.1155/2019/2730370

Fajardo-Ortiz, D., Lopez-Cervantes, M., Duran, L., Dumontier, M., Lara, M., Ochoa, H., & Castano, V. M. (2017). The emergence and evolution of the research fronts in HIV/AIDS research. *PLOS ONE*, *12*(5), e0178293. https://doi.org/10.1371/journal.pone.0178293

Fiebig, E. W., Wright, D. J., Rawal, B. D., Garrett, P. E., Schumacher, R. T., Peddada, L., Heldebrant, C., Smith, R., Conrad, A., Kleinman, S. H., & Busch, M. P. (2003).

Dynamics of HIV viremia and antibody seroconversion in plasma donors: Implications for diagnosis and staging of primary HIV infection. *AIDS (London, England)*, *17*(13), 1871–1879. https://doi.org/10.1097/00002030-200309050-00005

Freeman, R., Gwadz, M., Francis, K., & Hoffeld, E. (2021). Forgetting to take HIV antiretroviral therapy: A qualitative exploration of medication adherence in the third decade of the HIV epidemic in the United States. *SAHARA J : Journal of Social Aspects of HIV/AIDS Research Alliance*, *18*(1), 113. https://doi.org/10.1080/17290376.2021.1989021

Ganser-Pornillos, B., M, Y., & Wi, S. (2008). The structural biology of HIV assembly. *Current Opinion in Structural Biology*, *18*(2). https://doi.org/10.1016/j.sbi.2008.02.001

Grazinoli Garrido, R., Gonçailves, G. da C., Grazinoli Garrido, R., & Gonçailves, G. da C. (2020). Forensics in HIV Transmission Crimes. *Medicina Legal de Costa Rica*, *37*(1), 101–113.

Hernández-Walias, F., Ruiz-de-León, M. J., Rosado-Sánchez, I., Vázquez, E., Leal, M., Moreno, S., Vidal, F., Blanco, J., Pacheco, Y. M., & Vallejo, A. (2020). New signatures of poor CD4 cell recovery after suppressive antiretroviral therapy in HIV-1-infected individuals: Involvement of miR-192, IL-6, sCD14 and miR-144. *Scientific Reports*, *10*(1), 2937. https://doi.org/10.1038/s41598-020-60073-8

Hirigo, A. T., Yilma, D., Astatkie, A., & Debebe, Z. (2023). Effect of dolutegravir-based first-line antiretroviral therapy on weight and body mass index among adult people living with HIV on follow up at health facilities in Hawassa city administration,

Southern Ethiopia: A retrospective cohort study. *Annals of Medicine, 55*(2). https://doi.org/10.1080/07853890.2023.2242250

Hokello, J., Tyagi, K., Owor, R. O., Sharma, A. L., Bhushan, A., Daniel, R., & Tyagi, M. (2024). New Insights into HIV Life Cycle, Th1/Th2 Shift during HIV Infection and Preferential Virus Infection of Th2 Cells: Implications of Early HIV Treatment Initiation and Care. *Life*, *14*(1), Article 1. https://doi.org/10.3390/life14010104

Holdsworth, S. R., & Gan, P.-Y. (2015). Cytokines: Names and Numbers You Should Care About. *Clinical Journal of the American Society of Nephrology: CJASN*, *10*(12), 2243–2254. https://doi.org/10.2215/CJN.07590714

Hughes, S. M., Levy, C. N., Calienes, F. L., Stekler, J. D., Pandey, U., Vojtech, L., Berard, A. R., Birse, K., Noël-Romas, L., Richardson, B., Golden, J. B., Cartwright, M., Collier, A. C., Stevens, C. E., Curlin, M. E., Holtz, T. H., Mugo, N., Irungu, E., Katabira, E., … Hladik, F. (2020). Treatment with Commonly Used Antiretroviral Drugs Induces a Type I/III Interferon Signature in the Gut in the Absence of HIV Infection. *Cell Reports Medicine*, *1*(6), 100096. https://doi.org/10.1016/j.xcrm.2020.100096

Iacob, S. A., Iacob, D. G., & Jugulete, G. (2017). Improving the Adherence to Antiretroviral Therapy, a Difficult but Essential Task for a Successful HIV Treatment—Clinical Points of View and Practical Considerations. *Frontiers in Pharmacology*, *8*, 831. https://doi.org/10.3389/fphar.2017.00831

Iketleng, T., Moyo, S., Gaseitsiwe, S., Nyombi, B., Mitchell, R. M., Makhema, J., Baum, M. K., Marlink, R., Essex, M., & Musonda, R. (2016). Plasma Cytokine Levels in Chronic Asymptomatic HIV-1 Subtype C Infection as an Indicator of Disease

Progression in Botswana: A Retrospective Case Control Study. *AIDS Research and Human Retroviruses*, *32*(4), 364–369. https://doi.org/10.1089/AID.2015.0163

Januškevica, I., Rozentāle, B., Hagina, E., Eglīte, J., Kolupajeva, T., Storozenko, J., Guseva, L., & Lejnieks, A. (2016). Role of Interferon-Gamma (Ifn-γ) in Immune Response Regulation in Hiv-1 and Hiv-1 + Mycobacterium Tuberculosis (Tb) Infected Patients / Interferona-gamma (Ifn-γ) Loma Imūnās Atbildes Regulēšanā Pacientiem Ar HIV-1 un HIV-1 + mycobacterium Tuberculosis. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences.*, *70*(4), 211–214. https://doi.org/10.1515/prolas-2016-0033

Ji, J., Sahu, G. K., Braciale, V. L., & Cloyd, M. W. (2005). HIV-1 induces IL-10 production in human monocytes via a CD4-independent pathway. *International Immunology*, *17*(6), 729–736. https://doi.org/10.1093/intimm/dxh252

Jianu, C., Itu-Mureşan, C., Drugan, C., Filipescu, I., Topan, A. V., Jianu, M. E., Morar, I. I., & Bolboacă, S. D. (2021). Evaluation of several serum interleukins as markers for treatment effectiveness in naïve HIV infected patients: A pilot study. *PLOS ONE*, *16*(11), e0260007. https://doi.org/10.1371/journal.pone.0260007

KAIS. (2017). *Kenya—Kenya Aids Indicator Survey 2017*. https://statistics.knbs.or.ke/nada/index.php/catalog/21

Katsikis, P. D., Mueller, Y. M., & Villinger, F. (2011). The Cytokine Network of Acute HIV Infection: A Promising Target for Vaccines and Therapy to Reduce Viral Set-Point? *PLOS Pathogens*, *7*(8), e1002055. https://doi.org/10.1371/journal.ppat.1002055

Kedzierska, K., & Crowe, S. M. (2001). Cytokines and HIV-1: Interactions and clinical implications. *Antiviral Chemistry & Chemotherapy*, *12*(3), 133–150. https://doi.org/10.1177/095632020101200301

Kioko, M. T., & Pertet, A. M. (2017). Factors contributing to antiretroviral drug adherence among adults living with HIV or AIDS in a Kenyan rural community. *African Journal of Primary Health Care & Family Medicine*, *9*(1), e1–e7. https://doi.org/10.4102/phcfm.v9i1.1343

Klatt, N. R., Chomont, N., Douek, D. C., & Deeks, S. G. (2013). Immune activation and HIV persistence: Implications for curative approaches to HIV infection. *Immunological Reviews*, *254*(1), 326–342. https://doi.org/10.1111/imr.12065

Künzli, M., & Masopust, D. (2023). CD4+ T cell memory. *Nature Immunology*, *24*(6), 903–914. https://doi.org/10.1038/s41590-023-01510-4

Kwon, Y.-S., Daley, C. L., & Koh, W.-J. (2019). Managing antibiotic resistance in nontuberculous mycobacterial pulmonary disease: Challenges and new approaches. *Expert Review of Respiratory Medicine*, *13*(9), 851–861. https://doi.org/10.1080/17476348.2019.1638765

Le Saout, C., Lane, H. C., & Catalfamo, M. (2012). The Role of Cytokines in the Pathogenesis and treatment of HIV Infection. *Cytokine & Growth Factor Reviews*, *23*(0), 207–214. https://doi.org/10.1016/j.cytogfr.2012.05.007

Leonard, R., Zagury, D., Desportes, I., Bernard, J., Zagury, J. F., & Gallo, R. C. (1988). Cytopathic effect of human immunodeficiency virus in T4 cells is linked to the last stage of virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(10), 3570–3574.

Levings, M. K., Bacchetta, R., Schulz, U., & Roncarolo, M. G. (2002). The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. *International Archives of Allergy and Immunology*, *129*(4), 263–276. https://doi.org/10.1159/000067596

Ling, B., Santiago, M. L., Meleth, S., Gormus, B., McClure, H. M., Apetrei, C., Hahn, B. H., & Marx, P. A. (2003). Noninvasive Detection of New Simian Immunodeficiency Virus Lineages in Captive Sooty Mangabeys: Ability To Amplify Virion RNA from Fecal Samples Correlates with Viral Load in Plasma. *Journal of Virology*, *77*(3), 2214–2226. https://doi.org/10.1128/JVI.77.3.2214-2226.2003

Liu, J., Ding, C., Shi, Y., Wang, Y., Zhang, X., Huang, L., Fang, Q., Shuai, C., Gao, Y., & Wu, J. (2024). Advances in Mechanism of HIV-1 Immune Reconstitution Failure: Understanding Lymphocyte Subpopulations and Interventions for Immunological Nonresponders. *The Journal of Immunology*, *212*(11), 1609–1620. https://doi.org/10.4049/jimmunol.2300777

Lopez, C. A., Vazquez, M., Hill, M. D., Del C. Colon, M., Porrata-Doria, T., Johnston, I. C. D., & Lorenzo, E. (2010). Characterization of HIV-1 RNA forms in the plasma of patients undergoing successful HAART. *Archives of Virology*, *155*(6), 895–903. https://doi.org/10.1007/s00705-010-0659-3

Maharaj, N. R., Phulukdaree, A., Nagiah, S., Ramkaran, P., Tiloke, C., & Chuturgoon, A. A. (2017). Pro-Inflammatory Cytokine Levels in HIV Infected and Uninfected Pregnant Women with and without Preeclampsia. *PloS One*, *12*(1), e0170063. https://doi.org/10.1371/journal.pone.0170063

Mahmood, S., Karanja, P. S., & Mwenga, M. A. (2020). FACTORS ASSOCIATED WITH TREATMENT SUCCESS AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED ADOLESCENTS AT THE COASTAL GENERAL HOSPITAL, MOMBASA COUNTY. *Journal of Health, Medicine and Nursing*, *5*(1), Article 1.

Maia, A., Tarannum, M., Lérias, J. R., Piccinelli, S., Borrego, L. M., Maeurer, M., Romee, R., & Castillo-Martin, M. (2024). Building a Better Defense: Expanding and Improving Natural Killer Cells for Adoptive Cell Therapy. *Cells*, *13*(5), Article 5. https://doi.org/10.3390/cells13050451

Maina, W. K., Kim, A. A., Rutherford, G. W., Harper, M., K'Oyugi, B. O., Sharif, S., Kichamu, G., Muraguri, N. M., Akhwale, W., De Cock, K. M., & KAIS Study Group. (2014). Kenya AIDS Indicator Surveys 2007 and 2012: Implications for public health policies for HIV prevention and treatment. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *66 Suppl 1*(Suppl 1), S130-137. https://doi.org/10.1097/QAI.0000000000000123

Mavarani, L., Albayrak-Rena, S., Potthoff, A., Hower, M., Dolff, S., Sammet, S., Maischack, F., Schadendorf, D., Schmidt, B., Esser, S., & on behalf of HIV HEART AGING Study. (2023). Changes in body mass index, weight, and waist-to-hip ratio over five years in HIV-positive individuals in the HIV Heart Aging Study compared to the general population. *Infection*, *51*(4), 1081–1091. https://doi.org/10.1007/s15010-023-02009-8

Mayer, K. H., & Venkatesh, K. K. (2010). Antiretroviral therapy as HIV prevention: Status and prospects. *American Journal of Public Health*, *100*(10), 1867–1876. https://doi.org/10.2105/AJPH.2009.184796

Mills, E. J., Nachega, J. B., Buchan, I., Orbinski, J., Attaran, A., Singh, S., Rachlis, B., Wu, P., Cooper, C., Thabane, L., Wilson, K., Guyatt, G. H., & Bangsberg, D. R. (2006). Adherence to Antiretroviral Therapy in Sub-Saharan Africa and North AmericaA Meta-analysis. *JAMA*, *296*(6), 679–690. https://doi.org/10.1001/jama.296.6.679

MoH. (2018). *Kenya HIV Estimates Report 2018. Kenya HIV Estimates.* https://www.health.go.ke/

Mohammadi, A., Etemad, B., Zhang, X., Li, Y., Bedwell, G. J., Sharaf, R., Kittilson, A., Melberg, M., Crain, C. R., Traunbauer, A. K., Wong, C., Fajnzylber, J., Worrall, D. P., Rosenthal, A., Jordan, H., Jilg, N., Kaseke, C., Giguel, F., Lian, X., … Li, J. Z. (2023). Viral and host mediators of non-suppressible HIV-1 viremia. *Nature Medicine*, *29*(12), 3212–3223. https://doi.org/10.1038/s41591-023-02611-1

Moro-García, M. A., Mayo, J. C., Sainz, R. M., & Alonso-Arias, R. (2018). Influence of Inflammation in the Process of T Lymphocyte Differentiation: Proliferative, Metabolic, and Oxidative Changes. *Frontiers in Immunology*, *9*, 339. https://doi.org/10.3389/fimmu.2018.00339

Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., & Coffman, R. L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology (Baltimore, Md.: 1950)*, *136*(7), 2348–2357.

Mukui, I. N., Ng'ang'a, L., Williamson, J., Wamicwe, J. N., Vakil, S., Katana, A., & Kim, A. A. (2016). Rates and Predictors of Non-Adherence to Antiretroviral Therapy among HIV-Positive Individuals in Kenya: Results from the Second Kenya AIDS

Indicator Survey, 2012. *PLOS ONE*, *11*(12), e0167465. https://doi.org/10.1371/journal.pone.0167465

Musa, F., Shaviya, N., Mambo, F., Abonyo, C., Barasa, E., Wafula, P., Sowayi, G., Barasa, M., & Were, T. (2021). Cytokine profiles in highly active antiretroviral treatment non-adherent, adherent and naive HIV-1 infected patients in Western Kenya. *African Health Sciences*, *21*(4), 1584–1592. https://doi.org/10.4314/ahs.v21i4.12

Nabukeera-Barungi, N., Elyanu, P., Asire, B., Katureebe, C., Lukabwe, I., Namusoke, E., Musinguzi, J., Atuyambe, L., & Tumwesigye, N. (2015). Adherence to antiretroviral therapy and retention in care for adolescents living with HIV from 10 districts in Uganda. *BMC Infectious Diseases*, *15*, 520. https://doi.org/10.1186/s12879-015-1265-5

NASCOP. (2018). *Kenya HIV estimates.* https://www.nascop.or.ke/

Nittayananta, W., Amornthatree, K., Kemapunmanus, M., Talungchit, S., & Sriplung, H. (2014). Expression of oral cytokines in HIV-infected subjects with long-term use of antiretroviral therapy. *Oral Diseases*, *20*(3), e57–e64. https://doi.org/10.1111/odi.12135

Oluoch, R. P., Mutinda, D., Karama, M., Oundo, J., & Nganga, Z. (2019). Community perceptions affecting uptake & retention on antiretroviral therapy by PLHIV: A qualitative study among residents of an urban informal settlement in Kenya. *African Journal of Health Sciences*, *32*(1), Article 1.

Osuji, F. N., Onyenekwe, C. C., Ahaneku, J. E., & Ukibe, N. R. (2018). The effects of highly active antiretroviral therapy on the serum levels of pro-inflammatory and

anti-inflammatory cytokines in HIV infected subjects. *Journal of Biomedical Science*, *25*(1), 88. https://doi.org/10.1186/s12929-018-0490-9

Pantazis, N., Papastamopoulos, V., Antoniadou, A., Adamis, G., Paparizos, V., Metallidis, S., Sambatakou, H., Psichogiou, M., Chini, M., Chrysos, G., Panagopoulos, P., Sipsas, N. V., Barbunakis, E., Gogos, C., & Touloumi, G. (2022). Changes in Body Mass Index after Initiation of Antiretroviral Treatment: Differences by Class of Core Drug. *Viruses*, *14*(8). https://doi.org/10.3390/v14081677

Phiri, M. M., Schaap, A., Simwinga, M., Hensen, B., Floyd, S., Mulubwa, C., Simuyaba, M., Chiti, B., Bond, V., Shanaube, K., Fidler, S., Hayes, R., Ayles, H., & Team, the H. 071 (PopART) S. (2022). Closing the gap: Did delivery approaches complementary to home-based testing reach men with HIV testing services during and after the HPTN 071 (PopART) trial in Zambia? *Journal of the International AIDS Society*, *25*(1), e25855. https://doi.org/10.1002/jia2.25855

Pillay, P., Moodley, K., Vatish, M., Moodley, J., Duarte, R., & Mackraj, I. (2020). Exosomal Th1/Th2 cytokines in preeclampsia and HIV-positive preeclamptic women on highly active anti-retroviral therapy. *Cytokine*, *125*, 154795. https://doi.org/10.1016/j.cyto.2019.154795

Poles, M. A., Elliott, J., Taing, P., Anton, P. A., & Chen, I. S. Y. (2001). A Preponderance of CCR5+ CXCR4+ Mononuclear Cells Enhances Gastrointestinal Mucosal Susceptibility to Human Immunodeficiency Virus Type 1 Infection. *Journal of Virology*, *75*(18), 8390–8399. https://doi.org/10.1128/JVI.75.18.8390-8399.2001

Reeves, D. B., Duke, E. R., Wagner, T. A., Palmer, S. E., Spivak, A. M., & Schiffer, J. T. (2018). A majority of HIV persistence during antiretroviral therapy is due to

infected cell proliferation. *Nature Communications*, *9*(1), 4811. https://doi.org/10.1038/s41467-018-06843-5

Reuter, M. A., Pombo, C., & Betts, M. R. (2012). Cytokine production and dysregulation in HIV pathogenesis: Lessons for development of therapeutics and vaccines. *Cytokine & Growth Factor Reviews*, *23*(0), 181–191. https://doi.org/10.1016/j.cytogfr.2012.05.005

Roff, S. R., Noon-Song, E. N., & Yamamoto, J. K. (2014). The Significance of Interferon-γ in HIV-1 Pathogenesis, Therapy, and Prophylaxis. *Frontiers in Immunology*, *4*, 498. https://doi.org/10.3389/fimmu.2013.00498

Schaecher, K. L. (2013). The importance of treatment adherence in HIV. *The American Journal of Managed Care*, *19*(12 Suppl), s231-237.

Seid, A., Seid, O., Workineh, Y., Dessie, G., & Bitew, Z. W. (2023). Prevalence of undernutrition and associated factors among adults taking antiretroviral therapy in sub-Saharan Africa: A systematic review and meta-analysis. *PLOS ONE*, *18*(3), e0283502. https://doi.org/10.1371/journal.pone.0283502

Shalev, I., Schmelzle, M., Robson, S. C., & Levy, G. (2011). Making sense of regulatory T cell suppressive function. *Seminars in Immunology*, *23*(4), 282–292. https://doi.org/10.1016/j.smim.2011.04.003

Shaw, G. M., & Hunter, E. (2012). HIV transmission. *Cold Spring Harbor Perspectives in Medicine*, *2*(11), a006965. https://doi.org/10.1101/cshperspect.a006965

Shebl, F. M., Yu, K., Landgren, O., Goedert, J. J., & Rabkin, C. S. (2012). Increased levels of circulating cytokines with HIV-related immunosuppression. *AIDS Research and Human Retroviruses*, *28*(8), 809–815. https://doi.org/10.1089/AID.2011.0144

Shoko, C., & Chikobvu, D. (2019). A superiority of viral load over CD4 cell count when predicting mortality in HIV patients on therapy. *BMC Infectious Diseases*, *19*(1), 169. https://doi.org/10.1186/s12879-019-3781-1

Siddiqui, J., Samuel, S. K., Hayward, B., Wirka, K. A., Deering, K. L., Harshaw, Q., Phillips, A., & Harbour, M. (2022). HIV-associated wasting prevalence in the era of modern antiretroviral therapy. *AIDS (London, England)*, *36*(1), 127. https://doi.org/10.1097/QAD.0000000000003096

Sivori, S., Vacca, P., Del Zotto, G., Munari, E., Mingari, M. C., & Moretta, L. (2019). Human NK cells: Surface receptors, inhibitory checkpoints, and translational applications. *Cellular and Molecular Immunology*, *16*(5), 430–441. https://doi.org/10.1038/s41423-019-0206-4

Stricker, S. M., Fox, K. A., Baggaley, R., Negussie, E., de Pee, S., Grede, N., & Bloem, M. W. (2014). Retention in Care and Adherence to ART are Critical Elements of HIV Care Interventions. *AIDS and Behavior*, *18*(5), 465–475. https://doi.org/10.1007/s10461-013-0598-6

Sun, W., Gao, C., Hartana, C. A., Osborn, M. R., Einkauf, K. B., Lian, X., Bone, B., Bonheur, N., Chun, T.-W., Rosenberg, E. S., Walker, B. D., Yu, X. G., & Lichterfeld, M. (2023). Phenotypic signatures of immune selection in HIV-1 reservoir cells. *Nature*, *614*(7947), 309–317. https://doi.org/10.1038/s41586-022-05538-8

Tesfaye, D. J., Hibistu, D. T., Abebo, T. A., Asfaw, F. T., Lukas, K., Laelago, T., Turuse, E. A., Kebede, H. G., Altaye, A. A., & Bekele, F. B. (2019). Option B plus antiretroviral therapy adherence and associated factors among HIV positive

pregnant women in Southern Ethiopia. *BMC Pregnancy and Childbirth*, *19*(1), 82. https://doi.org/10.1186/s12884-019-2228-4

Theron, A. J., Anderson, R., Rossouw, T. M., & Steel, H. C. (2017). The Role of Transforming Growth Factor Beta-1 in the Progression of HIV/AIDS and Development of Non-AIDS-Defining Fibrotic Disorders. *Frontiers in Immunology*, *8*, 1461. https://doi.org/10.3389/fimmu.2017.01461

Thet, D., & Siritientong, T. (2020). Antiretroviral Therapy-Associated Metabolic Complications: Review of the Recent Studies. *HIV/AIDS (Auckland, N.Z.)*, *12*, 507. https://doi.org/10.2147/HIV.S275314

Tsibris, A. M. N., & Hirsch, M. S. (2010). Antiretroviral therapy in the clinic. *Journal of Virology*, *84*(11), 5458–5464. https://doi.org/10.1128/JVI.02524-09

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., & Ugolini, S. (2008). Functions of natural killer cells. *Nature Immunology*, *9*(5), 503–510. https://doi.org/10.1038/ni1582

Ward, A. R., Mota, T. M., & Jones, R. B. (2021). Immunological approaches to HIV cure. *Seminars in Immunology*, *51*, 101412. https://doi.org/10.1016/j.smim.2020.101412

Watanabe, D., Uehira, T., Suzuki, S., Matsumoto, E., Ueji, T., Hirota, K., Minami, R., Takahama, S., Hayashi, K., Sawamura, M., Yamamoto, M., & Shirasaka, T. (2019). Clinical characteristics of HIV-1-infected patients with high levels of plasma interferon-γ: A multicenter observational study. *BMC Infectious Diseases*, *19*(1), 11. https://doi.org/10.1186/s12879-018-3643-2

Wekesa, E. (2019). HIV testing experiences in Nairobi slums: The good, the bad and the ugly. *BMC Public Health*, *19*(1), 1600. https://doi.org/10.1186/s12889-019-7975-7

Williams, A., Steffens, F., Reinecke, C., & Meyer, D. (2013). The Th1/Th2/Th17 cytokine profile of HIV-infected individuals: A multivariate cytokinomics approach. *Cytokine*, *61*(2), 521–526. https://doi.org/10.1016/j.cyto.2012.11.006

Younas, M., Psomas, C., Reynes, J., & Corbeau, P. (2016). Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. *HIV Medicine*, *17*(2), 89–105. https://doi.org/10.1111/hiv.12310

Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., & Li, Y. (2021). Inflammation and tumor progression: Signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy*, *6*(1), 1–46. https://doi.org/10.1038/s41392-021-00658-5

## APPENDICES

### APPENDIX I: CONSENT FORM
### CONSENT FORM (ENGLISH VERSION)

### INTRODUCTION

Good morning/afternoon, my name is..... I'm researching within the Siaya county referral hospital. The purpose of this research is to determine cytokines levels among ART defaulter's HIV infected patients attending Siaya county referral hospital. This information will assist the researcher from Masinde Muliro University of Science and Technology - Masters of Science in Medical Laboratory Sciences and also an employee of Kakamega county government, to understand the above aspects and advice accordingly.

Regarding this, I would like to ask you some questions. Some of the questions are personal, but the answers you give will be confidential. They will only assist the researcher to learn more about the benefits and challenges of ART programs. Your participation in the study is voluntary and therefore you are not obligated to participate. However, we would appreciate your participation. I would like to interview you and collect some blood from you and all the information you provide and also result in getting will be kept in strict confidence therefore not exposing you to any kind of victimization and will only be used for this study. I do appreciate your time and contribution.

Do I have your permission to continue?

..... Yes (proceed with interview)

..... No (end the interview)

Respondent's signature or thumbprint: .....

(Interviewer arranges for a private setting to conduct the interview)

Date: .....

Time start: .....

Time end: .....

**APPENDIX II: QUESTIONNAIRE**

**SECTION A: PERSONAL DATA**

1. Age - …….

2. Educational Level…… Non formal education [ ] Primary level [ ]

Junior high/JSS [ ] senior high/SSS/Secondary [ ] Tertiary/Post-secondary [ ]

3. Marital status Single [ ] Married [ ] Divorce [ ] Widow [ ]

4. Place of Residence

5. Clinic Where You Used to Take Drugs

**SECTION B: HIV KNOWLEDGE**

**To see how much, you know about the virus (HIV) and AIDS. This is not a test, so feel free to say you "don't know" if you do not know the answer.**

***Please answer either "true", "false" or "don't know" to the questions below***

| | | True | False | Don't Know |
|---|---|---|---|---|
| **1** | It is possible to get HIV from having many sexual partners | | | |
| **2** | It is possible to get HIV from a blood transfusion | | | |
| **3** | It is possible to get HIV from using public toilets | | | |
| **4** | It is possible to get HIV from donating blood | | | |
| **5** | It is possible to get HIV from mosquito bites | | | |
| **6** | It is possible to get HIV from sharing food/utensils with a person who has HIV/AIDS | | | |
| **7** | A person infected with HIV can transmit the virus to his/her sexual partner during sex without a condom | | | |
| **8** | Using a condom every time you have sex can protect against HIV transmission baby during pregnancy | | | |

| | | | | |
|---|---|---|---|---|
| **9** | A pregnant woman with HIV can transmit the virus to her baby during delivery | | | |
| **10** | A woman with HIV can transmit the virus to her baby during breastfeeding | | | |
| **11** | All pregnant women with HIV/AIDS will have babies born with HIV | | | |
| **12** | You can always tell that a person has HIV/AIDS by just looking at him/her | | | |
| **13** | There is a cure for HIV/AIDS | | | |
| **14** | Taking HIV medication (ARVs) can help reduce severity of HIV infection | | | |

**SECTION C: HIV Treatment Knowledge (TK)**

***Please answer either "true", "false" or "don't know" to the questions below***

| | | **True** | **false** | **I Don't Know** |
|---|---|---|---|---|
| **1** | Once the HIV viral load results are 'undetectable', HIV medications should be stopped | | | |
| **2** | If HIV medications are not taken at the right time of day, HIV drug resistance can occur | | | |
| **3** | HIV is cured when the HIV viral load blood test result is 'undetectable' | | | |
| **4** | Condoms during sex are not needed when the HIV viral load blood test results are at 'undetectable' levels | | | |

| | | | | |
|---|---|---|---|---|
| **5** | It is better to take a half dose of HIV medications than stopping HIV medications completely. | | | |
| **6** | Treatments are available to reduce HIV medication side effects. | | | |
| **7** | Recreational drugs (e.g., cocaine) can affect the effectiveness of HIV medications. | | | |
| **8** | There currently exists an HIV vaccine that prevents HIV infection | | | |

**SECTION D: HIV MEDICATION ADHERENCE**

Please tell us what you are doing. Don't worry about telling us that you don't take all your pills. We need to know what is happening, not what you think we "want to hear."

***The following questions are about your HIV medication regimen. If you took only a portion of a dose on one or more of these days, please report the dose(s) as being missed.***

**During the past 4 days, on how many days have you missed taking all your doses?**

0 □ None

1 □ One day

**Most HIV medications need to be taken on a schedule, such as "2 times a day" or "3 times a day" or "every 8 hours." How closely did you follow your specific schedule over the last four days?**

0 □ None of the days

1 □ 1 day

2 □ 2 days

3 □ 3 days

4 □ All 4 days

**Does any of your HIV medications have special instructions, such as "take with food" or "on an empty stomach" or "with plenty of fluids?"**

1 □ Yes

2 □ No

**If Yes**, how often did you follow those special instructions over the last **four** days?

0 □ None of the days

1 □ 1 day

2 □ 2 days

3 □ 3 days

4 □ All 4 days

**Some people find that they forget to take their pills on the weekend days. Did you miss any of your HIV medications last weekend— last Saturday or Sunday?**

1 □ Yes

2 □ No

**When was the last time you missed any of your HIV medications?** Check one.

0 □ Within the past **week**

1 □ 1-2 **weeks** ago

2 □ 2-4 **weeks** ago

3 □ 1-3 **months** ago

4 □ More than 3 **months** ago

5 □ **Never** skipped/missed medications

During the last 7 days how many times, in total did you take one or more of your ARV'S_____

How would you rate your adherence over the last months (tick one)?

0 □ Very Poor

1 □ poor

2 □ Fair

3 □ Good

4 □ Very Good

5 □ **Excellent**

**Section E. Morisky Medication Adherence Scale (NASCOP 2016)**

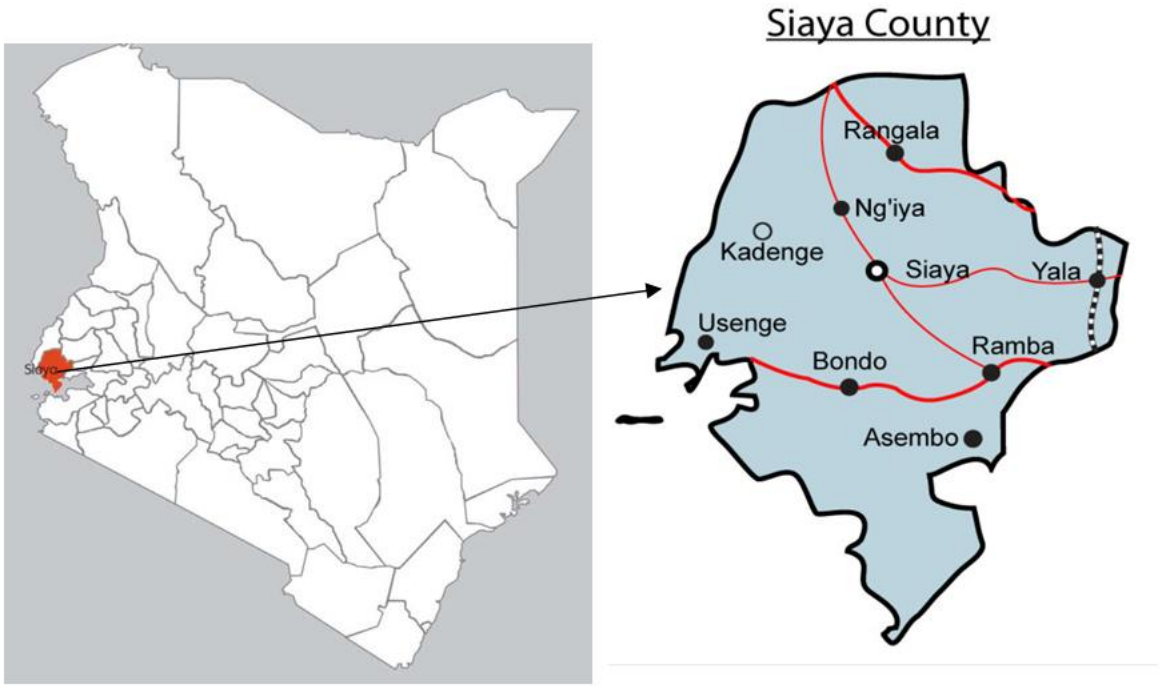
| **MMAS-8**: *Ask the patient each question below. Circle the corresponding score for each response. After completion of all questions, add up all the points you have circled for the total score.* | | | |
|---|---|---|---|
| **NO** | **Question** | **YES** | **NO** |
| 1. | Do you ever forget to take your medicine? | 1 | 0 |
| 2. | Are you careless at times about taking your medicine? | 1 | 0 |
| 3. | Sometimes if you feel worse when you take the medicine, do you stop taking it? | 1 | 0 |
| 4. | When you feel better, do you sometimes stop taking your medicine? | 1 | 0 |
| 5. | Did you take your medicine yesterday? | 0 | 1 |
| 6. | When you feel like your symptoms are under control, do you sometimes stop taking your medicine? | 1 | 0 |
| 7. | Taking medication every day is a real inconvenience for some people. Do you ever feel under pressure about sticking to your treatment plan? | 1 | 0 |
| 8. | How often do you have difficulty remembering to take all of your medications? (Please circle the correct answer below)<br>A. Never/rarely<br>B. Once in a while<br>C. Sometimes<br>D. Usually<br>E. All of the time | A. 0<br>B. ¼<br>C. ½<br>D. ¾<br>E. 1 | |
| 9. | Total Score (sum of all items) | | |

**Interpretation of MMAS-8 Score**

| **MMAS-8 Score** | **Adherence Rating** | **Action Required** |
|---|---|---|
| 0 | Good | Continue with routine monitoring, counseling, and support |
| 1-2 | Inadequate | • Discuss at MDT<br>• Assign a client-specific case manager<br>• Assess barriers to adherence |

| | | |
|---|---|---|
| | | • Engage treatment support staff in adherence counseling sessions<br>• Follow-up in two to four weeks |
| 3-8 | Poor | • Review client file at next MDT meeting<br>• Assign a client-specific case manager<br>• Assess for barriers to adherence, and develop solutions to address each<br>• Engage treatment support staff in adherence counseling sessions<br>• Implement DOT<br>• Follow-up in one to two weeks |

**APPENDIX III: STUDY SITE MAP**




Showing the map of Kenya and Siaya County

## APPENDIX IV: APPROVAL LETTER

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

**Ref:** MMU/COR: 509099

**Date:** 26th October, 2020

Fauzia N. Musa,
HML/G/01/2016,
P.O. Box 190-50100,
**KAKAMEGA.**

Dear Mr. Fauzia,

**RE: APPROVAL OF PROPOSAL**

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your masters proposal entitled: ***"Interferon-gamma and Interlukin-10 Derangements in HIV-1 Antiretroviral Non-adhearent Patients attending Siaya County Referral Hospital, Siaya County Kenya"*** and appointed the following as supervisors:

1. Dr. Nathan M. Shaviya - SPHBST, MMUST
2. Mr. George A. Sowayi - SPHBST, MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

SCHOOL OF GRADUATE STUDIES
MASINDE MULIRO UNIVERSITY
OF SCIENCE & TECHNOLOGY
Date:

Prof. John Obiri
**DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES**

## APPENDIX V: INSTITUTIONAL ETHICS COMMITTEE (IERC) LETTER

**MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY**

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega, Kenya

**Institutional Ethics Review Committee (IERC)**

**Ref:** MMU/COR: 403012 Vol 3 (01)

**Date:** 11th March, 2021

Fauzia Musa,
Masinde Muliro University of Science and Technology,
P.O. Box 190-50100,
Kakamega.

Dear Ms. Fauzia Musa,

**RE: Interferon-Gamma and Interleukin-10 Derangements in HIV-1 Antiretroviral Non-Adherent Patients Attending Siaya County Referral Hospital, Siaya County, Kenya. - MMUST/IERC/177/2021**

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with no further revisions) the above Referenced application for one year.

This approval is valid from **11th March, 2021**through to **11th March, 2022**. Please note that authorization to conduct this study will automatically expire on by **11th March, 2022.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **11th February, 2022.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC Secretariat by **11th February, 2022.** You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/event resulting from the conduct of this study must be reported to MMUST IERC. Also note that you are required to seek for research permit from NACOSTI prior to the initiation of the study.

Yours faithfully,

MAR 2020

Dr. Gordon Nguka (PhD)
**Chairman, Institutional Ethics Review Committee**

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

## APPENDIX VI: NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 620716

Date of Issue: 26/April/2021

### RESEARCH LICENSE

This is to Certify that Ms.. Fauzia Musa of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Siaya on the topic: INTERFERON –GAMMA AND INTERLUKIN -10 DERANGEMENTS IN HIV-1 ANTIRETROVIRAL NON-ADHEARENT PATIENTS ATTENDING SIAYA COUNTY REFERRAL HOSPITAL, SIAYA COUNTY, KENYA for the period ending : 26/April/2022.

License No: NACOSTI/P/21/10115

620716

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.